# Molecular and Physiological Consequences of Prematurity and Blood Pressure Elevation on the Young Adult Left Ventricular Stress Response

**ODARO J HUCKSTEP**
**BA, MSc**

**Thesis presented for Doctor of Philosophy degree in Cardiovascular Medicine**
**Supervisors: Professor Paul Leeson,**
**Dr Adam Lewandowski**
**September 2018**
**Approximate word count: ~40,000 words**




**Wadham College, University of Oxford**
**&**
**Cardiovascular Clinical Research Facility**
**Division of Cardiovascular Medicine**
**Radcliffe Department of Medicine**
**University of Oxford**

# ACKNOWLEDGEMENTS

I am deeply grateful for the wonderful personal and organisational support I received that was essential to allowing me to complete this research. The tireless dedication of my wife Amanda was absolutely vital to providing the encouragement and support I needed in addition to being a stalwart pillar of strength for our family during my very many hours away. To our two children, Jenna and Mason, I'm blessed to be your dad, every day you reminded me of what is important in life. I very much appreciate the United States Air Force Academy and Institute of Technology for allowing me to pursue this DPhil and for funding my research fellowship. My two supervisors Paul Leeson and Adam Lewandowski were instrumental in guiding my research efforts and training me to think like a researcher; as mentors, they provided necessary blend of motivation, support, patience and encouragement to keep me progressing and working through the excitement and challenges of research. Wilby Williamson, you were definitely my brother during my DPhil, thanks for teaching me the new skills I needed to successfully transition to the clinical research arena, it was great to run this race together. Afifah Mohamed, you were my sister in research and also a great colleague and encouragement, thanks for your positive attitude and incredible work ethic. Mariane, your positive energy and prowess in all things angiotensin are without equal. Harry Boardman and Christina Aye, I was fortunate to know you and learn from your exceptional examples of what it looks like to be a high quality DPhil student. Fernando Telles, you are a gifted clinician and researcher, your image analysis support is greatly appreciated. Yvonne, Nancy and Polly, thanks for keeping things both fun and productive and for answering just about every random question I came up with, crack on. Charlotte, thanks for all the echo work, wish you the best in Jersey. Cheryl and Maryam, I'm glad our time overlapped by almost a year, thanks for bringing new energy into CCRF, best of luck with your research. Svetlana Reilly and Oliver Rider, thank you for the very useful advice and guidance during my transfer of status. Scott, Cathy and Ben, you ensured my MSc experience prepared me for this later academic work. Dave, thanks for taking me fishing, those were great times. Holger, you are a terrific physiologist and a true renaissance man, it was great to work with you on the exercise physiology studies. To the entire team at OCMR, thanks for being outstanding professionals and colleagues. To Ashley, Grace, Phil, Aggy, Aiden, Annette, Jennie, Rachel, Maria, Laura, Linda, Yrsa, Timo, Rob, Angus, Ross, Adja, thank you. To my dear mom, thanks for all the prayers and always believing in me. And to the creator of all things, thank you giving me a thirst for knowledge and so many streams to drink from.

My graduate work for this thesis was funded by The United States Air Force Institute of Technology. The views expressed in this dissertation are my own and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the U.S. Government.

# DEDICATIONS

*For Isaac, Kent, and Thomas Stanton*

# ABBREVIATIONS

| | |
|---|---|
| ABP | Ambulatory blood pressure |
| AIx | Augmentation index |
| BMI | Body mass index |
| BP | Blood pressure |
| BPM | Beats per minute |
| BSA | Body surface area |
| DALY | Disability adjusted life year |
| DBP | Diastolic blood pressure |
| CMR | Cardiac magnetic resonance |
| EDV | End diastolic volume |
| ESV | End systolic volume |
| EF | Ejection fraction |
| FVC | Forced vital capacity |
| FEV1 | Forced expiratory volume in 1 second |
| HDL | High density lipoprotein |
| LDL | Low density lipoprotein |
| LV | Left ventricle |
| LVH | Left ventricular hypertrophy |
| MVV | Maximum ventilatory volume |
| PWV | Pulse wave velocity |
| SBP | Systolic blood pressure |
| SD | Standard deviation |
| TOD | Target organ damage |

# ABSTRACT

Cardiovascular disease is the leading cause of global mortality. Acute disease usually strikes later in life, but risk trajectories are established earlier and are impacted by factors including preterm birth and blood pressure elevation. Prematurity results in increased left ventricular (LV) mass and is a risk factor for hypertensive disorders and heart failure later in life. Blood pressure elevation also contributes to increased LV mass and cardiovascular risk, and is increasingly prevalent in young adults. This thesis used purposive recruitment of young adults with a range of blood pressures and birth histories (preterm-born and term-born) and employed deep baseline cardiovascular phenotyping combined with exercise stress assessments to characterise LV stress response phenotypes in young adults born preterm, or with blood pressure elevation. Those born preterm were found to have impaired, declining exercise ejection fraction with increasing workload. This defect related to shorter LV length, a characteristic structural feature in the preterm-born. Preterm-born young adults also had a lower aerobic capacity than controls, which was commensurate with their exercise systolic impairment. In young adults with elevated blood pressure, it was found that submaximal exercise produced greater fatigue and impaired LV systolic performance vs normotensive controls. Blood pressure elevation was also associated with lower exercise capacity and enjoyment of physical activity and also sub-clinical LV mass increase. Collectively, these findings suggest preterm-born young adults have reduced myocardial functional reserve which likely contributes to risk of future disease including heart failure. Continued research is needed to better understand risk stratification and

management for this population. In the blood pressure studies, the dysfunctional exercise stress response and lower aerobic capacity may indicate greater risk, while lower enjoyment of physical activity may confound exercise prescription. Future studies are needed to identify intervention strategies to optimally manage physiological and experiential challenges in this growing population.

# LIST OF PUBLICATIONS

## ORIGINAL MANUSCRIPTS PUBLISHED DURING DPHIL

**Huckstep O**, Williamson W, Telles F, Burchert H, Bertagnolli M, Herdman C, Arnold L, Smillie R, Mohamed A, Boardman H *et al*: Physiological Stress Elicits Impaired Left Ventricular Function in Preterm-Born Adults. *J Am Coll Cardiol* 2018, 71(12):1347-1356.

**Huckstep O**, Lewandowski AJ, Leeson P. Invited Commentary: Hypertension During Pregnancy and Offspring Microvascular Structure-Insights From the Retinal Microcirculation. *Am J Epidemiol*. 2016 Nov 1;184(9):616-618.

Williamson W, Foster C, Reid H, Kelly P, Lewandowski AJ, Boardman H, Roberts N, McCartney D, **Huckstep O**, Newton J *et al*: Will Exercise Advice Be Sufficient for Treatment of Young Adults With Prehypertension and Hypertension? A Systematic Review and Meta-Analysis. *Hypertension* 2016, 68(1):78-87.

Mohamed A, Lewandowski AJ, **Huckstep O**, Leeson P: Can diet influence cardiac geometry and function in young adults? *Eur J Prev Cardiol* 2018:2047487318796292.

Williamson W, Lewandowski AJ, Forkert ND, Griffanti L, Okell TW, Betts J, Boardman H, Siepmann T, McKean D, **Huckstep O** *et al*: Association of Cardiovascular Risk Factors With MRI Indices of Cerebrovascular Structure and Function and White Matter Hyperintensities in Young Adults. *Jama* 2018, 320(7):665-673.

## CONFERENCE ABSTRACTS DURING DPHIL

**Huckstep O**, Williamson W, Telles F, Verburg A, Han J, Herdman C, Arnold L, McCance A, Smillie R, Mohamed A, Boardman H, Lewandowski AJ, Leeson P. Young Adults with Elevated Blood Pressure have Reduced Cardiac Response and Greater Perceived Exertion at Low Intensity Exercise. *American Heart Association Scientific Sessions 2018.*

**Huckstep O**, Williamson W, Telles F, Verburg A, Han J, Herdman C, Arnold L, McCance A, Smillie R, Mohamed A, Boardman H, McCormick K, Leeson P, Lewandowski AJ. Blunted Systolic Response to Exercise Underlies Reduced Exercise Capacity in Preterm-born Young Adults. *American Heart Association Scientific Sessions 2018.*

Verburg A, Williamson W, **Huckstep O**, Yu GZ, Mohamed A, Herdman C, Whitworth P, Leeson P, Lewandowski AJ. Soluble Endoglin is an Independent Predictor of Blood Pressure Response to Hemodynamic Stress in Young Adults. *American Heart Association Scientific Sessions 2017.*

# CONTENTS

**ACKNOWLEDGEMENTS** ..... **2**

**DEDICATIONS** ..... **4**

**ABBREVIATIONS** ..... **5**

**ABSTRACT** ..... **6**

**LIST OF PUBLICATIONS** ..... **8**

**LIST OF FIGURES** ..... **20**

**LIST OF TABLES** ..... **22**

**CHAPTER 1 INTRODUCTION** ..... **23**

**1.1 Left Ventricular Structure and Pump Function** ..... **24**

1.1.1 Elements of Myocardial Structure that Affect Cardiac Mechanics 24

1.1.2 Elements of Ventricular Geometry that Affect Systolic Pumping Function ..... 30

**1.2 Cardiovascular Stress** ..... **35**

1.2.1 A Working Definition of Cardiovascular Stress ..... 35

1.2.2 Nominal Cardiovascular Response to Increased Workload ..... 36

1.2.3 Measurements of Cardiovascular Stress ..... 38

**1.3 Relevant Exposures That Impact Left Ventricular Structure and Function……44**

1.3.1 Developmental Exposure ..... 44

1.3.2 Disease ..... 47

1.3.3 Lifestyle ..... 54

**1.4 Thesis Hypotheses and Objectives ..... 62**

**CHAPTER 2 STUDY POPULATION ..... 65**

**2.1 Overview ..... 66**

2.1.1 Personal Contribution ..... 68

2.1.2 Allocation of Study Groups ..... 71

**2.2 Group 1 - Term-born Young Adults ..... 72**

2.2.1 Inclusion Criteria ..... 72

2.2.2 Exclusion Criteria ..... 72

2.2.3 Characteristics ..... 73

**2.3 Group 2 - Preterm-born Young Adults ..... 73**

2.3.1 Inclusion Criteria ..... 73

2.3.2 Exclusion Criteria ..... 73

2.3.3 Characteristics ..... 74

**2.4 Subject Recruitment for Groups 1 and 2 ..... 76**

**2.5 Group 3 - Young Adults with Elevated Blood Pressure ..... 77**

2.5.1 Inclusion Criteria ..... 77
2.5.2 Exclusion Criteria ..... 77
2.5.3 Characteristics ..... 78

**2.6 Group 4 – Young Adults with Normal Blood Pressure ..... 78**
2.6.1 Inclusion Criteria ..... 78
2.6.2 Exclusion Criteria ..... 79

**2.7 Subject Recruitment for Groups 3 and 4 ..... 79**
2.7.1 Characteristics of Groups 3 and 4 ..... 79

**CHAPTER 3 GENERAL METHODS ..... 82**

**3.1 Overview ..... 83**
3.1.1 Study Visit Overview ..... 83

**3.2 Ethical Standard ..... 86**

**3.3 Anthropometry ..... 86**

**3.4 Cardiac Magnetic Resonance (CMR) Imaging ..... 87**
3.4.1 Left Ventricular Structure Quantification ..... 87

**3.5 Venous Blood Sampling ..... 88**
3.5.1 Biochemistry Analysis ..... 89
3.5.2 Copeptin Analysis ..... 89

**3.6 Blood Pressure Measurements ..... 90**

3.6.1 Aortic (Central) Blood Pressure Measurement ..... 90
3.6.2 Resting Peripheral Blood Pressure ..... 91

**3.7 Resting Echocardiography ..... 91**

3.7.1 Echocardiography Analysis ..... 93

**3.8 Spirometry Testing ..... 94**

3.8.1 Cardiopulmonary Exercise Testing (CPET) ..... 95
3.8.2 Stress Echocardiography ..... 96

**3.9 Fitting of Monitoring Devices ..... 97**

3.9.1 24 Hour Ambulatory Blood Pressure Monitoring ..... 97
3.9.2 Activity Monitoring ..... 98

**3.10 Statistical Analysis ..... 98**

**CHAPTER 4 STUDY 1 RESULTS LEFT VENTRICULAR SYSTOLIC RESPONSE TO PHYSIOLOGICAL EXERCISE STRESS IN PRETERM-BORN YOUNG ADULTS ..... 100**

**4.1 Abstract ..... 101**

**4.2 Introduction ..... 102**

**4.3 Methods ..... 103**

4.3.1 Study Population ..... 104
4.3.2 Study Visit ..... 104

4.3.3 Statistical Analysis ..... 105

**4.4 Results ..... 105**

4.4.1 Group Characteristics ..... 105

4.4.2 Resting Echocardiography ..... 108

4.4.3 Exercise Stress Echocardiography ..... 110

**4.5 Discussion ..... 118**

4.5.1 Potential Mechanistic Links between Preterm Birth and Heart Failure ..... 118

4.5.2 Left Ventricular Structure and Function, Early Life History, and Cardiac Risk in the Preterm-born Population ..... 123

4.5.3 Study 1 Limitations ..... 127

**4.6 Conclusions ..... 130**

**CHAPTER 5 STUDY 2 RESULTS EXERCISE CAPACITY AND LEFT VENTRICULAR FUNCTION IN THE PRETERM-BORN YOUNG ADULT CARDIAC PHENOTYPE ..... 132**

**5.1 Abstract ..... 133**

**5.2 Introduction ..... 134**

**5.3 Methods ..... 136**

5.3.1 Study Population ..... 136

5.3.2 Study Visit ..... 136

5.3.3 Statistics ..... 137

**5.4 Results ..... 138**

5.4.1 Group Characteristics ..... 138

5.4.2 Exercise Characteristics ..... 140

**5.5 Discussion ..... 146**

5.5.1 Impaired Systolic Response to Exercise Underlies Reduced Exercise Capacity in Preterm-born young Adults ..... 146

5.5.2 Pulmonary Capacity does not Explain Reduced Exercise Capacity in the Typical Preterm-born Young Adult Population ..... 148

5.5.3 Clinical Implications ..... 150

5.5.4 Study 2 Limitations ..... 150

**5.6 Conclusions ..... 151**

**CHAPTER 6 STUDY 3 RESULTS LEFT VENTRICULAR REMODELLING AND PHYSIOLOGICAL STRESS RESPONSE IN YOUNG ADULTS WITH MODEST BLOOD PRESSURE ELEVATION ..... 153**

**6.1 Abstract ..... 154**

**6.2 Introduction ..... 155**

**6.3 Methods ..... 157**

6.3.1 Study Population ..... 158

6.3.2 Study Visit ..... 158

6.3.3 Assessment of Physical Activity Enjoyment ..... 159
6.3.4 Plasma Copeptin Measurement ..... 159
6.3.5 Statistics ..... 159

**6.4 Results ..... 160**
6.4.1 Group Characteristics ..... 160
6.4.2 Resting Cardiac Profile ..... 162
6.4.3 Physiological Exercise Stress Response ..... 166

**6.5 Discussion ..... 175**

**6.6 Reduced Systolic Performance during Sub-maximal Exercise may Indicate Risk – Is this Response Reversible? ..... 175**

**6.7 Elevated Post-exercise Circulating Stress Signal Follows Poor Systolic Response to Exercise ..... 176**

**6.8 Possible Mechanism Underlying Impaired Left Ventricular Exercise Stress Response ..... 177**

**6.9 Clinical Implications ..... 178**

**6.10 Study 3 Limitations ..... 180**

**6.11 Conclusions ..... 181**

**CHAPTER 7 STUDY 4 RESULTS EXERCISE CAPACITY AND LEFT VENTRICULAR FUNCTION IN YOUNG ADULTS WITH MODEST BLOOD PRESSURE ELEVATION ..... 182**

**7.1 Abstract ..... 183**

**7.2 Introduction ..... 184**

**7.3 Methods ..... 185**

7.3.1 Study Population ..... 186

7.3.2 Study Visit ..... 186

7.3.3 Statistics ..... 186

**7.4 Results ..... 187**

7.4.1 Group Characteristics ..... 187

7.4.2 Baseline Left Ventricular Structure and Function ..... 189

7.4.3 Exercise Characteristics ..... 191

**7.5 Discussion ..... 200**

7.5.1 Possible Mechanisms Underlying Aortic Diastolic Pressure as a Highly SIgnificant Correlate of Exercise Capacity ..... 200

7.5.2 Clinical Implications ..... 203

7.5.3 Study 4 Limitations ..... 203

**7.6 Conclusions ..... 204**

**CHAPTER 8 CONCLUSIONS ..... 205**

**8.1 Future Questions ..... 210**

**8.2 Future Work ..... 212**

**REFERENCES ..... 216**

**APPENDICES ..... 241**

# LIST OF FIGURES

Figure 1.1. Depiction of Frank-Starling Mechanism Relationship between End Diastolic Volume and Left Ventricular Pressure ..... 31

Figure 1.2 Simplified Depiction of Laplace's Law ..... 33

Figure 1.3 Summary of Global Rates of Preterm Birth ..... 46

Figure 2.1. Integrated Progressive Protocol Structure ..... 67

Figure 2.2 Summary of Group 1 and Group 2 ..... 75

Figure 2.3. Summary of Group 3 and Group 4 ..... 81

Figure 3.1. Study Visit Flow Diagram ..... 85

Figure 3.2. Apical Four-chamber Echocardiographic Image Collected at Rest ..... 92

Figure 3.3. Spirometry and Cardiopulmonary Exercise Testing (CPET) ... 96

Figure 4.1. Ejection Fracton with Increasing Exercise Intensity in Preterm-born Versus Term-born Young Adults ..... 112

Figure 4.2. Sub-Maximal Exercise Cardiac Index Reserve with Increasing Exercise Intensity in Preterm-born vs Term-born Adults..... 115

Figure 4.3. Relationship between LV Length and Change in EF when Going from Rest to 60% Exercise Intensity in Preterm-born (Panel A) Versus Term-born (Panel B) Young Adults. ..... 117

Figure 5.1. Reduced Aerobic Exercise Capacity in Preterm-born Young Adults..... 141

Figure 5.2. Increase in EF from baseline to 40% and 60% Exercise Intensity Predicts Exercise Capacity in Preterm-born (panels A & C), but not Full-Term born (panels B & D) Young Adults ..... 144

Figure 6.1. 2-D echocardiography ejection fraction (EF) with increasing exercising intensity in young adults with elevated blood pressure vs controls. ... 169

Figure 6.2. Functional and Physiological Phenotyping of 40% Exercise Load Stress Response Profile ..... 170

Figure 6.3. Greater Resting Systolic Blood Pressure and Perceived Exertion during Low Intensity Exercise are Associated with Decreased Enjoyment of Physical Activity in Young Adults ..... 173

Figure 7.1. Decreasing Exercise Capacity with Increasing Aortic Diastolic Pressure in Young Adults. ..... 196

Figure 8.1. Trial of Exercise to Prevent HypeRtension in Young Adults (TEPHRA) Overview ..... 214

# LIST OF TABLES

Table 2.1 Comparison of key Characteristics of Preterm (Group 2) and Term-born (Group 1) Young Adult. ..... 74

Table 2.2. Comparison of key Characteristics of Young Adults with Normal Blood Pressure (Group 3) and Elevated Blood Pressure (Group 4) ..... 80

Table 4.1. Group Characteristics ..... 107

Table 4.2. Left Ventricle Resting Echocardiography ..... 109

Table 4.3. Left Ventricle Exercise Echocardiography ..... 113

Table 5.1. Group Characteristics ..... 139

Table 5.2. Exercise Characteristics ..... 141

Table 6.1. Group Characteristics ..... 161

Table 6.2. Magnetic Resonance Imaging Results ..... 163

Table 6.3. Exercise Testing Group Characteristics ..... 165

Table 7.1. Group Characteristics ..... 188

Table 7.2. Baseline Structure and Function Characteristics ..... 190

Table 7.3. Exercise Characteristics ..... 192

Table 7.4. Multivariable Regression Model Results Comparing Alternate BP Measures vs Aortic Diastolic BP in Predicting Exercise Capacity ..... 195

Table 7.5. Multivariable Regression Models Assessing Correlates of Exercise Capacity ..... 199

# Chapter 1 INTRODUCTION

## 1.1 Left Ventricular Structure and Pump Function

Adequate left ventricular (LV) pumping function, while essential for survival is nonetheless physiologically challenging. This is demonstrated by the global burden of heart failure which is estimated to exceed 37.7 million cases worldwide with an annual economic impact of $108 billion, and the vast majority of cases attributable to pathological declines in LV pump function (1-3). Additionally, LV pump function plays an important role in the overall pathology of other cardiovascular disease states as well as normal, healthy physiological adaptations to stressors such as physical exercise. The cardiovascular system demonstrates robust relationships between form and function and in the left ventricle, these structure-function linkages are particularly strong. Three powerful drivers of these strong structure-function linkages are the physical laws and relationships that govern hemodynamics, tissue physiology and function, and also the alterations which commonly accompany disease states as well as healthy physiological adaptations (4-6). In the introduction to this thesis, I will describe these key relationships, discuss some of the commonest pathological and physiological conditions in which they are altered, and address how those alterations subsequently relate to cardiovascular function and risk.

### 1.1.1 Elements of Myocardial Structure that Affect Cardiac Mechanics

As previously noted, the structural makeup of the LV has a strong bearing on its functional performance parameters. For this thesis, the most relevant elements of the myocardium itself are the wall thickness, fibre orientation and the physical

properties of the actual tissue, hence, these elements will be included in the introduction. Myocardial wall thickness has a direct relationship on the ability of the LV to develop wall tension and chamber pressure, but may also be abnormally increased in the hypertensive or preterm-born phenotypes (6,7). Myocardial fibre orientation has subtle yet important effects on myocardial deformation which are particularly relevant in revealing subclinical dysfunction that the study populations in this thesis are more likely to display. Finally, the physical tissue qualities, most notably the compliance and elasticity of the LV which relate to diastolic function, can be sensitive indicators of sub-clinical target organ damage (TOD) and premature cardiovascular aging (8-10).

#### 1.1.1.1 Wall Thickness

Necessarily, the right and left ventricles maintain identical output volumes, and in healthy adult males, flows of roughly 5L per minute are typical. Despite delivering equal output volumes, the overall workload of the LV is much greater than that of the right ventricle. This is due to the greater resistance of the systemic vs pulmonary circuit, which requires the left ventricle to produce nominal systolic pressures in excess of 100 mmHg whereas systolic pressures of 14 mmHg are typical for the right ventricle (11). The muscular mass required to produce these pressures necessitates a thicker myocardium, with nominal average wall thickness in healthy adult males ranging from 6-10 mm in the LV, vs 3-4 mm in the right ventricle (12,13). A further consequence of the systolic pressure developed by the LV is that even in healthy, normotensive individuals, the transmural systolic tension in the LV is too great to permit continuous myocardial perfusion throughout systole.

This results in intermittent LV perfusion, with continuous positive perfusion sustained only during diastole. During systole however, LV flow may be halted entirely, with retrograde flow possible due to dynamic compression of the small vessels and a simple downstream pressure gradient from the myocardium, through the coronary arteries and back to the aorta (14).

The deepest portion of the wall, the subendocardium, experiences greater wall tension than the subepicardium and consequently has a higher metabolic workload relative to the more superficial midwall and subepicardial layers. These and other factors subsequently contribute to the subendocardial layer having increased oxygen and perfusion demands. As a result, the subendocardial layer experiences increased susceptibility to ischemia (14,15). While direct measurement and quantification of the contributions of each mechanism contributing to the subendocardial perfusion challenge is difficult, Toyota et al's 2005 paper reporting active imaging of dynamic coronary arteriole flow in the canine model provided an insightful view of the collective summary effect. In this study, the "slosh ratio" which is an index measure of systolic retrograde flow, was 84% in subendocardial arterioles vs only 25% in subepicardial arterioles. This demonstrated that the subendocardial flow was predominantly retrograde during systole, and though the subepicardial arterioles experienced some systolic retrograde flow, the net systolic arteriolar flow in this layer remained downstream (16). Alternatively, the right ventricle, with its thinner walls and lower systolic pressure has commensurately lower wall tension and is continuously perfused through the cardiac cycle. Not surprisingly, the thick-walled LV, with its high workload and metabolic demand, and

inherent perfusion challenges, accounts for the majority of cases of myocardial ischemia. Therefore, a discussion of how wall thickness impacts, and may be altered by disease states and physiological stressors such as physical exercise, will also be presented as part of this introduction.

#### 1.1.1.2 Fibre Orientation

The myocardium includes three basic muscle fibre layers. The anatomically deepest subendocardial layer is composed of primarily longitudinally oriented fibres. Fibre orientation transitions to a circumferential orientation in the midwall layer, then back to a longitudinal orientation in the outer, epicardial layer (4,5,17). The tethering of the myocardium to the fibrocartilage skeleton of the heart and interplay of amount, rate, and synchronicity of contraction between these layers produces the characteristic twist and torsion that occurs in the LV during systole and diastole (18,19). As mentioned above, the deep, subendocardial tissue is subjected to the greatest perfusion challenge and correspondingly, human studies as well as experimental models demonstrate that the deep longitudinal fibres residing there are at greater risk for ischemic burden (14,20). This has an important consequence in that even in relatively healthy subjects, subclinical hypoperfusion resulting from early myocardial dysfunction or disease often first manifests via subtle impairment of longitudinal myocardial deformation which can be detected by echocardiographic strain imaging techniques (4,14,21,22). In addition to the characteristic fibre orientation, the basic physical properties of the myocardial tissue also bear important relationships to cardiac function.

#### 1.1.1.3 Myocardial Tissue Properties

The physical properties of the myocardium significantly contribute to effective and efficient cardiac function. In the healthy heart, the myocardium has a high amount of intact elastin, a structural protein which provides both strength and resilience, but does not contain excess fibrous collagen which is structurally stiff with poor resilience (23). This elastic and compliant myocardium works synergistically with the springy fibrocartilage skeleton of the heart to optimise LV function. Such myocardium provides minimal physical resistance to deformation during systole which reduces parasitic loss of work effort, then during diastole, the restoring force of the LV produces suction which allows 60-70% of LV filling to occur passively as the LV springs back near to its filled dimensions. Atrial kick then provides the final filling of the LV. Normal aging and also pathology can alter the physical properties of the myocardium and thereby impact LV function (24).

Normal aging results in a slow but progressive breakdown of the elastic proteins of the myocardium and a relative increase in collagenous fibre deposition. This results in the LV slowly becoming stiffer with age and drives corresponding reductions in end diastolic volume (24). Additionally, these tissue changes reduce diastolic function, and as the diastolic restoring forces of the LV diminishes, passive LV filling declines and the left atrium must work harder to facilitate sufficient LV filling. The effects of myocardial aging are accelerated by common risk factors including blood pressure elevation, sedentary lifestyle, oxidative stress and a pro-inflammatory profile. Accordingly, the relationship between chronic pathologies

such as cardiomyopathies, arrhythmias and LVH, and increased cardiac fibrosis is well documented in both human studies and animal models (25-31).

Developmental events and exposures can also have lasting impacts on the myocardium. In one rat model, prematurity was associated with increased myocardial mass and fibrosis triggered through increased angiotensin II signaling (32,33). Severe, acute pathologies such as myocardial infarction result in myocardial scarring and are associated with rapid collagen deposition in the myocardium, however rat experiments indicate that up-regulating elastin expression may result in more stable and resilient post infarct myocardium (34). Regardless of the underlying mechanism, excess myocardial stiffening directly contributes to risk of heart failure with preserved ejection fraction (35), a pathology which will be discussed further later in this introduction.

Myocardial tissue properties are also related to arterial properties, both functionally and histologically, with adverse and beneficial histological indicators often correlated between the two (36-41). Functionally, optimal arterio-ventricular coupling occurs when the myocardium and arteries are healthy and compliant, with a degradation of this relationship occurring in heart failure, cardiomyopathy and other pathologies (42-45). A healthy, compliant aorta readily expands to receive the blood ejected during systole. However, as with the LV, arterial aging produces histological alterations which stiffen the arteries, commonly known as arteriosclerosis. Increased aortic stiffness is associated with a wide range of pathologies in both clinical and experimental research and is a common indicator of

increased cardiovascular risk (29,46-49). A hemodynamic consequence of reduced myocardial and aortic compliance is that the systolic pressure wave is more readily reflected off the arterial tree and back to the ventricle in an effect called augmentation (50-52). Augmentation causes increased systolic pressures and myocardial workload as the LV has to work against a portion of its own pressure wave.

While the targeted study populations in this DPhil are absent of cardiomyopathies or history of myocardial infarction, the risk factors of preterm birth and blood pressure elevation may produce structural and functional states which contribute to subtle LV dysfunction including detectable levels of diastolic impairment through degraded myocardial tissue quality. Such physical characteristics of the myocardium are fully integrated with LV geometry in contributing to the overall functional profile of the LV.

### 1.1.2 Elements of Ventricular Geometry that Affect Systolic Pumping Function

#### 1.1.2.1 Chamber Volume

Historically, the essential role of the left ventricle as a pump has motivated efforts to understand and characterise LV systolic function. The Frank-Starling mechanism or “law” of the heart combines multiple fundamental concepts that collectively characterise basic elements of systolic function based on end diastolic chamber volume. First, Otto Frank’s groundbreaking 1895 experiment conducted

on the frog heart revealed the direct relationship between end diastolic volume (and subsequently cavity size) and the peak systolic pressure as shown in **Figure 1.1**.

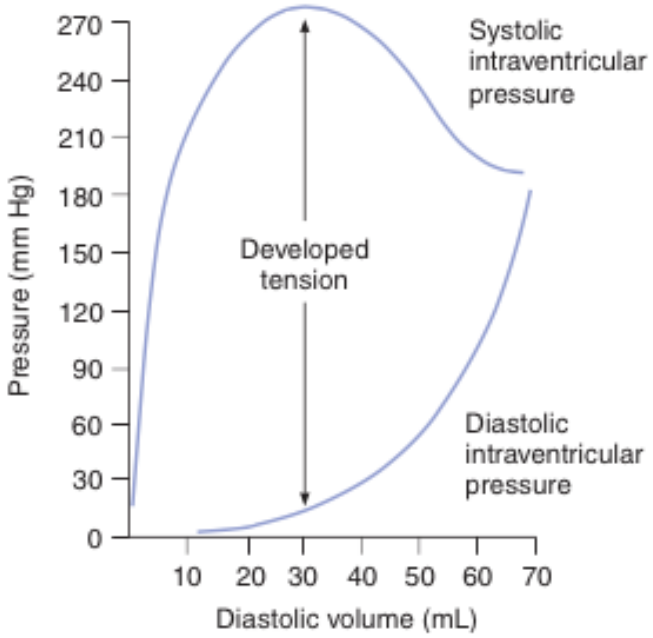



**Figure 1.1. Depiction of Frank-Starling Mechanism Relationship between End Diastolic Volume and Left Ventricular Pressure**

A special length-tension relationship exists in cardiac muscle. Contributing mechanisms include sarcomere length and calcium storage and release kinetics. The net effect is that the increased myocardial stretch associated with greater end diastolic volume results in more forceful systolic contraction. This "Frank-Starling mechanism" remains effective across the range of ventricular filling volumes. Figure adapted from http://www.tomhsiung.com/wordpress/2015/02/the-regulation-of-circulation-heart-neurohormons-local/.

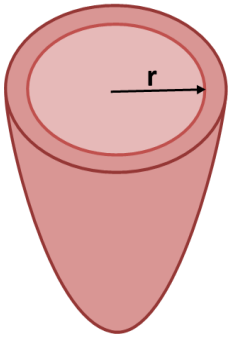
This concept was combined with further work in the early 20th century by Ernest Starling and others to describe the mechanism that allows the heart to compensate for variations in end diastolic volumes through beat to beat adjustments

of stroke volume (53,54). Further experimentation has increased the mechanistic understanding of the Frank-Starling relationship through sarcomere length-tension relationships, calcium based excitation contraction coupling and mechanical elastic characteristics of the myocardium (54). However, no findings have necessitated a deviation from the originally described Frank-Starling law of the heart which remains highly relevant in modern clinical practice, and as Borlaug et al editorialised in 2017, “The fundamental importance of this Frank-Starling relationship in cardiovascular physiology cannot be overstated” (55).

#### 1.1.2.2 Chamber Width

Because LV end diastolic volume bears a direct relationship to cavity radius, the Frank-Starling mechanism has in important interplay with a second fundamental geometric characterisation of LV systolic function, Laplace’s law of the heart. Pierre-Simon Laplace, a French mathematician born in 1749, derived the equations that govern wall stress in a pressurized sphere based on chamber pressure, chamber radius and wall thickness. These relationships can generally be applied to the LV and are represented below in **Figure 1.2**. Laplace’s equation conveys the direct linear relationship that chamber pressure and radius have on wall tension, which also reveals an inherent geometric limitation in the Frank-Starling mechanism. The Frank-Starling mechanism relies on increasing end diastolic volume to produce greater myocardial stretch and subsequently increased force of contraction. However, this increased end diastolic volume also results in increased chamber radius, and therefore, by Laplace’s law, the heart must produce greater wall tension to develop the same pressure in the ventricle. However, as blood is expelled during

the ejection phase, ventricle radius diminishes, allowing for a decline in the wall tension required to sustain pressure as systole progresses.




$$P = \frac{2T}{r}$$

**Figure 1.2 Simplified Depiction of Laplace's Law**

In Laplace's law, P represents pressure, T represents wall tension, and r represents the radius (of a sphere). These relationships generally apply to the left ventricle despite its more conical shape. Two physiologically and clinically important relationships revealed by Laplace's Law are that: 1) Ventricular pressure is proportional to myocardial wall tension, and 2) Ventricular pressure is inversely proportional to chamber radius. These relationships determine fundamental consequences of physiological adaptations as well as pathologies that alter chamber size and or pressure.

At a simplified level, the work of the myocardium can be considered as a sustained, repetitive cycle of rapidly producing and then releasing myocardial wall tension. This development of wall tension has direct consequences in tissue metabolism and morphology. Metabolically, increased myocardial wall tension requires greater myocardial workload and commensurate increases in oxygen demand. This has been demonstrated in hypertensive diseases and dilated cardiomyopathies, where resting cardiac metabolic demands increase with increasing pressure and chamber size respectively (56,57). Additionally, in conditions where elevated pressures are developed by the LV such as hypertension or aortic stenosis, greater wall tension is required. In response to such increased hemodynamic loading and the signaling cascades associated with these conditions,

over time, the myocardium frequently undergoes a pathological increase in mass and thickness called left ventricular hypertrophy (LVH) (58-61). LVH is a common sequela in which the thicker, remodelled myocardium has greater muscle (and fibrotic) tissue mass adapted to elevated systolic workloads. However, LVH is ultimately maladaptive, and a strong indicator of increased cardiovascular risk that most commonly follows years of exposure to adverse cardiovascular conditions such as hypertension (61-64). LVH and other forms of pathological remodelling will be addressed in greater detail later in this chapter, but in regard to Laplace's law of the heart, conditions that result in increased myocardial thickness reveal a significant limitation of the law. Laplace's law, while immensely useful in understanding geometric relationships to myocardial tension, does not account for a clinically relevant physiological reality which is the gradient in wall tensions across the myocardium, with the greatest tension in the deeper sub-endocardium and comparatively lower tension in the outer layers. This phenomenon and its impacts will be discussed in further detail in the section regarding wall thickness.

#### 1.1.2.3 Chamber Length

Although length is a fundamental element of LV geometry and sometimes collaterally reported with other basic elements of LV structure, LV length is rarely a central investigative focus. In analysing echocardiographic and cardiac magnetic resonance images, LV length is often measured and used to calculate values such as chamber volume, ejection fraction and mass which are commonly employed clinical measures (65). However, LV length as a raw value is rarely used in nominal clinical practice (66-68).

As is generally the case for cardiac structural measures, LV length is measured at end-diastole, and typical values in adult female subjects hover around 9 cm with lengths of roughly 10 cm typical in males (7,65,66). As the heart ages, the myocardium slowly becomes stiffer, primarily through increased fibre deposition as part of the aging process which may be accelerated secondary to pathologies such as diabetes or hypertension (69,70). As a result, diastole does not result in the same degree of LV lengthening in the elderly as it does in younger adults, and cross-sectional study data on LV geometry shows that adults below 40 years of age have LVs that are on average, 4-5 mm longer than adults over 60 years of age (66). While aging and disease may alter the LV length to some degree, it is generally considered to be a relatively "fixed" cardiac geometric parameter in most subjects.

## 1.2 Cardiovascular Stress

### 1.2.1 A Working Definition of Cardiovascular Stress

The term "cardiac stress" is frequently used in literature and clinical practice to describe a state of increased myocardial challenge or workload brought on by a stimulus or "stressor" such as physical exercise, exposure to a β receptor agonist or coronary vasodilator, or pathology such as hypertension or aortic stenosis (71,72). While the heart itself, specifically the LV is the focus of this DPhil, I will use the general working definition given above, but also use the term "cardiovascular stress" at times when there is specific intent to consider or address the integrated nature of the cardiovascular system. Hence, use of the term cardiovascular stress

implies the intent to address both the central and peripheral elements of the cardiovascular system experiencing the effect of various stressors and contributing to an integrated response to cardiovascular stress. This response to acute cardiovascular stress is complex, including classic central cardiac elements such as increased chronotropy, inotropy, lusitropy, dromotropy, and coronary blood flow, as well as peripheral and systemic effects such vasomotive actions and also the release of biomarkers into the plasma (73,74).

### 1.2.2 Nominal Cardiovascular Response to Increased Workload

#### 1.2.2.1 Physiological Stress

In a cardiovascular sense, a physiological stressor is generally understood as physical exertion or exercise. In a typical day, healthy people experience wide variations in physiological stress which can range from sleep and sedentary awake time at the low end, to highly vigorous activities including physical labor, recreation, or other demanding activities toward the maximal end of the spectrum. An extensive concert of rapid physiological response mechanisms facilitates dynamic and effective transitions through these various states. This response to physiological stress is highly integrated and largely regulated through stimulation of the sympathetic nervous system (SNS) via the release of catecholamines into the blood stream and also through direct sympathetic innervation (75). An abbreviated overview of this response, with a focus on the LV stress response, follows.

The LV is extensively innervated by post-ganglionic sympathetic fibres, which act primarily through stimulation of the many $\beta_1$ adrenergic receptors throughout the heart. These same cardiac $\beta_1$ receptors also respond to circulating catecholamines released from the adrenal glands as part of the exercise response (76). Sympathetic stimulation of the heart results in increased chronotropy (heart rate), dromotropy (conduction rate through the atrioventricular node), inotropy (strength of contraction), and lusitropy (myocardial relaxation rate). In the physiological exercise stress response, a healthy young adult may increase their heart rate from a resting value of 70 beats per minute to a peak rate of 190 beats per minute. Additionally, stroke volume may increase 20% or more during physical exercise. Collectively, these increases in heart rate and stroke volume allow a typical healthy young adult to produce a three-fold increase in cardiac output during exercise (77). In highly trained aerobic athletes, five to seven-fold increases in cardiac output may be achieved during peak exercise (77,78).

Large gains in LV perfusion are required to support the metabolic demands of the cardiac stress response. Unlike skeletal muscles which have relatively low baseline metabolic demands, at a typical resting heart rate, the myocardium of the LV sustains a metabolic workload roughly 20 times greater than an equivalent mass of resting skeletal muscle (79,80). Additionally, energy demands of the myocardium are provided almost exclusively through aerobic metabolic pathways and oxygen uptake and utilization in the LV nominally consumes 70-80% of the total oxygen carried by blood that passes through its tissue. Due to the kinetics that govern the oxyhemoglobin dissociation curve and the partial pressures of oxygen in the plasma

and tissue of the working myocardium, this 70-80% utilisation of oxygen represents almost 100% of oxygen that is available for delivery to working tissue. As a result, increases in LV workload must be met with commensurate increases in LV perfusion and three to six-fold increases in LV perfusion may occur during maximal exercise (76). If myocardial perfusion demands are not met, ischemic dysfunction onsets swiftly, with minor dysfunction commonly detectable by ST segment depression in the ECG or by reduced wall motion (hypokinesis) observed in the affected regions during echocardiography. More severe perfusion deficits can result in complete loss of motion in affected wall segments (akinesis) or in the most acute cases, myocardial infarction (71).

### 1.2.3 Measurements of Cardiovascular Stress

Observing the cardiovascular system under stress provides insights into cardiovascular health and function that cannot be gained through resting assessments alone. For example, a marginalised system such as in the preterm-born phenotype, an otherwise healthy hypertensive patient, or an individual with stable angina, may show biphasic response characteristics where the system is well compensated at baseline, but susceptible to stressors which may drive the system out of homeostatic control and into dysfunction. Hence, investigating the cardiac stress response and profile may be central to understanding how various structure and function LV phenotypes may have specific predictive or explanatory power in cardiovascular disease risk assessment.

In addition to administering prescribed levels of stress and assessing the cardiac stress response, the resting indicators of overall cardiac stress can be useful as well. In the well-reported Framingham Heart Study, adding measurements of myocardial stress biomarkers provided significantly improved prognostic value, with those in the least favorable quartile for biomarker scores having a three-fold increase in mortality and six-fold increase in heart failure in an 11 year follow-up (81). Similarly, cardiac troponin assays have also shown value in guiding care and predicting morbidity and mortality in highly distressed neonates (82), heart failure patients (83,84) and acute coronary syndromes (85). Such studies highlight informative and actionable insights which may be gained by measuring indicators of the baseline cardiac stress profile, however, prescribed stress testing may provide even further insights.

Because of its wide-ranging utility, cardiac stress testing is employed across many applications from evaluating levels of desirable physiological adaptation in trained athletes to determining the degree of maladaptive pathology in CAD and heart failure patients. Cardiovascular stress testing offers significant advantages over resting assessments as the cardiovascular system can be observed under increased load where sub-acute pathology may manifest more clearly and physiological reserve capacities can be challenged and assessed. An overview of common cardiac stress measurement follows below.

### 1.2.3.1 Non-Circulating Measurements of Cardiovascular Stress

#### *1.2.3.1.1 Imaging Measurements of Myocardial Stress*

Stress echocardiography is a popular stress testing technique which allows cardiac function to be directly observed while a patient is subjected to prescribed levels of increased cardiac stress (71). Under increased cardiac stress, coronary artery disease (CAD) will often manifest as decreased wall motion (hypokinesis) or absence of wall motion (akinesis) in the diseased portions of the myocardial wall. In addition to acute, stress induced wall motion abnormalities, measures such as ejection fraction, stroke volume, and indicators of diastolic function can also be measured and graded to provide a useful characterisation of a range of cardiac performance indicators in the stressed state.

Echocardiographic stress testing may employ electrical stimulation to increase heart rate (in paced patients only), pharmacological stressors, or physical exercise to provide a stress challenge to the myocardium. Pharmacological stimulation most commonly employs dobutamine or dipyridamole whereas physiological exercise stress testing most commonly employs a treadmill or stationary cycle ergometer to allow cardiac performance and function to be evaluated under the stress of an increased myocardial workload. Stress echocardiography is highly accessible in modern clinical settings and meta-analyses indicate that detection rates of 72%-83% are typically achieved for CAD (71,86). As a result, stress echocardiography has earned frontline clinical standing, offering rapid assessment of physiological parameters of cardiac function, modest

cost, and substantial clinical value in identification of CAD and also in monitoring the progression of diseases such as heart failure (71,87). The use of contrast extends the capabilities of echocardiography, and with appropriate protocols can permit a usable assessment of myocardial perfusion in many subjects (88,89). Still, early disease that does not produce hypokinesis or other wall motion anomalies may not be clearly detectable by stress echo. Nevertheless, general cardiac performance in the stressed state can still be evaluated, and even apart from overt wall motion anomalies, a reduced functional response to stress is indicative of increased risk (90). In instances where further, non-invasive investigation is desired, the more capable stress Cardiac Magnetic Resonance (CMR) imaging may offer additional clinical benefit.

Stress CMR imaging almost exclusively uses pharmacological stressors such as dobutamine or adenosine to induce cardiac stress while CMR imaging is accomplished. Stress CMR provides information outside the reach of stress echocardiography including more competent detection of myocardial perfusion compromise when there is no associated wall motion or ECG defect observed (91). Additionally, stress CMR can also be used to investigate tissue composition and other clinically relevant measures that can inform patient care. Indeed, a recent review reported that 70% of patients who underwent stress CMR had their clinical care revised as a result (91). However, stress CMR is often cost-prohibitive, and like other imaging methods, is not well suited to detecting circulating indicators of cardiovascular stress or function. Not surprisingly, the efforts to identify circulating

biomarkers that provide novel or augmented diagnostic and prognostic capability is a dynamic area of inquiry (92).

#### 1.2.3.2 Circulating Measurements of Cardiovascular Stress

Extensive research efforts have yielded a “biomarker tsunami” (93) though few have gained consensus acceptance as mainstream clinical tools (94). In measuring cardiac stress, the primary constituents of this “tsunami” can be summarised by grouping the biomarkers into biological categories. Using this approach, two relevant categories would include cellular proteins and peptide biomarkers.

##### *1.2.3.2.1 Cellular Proteins*

Cardiac troponins remain the clinical standard for myocardial stress measurement and will be discussed first. Cardiac specific troponin I and T are widely used in clinical and research applications and are the clinical biomarkers of choice for diagnosing myocardial infarction (95). Early assays lacked the sensitivity to detect circulating troponins without substantial release triggers generally associated with myocardial damage such as occurs in acute coronary syndromes, however, modern high sensitivity assays permit the measurement of circulating cardiac troponins even in healthy individuals at baseline, and in some cases can quantify small increases following intense physical exercise or stress testing (96). Sub-acute conditions, some of which include modest but persistent sub-clinical dysfunction, have been associated with detectable elevations of cardiac troponins at baseline. For example, compared to controls, studies have reported higher

resting cardiac troponins with increased BMI (97), hypertension (98), and interestingly, even in the cord blood following preeclamptic pregnancy (99). While troponin measurements are well established and provide both diagnostic and prognostic value, novel biomarkers including other cellular proteins and extracellular vesicles are under investigation to determine if they may be measured to improve the timeliness and specificity of clinical stress assessments and facilitate earlier detection of nascent disease.

#### *1.2.3.2.2 Peptides*

Circulating peptides have become popular biomarkers. These are generally either active hormones, pro-hormones, or the relatively inactive C or N terminal fragments that are cleaved from a pro-hormone upon hormone activation. In most cases, the active hormones have a shorter half-life and more variable overall kinetics in the plasma, as a result, pro-hormone and N or C terminal fragments are often used as surrogate analytes and may offer more consistent and reliable measurements of bioactivity than the active hormone itself. Such is the case with N-terminal pro B-type natriuretic peptide (NT-pro BNP), the current gold standard biomarker for heart failure assessment (100). Another peptide biomarker of great interest is copeptin, a neuropeptide which peaks more quickly in response to cardiac stress than troponin and has been recognized by the European Society of Cardiology as clinically useful in ruling out non-ST-elevation myocardial infarction (101). Copeptin is the inactive C-terminus peptide fragment that is co-released in equal molecular proportions to antidiuretic hormone. Antidiuretic hormone has a very short half-life that has been measured at 24 minutes in-vivo, and degradation

continues at a high rate in collected samples (102), however, copeptin levels provide accurate, dynamic assessment of antidiuretic hormone activity, and collected samples can be readily frozen for long term-storage and even remain stable for several days at room temperature (103).

## 1.3 Relevant Exposures That Impact Left Ventricular Structure and Function

### 1.3.1 Developmental Exposure

#### 1.3.1.1 Preterm Birth

Preterm birth is associated with long-term cardiovascular alterations including microvascular rarefaction, increased blood pressure, and cardiac remodelling (7,104,105). The specific cardiac alterations in those born preterm are increasingly well characterized. In the LV, the main structural alterations observed are reduced length, increased overall mass and lesser cavity size. This unique morphology was first described in a CMR imaging study of 102 young adults with an average gestational age of 30 weeks. A central finding in this study was that LV mass index was 20% greater in the preterm-born cohort than controls. Additionally, resting longitudinal myocardial deformation and diastolic function were slightly impaired though resting ejection fraction was completely preserved (7). These structural modifications are understood to be triggered as a result of truncated third

trimester development where the heart experiences an early transition from the fetal to neonate circulatory system prior to completing typical in-utero development (106).

The increase in LV mass in the preterm-born population shares some morphological similarities with hypertensive concentric remodeling, however the phenotypic trait generally manifests in those born preterm even apart from blood pressure elevation. Indeed, multiple studies have observed increased blood pressure in preterm-born adults, although the mass increase seen in those born preterm cannot be accounted for by blood pressure alone (7). The degree to which the specific modifications in the preterm-born LV phenotype may be maladaptive is poorly understood and investigated, therefore, I chose to further investigate the consequences of these structural and functional alterations as a central aim of this thesis.

#### *1.3.1.1.1 Epidemiology*

Prematurity is the leading global cause of perinatal morbidity and mortality worldwide and effects 5-18% of global births (107). In many cases, it is equally common in developed and developing countries alike, as indicated by **Figure 1.3** below, adapted from Blencowe et al's Lancet report on preterm birth from 1990-2010 (107). In the UK, 6-12% of the population are born preterm, and the in US, approximately 12% of births are preterm (107-109).

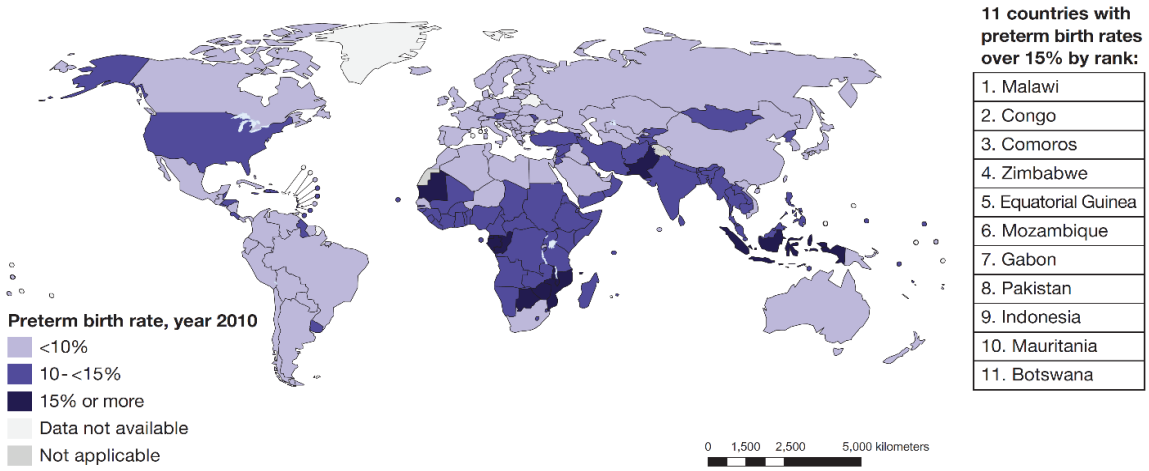



**Figure 1.3 Summary of Global Rates of Preterm Birth**

Rates of preterm birth vary by country with approximately 5-18% babies born before 37 weeks gestation (preterm). Image adapted from Blencowe et al, *The Lancet,* 2013.

Prior to the widespread clinical implementation of pulmonary surfactant in the 1980s, neonatal respiratory distress syndrome had a devastating effect on the survivability of preterm birth and mortality rates often exceeded 30% for those born moderately premature (28-34 weeks' gestation). Mortality was even higher for those born before 28 weeks gestational age (110,111). However, the combination of surfactant administration when required (112) and modern perinatal care has driven dramatic improvements, with overall 95% survival rates common in developed countries (113). As a result, the global population of preterm-born adults has steadily increased in recent decades and is presently higher than ever recorded (114). While excellent neonatal survival rates represent a great achievement, the longer term epidemiological consequences of prematurity are beginning to unfurl as now for the first time, a substantial portion of the young and middle aged adult population was born preterm. Rising to understand and meet these challenges

which are now understood to include increased risk of hypertensive disease and heart failure, is an imminent task for the global clinical research community. In support of this end, I chose to dedicate a significant portion of this thesis to elucidating mechanistic linkages of the preterm-born cardiac phenotype to increased risk in this population.

### 1.3.2 Disease

#### 1.3.2.1 Hypertension

In hypertension, the increased hemodynamic loading of the LV often produces a remodelling of the myocardium (58,60,115-117). The elevated working pressures stimulates the release of growth factors which results in cardiomyocyte hypertrophy which is often associated with increased collagenous fibre deposition (59,118,119). In some ways, this remodeling is analogous to skeletal muscle hypertrophy in response to resistance training, however, LV remodelling secondary to hypertension is always considered pathological, generally results in reduced myocardial tissue quality, and is a significant indicator of increased cardiovascular risk (62,64,120-122). Interestingly, LVH is uncommon in young adult hypertensives and longitudinal and cross-sectional studies indicate that exposure to elevated blood pressure often does not result in clinical LVH until later in life (41,64,123-125).

LVH diagnosis is generally accomplished using cardiac imaging, most frequently via echocardiography, although ECG may provide a rudimentary, low specificity assessment (126-129). There is some variation in agreed upon cut-points

for clinical LVH (12,130), however, the following are common and will be used in this thesis: In males, an LV mass that exceeds 102 grams per square meter of body surface area, and in females, an LV mass that exceeds 88 grams per square meter of body surface area (12). The two commonest forms of LVH seen in hypertensives are concentric hypertrophy where the majority of the LV mass increase is radially inward, resulting in a smaller chamber diameter, and eccentric hypertrophy, where the LV expands radially outward and chamber diameter may increase (124,131-135). Propensity for a particular variety of LVH (or no LVH) is not fully understood, though genetic markers related to growth and proliferation of structural proteins and cardiomyoctes are generally understood to play a significant role (28,115,136-139).

Another, less extensive form of LV remodelling that is common secondary to hypertension is concentric remodelling. In concentric remodelling, the LV experiences a significant increase of wall thickness relative to cavity volume, but may not have sufficient overall mass increase to be categorized as LVH. Specifically, concentric remodelling is diagnosed when LV mass is within the normal range, but relative wall thickness (RWT) is greater than 0.42 (12). The calculation for RWT is (2 x inferior LV wall thickness) / (end-diastolic diameter), and although concentric remodelling is technically an echocardiographic standard, it is used in magnetic resonance studies as well (135). While the imaging modalities and associated measurements are not identical, RWT values are similar across echocardiography and CMR, which are the two most common imaging modalities.

Over the last decade, studies have increasingly identified associations between concentric remodeling and increased risk of stroke and other cardiovascular events (140-144). Indeed, a 2015 report of echocardiography conducted on 368 non-cardioembolic stroke patients, abnormally high relative wall thickness was present in 80.4% of patients, but LVH was only identified in only 35.3% (143). These data indicating that concentric remodeling may appropriately play a larger role in prognosis and intervention targeting are gaining greater translational interest. This is part of the growing body of evidence that in those with blood pressure elevation, sub-clinical target organ damage (TOD) often predicts acute cardiovascular disease and events and may warrant greater consideration as a clinical risk factor (145,146). Presently however, concentric remodeling alone is not generally targeted by standard clinical intervention practices which are more proactive in targeting LVH (147).

#### *1.3.2.1.1 Epidemiology*

High blood pressure has an immense global impact. It directly contributes to 9 million global deaths (148) per year and more disability-adjusted life years (DALYs) lost than any other single factor (149). In addition to mortality, there are many living with the disease and associated morbidity and increased cardiovascular risk. In the United Kingdom, 1 in 4 adults over the age of 25 are hypertensive and the incidence in the 18 to 40 year old population may be increasing (150). Commensurate with the overall increase in the prevalence of hypertension is an increased disease load in the younger population. In North America, population studies report incidence as high as 20% in adults under 40 years of age (151).

Prevalence of hypertension is higher in young adults with history of preterm birth and exposure to maternal pregnancy complications such as pre-eclampsia (152,153). Our recent work identified distinct etiological pathways linked to preterm birth and pregnancy complications including impaired angiogenesis (154,155). In most cases, hypertension can be effectively managed and treated, commonly through drugs that inhibit the renin angiotensin system such as ACE inhibitors or angiotensin receptor blockers, diuretics such as chlortalidone or hydrochlorothiazide, calcium channel blockers such as amlodipine, antimineralocorticoids such as spironolactone, or β receptor antagonists such as atenolol.

Despite numerous efforts to curb the increasing prevalence of high blood pressure, blood pressure elevation remains on the rise globally (156). New estimates based on 8.69 million study participants across 154 countries show that global BP elevation is increasing and further, that systolic BP in the 110-115 mmHg range accounted for 48% more DALYs lost from 1990-2015 than did systolic BP > 140 mmHg (149). In late 2017, the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines extensive evidentiary review resulted in lowering the clinical thresholds for Stage I hypertension from 140 to 130 mmHg and categorizing systolic BP of 120-129 as “elevated” (147). While the clinical and epidemiological impacts of these updated guidelines remain to be determined, they indicate a strong recognition of the extensive impact of blood pressure on global health. In particular, there is growing recognition of the evidence

that modest blood pressure increases which have traditionally not been addressed clinically, have substantial fiscal and human consequences (147,156-158).

#### 1.3.2.2 Heart Failure

Heart failure is a common disease that most frequently presents with a classic range of symptoms which may include dyspnea, general and exertional fatigue, nocturnal cough, and edema. Manifold causes including structural and functional defects, CAD, myocardial infarction, hormone imbalance, hypertension and drug toxicity may contribute to the development of heart failure (159,160). Nevertheless, regardless of the particular etiology for a given case, heart failure is characterised by cardiac output insufficiency, most frequently due to LV dysfunction (159-162).

##### *1.3.2.2.1 Epidemiology*

Heart failure poses a widespread cardiovascular disease burden with over 37 million global cases estimated worldwide which includes roughly 1-2% of the adult population and at least 10% of those over 70 years in age (1,2,163,164). Although heart failure predominantly afflicts those in the more mature adult population, it also occurs in pediatric and young adult populations although outcomes and prevalent etiologies differ significantly by age (165,166). A 2006 report published by Webster et al. analysed US databases including 5,610 pediatric and 732,752 adult cases of heart failure and found that 61% of pediatric cases were due to congenital defects vs only 0.3% of adult cases (167). Interestingly, although LV dysfunction is generally more greatly impaired in younger cases, the associated

symptoms may be subdued as compared to the symptoms that are likely to occur in older adult cases. Correspondingly, mortality rates for those diagnosed with heart failure increase with age. A large meta-analysis of over 41,000 US new heart failure cases demonstrates this, with reported three year mortality rates for those under 40 years of age averaging 7.5%, but increasing steadily all the way to 57.2% for those over 80 years of age (168).

Extensive research and national and global efforts have made steady incremental advances in modern, evidence-based care in the past three decades. These gains have improved treatment outcomes and post-diagnosis longevity where modern care is available, however, because relative risk of heart failure increases with age, rising life expectancy in developed countries carries a forecast of growing heart failure burden (162,169-172). In the US, the total number of people living with diagnosed heart failure is projected to increase by 46% between 2012 and 2030, with most of the additional cases expected in older adults (173). Additionally, advances in treatment significantly improve function and lifespan but in most cases does not provide a cure, and the majority of improvements have come in treating heart failure with reduced ejection fraction (HFrEF) with less progress made against heart failure with preserved ejection fraction (HFpEF) (162,174,175).

#### *1.3.2.2.2 Etiology*

Although general cardiac insufficiency is common across all cases of heart failure, a very wide range of etiologies may lead to the disease and in many instances, patients have multiple contributing risk factors. Further complicating an

optimal and precise characterisation of etiologies and their prevalence are the continuously evolving definitions and diagnostic criteria, as well as risk factors that vary significantly by region (2,162). I will however, briefly introduce two basic categories of heart failure, HFrEF and HFpEF, and some of the etiologies and risk factors most relevant to this dissertation.

HFrEF can be considered as the “traditional” variety of heart failure, which develops as a result of systolic dysfunction. In HFrEF, the LV is unable to expel a physiologically sufficient fraction of its end diastolic volume during systole to meet metabolic needs. There is generally agreement that heart failure cases with an LV ejection fraction below 40% should be classed as HFrEF. HFpEF on the other hand is diagnosed when cardiac insufficiency occurs despite an ejection fraction that is 50% or greater (161,162). HFpEF is characterized by diastolic dysfunction because LV filling is impaired during diastole, resulting in a low end diastolic volume, however, the LV ejects a “normal” fraction of its end diastolic volume during systole, but due to reduced filling, stroke volume remains low. Heart failure with middling ejection fraction in the 40-49% range is often categorized as heart failure with midrange ejection fraction (HFmrEF), but consensus is still developing regarding categorizations in this area (161,162,176,177). HFrEF and HFpEF share some common risk factors, though etiology and treatment differ significantly.

A major risk for heart failure that is directly relevant to this dissertation is persistent hemodynamic overload due to hypertension, which is a leading antecedent cause of both HFrEF and HFpEF and was found to contribute to over

70% of heart failure cases in a recent African study (178-182). Myocardial tissue quality is often degraded in both varieties of heart failure, with numerous studies reporting increased fibrosis, myocardial thickening and reduced myocardial compliance in both varieties of heart failure (2,169,183). HFpEF however differs from HFrEF in that myocardial stiffening and loss of compliance are central to the etiology of HFpEF. Because myocardial stiffening and loss of compliance occur as part of the aging process, in regions with increasing lifespans, there is a gradual shift toward this variety of heart failure. This is demonstrated by epidemiological data which reveals that in 1980, less than 50% of new cases were classed as HFpEF, whereas presently, approximately 60% of new cases are classed as HFpEF (162,174,184). In addition to traditional risk factors, a newly identified risk factor for heart failure is history of preterm birth (185), this risk factor will be investigated and developed in greater detail in the results portion of this dissertation.

### 1.3.3 Lifestyle

#### 1.3.3.1 Body Composition

Many genetic factors are understood to impact an individual's propensity toward a particular body composition (186,187). Nevertheless, lifestyle changes such as altering caloric intake and type and amount of physical activity can significantly alter body composition (188,189). Increased adiposity is associated with greater cardiovascular risk and interestingly, the degree of risk can vary by body region. Excess abdominal adiposity is generally associated with greater risk than excess adiposity in the hip and buttock region (190,191). Most common

cardiac structure and function measurements such as mass and stroke volume are indexed by dividing the raw cardiac measurement by the individual's body surface area (BSA). This allows more equitable comparison of measures across a relatively wide range of heights and weights. When BMI is highly elevated, cardiac measures may benefit by indexing vs height. This is relatively common in obesity studies where nominal BMI values exceed 40.

#### 1.3.3.2 Age

Age is associated with variations in left ventricular structure and function. The aging process results in a gradual decline in ventricular compliance and overall tissue quality, including the extracellular matrix, which causes end diastolic volume and overall diastolic function to slowly decline with age (24,37,192-195). LV length also decreases slightly with age although this is generally not considered clinically consequential (24). LV mass tends to increase with age, particularly in middle age and beyond. Even in cases of hypertension, Clinical levels of LV mass increase are uncommon in young adults, with greater mass increase often beginning to onset after the age of 40 (8,41,124,131,196). Because of the alterations that can occur with normal aging, analyses in this thesis were adjusted for age where appropriate.

#### 1.3.3.3 Sex

Sex is also associated with variation in the LV. The greatest difference is in the gross structural components, with LV size and mass generally greater in males (12,197,198). Studies have identified a range of sex-based functional differences,

including relative risk assessments in specific and populations or pathologies (188,199-203). Overall, similar to aging, sex accounts for a degree of variance in LV parameters. Where appropriate, analyses in this thesis were adjusted for sex.

#### 1.3.3.4 Physical Training

The recurring stressor of chronic physical training exerts a wide range of alterations across the cardiovascular system (77,204,205). I will provide a summary overview of the type of stress produced by two common forms of physical training and then will describe the key LV alterations that most commonly result from each type of type training. The first type of training is resistance training. The second type of training is aerobic training. These two forms of physical training are characterized by different physical activities, produce different challenges to the LV and can drive varying adaptations on LV structure and function.

##### *1.3.3.4.1 Resistance Training*

Resistance training (also frequently called strength training) occurs when skeletal muscles are exercised repeatedly against an increased resistance, with the most well-known form of resistance training being weight lifting. Various forms of resistance training produce increases in physical strength and power and are commonly employed in efforts to enhance athletic performance and physical appearance. Although aerobic pathways are used to some extent, resistance training primarily challenges anaerobic capacity and metabolic pathways, with each individual set of repetitions usually lasting under one minute then followed by a rest period of a few minutes (189). Resistive load frequently ranges between 50 and

70% of the peak achievable load in the most common strength and resistance training regimens, with 8-15 repetitions of a particular movement being typical (189). Powerlifting represents a form of maximal resistance training and loads approaching or even exceeding the maximum single repetition capabilities are frequently used, albeit with correspondingly fewer repetitions accomplished.

Resistance training subjects the LV to specific hemodynamic challenges. Although vasodilation occurs in working skeletal muscle, which reduces flow resistance and facilitates perfusion between repetitions, during active contraction against a load, the force and duration of contraction increases resistance to flow dramatically (189). During active resistance training, a strong, sympathetically mediated inotropic and chronotropic effect occurs, which aids in driving blood into working skeletal muscle tissue (206-208). The associated temporary increases in blood pressure can be extreme, especially during maximal effort which often occurs with a valsalva maneuver. Strikingly, intra-arterial monitors in powerlifters have recorded blood pressure spikes as high as 345/245 mmHg during active lifts (206-208).

Chronic exposure to this hemodynamic loading frequently drives physiological adaptations in the LV. In most cases, resting heart rate and stroke rate do not change substantially, however, contractile and cardiac output reserve may increase in some cases, and modestly increased LV mass is common (205,209,210). Unlike cardiac hypertrophy secondary to hypertension, studies indicate that the increases in LV mass associated with resistance training generally

occur without degradation of myocardial tissue quality. That is, such mass increases are understood to occur without fibrosis or loss of compliance associated with pathological increases in LV mass. This is demonstrated by a study published in 2008 by Adler et al in which peak early transmitral acceleration time (a common indicator of diastolic function) was 40% higher in trained weightlifters than sedentary controls. During exercise, the same measure increased to 46% higher in the weightlifters (211).

Resistance training may be prescribed for some patients with cardiovascular disease. It has shown benefit in heart failure patients, including those with HFpEF (212). That said, evidence on resistance training is mixed for those with cardiovascular disease, particularly for those with existing blood pressure elevation (189,213,214). In resistance training, post-exercise blood pressure is generally reduced, though training parameters including time of day, intensity, and duration are thought to affect this response (215-217). In most cases of cardiovascular disease however, some resistance training at moderate intensity may be beneficial and prescribed once patients are medically cleared. Nevertheless, standard clinical guidance broadly recommends low to moderate intensity aerobic training for those with cardiovascular disease (204,212,218-223), the effects of which shall be discussed next.

#### *1.3.3.4.2 Aerobic Training*

Chronic aerobic training is commonly prescribed for weight loss, blood pressure management, stress reduction and for reducing overall cardiovascular risk

(222,223). Aerobic training is also undertaken for leisure, recreation, and competitive sporting activities as well, with the primary physical performance gains centered around endurance and lesser gains developed in strength and power (189). Common activities rhythmically engage large muscle groups and include walking, jogging, cycling, swimming and rowing. Aerobic training activities are usually sustained for several minutes up to several hours, with 30-60 minute sessions being typical in exercise prescription (189). Exercise intensity is most frequently based on a percentage of measured or estimated peak heart rate, with low intensity exercise occurring around 40-50% of peak heart rate and moderate intensity exercise generally occurring at 50-80% of peak heart rate (189). Unlike resistance training, aerobic training is usually continuous, with skeletal muscles working well below their maximal workload, but doing so for extended periods of time. Energy needs are supplied predominantly via aerobic pathways and maximum sustainable workload is heavily dependent on a combination of oxygen delivery through increased cardiac output and muscle perfusion, and up-regulation of aerobic metabolic pathways allowing oxygen uptake and utilization in skeletal muscles. Not surprisingly, aerobic training produces substantially different hemodynamic challenges to the LV as compared to resistance training (77).

In aerobic training, skeletal muscles generally work in rhythmic cycles against modest resistance. As such, the LV meets an overall reduced resistive load in working muscle during aerobic exercise, and the rhythmic muscle contractions aid in venous return via the skeletal muscle pump effect, with vasodilated working muscles providing minimal resistance between contractions (224). Aerobic training

challenges the LV by demanding high cardiac output for extended periods of time but at moderate systolic pressure increases, with exercise systolic pressures in normotensive subjects commonly ranging from 140-170 mmHg (189,223,225,226). Although the range of LV adaptations that may occur varies widely based on the particular individual and training regimen, the commonest changes in the LV are reduced resting heart rate, increase in resting and exercise stroke volume, increased exercise cardiac output and improved diastolic function (77,227). Resting and exercise stroke volume may increase by well over 20%, and an echocardiographic study on endurance athletes found that 70% of stroke volume increase during upright exercise is attributable to increases in end-diastolic volume, with only a small amount of the additional stroke volume due to decrease in exercise end-systolic volume (228). Further, as LV length did not change appreciably, most of the increase in end-diastolic volume during exercise was related to short axis exercise expansion, predominantly in the mid and apical portions of the LV.

Changes in the overall LV mass are variable in response to aerobic training with some studies showing a net mass increase, others a decrease, and still others no change (205,229-231). However, in the cases where overall mass increased, a commensurate increase in end-diastolic volume was typically reported, indicative of physiological adaptation to aerobic training rather than pathological changes (229,230). When considering the populations of the studies above, net LV mass reductions can generally be expected in hypertensive individuals who undertake aerobic training programs. Alternatively, untrained, but healthy and normotensive

subjects are more likely to experience overall LV mass increase in response to chronic aerobic training.

In cases of cardiovascular disease, aerobic training is commonly prescribed. It is a front line prescription for blood pressure reduction, and in the case of young hypertensives, aerobic exercise and lifestyle change are often exclusively prescribed despite a limited evidence-base to support this guidance (232). The American College of Sports Medicine notes that once medically cleared, aerobic training is beneficial for hypertensives, but exercise blood pressure should be kept below 220 mmHg systolic pressure and below 105 mmHg diastolic pressure (189,223). In broad terms, for middle aged and older hypertensives, chronic, moderate intensity aerobic training has been associated with LVH regression, 5-7 mmHg systolic blood pressure reduction and fewer cardiovascular events (233-238). Moderate aerobic training has benefits in heart failure patients as well, where training regimens have been shown slow the progression of disease, and in some cases is associated with a regression of fatigue or other adverse symptoms (161,212,239,240). Overall, chronic aerobic training is generally considered safe and beneficial for most populations, and in the LV produces common physiological adaptations characterised by increases in resting and exercise stroke volume, primarily through greater radial expansion and associated increases in end-diastolic volume. These changes facilitate increases in exercise cardiac output, which in elite athletes during peak exercise may be 500% or more of resting cardiac output (77).

## 1.4 Thesis Hypotheses and Objectives

The Aim of this thesis is to characterise the LV exercise stress response of two prevalent young adult populations with increased cardiovascular risk: 1) individuals born preterm and 2) individuals with elevated blood pressure. Furthermore, I will investigate potential links between baseline phenotypic traits and dysfunctional elements of the stress response. I predict that the structural and functional remodelling associated with the preterm-born cardiac phenotype will adversely impact the cardiac stress response in young adults. I also predict that blood pressure elevation will be associated with defects in the cardiac stress response in these individuals during young adulthood. My specific hypotheses are:

1. Preterm-born young adults will have impaired LV response to the physiological stress of physical exercise that is proportional to their degree of LV remodelling.

2. Preterm-born young adults will have reductions in aerobic exercise capacity that is proportional to their degree of left ventricular remodelling.

3. Young adults with elevated blood pressure will have impaired LV response to the physiological stress of physical exercise that is proportional to their degree of blood pressure elevation.

4. Young adults with elevated blood pressure will have reductions in exercise capacity that is proportional to their degree of blood pressure elevation.

As a means to investigate these hypotheses, my thesis objectives are to:

1. Perform baseline cardiac phenotyping and exercise stress echocardiography at prescribed exercise intensities to quantify variations in the left ventricular exercise stress response of preterm-born young adults vs term-born controls and investigate how left ventricular remodelling in those born preterm relates to variations in physiological stress response;

2. Perform maximal cardiopulmonary exercise testing to quantify variations in aerobic exercise capacity between preterm-born young adults vs term-born controls and investigate how left ventricular remodelling in those born preterm relates to variations in exercise capacity;

3. Perform baseline left ventricular structural phenotyping and exercise stress echocardiography at prescribed exercise intensities to quantify variations in the left ventricular structure and exercise stress response in young adults with elevated blood pressure vs normotensive controls and investigate how blood pressure relates to variations in left ventricular structure and physiological stress response;

4. Perform maximal cardiopulmonary exercise testing to quantify variations in aerobic exercise capacity between young adults with elevated blood pressure vs normotensive controls and investigate how blood pressure relates to variations in exercise capacity; and

5. Discuss the findings of my thesis investigations including possible mechanisms, clinical implications and future work.

# Chapter 2 STUDY POPULATION

## 2.1 Overview

The University of Oxford Cardiovascular Clinical Research Facility (CCRF), where I conducted my DPhil research, employs an integrated protocol structure to develop and conduct clinical investigations relevant to birth history and blood pressure in the young adult population. These protocols are conducted under the auspices of principle investigator Prof Paul Leeson and Dr Adam Lewandowski, with close collaboration with the Oxford Centre for Clinical Magnetic Resonance (OCMR) for the magnetic resonance imaging components. This protocol structure uses a standardised core set of deep phenotyping cardiovascular measures across the protocols to facilitate linking and comparing of baseline data across the protocols. As new protocols are developed, additional components or measures can be added in a modular fashion in order to pursue new investigative targets that have been informed by our previous protocols and relevant published research.

Currently, this overall structure includes two completed protocols, the Early Vascular Study (EVS) and the Young Adult Cardiovascular Health sTudy (YACHT) as well as one active protocol, the Trial of Exercise to Prevent HypeRtension in young Adults (TEPHRA). This thesis primarily employs data collected from YACHT, with some baseline structural phenotyping in the blood pressure investigations of analyses for Study 3 including EVS data. The immediate focus of my future research work will be based on the TEPHRA trial. Briefly, in addition to the core measures, YACHT adds an exercise testing component which was carried into the TEPHRA trial. TEPHRA also includes a randomized control exercise intervention

component. A summary of the integrated progressive protocol structure is provided in **Figure 2.1.**

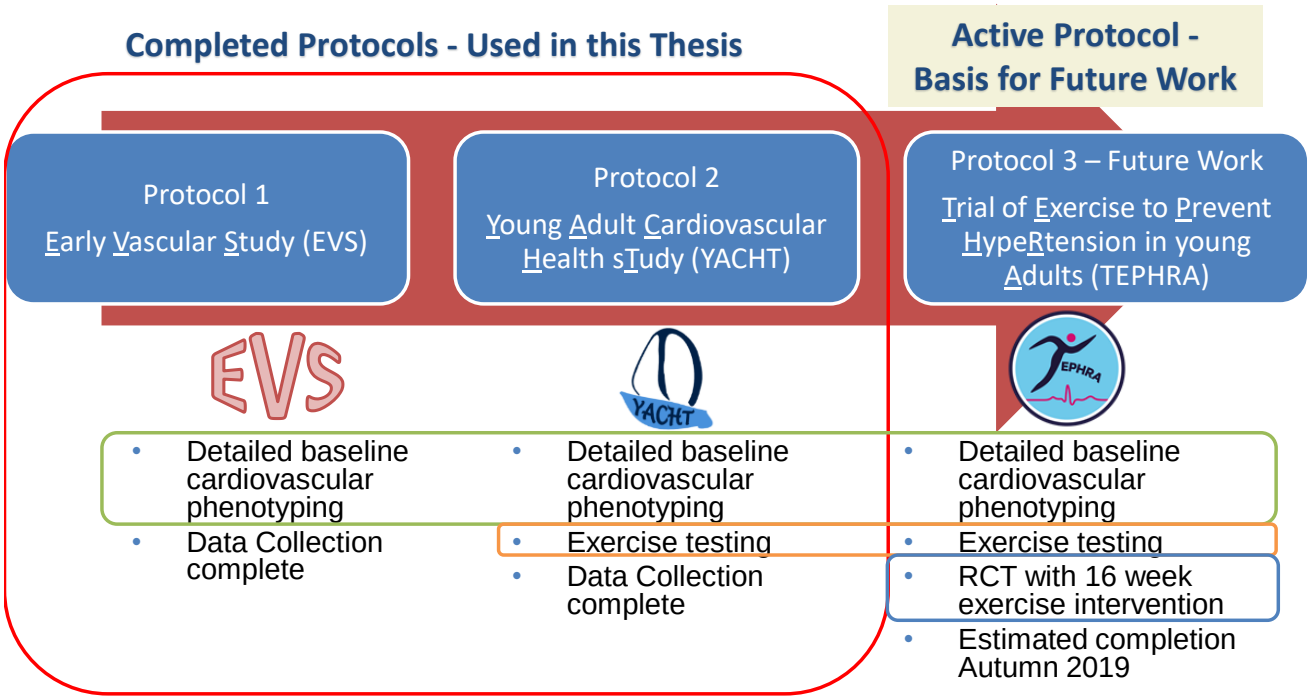



**Figure 2.1. Integrated Progressive Protocol Structure**

An integrated progressive structure of three protocols have been employed at the University of Oxford CCRF as a vehicle to conduct targeted young adult investigations relevant to birth history and blood pressure. Protocol 1 and Protocol 2 are complete, and provided the analytical basis for this thesis whereas Protocol 3 is ongoing and provides a foundation for my future research work.

The YACHT study was initiated by PI Leeson, Dr Adam Lewandowski (presently an intermediate research fellow) and sports medic Dr Wilby Williamson (who is presently a DPhil candidate at the time of this thesis drafting). Approval for YACHT was granted by the South Central Berkshire Research Ethics Committee (14/SC/0275). Study registration was completed via www.clinicaltrials.gov (NCT02103231). YACHT was a cross-sectional, case-control study which

investigated cardiovascular structure, function and physiological exercise stress response in preterm-born, term-born, and hypertensive young adults aged 18-40. YACHT was ongoing when I began my DPhil and I was actively engaged across the range of study activities from the time of my arrival through to study completion, which covered the majority of the study.

EVS was registered with http://www.clinicaltrials.gov (NCT01487824), and the study was approved by the Oxfordshire Research Ethics Committee A (06/Q1604/118). Data collection for EVS had already been completed by Dr Adam Lewandowki with Prof Paul Leeson as PI when I began my DPhil. EVS primarily followed up on multiple birth registry cohorts and accomplished cardiovascular phenotyping of participants with a range of birth histories and blood pressures. YACHT was designed as a follow-on to EVS within the progressive protocol structure. Eligibility criteria for the two studies were similar with. A summary of study measures is presented in **Figure 3.1**.

### 2.1.1 Personal Contribution

I conducted my own specific, targeted DPhil investigations as a member of an integrated research team. Overall, I was extensively involved in frontline study activities and data collection for approximately 85 of the 149 YACHT study visits. A more detailed accounting of my personal contributions to the recruitment, investigative data collection, data processing and data analysis from the YACHT and EVS studies which were used in this dissertation follows below.

#### 2.1.1.1 Personal Contribution to the YACHT study

For YACHT study recruitment, I performed approximately 35% percent of the email and telephone correspondence activities for recruitment of the last 85 study participants. This included answering questions and providing information about the study to those who had responded to flyers or mail shots, as well as scheduling study visits.

For YACHT Cardiac Magnetic Resonance (CMR) imaging, Dr Lewandowski performed all image acquisition and processing. For approximately 40 CMR scans, I performed: participant scheduling, pre-imaging safety screening and briefing, MRI coil and hardware positioning and monitoring of the scan to ensure participant safety. Following image acquisition and processing, I performed all analyses of CMR measures and parameters that are presented in this thesis.

For YACHT venous blood sampling, I collected and or processed samples on approximately 85 of the 149 study visits. Upon study completion, I sorted and tallied all study samples and established the working database used for planning and tracking of all assays. I provided samples to the John Radcliffe Hospital Biochemistry Lab for all lipid and glucose assays. I performed approximately 60% the copeptin assays on my own and was assisted by Ms Ashley Verburg and Ms Jennie Han for the remaining copeptin assays. I performed all analyses of biochemistry measures and parameters that are presented in this thesis.

For YACHT resting echocardiography, image acquisition and processing was performed by research team members trained in echocardiographic image acquisition and processing. I performed all analyses of resting echocardiographic measures and parameters that are presented in this thesis.

For YACHT cardiopulmonary exercise testing (CPET) and spirometry assessments, I conducted approximately 85 CPETs either on my own or with the aid of another research team member and I personally conducted approximately 45 spirometry assessments. I performed all analyses of CPET and spirometry measures and parameters that are presented in this thesis.

For YACHT stress echocardiography, image acquisition and processing was performed by research team members trained in echocardiographic image acquisition and processing. I performed the non-imaging components of the test for approximately 40 exercise stress assessments. I performed all analyses of stress echocardiographic measures and parameters that are presented in this thesis.

Finally, for routine baseline assessments on approximately 85 YACHT study visits, I regularly performed anthropometric measures, collected peripheral and aortic blood pressure measures and also fitted 24 hour blood pressure monitors and wrist-worn logging accelerometers. I also downloaded data collected from monitoring devices. Raw accelerometry data was processed by Dr Aiden Doherty at the University of Oxford Big Data Institute. I performed all analyses of the above measures and parameters that are presented in this thesis.

#### 2.1.1.2 Personal Contribution to the EVS study

Data collection as well as image and sample processing for the EVS study were completed prior to my arrival at the University of Oxford. I used the existing baseline physiological, anthropometric and CMR measures from this study and then performed my own analyses on these data. These data contributed to the baseline analyses of Study 3 presented in this thesis.

### 2.1.2 Allocation of Study Groups

To perform the target investigations of this dissertation, participants were divided into the following four study groups:

- Group 1 – Term-born Young Adults
- Group 2 – Preterm-born Young Adults
- Group 3 – Young Adults with Elevated Blood Pressure
- Group 4 – Young Adults with Normal Blood Pressure

The group descriptions follow below and a summary of the groups is provided at **Figure 2.2 and Figure 2.3**. The groups were analysed in four distinct investigations or "Studies". Four results chapters are presented in this thesis, in each results chapter I present the results of an individual Study.

## 2.2 Group 1 - Term-born Young Adults

### 2.2.1 Inclusion Criteria

The term-born young adult control group for this thesis was derived of participants in the YACHT study who met the following inclusion criteria:

- Aged 18 to 40 years
- BMI < 40 kg/$m^2$
- Awake ambulatory systolic blood pressure < 135 mmHg
- Awake ambulatory diastolic blood pressure < 85 mmHg
- Verified history of full-term birth
- Able and willing to give informed consent for participation in the YACHT study

### 2.2.2 Exclusion Criteria

- Unwilling or unable to give informed consent
- Pregnant or lactating
- Prior history of acute cardiac or cerebrovascular event
- Diagnosis with a disease or disorder which could influence study participation or outcome measures
- Hypertension diagnosis
- Treatment with antihypertensive medication

### 2.2.3 Characteristics

The mean gestational age of Group 1 was 39.5 weeks. A comparison of key characteristics of Group 1 and Group 2 is provided at **Table 2.1**.

## 2.3 Group 2 - Preterm-born Young Adults

### 2.3.1 Inclusion Criteria

The preterm-born control group for this thesis was derived of participants in the YACHT study who met the following inclusion criteria:

- Aged 18 to 40 years
- BMI < 40 kg/$m^2$
- Awake ambulatory systolic blood pressure < 135 mmHg
- Awake ambulatory diastolic blood pressure < 85 mmHg
- Verified history of preterm birth
- Able and willing to give informed consent for participation in YACHT

### 2.3.2 Exclusion Criteria

- Unwilling or unable to give informed consent
- Pregnant or lactating
- Prior history of acute cardiac or cerebrovascular event
- Diagnosis with a disease or disorder which could influence study participation or outcome measures

- Hypertension diagnosis
- Treatment with antihypertensive medication

### 2.3.3 Characteristics

The mean gestational age of the preterm-born group was 32.8 weeks. A comparison of key characteristics of Group 1 and Group 2 is provided at **Table 2.1** below.

**Table 2.1 Comparison of key Characteristics of Preterm-born (Group 2) and Term-born (Group 1) Young Adult.**

| | Preterm-born Young Adults (N=47) | Term-born Young Adults (N=54) | P Value |
|---|---|---|---|
| **Demographics and Anthropometrics** | | | |
| Age (years) | 22.7±3.04 | 23.6±3.77 | 0.239 |
| Male, n (%) | 14 (30) | 26 (48) | 0.061 |
| Height (cm) | 167±8.6 | 175±10.0 | **<0.001** |
| Weight (kg) | 65.3±13.5 | 70.0±13.0 | 0.299 |
| Body surface area ($m^{2)}$ | 1.73±0.19 | 1.84±0.21 | **0.010** |
| BMI ($kg/m^2$) | 23.3±4.52 | 22.7±2.68 | 0.401 |
| Birth Weight (grams) | 1916±806 | 3390±424 | **<0.001** |
| Birth Weight Range (grams) | 595-3203 | 2640-4536 | **<0.001** |
| Gestational age (weeks) | 32.8±3.22 | 39.5±1.37 | **<0.001** |
| Gestational age range (weeks) | 23-36 | 38-42 | **<0.001** |
| Gestational Hypertension, n (%) | 8 (17.0) | 0 (0) | **0.002** |
| Small For Gestational Age, n (%) | 2 (4.3) | 0 (0) | 0.214 |
| **Brachial Blood Pressure (mmHg)** | | | |
| Resting Systolic | 119±9.35 | 115±7.68 | **0.014** |
| Resting Diastolic | 70±8.07 | 66 ±4.82 | **0.014** |
| Awake Average Ambulatory Systolic | 119±6.27 | 119±7.62 | 0.610 |
| Awake Average Ambulatory Diastolic | 71±4.90 | 69±4.54 | 0.058 |

Group Characteristics presented as mean±standard deviation. P values were adjusted for sex. BMI, Body Mass Index. P values below 0.05 depicted in bold.

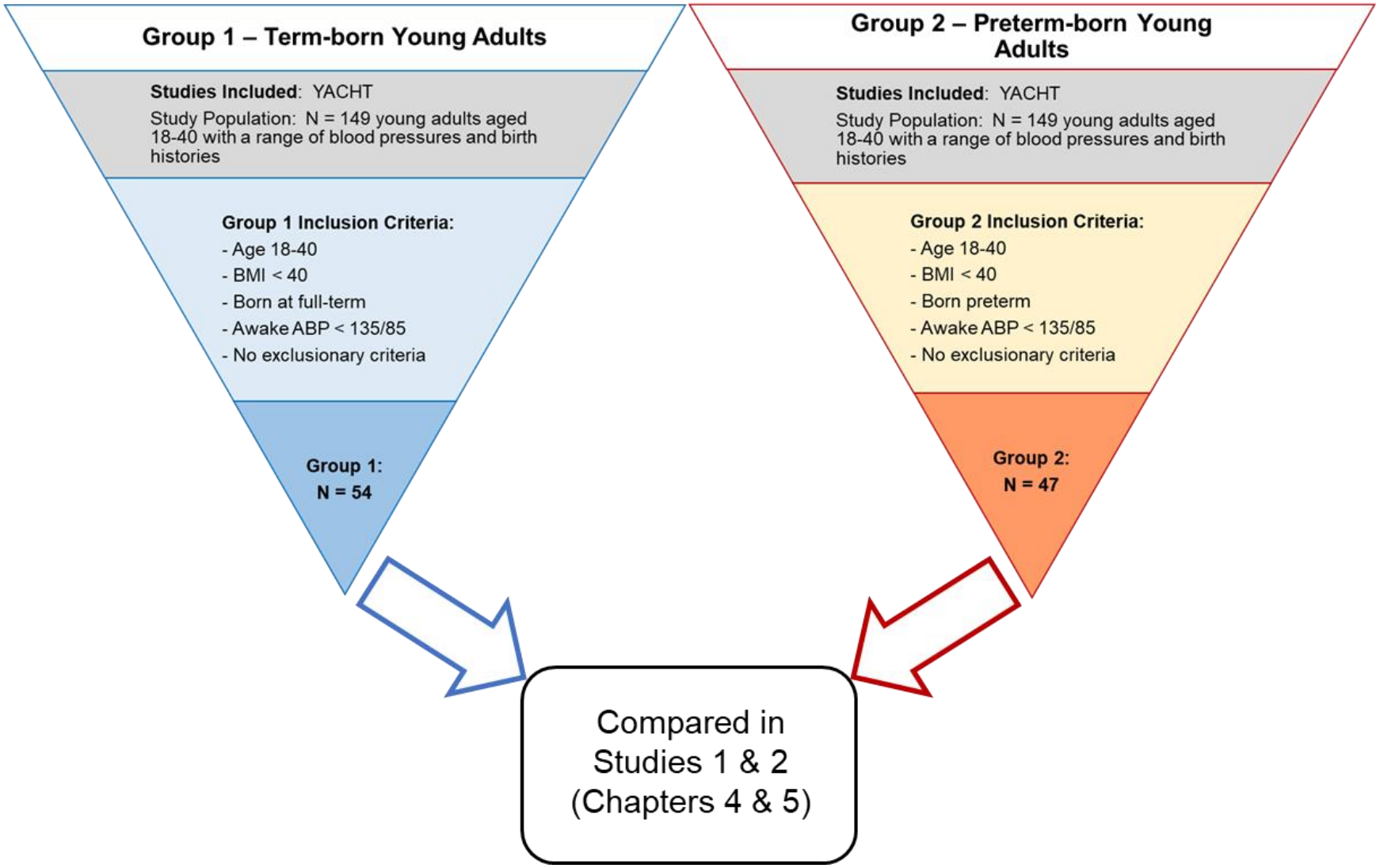



**Figure 2.2 Summary of Group 1 and Group 2**

Groups 1 and 2 were used for all comparisons and analyses between preterm and term-born young adults in this dissertation. All investigations were conducted at the University of Oxford Cardiovascular Clinical research Facility and Oxford Centre for Magnetic Resonance. The results of the investigations carried out on these groups are presented in Chapters 4 and 5.

## 2.4 Subject Recruitment for Groups 1 and 2

Identification and recruitment of subjects for YACHT was accomplished through a multi-tiered recruitment campaign optimized to produce heterogeneity across two key risk factors of preterm birth and blood pressure elevation. YACHT recruitment initiated in August 2014 and continued until completion of study recruitment and data collection on 31 May 2016. Recruitment was accomplished through purposive active recruitment of young adults with varying blood pressure by inviting young adults who had visited the John Radcliffe Hospital Hypertension Services Clinic in Oxford, UK to take part in the study, passive open recruitment in the local Oxford community using posters, mailed invitations from the John Radcliffe Hospital birth registries, word of mouth, and invitations to previous study participants who had indicated interest in future study participation.

In total, N=149 participants were recruited to take part in YACHT. Of the N=149 recruited individuals who completed YACHT, 48 participants were excluded from Groups 1 and 2 for failing to meet the blood pressure criteria. A summary of those excluded follows below:

- 32 diagnosed hypertensive participants recruited through the John Radcliffe Hospital Hypertension Clinic
- 16 participants with awake ambulatory systolic and/or diastolic blood pressure ≥ 135/85 mmHg

After excluding the above 48 participants from groups 1 and 2 to reduce confounding due to hemodynamic alterations secondary to hypertension, 101 non-hypertensive young adult participants (47 preterm-born, 54 term-born) meeting inclusion criteria were included in groups 1 and 2 for analysis. A summary of Group 1 and Group 2 is included in **Figure 2.2.**

## 2.5 Group 3 - Young Adults with Elevated Blood Pressure

The group of young adults with elevated blood pressure studied in this thesis consists of participants from the YACHT and EVS studies. Participants from the EVS study meeting the inclusion criteria below were frequency matched for age and sex into the YACHT elevated blood pressure group. Though not the focus of this portion of analyses, members of this group were all born at term.

### 2.5.1 Inclusion Criteria

- Aged 18 to 40 years
- BMI < 40 kg/m$^2$
- Resting systolic and/or diastolic blood pressure ≥ 120/80 mmHg
- Able and willing to give informed consent for study participation

### 2.5.2 Exclusion Criteria

- Unwilling or unable to give informed consent
- Pregnant or lactating
- Prior history of acute cardiac or cerebrovascular event

- Diagnosis with a disease or disorder which could influence study participation or outcome measures

### 2.5.3 Characteristics

Group 3 consisted of 62 young adults (mean age 26.8±5.51 years) with elevated blood pressure. Mean systolic pressure was 129±9.5 mmHg and mean diastolic pressure was 77±8.0 mmHg. A comparison of key characteristics between groups 3 and 4 is provided at **Table 2.2**.

## 2.6 Group 4 – Young Adults with Normal Blood Pressure

The group of young adults with normal blood pressure studied in this thesis consists of participants from the YACHT and EVS studies. This group was frequency matched for age and sex to the YACHT elevated blood pressure group. Though not the focus of this portion of analyses, all members of this group were born at term.

### 2.6.1 Inclusion Criteria

- Aged 18 to 40 years
- BMI < 40 kg/m$^2$
- Resting systolic blood pressure < 120 mmHg
- Resting diastolic blood pressure < 80 mmHg
- Able and willing to give informed consent for study participation

### 2.6.2 Exclusion Criteria

- Unwilling or unable to give informed consent
- Pregnant or lactating
- Prior history of acute cardiac or cerebrovascular event
- Diagnosis with a disease or disorder which could influence study participation or outcome measures

## 2.7 Subject Recruitment for Groups 3 and 4

Groups 3 and 4 included term-born participants from the YACHT and EVS studies. A description of the recruitment campaign was provided at on page 76.

In total, we recruited N=149 participants who took part in YACHT and N=244 participants who took part in EVS. After excluding those born preterm and completing frequency matching for age and sex, 71 participants from YACHT and 75 participants from EVS were included in Group 3 (N=62) and Group 4 (N=84) and subsequently analysed in studies 3 and 4 which are presented in results chapters 6 and 7 respectively. A summary of groups 3 and 4 is provided in **Figure 2.3.**

### 2.7.1 Characteristics of Groups 3 and 4

Group 4 consisted of 84 young adults (mean age 26.5±4.51 years) with normal blood pressure. Mean systolic pressure was 110±6.5 mmHg and mean diastolic pressure was 66±5.7 mmHg. A comparison of key characteristics between groups 3 and 4 is provided here at **Table 2.2**.

**Table 2.2. Comparison of key Characteristics of Young Adults with Normal Blood Pressure (Group 3) and Elevated Blood Pressure (Group 4)**

| | Elevated Blood Pressure (N=62) | Normal Blood Pressure (N=84) | P value |
|---|---|---|---|
| **Demographics and Anthropometry** | | | |
| Age | 26.8±5.51 | 26.5±4.51 | 0.681 |
| Male n (%) | 31 (50) | 42 (50) | >0.999 |
| Height (cm) | 173.6±9.86 | 174.0±9.92 | 0.807 |
| Weight (kg) | 73.9±14.04 | 70.2±13.35 | 0.113 |
| BMI ($kg/m^2$) | 24.4±3.80 | 23.0±2.93 | **0.014** |
| **Brachial Blood Pressure (mmHg)** | | | |
| Resting Systolic | 129±9.5 | 110±6.5 | **<0.001** |
| Resting Diastolic | 78±8.0 | 66±5.7 | **<0.001** |
| Awake Average Ambulatory Systolic | 133±13.7 | 120±10.3 | **<0.001** |
| Awake Average Ambulatory Diastolic | 79±9.6 | 71±5.9 | **<0.001** |
| Antihypertensive Drugs n (%) | 15 (24) | 0 (0) | **<0.001** |

Group characteristics presented as mean±standard deviation. BMI, Body Mass Index. P values below 0.05 depicted in bold.

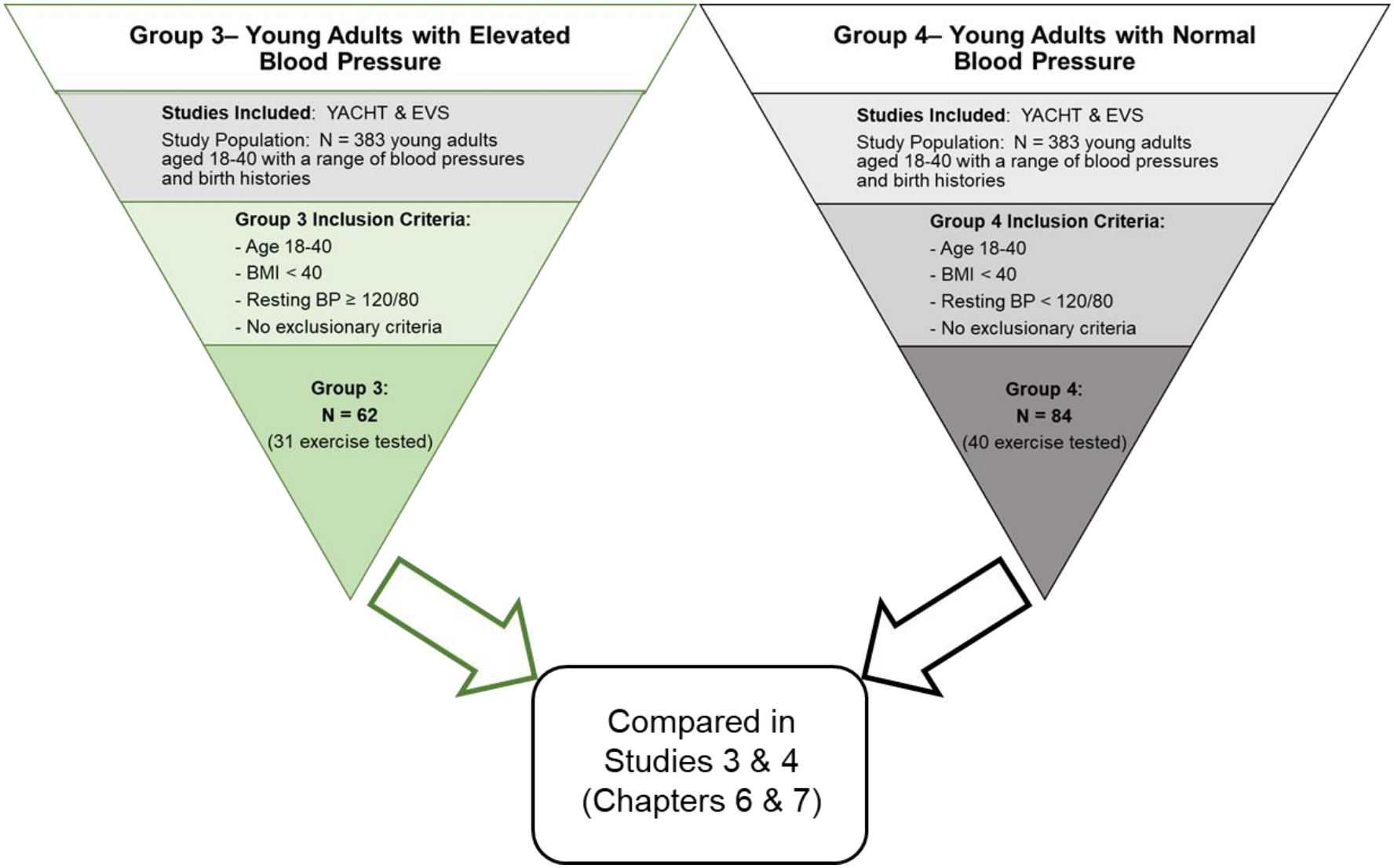



**Figure 2.3. Summary of Group 3 and Group 4**

Cohorts 3 and 4 were used for all comparisons and analyses between young adults with elevated blood pressure and normal blood pressure in this dissertation. All investigations were conducted at the University of Oxford Cardiovascular Clinical research Facility and Oxford Center for Magnetic Resonance. Groups 3 and 4 were all born at term and were frequency matched for age and sex. The results of investigations carried out on these cohorts are presented in chapters 6 and 7.

# Chapter 3 GENERAL METHODS

## 3.1 Overview

This chapter includes a description of the methods employed to perform the investigations used for this dissertation. I will begin with an overview of the YACHT and EVS study visits then progress to a more detailed description of the assessments and measurements included for analyses in this thesis. I will then describe the processes and methodologies used for analysis of collected data, measurements, and samples and provide a description of the statistical methods used for the statistical analysis.

### 3.1.1 Study Visit Overview

Potential recruits were instructed to fast overnight for 12 hours but encouraged to drink water to remain hydrated and continue taking any prescribed medications prior to attending a study visit at the University of Oxford OCMR and CCRF in the John Radcliffe Hospital in Oxford, UK. All individuals had previously received the study participant information leaflet and been encouraged to ask further questions regarding the study should they have any. Upon arrival, we completed an informed consent process which included providing a study overview and allowing for any further questions to be asked by potential recruits. Once written informed consent was completed, the study measures portion of the visit was initiated.

The visit began with the collection of a fasting, at-rest blood sample and a multi-organ MRI of the heart, brain, and liver. After initial blood sampling and MRI

imaging were completed, participants were provided with a standard light snack of two cereal bars after which they completed the remaining cardiovascular assessments. Exercise stress assessments were performed in the YACHT study only, and took place at the end of the study visit so as not to confound other measurements. At the end of the study visit, participants were fitted with blood pressure and physical activity monitoring devices and completed a standardised study questionnaire. Monitoring devices were posted back to the University of Oxford Cardiovascular Clinical Research Facility in postage paid envelopes which we provided to the participants. A consolidated flow diagram of the YACHT and EVS study visits is provided at **Figure 3.1**.

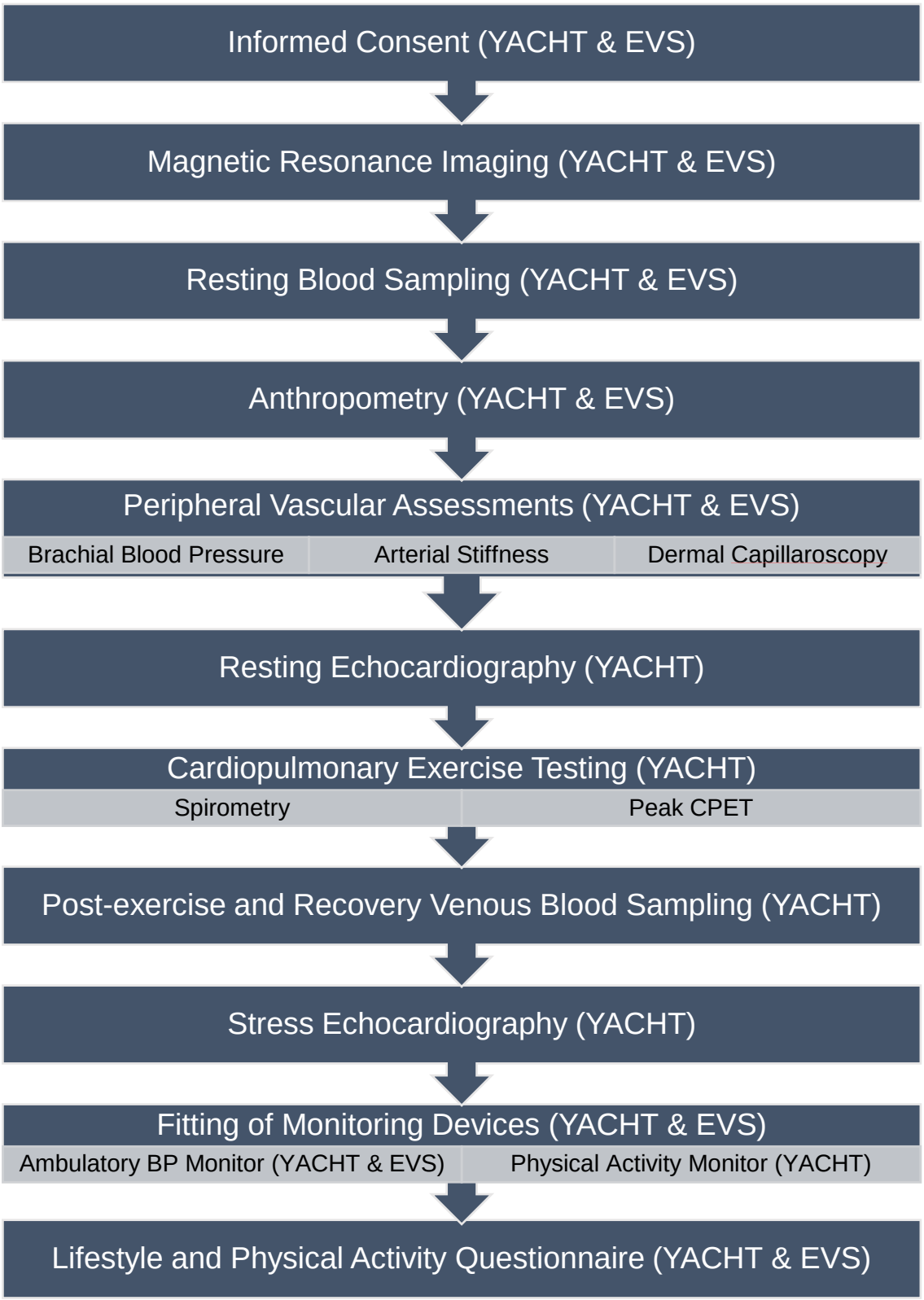



**Figure 3.1. Study Visit Flow Diagram**

Consolidated study visit flow diagram for the Early Vascular Study (EVS) and Young Adult Cardiovascular Health sTudy (YACHT). Baseline measurements were similar across studies. YACHT alone included echocardiography and exercise testing. CPET: cardiopulmonary exercise testing; BP: blood pressure.

## 3.2 Ethical Standard

Approval for YACHT was granted by the South Central Berkshire Research Ethics Committee (14/SC/0275) with study registration completed via www.clinicaltrials.gov (NCT02103231). EVS was approved by the Oxfordshire Research Ethics Committee A (06/Q1604/118) and was registered with http://www.clinicaltrials.gov (NCT01487824). Written informed consent was obtained from all potential participants prior to start of the study.

## 3.3 Anthropometry

Using an integrated height and weight measurement station (Sema, UK), height was measured to the nearest centimeter and weight measured to the nearest 0.1 Kg with footwear removed and participants wearing light clothing. Manual waist circumference was measured 2 cm above the iliac crest, and manual hip measurements were taken at the point of widest overall girth near the level of the greater trochanter of the femur or mid-buttock. Body Mass index (BMI) was calculated using the following standard formula:

$$BMI\ (kg/m2) = Weight\ (kg)/[height\ (m)]^{2}$$

Body Surface Area (BSA) was calculated using the Mosteller formula below:

$$BSA\ (m^2) = \sqrt{[Height\ (cm) \times Weight\ (kg)]/3600}$$

## 3.4 Cardiac Magnetic Resonance (CMR) Imaging

At-rest CMR imaging was performed on either a 1.5-T Seimens Sonata or 3-T Siemens Trio Tim scanner with a 12 channel receiver coil using a standardised imaging protocol. All CMR imaging was prospectively ECG gated with a precordial three lead electrocardiogram (ECG) and acquired during end-expiration breath hold. Image acquisition parameters for the steady-state free-precession images were as follows: echo time, 1.5 milliseconds; temporal resolution 47.8 ms; repetition time, 3.0 milliseconds; and flip angle, 60°. Localization images (horizontal long axis, vertical long axis and short axis) were acquired first via steady-state free precession cine sequences, followed by optimised LV horizontal long axis and vertical long axis cines. These images were then used to acquire an optimally aligned, steady-state free precession cine LV short axis stack with a 7.0 mm slice thickness and a 3.0 mm inter-slice gap. Images were immediately archived and LV structural analysis was performed offline using Siemens image analysis software (Argus; Siemens Medical Solutions, Erlangen, Germany).

### 3.4.1 Left Ventricular Structure Quantification

Image analysis was accomplished off-line by a single blinded investigator. LV short axis epicardial and endocardial borders were manually contoured at end-diastole as well as end-systole for mass and volume quantification. Image contrast was adjusted for each slice to facilitate delineation of epicardial fat from myocardium. End systole was visually determined by the image with the smallest cavity area. The basal slice was selected as the first slice in the short axis stack with 50% or more of the circumference surrounded by ventricular myocardium.

Additional verification of the basal slice was accomplished by comparing the end-diastolic image with subsequent systolic frames and by comparison of the above and below slices, as well as by reviewing the cine loops.

Papillary muscles were included for mass calculation and excluded for volume measurement. The interventricular septum was included as part of the LV. 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: ejection fractioN=[(stroke volume) / (end diastolic volume)]*100%. LV mass was calculated at end diastole by subtracting the endocardial volume from the epicardial volume. LV mass in grams was calculated based on standard values for myocardial specific gravity (1.05 g/cm$^3$).

## 3.5 Venous Blood Sampling

All blood samples were collected from the antecubital fossa by venipuncture or indwelling venous catheter. Water was offered and available to participants throughout their study visit to ensure euhydration. After collecting fasting, at-rest blood samples, participants were provided a standardised snack of two cereal bars. For YACHT participants, post-exercise samples were collected immediately following exercise testing and recovery samples were collected 20 minutes post exercise test completion. Serum collection tubes included a clotting activator to facilitate rapid clotting. Samples were centrifuged within 15 minutes of collection. Separated plasma and serum were then pipetted and stored at -80ºC for future analysis.

### 3.5.1 Biochemistry Analysis

Fasting blood biochemistry was measured at the Oxford John Radcliffe Hospital Biochemistry Laboratory through the use of routine validated assays with clinical level quality controls. Lipid profiling included high density lipoprotein (HDL) cholesterol, total lipoprotein cholesterol, low density lipoprotein (LDL) cholesterol and triglycerides. HDL was directly measured using routine validated assays. LDL cholesterol was calculated using the Friedewald formula:

$$\textit{(LDL = total cholesterol – high HDL – (triglycerides / 2.17))}$$

Homeostatic model assessments for insulin resistance (HOMA-IR) was calculated based on fasting insulin and glucose levels using a HOMA calculator (www.dtu.ox.ac.uk/homacalculator/index.php) using the following formula:

$$\textit{HOMA-IR} = \frac{\textit{Glucose (mmol/L) X Insulin (mU/L)}}{\textit{22.5}}$$

### 3.5.2 Copeptin Analysis

Copeptin levels were measured using a commercially available sandwich immunoassay for C-terminal ProAVP (Copeptin) on the BRAHMS KRYPTOR Compact Plus platform (ThermoFisher Scientific, Paisley, UK). Assays were completed by trained researchers who completed a hands-on vendor training course on the KRYPTOR Compact Plus instrument.

Assays were carried out according to vendor guidelines with researchers blinded to study participant sample allocation. Briefly, prior to running samples, instrument calibration and control runs were completed according to vendor

guidelines. Covariance during calibration was < 1%. Samples were thawed at room temperature and then kept on ice until they were loaded into the instrument for analysis.

Once instrument set up and calibration were complete and samples loaded, I initiated the KRYPTOR automated assay sequence. The instrument performed automated pipetting and mixing of sample aliquots with reagents including an analyte specific fluorophore to generate an immunocomplex with the target analyte (copeptin). The sample was then excited by a 337 nm nitrogen laser, causing the bound immunocomplex to produce a long-life emission which was measured by the device sensor and used for analyte quantification.

## 3.6 Blood Pressure Measurements

### 3.6.1 Aortic (Central) Blood Pressure Measurement

Aortic blood pressure was measured at time of the cardiac magnetic resonance scan with the Vicorder oscillometric device (Skidmore Medical, Taunton, UK), which uses a validated general transfer function for the estimation of aortic (central) blood pressure. The Vicorder, and other similar devices, have been independently validated and found to have similar accuracy to standard clinical oscillometric brachial blood pressure measurement devices (241,242).

A single brachial cuff was placed mid arm and measurements were taken supine with participants having relaxed quietly for at least five minutes. The first inflation of the cuff was used to record a traditional oscillometric brachial blood pressure reading. These readings were entered into the Vicorder and the cuff

was inflated a second time with the baseline measurements used to measure aortic blood pressure by application of a general transfer function algorithm.

### 3.6.2 Resting Peripheral Blood Pressure

After a five-minute quiescent acclimation period, three resting peripheral blood pressure measurements were recorded using a digital blood pressure monitor (Dinamap V100, GE Healthcare, Chalfont St. Giles, United Kingdom) on the left arm with the last two readings averaged and subsequently used for analysis.

## 3.7 Resting Echocardiography

Resting transthoracic echocardiography was performed in the left lateral decubitus position using a commercially available Philips iE33 or Philips EPIQ 7C cardiology ultrasound machine. British Society of Echocardiography guidelines were followed for collection of a standard clinical imaging dataset to quantify left ventricular dimensions and systolic and diastolic function, as well as review valvular function (65).

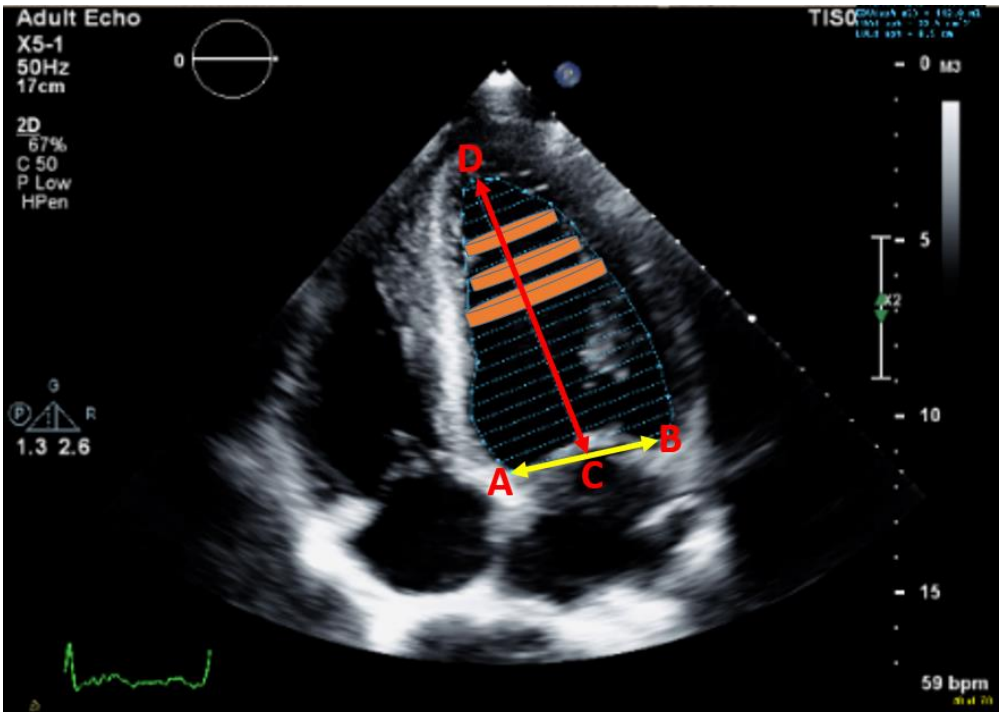



**Figure 3.2. Apical Four-chamber Echocardiographic Image Collected at Rest**

Resting echocardiography included a full standard imaging dataset. Exercise stress echocardiography included only apical 4-chamber views which were optimised for viewing of the left ventricle (LV). LV length and end diastolic volume (EDV) were calculated in the first frame showing full mitral valve closure as depicted above. The contour lines delineating the endocardial border from the cavity were manually optimised. The yellow line passing through the mitral valve leaflets connects the two opposing sides of the mitral valve annular ring (points A and B). The distance from the midpoint of the yellow line (C) to the most distant internal point of the LV apex (D) was recorded as LV length. EDV for sub-maximal exercise imaging was calculated by the single-plane Modified Simpson's Method (modified method of discs) which estimates EDV by integrating the volume of a theoretical series of stacked discs or short cylinders between the endocardial border which completely fill the LV (three discs represented in orange to allow for visualization). The method was repeated on images at maximal ventricular compaction (smallest cavity size) to calculate end systolic volume (ESV). Stroke volume (SV) was then calculated as: SV=EDV-ESV, and ejection fraction (EF) was calculated as: EF=(SV/EDV×100).

### 3.7.1 Echocardiography Analysis

Echocardiography analysis was completed off-line in accordance with standard guidelines from the European Association of Echocardiography and the American Society of Echocardiography (198,243,244) by a single blinded investigator to minimise bias and intra-observer variation. The Philips Xcelera 3.3 (Philips Healthcare Informatics, Belfast, Ireland) and TomTec Image Arena 4.6 (Chicago, IL, United States) software suites were utilised for image analysis. End-diastole was set at the point of mitral valve closure and end systole was set at the point of minimum LV cavity size, LV endocardial borders were manually contoured at end-diastole and end-systole to allow calculation of end diastolic volume (EDV) and end systolic volume (ESV). Resting ejection fractions were then calculated via the bi-plane Modified Simpson's Method using apical 4-chamber and apical 2-chamber views while sub-maximal exercise ejection fractions were calculated by the single-plane Modified Simpson's Method using apical 4-chamber views.

#### 3.7.1.1 Echocardiography Variability

Ten imaging datasets were randomly selected and re-evaluated for intra- and inter-observer variability. Intraclass Correlation Coefficients (ICC) for LV mass, ejection fraction, stroke volume, end-diastolic volume, longitudinal strain, longitudinal systolic strain rate and longitudinal diastolic strain rate for intra/inter-observer variability yielded: 0.99/0.96; 0.89/0.85; 0.92/0.88; 0.94/0.91; 0.93/0.86; 0.86/0.81; and 0.95/0.90 respectively.

## 3.8 Spirometry Testing

Prior to peak cycle ergometry testing, standard spirometry measurements were performed to assess participant pulmonary function. Volumetric and gas standard calibration was accomplished prior to each test and the room temperature was maintained between 18-19 degrees Celcius. The British Thoracic Society and Association of Respiratory Technicians and Physiologist Guidelines for the Measurement of Respiratory Function (245) were followed to collect forced vital capacity (FVC) and maximum ventilatory volume (MVV) and associated sub-measures. Subject age, sex, height and weight were first entered into the system and then participants were instructed on proper technique for each assessment. Subjects were seated and wore comfortable exercise clothing for the assessments. Each participant was guided through three acceptable FVC maneuvers as follows:

- Subject sitting comfortably and breathing normally with mouthpiece in and nose clipped
- Subject maximally inhales
- Subject exhales as forcefully and completely as possible, continuing to exhale for 15 seconds
- Subject maximally inhales to complete flow loop
- Subject allowed to recover for at least 30 seconds then repeats until three acceptable maneuvers obtained

After FVC measurement, participants were instructed on the MVV maneuver and completed 1 valid MVV maneuver as follows:

- Subject sitting comfortably and breathing normally with mouthpiece in and nose clipped
- When cued by investigator, subject breathes as deeply and rapidly as possible for 12-15 Seconds
- Investigator coaches subject to breath deeper or faster as appropriate
- Complete once one valid measure collected

From the FVC maneuvers, FVC, Forced Expiratory Volume in 1 second ($FEV_1$) and Peak Expiratory Flow Rate (PEFR) were automatically calculated by the software. MVV was also directly calculated from within the software.

### 3.8.1 Cardiopulmonary Exercise Testing (CPET)

Participants completed a peak CPET on a seated stationary cycle ergometer (Ergoline GmbH, Bitz, Germany) using a validated incremental protocol with respiratory gases collected and measured (Metalyzer 3B, Cortex Biophysik, Leipzig, Germany). Masks were properly fitted and checked to ensure no leaks and then adjusted as needed. Seat height was set to allow for near full extension of the leg when the pedal was closest to the ground to minimize premature fatigue of the quadriceps muscles. Heart rate was recorded using continuous electrocardiogram monitoring, while rate of perceived exertion (RPE) on a standard Borg scale (246,247) was recorded every two minutes and blood pressure was recorded every four minutes using a manual mercury sphygmomanometer (ACCOSON Freestyle, Essex, UK). Participants were instructed to maintain a rate of 60 revolutions per minute during the test, which began with one quiescent minute of resting measurements followed by a two-minute warmup against a 20 watt workload. After the warmup period, workload

increased to 35 watts. To normalise test duration to approximately 8-12 minutes, participants who reported higher activity or fitness levels had their workload increased to 75 watts after the warmup period. Workload then incremented by 15 watts each minute and participants cycled continuously until exhaustion prevented them from maintaining at least 50 revolutions per minute or safety termination criteria were met (248,249). Participants then completed a two-minute cool down period at 35 watts and the revolutions per minute of their preference. Following the cool down period, participants were transferred to a reclined treatment bed for post-exercise venous blood sampling.



**Figure 3.3. Spirometry and Cardiopulmonary Exercise Testing (CPET)**

Resting spirometry was performed prior to CPET to assess baseline pulmonary function. CPET testing was used to measure exercise capacity as well as cardiovascular and pulmonary response to exercise.

### 3.8.2 Stress Echocardiography

After completing CPET testing, participants had a 30-minute recovery period during which they were offered water and an additional cereal bar. Sub-

maximal exercise stress echocardiography was then performed on study subjects for three minutes at 40%, 60%, and 80% of their peak exercise wattage achieved during CPET testing. After participants exercised at each prescribed workload for two minutes and steady-state heart rate was verified, apical 4-chamber views oriented for optimal imaging of the left ventricle as well as pulsed wave Doppler imaging of mitral inflow were collected at each stage using either a Philips iE33 or Philips EPIQ 7C cardiology ultrasound machine. Participants were given a three-minute rest period between each stage. Off-line image analysis was only performed on images where heart rate was within 5% of maximum achieved steady-state heart rate for a given stage.

## 3.9 Fitting of Monitoring Devices

At the end of the study visit, participants were fitted with an ambulatory blood pressure monitor and wrist-worn activity monitor by a trained study investigator. The procedures for each device follow below.

### 3.9.1 24 Hour Ambulatory Blood Pressure Monitoring

24-hour ambulatory blood pressure monitoring was initiated at the end of the study visit using oscillometric, ambulatory devices (TM-2430, A&D Instruments, Abingdon, United Kingdom). Correct cuff size was chosen based on arm circumference. Subjects were instructed to remain still during measurements. Measurements were automatically taken every 30 minutes during daytime and then hourly from 11:00PM to 7:00AM. Subjects completed a diary documenting hours asleep and awake. Monitors were posted back to the University of Oxford Cardiovascular Clinical Research Facility where

measurements were downloaded using the Doctor Pro 2 or Doctor Pro 3 software for Windows (A&D Company Limited, Tokyo, Japan) and awake and asleep times were recorded.

### 3.9.2 Activity Monitoring

At the end of the study visit, participants were properly fitted with AX3 wrist-worn, three-axis logging accelerometers (Axivity, Newcastle upon Tyne, United Kingdom). The monitors were worn for nine days then mailed back in envelopes provided by the study team. The first seven days of wear data were analysed for total hours of Moderate to Vigorous Physical Activity (MVPA) with acceleration thresholds set to include activities equivalent to approximately three or more METs (ie walking, leisurely cycling) and also vigorous physical activity (VPA) with acceleration thresholds set to include activities exceeding approximately 6 METs (ie jogging, running, active sport) (250).

## 3.10 Statistical Analysis

Statistical analysis was performed using SPSS Version 23. Shapiro-Wilk testing and visual inspection were used to assess normality of variable distribution. Direct between-group comparisons were performed using independent-samples Student t-tests for normally distributed data and Mann-Whitney and Kruskal-Wallis tests for non-normally distributed data. Linear regression modelling was completed using forced entry with missing data excluded pairwise. Standardised regression coefficients (β) are presented for multivariable linear regressions. Pearson correlations (*r*) are presented for simple bivariate and also partial correlation analyses. Where appropriate and

indicated, multivariable linear regression modelling was used to adjust for differing age and or sex distributions between analysis groups. p values below 0.05 were considered statistically significant.

# Chapter 4 Study 1 Results Left Ventricular Systolic Response To Physiological Exercise Stress In Preterm-Born Young Adults

## 4.1 Abstract

**Background** - Experimental and clinical studies show that prematurity leads to altered left ventricular (LV) structure and function with preserved resting LV ejection fraction (EF). Large-scale epidemiological data now links prematurity to increased early heart failure risk.

**Objectives –** We performed echocardiography imaging at prescribed exercise intensities to determine whether preterm-born adults have impaired LV functional response to physical exercise.

**Methods** – We recruited 101 normotensive young adults born preterm (N=47 mean gestational age 32.8±3.2 weeks) and term (N=54) for detailed cardiovascular phenotyping. Full clinical resting and exercise stress echocardiograms were performed, with apical four-chamber views collected while exercising at 40%, 60%, and 80% of peak exercise capacity, determined by maximal cardiopulmonary exercise testing.

**Results** – Preterm-born individuals had greater LV mass (P=0.015) with lower peak systolic longitudinal strain (P=0.038) and similar EF to term-born controls at rest (P=0.62). However, by 60% exercise intensity, EF was 6.7% lower in those born preterm (71.9±8.7 versus 78.6±5.4%, P=0.004) and further declined to 7.3% below the term-born group at 80% exercise intensity (69.8±6.4 versus 77.1±6.3% P=0.004). Sub-maximal cardiac index reserve was 56% lower in preterm-born versus term-born controls at 40% of peak exercise capacity (729±1162 versus 1669±937ml/min/$m^2$, P=0.021). LV length and resting peak systolic longitudinal strain predicted EF increase from rest to 60% exercise

intensity in the preterm-born group (r=0.68, P=0.009 and r=0.56, P=0.031 respectively).

**Conclusions** – Preterm-born young adults have impaired LV response to physiological stress when subjected to physical exercise, suggesting a reduced myocardial functional reserve that may help explain their increased risk of early heart failure.

**Publication Status:** The results presented in Chapter 4 have been published at: Huckstep et al, *J am Coll Cardiol.* 2018; 71(12): 1347-1356

## 4.2 Introduction

Preterm birth rates range from 5-18% worldwide (251), and with modern perinatal care often achieving 95% survival rates (113), the population of adults born preterm has risen sharply in recent decades. Multiple studies have described a distinct preterm-born cardiovascular phenotype, including altered cardiac structure and function (7,104,252) and impaired exercise capacity (253), as well as increased risk for hypertensive disorders and stroke (254). However, as the modern cohorts of preterm-born survivors now approach middle age, the long-term epidemiological consequences of prematurity remain largely unknown. A recently reported study of over 2.6 million individuals born between 1987 and 2012 identified preterm birth as a novel risk factor for incident heart failure in childhood and adolescence (185), emphasising the importance of research into underlying mechanisms responsible for increased risk in this population.

Acute cardiac insufficiency is uncommon in young adults (165) and our previous cardiovascular magnetic resonance (CMR) study confirmed that preterm-born young adults maintain a fully preserved resting left ventricular (LV) ejection fraction (EF) despite significant functional and anatomical LV remodelling (104). Stress echocardiography is a powerful tool used to unmask underlying cardiac dysfunction that is well-compensated at rest by assessing the cardiac response to physiological stressors such as exercise (255). Since those born preterm have well compensated resting LV function despite significant structural modifications and moderate changes in myocardial deformation at rest (104), investigating cardiac functional responses to physiological stress is essential to more fully understand the impact of being born preterm on LV function (255). We therefore used two-dimensional echocardiography to test the hypothesis that the LV of preterm-born young adults has reduced myocardial reserve, which results in functional impairment in response to the physiological stressor of physical exercise.

## 4.3 Methods

Study 1 utilises participants and measures exclusively from the YACHT study. Methods are described in detail in Chapter 3 with a summary overview of study measures provided in **Figure 3.1.** The central investigations of this chapter focus on analyses of the resting and exercise stress echocardiographic data collected in the YACHT study.

### 4.3.1 Study Population

The study population for this chapter includes: Group 1, normotensive full-term born young adult controls, and Group 2, normotensive preterm-born young adults. The groups are described in detail in Chapter 2, with a summary of the groups provided in **Figure 2.2**.

### 4.3.2 Study Visit

All data for this analysis was taken from the YACHT study. The study visit flow diagram and associated procedures and measures is provided in **Figure 3.1**.

#### 4.3.2.1 Data Processing

##### *4.3.2.1.1 Sub-maximal Cardiac Index Reserve Calculation*

Sub-maximal cardiac index reserve was calculated similarly to previous studies (256). Briefly, cardiac output indexed to body surface area (BSA) was calculated at rest ($CI_{rest}$) and also at 40, 60, and 80% exercise intensity ($CI_{40}$, $CI_{60}$, $CI_{80}$) by multiplying heart rate in beats per minute by stroke volume indexed to BSA, measured using echocardiography. Sub-maximal cardiac index reserve values at 40, 60, and 80% exercise intensity ($CIR_{40}$, $CIR_{60}$, $CIR_{80}$) were then calculated by subtracting $CI_{rest}$ from the calculated cardiac index at each exercise intensity ($CI_{40}$, $CI_{60}$, $CI_{80}$). The units for both CI and CIR are mL/min/$m^2$. The sub-maximal cardiac index reserve equations are as follows: $CIR_{40}=CI_{40} - CI_{rest}$; $CIR_{60}=CI_{60} - CI_{rest}$; and $CIR_{80}=CI_{80} - CI_{rest}$.

### 4.3.3 Statistical Analysis

Statistical analysis was performed using SPSS Version 23. Shapiro-Wilk testing and visual inspection were used to assess normality of variable distribution. Direct between-group comparisons were performed using independent-samples Student t-tests for normally distributed data and Mann-Whitney and Kruskal-Wallis tests for non-normally distributed data. Linear regression modelling was completed using forced entry with missing data excluded pairwise. Standardised regression coefficients (β) are presented for multivariable linear regressions. Pearson correlations (*r*) were used for bivariate correlations. The study was powered at 80% and P=0.05 to detect a 0.77 standard deviation difference between groups for LV exercise echocardiography measures. Where appropriate, comparisons between term-born and preterm-born adults were adjusted for sex by multivariable linear regression modelling to account for differing sex distributions between groups. This includes demographic, anthropometric, biochemistry, blood pressure and LV echocardiography measures. P values below 0.05 were considered statistically significant.

## 4.4 Results

### 4.4.1 Group Characteristics

The gestational age of the preterm-born group was lower than the term-born controls (32.8±3.22 vs 39.5±1.37 weeks, P<0.0001). The gestational age range for the preterm-born group was 23-36 weeks. However, 80.9% (N=38/47) were born moderate to late preterm (32-36 weeks), 10.6% (N=5/47) were born

very preterm (28-31 weeks), and 8.5% (N=4/47) born extremely preterm (less than 28 weeks). Height and body surface area were lower in the preterm-born group. There were no differences in BMI or age, but between group sex distribution approached significance with a 30% male preterm-born group vs a 48% male term-born group, P=0.061. Both groups had similar average awake ambulatory systolic and diastolic pressure, however, the preterm-born group had higher resting systolic (119±9.35 vs 115±7.68mmHg, P=0.014) and resting diastolic (70.0±8.07 vs 66.3±4.82mmHg, P=0.014) clinic blood pressure readings. Group characteristics are presented in **Table 4.1**.

**Table 4.1. Group Characteristics**

| | Preterm-born Adults (N=47) | Term-born Adults (N=54) | P Value |
|---|---|---|---|
| **Demographics and Anthropometrics** | | | |
| Age (years) | 22.7±3.04 | 23.6±3.77 | 0.239 |
| Male, n (%) | 14 (30) | 26 (48) | 0.061 |
| Height (cm) | 167±8.6 | 175±10.0 | **<0.001** |
| Weight (kg) | 65.3±13.5 | 70.0±13.0 | 0.299 |
| Body surface area ($m^2$) | 1.73±0.19 | 1.84±0.21 | **0.010** |
| BMI ($kg/m^2$) | 23.3±4.52 | 22.7±2.68 | 0.401 |
| Birth Weight (grams) | 1916±806 | 3390±424 | **<0.001** |
| Birth Weight Range (grams) | 595-3203 | 2640-4536 | **<0.001** |
| Gestational age (weeks) | 32.8±3.22 | 39.5±1.37 | **<0.001** |
| Gestational age range (weeks) | 23-36 | 38-42 | **<0.001** |
| Gestational Hypertension, n (%) | 8 (17.0) | 0 (0) | **0.002** |
| Small For Gestational Age, n (%) | 2 (4.3) | 0 (0) | 0.214 |
| **Biochemistry** | | | |
| Total Cholesterol (mmol/L) | 4.72±0.65 | 4.18±0.77 | **0.001** |
| HDL (mmol/L) | 1.49±0.31 | 1.47±0.26 | 0.953 |
| LDL (mmol/L) | 2.80±0.71 | 2.32±0.60 | **0.001** |
| Triglycerides (mmol/L) | 1.12±0.66 | 0.87±0.36 | **0.031** |
| High Sensitivity CRP (mg/L) | 1.57±2.42 | 1.14±1.96 | 0.412 |
| Glucose (mmol/L) | 5.02±0.41 | 4.82±0.51 | **0.030** |
| Insulin (pmol/L) | 51.1±29.0 | 35.8±29.4 | **0.012** |
| Insulin Resistance | 0.96±0.54 | 0.68±0.59 | **0.020** |
| **Brachial Blood Pressure (mmHg)** | | | |
| Resting Systolic | 119±9.35 | 115±7.68 | **0.014** |
| Resting Diastolic | 70.0±8.07 | 66.3±4.82 | **0.014** |
| Awake Average Ambulatory Systolic | 119±6.27 | 119±7.62 | 0.610 |
| Awake Average Ambulatory Diastolic | 71.3±4.90 | 69.4±4.54 | 0.058 |

Group Characteristics presented as mean±standard deviation. P values were adjusted for sex. BMI, Body Mass Index; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein; CRP, C-Reactive Protein. Insulin Resistance was calculated using the Homeostatis Model Assessment (HOMA) calculator (www.dtu.ox.ac.uk/homacalculator/). P values < 0.05 depicted in bold.

### 4.4.2 Resting Echocardiography

#### 4.4.2.1 Altered Left Ventricular Structure In Preterm-born Young Adults

2-Dimensional echocardiography of the LV showed the preterm-born group to have greater LV mass indexed to body surface area (BSA: 69.1±13.5 vs 63.7±14.7g/m², P=0.015) as well as greater LV mass indexed to EDV (1.54±0.33 vs 1.22±0.27g/mL, P<0.001). Additional adjustment for systolic blood pressure did not affect these between group differences for LV mass indexed to body surface area (P=0.022) and LV mass indexed to EDV (P<0.001). Those born preterm had smaller LV internal cavity diameters at end-diastole (4.50±0.41 vs 4.82±0.51 cm, P=0.007), with lower EDV indexed to BSA (46.4±9.7 vs 52.5±12.7mL/$m^2$, P=0.037, **Table 4.2**).

#### 4.4.2.2 Altered Resting Left Ventricular Function with Preserved EF In Preterm-born Young Adults

The preterm-born group had a higher resting heart rate (68.8±24.4 vs 58.8±16.3 beats per minute, P=0.023) but a similar resting EF compared to those born term (63.1±5.4 vs 63.4±4.7%, P=0.62). However, the preterm-born group had a lower E to A ratio (1.52±0.38 vs 1.75±0.42, P=0.013), A velocity time integral (4.93±1.18 vs 4.38±1.12cm, P=0.035) as well as E to A velocity time integral (2.77±0.95 vs 4.38±0.74cm, P=0.021). Peak systolic longitudinal strain was also lower in the preterm-born group at rest (-19.7±2.7 vs -20.6±3.0%, P=0.038) (**Table 4.2**).

**Table 4.2. Left Ventricle Resting Echocardiography**

| | Preterm-born Adults (N=47) | Term-born Adults (N=54) | P Value |
|---|---|---|---|
| **Structure** | | | |
| Mass Index (g/m²) | 69.1±13.5 | 63.7±14.7 | **0.015** |
| Mass/EDV (g/ml) | 1.54±0.33 | 1.22±0.27 | **<0.001** |
| End-Diastolic Diameter (cm) | 4.50±0.41 | 4.82±0.51 | **0.007** |
| End-Diastolic Length (cm) | 7.75±0.81 | 8.15±0.97 | 0.169 |
| EDV (ml) | 80.4±21.5 | 97.5±28.7 | **0.010** |
| EDV/BSA (ml/m²) | 46.4±9.7 | 52.5±12.7 | **0.018** |
| ESV/BSA (ml/m²) | 16.9±4.7 | 20.3±6.4 | **0.037** |
| **Function** | | | |
| Heart Rate (BPM) | 68.8±24.4 | 58.8±16.3 | **0.023** |
| Ejection Fraction (%) | 63.1±5.4 | 63.4±4.7 | 0.624 |
| Stroke Index (ml/m²) | 29.6±6.4 | 32.2±7.8 | 0.173 |
| Fractional Area Change (%) | 48.9±5.6 | 49.4±5.7 | 0.396 |
| Cardiac Output (L/min) | 3.78±0.89 | 3.62±0.56 | 0.224 |
| IVRT (s) | 0.085±0.019 | 0.088±0.022 | 0.517 |
| E/A ratio | 1.52±0.38 | 1.75±0.42 | **0.013** |
| E (cm/s) | 82.1±13.1 | 86.1±15.5 | 0.125 |
| A (cm/s) | 56.3±12.5 | 51.0±12.5 | 0.096 |
| E-wave Deceleration Time (ms) | 0.23±0.05 | 0.23±0.05 | 0.723 |
| Diastolic Filling Time (s) | 0.46±0.13 | 0.61±0.15 | **<0.001** |
| E-VTI (cm) | 12.8±2.5 | 13.6±2.1 | 0.163 |
| A-VTI (cm) | 4.93±1.18 | 4.38±1.12 | **0.035** |
| E-VTI/A-VTI | 2.77±0.95 | 3.22±0.74 | **0.021** |
| **2-Dimensional Peak Strain** | | | |
| Longitudinal | | | |
| Systolic Strain (%) | -19.7±2.7 | -20.6±3.0 | **0.038** |
| Systolic Strain Rate (%/s) | -1.17±0.19 | -1.11±0.37 | 0.590 |
| Diastolic Strain Rate (%/s) | 1.62±0.48 | 1.77±0.58 | 0.082 |
| Radial | | | |
| Systolic Strain (%) | 49.2±17.5 | 45.1±12.7 | 0.285 |
| Systolic Strain Rate (%/s) | 2.50±1.03 | 2.36±0.73 | 0.585 |
| Diastolic Strain Rate (%/s) | -3.11±1.41 | -2.88±1.15 | 0.555 |
| Circumferential | | | |
| Systolic Strain (%) | -26.6±4.0 | -27.0±3.8 | 0.321 |
| Systolic Strain Rate (%/s) | -1.67±0.30 | -1.57±0.28 | 0.156 |
| Diastolic Strain Rate (%/s) | 2.26±0.70 | 2.14±0.58 | 0.706 |

Data presented as mean±standard deviation. P values were adjusted for sex. EDV, end diastolic volume; BSA, body surface area; IVRT, isovolumetric relaxation time; E, peak early diastolic velocity of transmitral flow; A, peak atrial

systolic velocity of transmitral flow; VTI, velocity time integral. P values < 0.05 depicted in bold.

### 4.4.3 Exercise Stress Echocardiography

#### 4.4.3.1 Participant Completion of Stress Echocardiography

Following the recovery period after peak exercise testing, all participants were encouraged to carry out cycle ergometry stress echocardiography at 40, 60 and 80% of their peak achieved exercise intensity. Consistent with study protocol presented at consent, participants understood that all assessments were voluntary. Of 47 preterm-born participants, 21 (mean gestational age 33.0±3.4 weeks) performed the stress echocardiography assessment while the remaining 26 elected not to attempt stress echocardiography due to existing fatigue. All 21 preterm-born participants who performed stress echocardiography completed the 40 and 60% exercise intensity stages, but four did not perform the final stage at 80% intensity due to fatigue. Of 54 control group participants, 37 performed the stress echocardiography assessment while the remaining 17 elected not to attempt stress echocardiography due to existing fatigue. All 37 control group participants who performed stress echocardiography completed the 40 and 60% exercise intensity stages, but six did not attempt the final stage at 80% intensity due to fatigue.

#### 4.4.3.2 Stress Echocardiography Image Quality

Due to challenges with imaging quality, some stress echocardiography scans were not of sufficient quality for analysis. In the preterm-born group, image quality allowed for determination of EF for 13 scans at 40% intensity, 15 scans at 60% intensity and 12 scans at 80% intensity. In the preterm-born group, the

mean gestational age for those scanned at each intensity was: 32.3±4.2 weeks for 40%, 32.4±2.8 weeks for 60% and 32.7±4.2 weeks for 80%. In the control group, image quality allowed for determination of EF for 28 scans at 40% intensity, 30 scans at 60% intensity and 23 scans at 80% intensity.

#### 4.4.3.3 Impaired Systolic Response to Exercise in Preterm-born Adults

LV function was not significantly different between groups at 40% of peak exercise intensity (EF: 75.6±8.5 vs 79.0±5.2%, P=0.115; peak systolic longitudinal strain: -21.3±3.1 vs -22.7±2.7%; P=0.103). However, at 60% exercise intensity, the EF of the preterm-born group dropped to 6.7% below controls (71.9±8.7 vs 78.6±5.4%, P=0.004), with lower peak systolic longitudinal strain in the preterm-born group as well (-19.3±2.5 vs -21.6±3.3%, P=0.004). Additionally, within the preterm-born group, there was a statistically significant 3.7% decline in EF when going from 40 to 60% exercise intensity (P=0.039). EF remained lower in the preterm-born group compared to the term group at 80% exercise intensity (69.8±6.4 vs 77.1±6.3%, P=0.004). Stress echocardiography data are presented in **Table 4.3** and EF from rest through all stages of exercise are presented in **Figure 4.1**. Comparisons between preterm-born and term-born individuals for EF at 40%, 60% and 80% exercise intensity were repeated, adjusting for both sex and systolic blood pressure. EF at 40% exercise intensity did not differ between preterm-born and term-born adults (P=0.387), but was significantly different at 60% (P=0.017) and 80% (P=0.016).

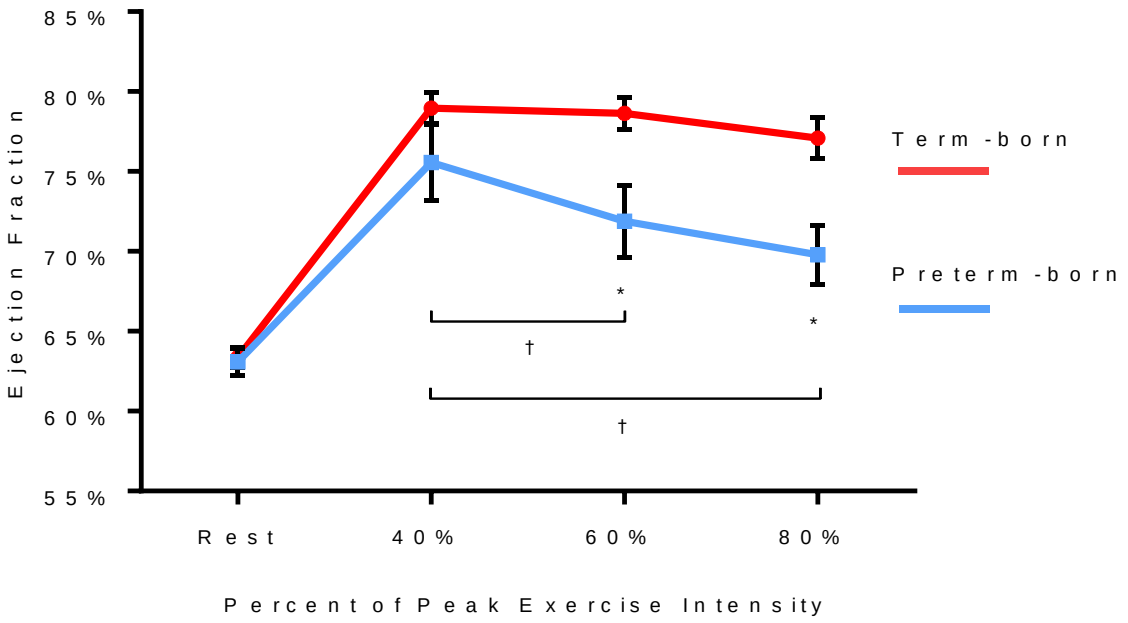



**Figure 4.1. Ejection Fracton with Increasing Exercise Intensity in Preterm-born Versus Term-born Young Adults**

EF with increasing exercise intensity in preterm versus term-born young adults. Preterm-born young adults (blue) had a lower EF than term-born young adults (red) at 60% and 80% of peak exercise intensity (71.9±8.7, N=15 vs 78.6±5.4%, N=30 & 69.8±6.4, N=12 vs 77.1±6.3%, N=23 respectively, P<0.005). In the preterm-born group, EF at 60% and 80% exercise intensity were both significantly lower than within group EF at 40% intensity (3.7% & 5.8% lower respectively, P<0.05). This declining EF with increasing exercise intensity was not observed in term-born controls. Error bars represent SEM. *Denotes significantly lower EF than term-born group (P<0.005), †denotes significantly lower than within group EF at 40% (P<0.05).

**Table 4.3. Left Ventricle Exercise Echocardiography**

| | Preterm-born Adults (N=21) | Term-born Adults (N=37) | P Value |
|---|---|---|---|
| **40% Exercise Load** | | | |
| Heart Rate (BPM) | 135±12 | 129±11 | 0.073 |
| Ejection Fraction (%) | 75.6±8.5 | 79.0±5.2 | 0.115 |
| Stroke Volume Index (ml/m²) | 21.4±8.8 | 26.9±7.6 | 0.104 |
| EDV/BSA (ml/m²) | 27.6±10.4 | 33.6±10.0 | 0.760 |
| ESV/BSA (ml/m²) | 6.68±2.79 | 7.40±3.34 | 0.147 |
| Peak Systolic Longitudinal Strain (%) | -21.3±3.1 | -22.7±2.7 | 0.103 |
| **60% Exercise Load** | | | |
| Heart Rate (BPM) | 159±11 | 151±14 | 0.055 |
| Ejection Fraction (%) | 71.9±8.7 | 78.6±5.4 | **0.004** |
| Stroke Volume Index (ml/m²) | 21.5±10.6 | 25.6±6.8 | 0.218 |
| ESV/BSA (ml/m²) | 29.2±11.5 | 32.8±9.5 | 0.500 |
| EDV/BSA (ml/m²) | 7.61±2.27 | 7.20±3.42 | 0.430 |
| Peak Systolic Longitudinal Strain (%) | -19.3±2.5 | -21.6±3.3 | **0.004** |
| **80% Exercise Load** | | | |
| Heart Rate (BPM) | 175±13 | 167±12 | 0.099 |
| Ejection Fraction (%) | 69.8±6.4 | 77.1±6.3 | **0.004** |
| Stroke Volume Index (ml/m²) | 19.9±7.6 | 25.1±6.9 | 0.094 |
| ESV/BSA (ml/m²) | 28.1±9.7 | 32.6±8.7 | 0.154 |
| EDV/BSA (ml/m²) | 8.24±2.60 | 7.52±3.12 | 0.420 |
| Peak Systolic Longitudinal Strain (%) | -20.2±2.9 | -20.1±2.4 | 0.592 |

Sample for exercise stress imaging values were: In the preterm-born group, N=13, 15, & 12 for 40, 60, and 80% respectively. For controls, N=28, 30, & 23 for 40, 60, & 80% respectively. Data presented as mean±standard deviation. P values adjusted for sex. BPM, beats per minute; EDV, end diastolic volume; ESV, end systolic volume; BSA, body surface area; E, peak early diastolic velocity of transmitral flow; A, peak atrial systolic velocity of transmitral flow. P values < 0.05 depicted in bold.

#### 4.4.3.4 Reduced Submaximal Cardiac Index Reserve in Preterm-born Young Adults

Between-group comparison at 40% exercise intensity showed the preterm-born group to have a 56% lower sub-maximal cardiac index reserve than term-born participants (729±1162, N=13 vs 1669±937ml/min/$m^2$, N=28 P=0.021). Mean sub-maximal cardiac index reserve at 60 and 80% intensity were lower in the preterm-born group (41 and 45% lower respectively) but were not statistically significant (P=0.057 and 0.071 respectively, **Figure 4.2**).

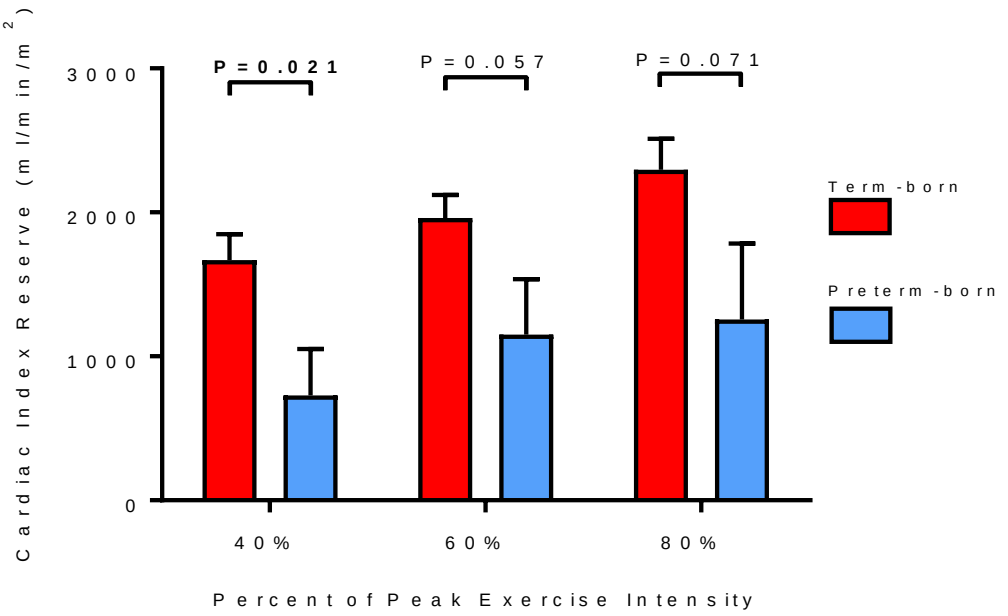



**Figure 4.2. Sub-Maximal Exercise Cardiac Index Reserve with Increasing Exercise Intensity in Preterm-born vs Term-born Adults.**

Sub-maximal cardiac index reserve values at 40, 60, and 80% exercise intensity were calculated by subtracting resting cardiac index from the cardiac index at each exercise intensity level. Sub-maximal exercise cardiac output reserve at 40% of peak exercise intensity was 56% lower in the preterm-born group vs term-born controls (P=0.021). In the preterm group, N=13, 15, & 12 for 40, 60, and 80% respectively. For controls, N=28, 30, & 23 for 40, 60, & 80% respectively. Error bars represent SEM. P value < 0.05 depicted in bold.

### 4.4.3.5 Predictors of Impaired Systolic Exercise Response in Preterm-born Young Adults

With increasing workload from rest to 60% exercise intensity, the preterm-born group increased their EF by 8.1% compared to 15.2% in the term-born group (P=0.039). To identify cardiac morphological predictors of this impaired systolic response to exercise, I selected resting structural and functional measures known to be modified in the preterm-born cardiac phenotype and

performed correlation analyses of these parameters against the change in EF from rest to 60% exercise load. Bivariate correlation showed that LV length and peak systolic longitudinal strain correlated to acute exercise-induced EF gains in the preterm-group but not in term-born controls. However, in a multivariable model including both LV length and peak systolic longitudinal strain, LV length remained the only significant predictor of exercise systolic response in the preterm-born group (β=0.51, P=0.036). A linear regression plot of LV length versus EF change from rest to 60% exercise load in the preterm-born group is presented in **Figure 4.3, Panel A** ($r^2$=0.46, P<0.01) while the same values for the term-born group are plotted in **Figure 4.3, Panel B** and show no significant relationship ($r^2$=0.02, P=0.46). Gestational age was also related to LV length and exercise systolic response in bivariate correlations in the preterm-born group (r=0.29, P=0.059 and r=0.67, P=0.011) and therefore, an additional multivariable model was performed to explore the independent relationship with exercise systolic response. In this model, LV length and gestational age were included as independent variables, as was systolic blood pressure due to its potential confounding effect on the EF change from rest to 60% exercise intensity. Only LV length (β=0.53, P=0.016) and gestational age (β=0.47, P=0.027) remained as independent predictors of exercise systolic response in the preterm-born adults.

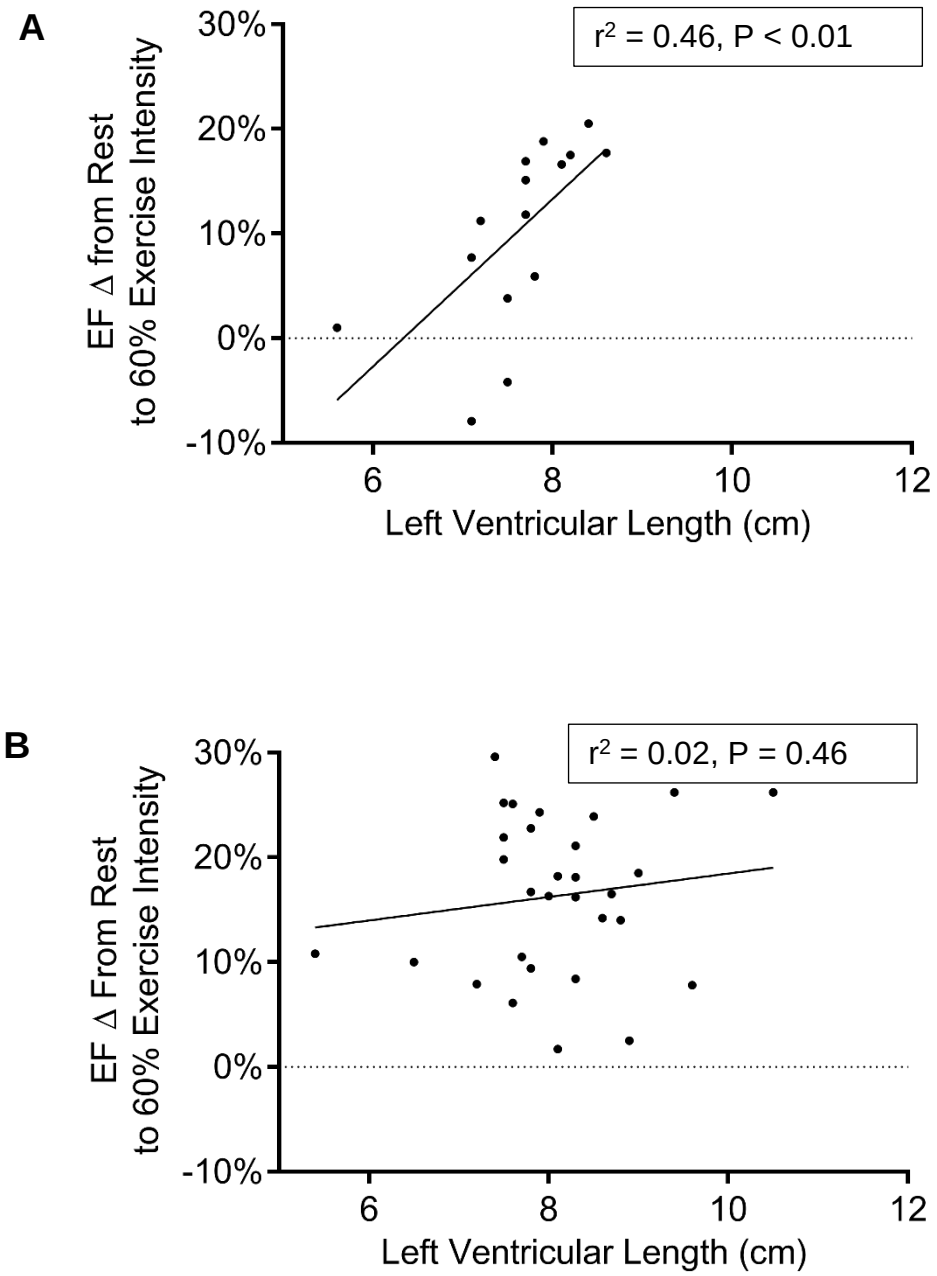



**Figure 4.3. Relationship between LV Length and Change in EF when Going from Rest to 60% Exercise Intensity in Preterm-born (Panel A) Versus Term-born (Panel B) Young Adults.**

**A.** In preterm-born adults, greater LV length was strongly correlated with increasing EF when going from rest to 60% exercise intensity (N=15, P=0.009). **B**. In term-born adults, LV length was not correlated with change in EF when going from rest to 60% exercise intensity (N=30, P=0.46).

## 4.5 Discussion

Study 1 of this thesis demonstrates for the first time that preterm-born young adults have significantly impaired LV systolic response to physical exercise stress. Structurally, echocardiography revealed that prematurity was associated with greater LV mass and lower LV volumes indexed for body size, as previously shown by CMR (7). At rest, EF remained fully preserved, but increasing exercise intensity elicited a decline in EF, resulting in an overall lower EF than term-born controls. Sub-maximal cardiac index reserve was also lower, and LV length and degree of prematurity were highly predictive of this adverse systolic response in preterm-born individuals.

### 4.5.1 Potential Mechanistic Links between Preterm Birth and Heart Failure

Recent large-scale epidemiological data provides evidence that being born preterm increases ones risk of early heart failure (185). In line with this, preterm-born experimental models in rats show prematurity-related stress can elicit LV remodelling and impaired systolic function, prior to blood pressure elevation. Moreover, these experimental models found early these cardiac to alterations increase susceptibility to developing heart failure secondary to the insult of persistent hemodynamic overload induced by angiotensin II infusion (32,257).

It is consequential that the systolic deficits we observed manifested under moderate intensity physical exercise, and were clearly evident in this group of healthy, normotensive, preterm-born young adults, indicating that apart from

subsequent pathology or insult, prematurity alone confers this systolic impairment and diminished myocardial functional reserve. Further decrements over time to this already marginalised reserve capacity in this normotensive population would bring peak achievable systolic function increasingly nearer to baseline requirements (258). Hypertensive individuals were excluded from this study to avoid potential confounding from altered hemodynamics, treatment with antihypertensive medications or other underlying cardiovascular risk factors. Despite the independence of the changes in LV systolic function during exercise from blood pressure, exposure to sustained blood pressure elevation or hypertension may have a greater cardiovascular impact over time in preterm-born individuals due to this reduced myocardial reserve (258). These new findings of impaired systolic response to exercise in preterm-born adults offer important clues, which may help elucidate the mechanisms linking preterm birth to increased heart failure risk.

#### 4.5.1.1 Early Postnatal Modifications in Preterm-born Infants

Recent clinical research indicates that even modest prematurity can result in significant early postnatal cardiac adaptations which may impact long-term cardiac resilience and vulnerability. A 2017 echocardiographic study of postnatal development in preterm-born (33.9±2.2 weeks' mean gestation) and term-born (39.4±1.3 weeks' mean gestation) infants (252) found that at birth, LV EF and mass index were both lower in the preterm-born group ($P<0.001$ and $P<0.01$ respectively). However, three month follow-up scans found that the EF difference was abolished, but the preterm-born infants had developed a greater LV mass index than controls ($P<0.001$). Because these postnatal LV mass

increases coincided with a normalisation of resting EF, this disproportionate postnatal LV myocardial mass gain in the preterm-born infants may represent an adaptation that in-part, offsets early postnatal deficits and restores baseline systolic function. Similarly, in Study 1, the preterm-born group had largely normalised baseline function, however, they showed significantly impaired LV stress response. Experiments in the porcine and ovine models reveal that poor stress response and also reduced capacity to recover from cardiac insults may stem from the early termination of normal fetal cardiomyocyte proliferation and development as well as the abrupt, early hemodynamic switch from fetal to postnatal ventricular loading.

#### 4.5.1.2 Insights from Animal Models

Eiby et al tested the hypothesis that in the preterm-born piglet LV, functional immaturity would manifest through poor response to elevated afterload. In this experiment on cardiac explants from newborn piglets, hearts from piglets delivered preterm produced lower baseline output (indexed to body weight) but had typical response to changes in preload. However, when challenged with increased afterload, they had significantly impaired systolic performance (259). Here, the preterm-born piglet hearts maintained stable LV performance as afterload was increased from 20 to 45mmHg, but LV systolic performance declined significantly above 45mmHg afterload, with less than 30% of baseline output preserved at 55mmHg afterload (the greatest afterload reported). Conversely, term-born piglet hearts had stable systolic performance up through 50mmHg afterload, which subsequently declined slightly with roughly 80% of baseline output preserved at 55mmHg afterload. In Study 1, baseline

systolic function was preserved and LV mass was elevated in the preterm-born group, which suggests the previously cited alterations observed at the three-month point in preterm-born infants (252) contribute to long-term preservation of baseline function through LV mass increase. Nevertheless, when preterm-born participants in Study 1 were challenged with increasing exercise intensity, a classic physiological stress mechanism that increases afterload, the preterm-born group showed declining EF above 40% workload while controls preserved EF through all stages of exercise. These new results indicate that in those born preterm, early deficits in resting LV systolic function may be palliated through myocardial mass increase, while marginalised functional response to increasing afterload persists through development and into adulthood.

Sheep research provides useful insight into the consequences of arrested fetal development on cardiomyocytes. These experiments show that maturing fetal cardiomyocytes undergo proliferative growth which transitions to primarily hypertrophic patterns by late in the normal gestational period as cardiomyocytes are increasingly terminally differentiated to their mature binucleated state (260,261). The effect of prematurity on cardiomyocyte development was studied in a late preterm-born sheep model (equivalent to 36 weeks' gestation in humans) at which point ~50% of cardiomyocytes would be expected to be binucleated (262). This experiment showed that at nine weeks post term equivalence age, the preterm-born lambs had greater LV cardiomyocyte volume with increased fibrosis in the extracellular matrix compared to controls. This increased cell volume and fibrosis indicates that normal late gestational cardiomyocyte proliferation was likely halted, resulting in fewer cardiomyocytes at birth, which upon being exposed to the increased LV loading of the ex-utero

circulatory system, experienced an exaggerated hypertrophic response (263). Additionally, the preterm-born sheep LVs had 12% fewer binucleated cardiomyocytes, more immature mononucleated cardiomyocytes, and the presence of trinucleated cardiomyocytes (absent in the full-term lambs). Bensley et al. hypothesised that this truncated cardiomyocyte development with subsequent remodelling and ploidy anomalies would result in long-term cardiac vulnerability (263).

#### 4.5.1.3 A Note on Cardiomyocyte Turnover

Interestingly, evidence from human studies challenges the traditional view that the heart is a completely post-mitotic organ and instead indicates that a small amount of cardiomyocyte renewal occurs at least through early adulthood. Above-ground nuclear detonations from weapons testing in the 1950s and 1960s increased atmospheric levels of Carbon-14 to ranges that allow radiometric dating of cardiomyocyte DNA to within two years (264,265). Carbon-14 studies and cell cycle studies report that in children and adolescents, as much as 2% of cardiomyocytes may renew in a year (266,267), however, these studies may be prone to confounding from variations in cardiomyocyte ploidy and cell cycle activities that are terminated prior to actual cell division (265). A study of human cardiac tissues reported in 2013 clarifies further. Here, Mollova et al quantified cardiomyocytes in active cytokinesis, the final stage of cell division, and calculated that a 1-2% annual turnover rate of cardiomyocytes occurs in humans below 20 years of age (267). Whether this cell renewal exclusively supports homeostatic maintenance or may contribute to any regenerative capacity in some instances could not be assessed in this study. However, while Mollova et

al and others (265,267,268) agree that these renewal rates are wholly insufficient to significantly offset acute pathologies, they also hypothesised that normal mechanisms of cardiomyocyte renewal may offer insights or avenues into future therapies for cardiac repair. As these data relate to those born preterm, due to truncated cardiomyocyte development, it is possible that functional myocardial reserve deficits may combine with reduced capacity for early life myocardial healing and cell renewal, leading to a compounding of vulnerabilities for future heart failure.

### 4.5.2 Left Ventricular Structure and Function, Early Life History, and Cardiac Risk in the Preterm-born Population

Our previously reported CMR study of the LV in 102 preterm-born adults (mean gestational age 30.3±2.5 weeks) versus 132 term-born controls identified a unique cardiac morphology in the preterm-born group with lower LV length being a major anatomical feature (7). While this current study showed a similar percentage difference in LV length in preterm-born offspring compared to term-born individuals, the slightly later mean gestational age of the preterm-born group (32.8±3.2 weeks), smaller sample size, and increased variance of echocardiography versus CMR (particularly with apical views) account for the lack of statistical significance. Nevertheless, the correlation between LV length and exercise systolic response was only evident in the preterm-born group and may play a future role as a structural biomarker for risk in preterm-born offspring.

Interestingly, in those born preterm, exclusive human milk feeding in early postnatal development has shown to relate to greater LV length, end diastolic volume and diameter in adulthood, though these values remain lower than

controls (269). While these and other findings may help develop and direct future targeted intervention strategies, existing data may be of some use in addressing the growing population of preterm-born adults with increased cardiovascular disease burden (105,185,251,270,271). Future studies however, must deliver a more complete evidence base that facilitates modification of clinical risk stratification and prevention guidelines to identify risk factors associated with prematurity and provide targeted guidance for preterm-born individuals.

#### 4.5.2.1 Considerations for Current and Future Risk Guidelines

In current practice, standard guidance identifies history of complicated pregnancy as a cardiovascular risk modifier for women, however, history of preterm birth is not yet highlighted as a cardiovascular risk modifier for offspring (147,158,272). This presents a challenge in appropriately addressing risk in the preterm-born population in the near-term, but the 2016 European guidelines on cardiovascular disease prevention in clinical practice provides useful direction for instances when increased risk is suspected but gaps in evidence preclude more specific guidance (272). This guidance recommends that “other” risk modifiers be considered when appropriate, particularly when an individual has a clinical risk level near to a treatment threshold (272). Considering Study 1, which provides evidence for reduced myocardial reserve in those born preterm, along with the recently reported four-fold or greater risk of early heart failure for those born before 32 weeks’ gestation (185), preterm birth could conservatively be included as a risk modifier within current European guidelines (272). Specifically, as high blood pressure and hyperlipidemia are leading treatable risk factors for heart failure (2,162,163,273), until more specific evidence based guidance is

available, it may be reasonable for clinicians to consider initiating antihypertensive or lipid managing treatment in those born before 32 weeks' gestation whose risk profiles fall slightly below current treatment thresholds.

#### 4.5.2.2 Considerations for Common Metabolic Risk Factors in the Preterm-born Population

A final risk consideration that I will discuss are the indications of relative metabolic dysfunction in the preterm-born group and whether prematurity may confer a form of metabolic syndrome. In this study, I observed greater plasma low density lipoprotein cholesterol, plasma triglycerides and insulin resistance in the preterm-born group which is consistent with our previously reported work and that of others (7,274,275). Additionally, systolic and diastolic blood pressure were roughly 4mmHg greater in the preterm-born group, which is also similar to previous findings (154,276) including a 2012 systematic review by de Jong et al where meta-analysis of high quality studies identified 3.8mmHg greater systolic blood pressure in those born preterm. However, the blood pressure difference in Study 1 may be subject to confounding as the inclusion criteria required 24-hour ambulatory blood pressure < 135/85mmHg and therefore, the blood pressure within the study groups cannot be considered representative of the larger population.

Despite the repeat findings of metabolic dysfunction noted above, there is evidence that prematurity may relate to some but not other characteristics of the metabolic syndrome according to most commonly used criteria. Particularly, excessive adiposity, a central hallmark of the metabolic syndrome (277-279) is not overly prevalent in the preterm-born population (280). While a complete

consensus does not exist on diagnostic criteria for the metabolic syndrome, a range of clusters of several common risk factors are often used to produce a diagnosis (277-279,281). One of the more widely used criteria is the National Cholesterol Education Program Adult Treatment Panel III (NCEP: ATPIII) diagnostic criteria (277) which requires three or more of the following to be verified for a positive diagnosis:

- Waist circumference ≥ 102 cm (males) or ≥ 88 cm (females)
- Triglygerides ≥ 1.7 mmol/L
- High density lipoprotein cholesterol < 1.0 mmol/L (males)
  or < 1.3 mmol/L (females)
- Systolic and or diastolic blood pressure ≥ 130/85 mmHg
- Fasting glucose > 5.5 mmol/L

Previous work in this area provides some illumination regarding the preterm-born population. A useful systematic review and meta-analysis of preterm birth and the metabolic syndrome in adult-life performed by Parkinson et al analysed a broadly inclusive set of parameters related to the metabolic syndrome across 27 studies which included 17,030 preterm-born adults and 295,261 term-born adults (282). Based on their analysis of BMI, waist-hip ratio, percentage fat mass, systolic and diastolic blood pressure, flow-mediated dilatation, carotid intima-media thickness and fasting glucose and lipid levels, they reported that on average, prematurity was associated with 3-4mmHg greater systolic blood pressure and 0.14 mmol/L greater plasma low density lipoprotein cholesterol. Their meta-analysis found no further relationships between prematurity and other assessed measures, however they noted limited

power to assess fasting glucose and insulin and were also unable to directly assess insulin resistance, hence their findings on glucose metabolism were largely inconclusive. However, a Helsenki birth cohort study of 12,813 individuals found that birth before 35 weeks of gestation resulted in greater risk (1.59 odds ratio) of type 2 diabetes (283), significantly indicating a connection between prematurity and impaired glucose metabolism. Collectively, the findings of my newly reported work and other discussed studies don't fully support a clear connection between prematurity and a more traditional view of the metabolic syndrome. Nonetheless, there is significant evidence linking prematurity to key features of the metabolic syndrome, namely blood pressure elevation, low density lipoprotein cholesterol elevation, and impaired glucose metabolism. Accordingly, a move to codify these elements as specific risk factors associated with prematurity seems appropriate. These factors are well-recognised in current practice, however blood pressure and lipid profiles are more commonly screened than insulin resistance (272,277) and future clinical guidelines that indicate testing of glucose metabolism for preterm-born patients with borderline risk profiles may prove beneficial.

### 4.5.3 Study 1 Limitations

We designed YACHT as an observational study around a single, full-day study visit. As the exercise stress testing came towards the end of the visit in order not to confound other measures, not all individuals were willing to continue with the sub-maximal exercise testing and echocardiography stress testing component due to existing fatigue. Accordingly, it is likely that this resulted in less stress imaging data collection on participants with reduced exercise

enjoyment or a compromised exercise response and recovery profile. Twenty one of 47 (45%) of preterm-born participants completed stress testing vs 37 of 54 (67%) of controls which could indicate slower recovery from physical exertion in the preterm-born group. Further, if those with poor physiological response to exercise were indeed less likely to complete stress testing, the lower completion rate in the preterm-born group would suggest that the actual between group difference in LV stress response could be greater than I was able to analyse and report. In order to address this limitation in future work, I revised the stress echocardiography protocol for the TEPHRA trial. In TEPHRA, stress imaging is collected during CPET testing at target heart rates which are calculated for each participant based on the relationship between heart rate and workload observed in YACHT. This approach should remove any selection bias in the measurement and produce a more complete dataset for TEPHRA.

Also, as noted in the results, due to challenges with imaging quality, not all scans were of sufficient quality for analysis. The overall sample size was modest with a lower percentage of males in the preterm-born group, and therefore, P values were adjusted for sex where appropriate. In spite of this, we were sufficiently powered to make between group comparisons for our primary outcome measure, LV EF, at rest, 40%, 60%, and 80% of maximal exercise capacity using echocardiography and all scans were anonymized to avoid bias during analysis, including image quality assessment.

Due to the retrospective nature of the perinatal data collection, we did not have complete information on perinatal characteristics and complications. Future prospective studies may be able to address whether specific early life insults

alter LV function. There was a wide gestational age range in our participants with the majority of our preterm-born individuals born moderate to late preterm, which reflects the demographic of the general preterm-born population (284). Although this makes our findings more relevant to a larger proportion of the population, our analyses did identify a significant correlation between gestational age and exercise systolic response. Accordingly, a study that excludes those born very or extremely preterm may observe less pronounced variations in the LV stress response. Looking forward, larger studies with a greater proportion of very and extremely preterm-born young adults will be needed to fully explore how and to what extent, varying degrees of prematurity relate to impairment of the LV systolic response to exercise.

This study primarily assessed physiological functions and parameters of the study participants. However, a limitation of this study is that potentially confounding lifestyle and demographic factors such as maternal smoking, current tobacco use, alcohol consumption, socioeconomic status, diet and race were not assessed. Smoking during pregnancy is a risk factor for preterm birth and other pregnancy complications, and maternal smoking rates from 13-24% are typically reported in western countries (285,286). However, it is also well reported that smoking is commonly associated with additional, compounding risk factors including diet, physical activity and education (287,288). Not surprisingly, the strongest relationships between lifestyle and demographic factors and cardiovascular risk are derived from multifactorial analyses that compare outcomes between those who maintain multiple beneficial indicators vs those who maintain few or none (272). Longitudinal studies ranging from 10-21 years indicate that maintaining four or more healthy lifestyle indictors may cut one's

risk of incident heart failure or cardiac event by more than half (272,289-291). Additionally, race, particularly African ancestry is associated with increased risk for heart failure and other cardiovascular disease (292,293). In Study 1, the fact that participants were recruited from the greater Oxford community may contribute to a degree of homogeneity in some of these potentially confounding factors. Nevertheless, future studies of young adults which include targeted assessment of lifestyle and demographics would be beneficial in identifying factors beyond prematurity that may associate with variations in the LV stress response.

The resting echocardiography was performed per clinical standards with subjects scanned in the lateral decubitus position (65), however, upright cycle ergometry and treadmill are the most common clinical exercise stress modalities (294). We selected upright, seated cycle ergometry for CPET and stress echocardiography to align with clinical norms and also minimise torso movement during scanning. While all participants received standardised, per-protocol measurements, the differing posture could confound the direct comparisons of some resting versus exercise echocardiography measures, such as stroke volume (295). Finally, as a single center study located in the Oxford area that included purposive recruitment of young adults with elevated risk factors, our study population may not reflect the broader general population.

## 4.6 Conclusions

Preterm-born young adults exhibit a significantly impaired LV functional response to physical exercise. This impairment is characterized by a lower

exercise EF, which declines with increasing exercise intensity, as well as a reduced sub-maximal cardiac index reserve. Resting echocardiography showed a significant relationship between birth history and LV structure and function that was consistent with our previous CMR findings. Most notably, the novel finding of impaired exercise response suggests that preterm-born young adults have a reduced myocardial functional reserve. These insights may help explain the recently verified relationship between preterm birth and increased risk of early heart failure. Given that more than 10% of young adults worldwide will now have been born preterm, there is an increasing need for further research into whether lifestyle and clinical interventions can beneficially modify cardiac morphology and function in this growing population of individuals.

# Chapter 5 Study 2 Results Exercise Capacity and Left Ventricular Function in the Preterm-born Young Adult Cardiac Phenotype

## 5.1 Abstract

**Background –** Prior studies have reported reduced exercise capacity in preterm-born adolescents and young adults, with arrested pulmonary development often suspected as the primary mechanism. However, severe neonate respiratory distress is uncommon, affecting <10% of preterm-born infants and premature cohort studies show normal lung capacity is typical by adolescence. Our recent clinical studies in preterm-born individuals have identified altered left ventricular (LV) structure and function including declining ejection fraction (EF) with increasing exercise intensity.

**Hypothesis -** Preterm-born young adults have reductions in exercise capacity proportional to their degree of remodelling in LV structure and function.

**Objectives -** To investigate whether previously reported reductions in exercise capacity in preterm-born young adults relate to an altered cardiac phenotype.

**Methods -** We recruited 101 normotensive young adults born preterm (N=47 mean gestational age 32.8±3.2 weeks) and term (N=54) for cardiovascular phenotyping. Baseline spirometry and echocardiography were performed and participants completed peak cardiopulmonary exercise testing to measure exercise capacity. Exercise stress echocardiograms were then performed with apical four-chamber views collected while exercising at 40%, 60%, and 80% of peak exercise workload.

**Results** - Exercise capacity was lower in preterm-born participants (94±20% vs 108±25% of predicted peak $VO_2$, P=0.001). Baseline EF was similar between groups. Change in EF from rest to 40% and 60% exercise intensity significantly associated with peak $VO_2$ utilisation rates in the preterm-born group ($r^2$=0.472, P=0.014 and $r^2$=0.462, P=0.007 respectively), but not controls ($r^2$=0.004, P=0.746 and $r^2$=0.008, P=0.644 respectively). Resting and dynamic pulmonary measures were similar between groups and did not explain differing exercise capacity.

**Conclusions -** Preterm-born young adults have reductions in exercise capacity that are proportional to their level of impairment in LV systolic response to exercise but not to pulmonary capacity. How prematurity impacts physiological adaptations to chronic aerobic training and associated alterations in exercise capacity and cardiovascular risk profile requires further investigation.

**Publication Status:** The results presented in Chapter 5 have been accepted as a primary research abstract for the American Heart Association Scientific Sessions, Nov 2018. A complete manuscript is in final preparation for journal submission.

## 5.2 Introduction

Prematurity is thought to be a risk factor for reduced exercise capacity during developmental years and on into adulthood (296). While altered metabolic risk has been reported in those born preterm (274,275,282,283), interrupted pulmonary development, which normally continues late into the third

trimester is generally imputed to contribute to potential exercise deficits (297-299). However, 80% or more of preterm births fall within the range of moderate to late prematurity (32-36 weeks gestational age) and acute neonatal respiratory distress syndrome (RDS) is uncommon by this gestational age range (300-302). Indeed, a 2012 study of 286,454 births reported an overall RDS rate of 3.22% for preterm-born infants who were born at 33 or more weeks' gestation (301).

To date, studies investigating exercise capacity in preterm-born subjects are limited, with the overall conclusions and associated underlying mechanisms uncertain (296). Additionally, premature groups selected for study have typically included compounding factors such as very-low-birth-weight (303), extreme prematurity (304) or history of severe pulmonary distress (298), and therefore, may have limited applicability to the majority of the preterm-born population. Finally, integrated investigation of cardiopulmonary function along with exercise load capacity is often limited or absent, making mechanistic insights difficult. One particularly large study of 218,820 Swedish conscripts reported reduced absolute exercise power in Watts in those born preterm (305). While providing valuable findings and insights, this study did not assess $O_2$ utilisation or cardiopulmonary function, and appropriate to the context of the exercise assessments (capacity for military duty), exercise power was not adjusted for body size which is known to be reduced in those born preterm.

Our previous investigations have identified an altered cardiac phenotype in preterm-born young adults that includes greater LV mass despite reduced cavity volume (7), and in Study 1, I reported impaired LV systolic response to exercise (306). However, whether, and to what extent, these alterations in the preterm-

born cardiac phenotype impact exercise capacity is unknown. We therefore used resting and exercise echocardiography, combined with cardiopulmonary exercise testing to test the hypothesis that preterm-born young adults have reduced exercise capacity that relates to the degree of LV structural and functional remodelling associated with the preterm-born cardiac phenotype.

## 5.3 Methods

Study 2 uses participants and data exclusively from the YACHT study. Methods are described in detail in Chapter 3 with a summary overview of study measures provided in **Figure 3.1.** The central investigations of this chapter focus on a combination of the resting and exercise stress echocardiographic data for LV structure and function assessments as well as cardiopulmonary exercise testing for exercise capacity measurement.

### 5.3.1 Study Population

The study population for this chapter includes: Group 1, normotensive term-born young adult controls, and Group 2, normotensive preterm-born young adults. The groups are described in detail in Chapter 2, with a summary of the groups provided in **Figure 2.2**. The same participants were investigated in Study 1.

### 5.3.2 Study Visit

All data for this analysis was taken from the YACHT study. The study visit flow diagram and associated procedures and measures was presented in **Figure 3.1**.

### 5.3.3 Statistics

Statistical analysis was performed using SPSS Version 23. Shapiro-Wilk testing and visual inspection were used to assess normality of variable distribution. Direct between-group comparisons were performed using independent-samples Student t-tests for normally distributed data and Mann-Whitney and Kruskal-Wallis tests for non-normally distributed data. Linear regression modelling was completed using forced entry with missing data excluded pairwise. Standardised regression coefficients (β) are presented for multivariable linear regressions. Pearson correlations (*r*) were used for simple and partial bivariate correlations. The YACHT study was designed around an estimated 6-10% between group difference in peak $VO_2$ and was conservatively powered at 80% to detect a 6% difference in percent of predicted peak $VO_2$ utilisation rates between 44 normotensive preterm-born and 44 normotensive term-born adults with 15 participants per sub-group needed to achieve the 80% power and p 0.05 to detect a 10% difference in percent of predicted peak VO2 utilisation rates. Where appropriate, comparisons between term-born and preterm-born adults were adjusted for sex by multivariable linear regression modelling to account for differing sex distributions between groups. This includes demographic, anthropometric, biochemistry, blood pressure and LV echocardiography results.

## 5.4 Results

### 5.4.1 Group Characteristics

The gestational age range for the preterm-born group was 23-36 weeks. However, 80.9% (N=38/47) were born moderate to late preterm (32-36 weeks), 10.6% (N=5/47) were born very preterm (28-31 weeks), and 8.5% (N=4/47) born extremely preterm (less than 28 weeks). Height was lower in the preterm-born group. There were no differences in BMI or age, but between group sex distribution approached significance with a 30% male preterm-born group vs a 48% male term-born group, P=0.061. Both groups had similar average awake ambulatory systolic and diastolic pressure, however, the preterm-born group had higher resting systolic (119±9 vs 115±7mmHg, P=0.014) and resting diastolic (70±8.07 vs 66±4.82mmHg, p= 0.014) clinic blood pressure readings. Resting heart rate was higher in the preterm-born group (69 vs 59 beats per minute, P=0.023). Ejection Fraction (EF) and stroke volume index were similar between groups. Baseline pulmonary function was also similar between groups with no significant differences in percent of predicted Forced Vital Capacity (FVC) or percent of FCV exhaled in first second of forced expiration ($FEV_1$/FVC). Baseline group characteristics are provided in **Table 5.1** below.

**Table 5.1. Group Characteristics**

| | Preterm-born Young Adults (N=47) | Term-born Young Adults (N=54) | P Value |
|---|---|---|---|
| **Demographics and Anthropometrics** | | | |
| Age (years) | 22.7±3.04 | 23.6±3.8 | 0.239 |
| Gestational age (weeks) | 32.8±3.22 | 39.5±1.37 | **<0.001** |
| Male, n (%) | 14 (30) | 26 (48) | 0.061 |
| Height (cm) | 167±9 | 175±10 | **<0.001** |
| BMI (kg/m$^2$) | 23.3±4.5 | 22.7±2.7 | 0.401 |
| **Biochemistry** | | | |
| Total Cholesterol (mmol/L) | 4.72±0.65 | 4.18±0.77 | **0.001** |
| HDL (mmol/L) | 1.49±0.31 | 1.47±0.26 | 0.953 |
| LDL (mmol/L) | 2.80±0.71 | 2.32±0.60 | **0.001** |
| Triglycerides (mmol/L) | 1.12±0.66 | 0.87±0.36 | **0.031** |
| High Sensitivity CRP (mg/L) | 1.57±2.42 | 1.14±1.96 | 0.412 |
| Glucose (mmol/L) | 5.02±0.41 | 4.82±0.51 | **0.030** |
| Insulin (pmol/L) | 51.1±29.0 | 35.8±29.4 | **0.012** |
| Insulin Resistance | 0.96±0.54 | 0.68±0.59 | **0.020** |
| **Brachial Blood Pressure (mmHg)** | | | |
| Resting Systolic | 119±9 | 115±8 | **0.014** |
| Resting Diastolic | 70±8 | 66±5 | **0.014** |
| Awake Average Ambulatory Systolic | 119±6 | 119±8 | 0.610 |
| Awake Average Ambulatory Diastolic | 71±5 | 69±5 | 0.058 |
| **Cardiac Function** | | | |
| Heart Rate (BPM) | 69±24 | 59±16 | **0.023** |
| Ejection Fraction | 63.1±5.4 | 63.1±4.7 | 0.624 |
| Stroke Index (ml/m$^2$) | 29.6±6.4 | 32.2±7.8 | 0.173 |
| **Pulmonary Function** | | | |
| FVC (% of predicted) | 105±15.8 | 105±12.9 | 0.870 |
| $FEV_1$/FCV (%) | 82.9±10.0 | 83.6±12.5 | 0.946 |

Group characteristics presented as mean±SD. Biochemistry, blood pressure, cardiac function, and pulmonary function P values adjusted for sex. BMI, Body Mass Index; HDL High Density Lipoprotein; LDL Low Density Lipoprotein; FVC, forced vital capacity; $FEV_1$, forced expiratory volume in 1s;. Insulin Resistance was calculated using the; Homeostatic Model Assessment Index (HOMA) calculator (www.dtu.ox.ac.uk/homacalculator/).

### 5.4.2 Exercise Characteristics

#### 5.4.2.1 Reduced Aerobic Exercise Capacity in Preterm-born Young Adults

Peak cardiopulmonary exercise testing showed lower aerobic exercise capacity in preterm-born young adults, who on average, achieved 94±20% of their predicted peak $VO_2$ utilisation rate vs 108±25% in term-born controls, P=0.001. The $O_2$ uptake efficiency slope (OUES) was lower in the preterm-born group as well (2.39±0.73 vs 3.05±0.89, P=0.001). Peak $VO_2$ measured as ml/kg/min was also lower in those born preterm (33.6±8.7 vs 40.1±9.0 ml/kg/min, P=0.003). Heart rate at the ventilatory aerobic threshold (VAT) was higher in the preterm-born group (132±18 vs 124±17 beats/min, P=0.038) but peak achieved heart rate was similar between groups. Ventilatory reserve at peak ventilation rate was similar between preterm-born and control subjects (23.3±17.8 vs 21.2±18.3 % of maximum minute ventilatory volume respectively, P=0.513) and wrist-worn accelerometry measures showed no between group differences in objective measures of physical activity. Exercise characteristics are presented in **Table 5.2.**

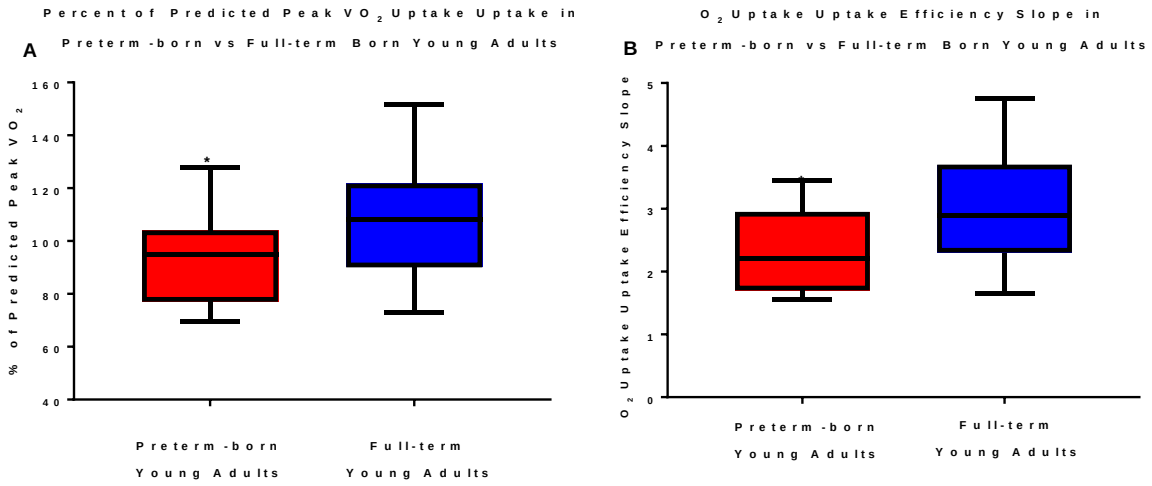



**Figure 5.1. Reduced Aerobic Exercise Capacity in Preterm-born Young Adults**

Peak aerobic capacity was assessed using upright cycle ergometry cardiopulmonary exercise testing. Preterm young adults achieved 94 ± 20% of their predicted peak $VO_2$ utilisation rate vs 108±25% in term-born controls, P=0.001. Oxygen uptake and efficiency slope was lower in the preterm group as well (2.39±0.73 vs 3.05±0.89, P=0.003). Error bars represent 95$^{th}$ percentile range. * Indicates significantly lower than controls (P<0.005)

**Table 5.2. Exercise Characteristics**

| | Preterm-born Young Adults (N=47) | Term-born Young Adults (N=54) | P Value |
|---|---|---|---|
| **Exercise Performance** | | | |
| Percent of Predicted Peak VO2 (%) | 94±20 | 108±25 | **0.001** |
| Peak VO2 (ml/kg/min) | 33.6±8.7 | 40.1±9.0 | **0.003** |
| OUE Slope | 2.39±0.73 | 3.05±0.89 | **0.001** |
| VE/VCO2 Slope | 28.1±3.3 | 25.9±3.8 | **0.011** |
| Peak RER | 1.19±0.06 | 1.19±0.06 | 0.738 |
| **Cardiovascular Function** | | | |
| Heart Rate at VAT | 132±18 | 124±17 | **0.038** |
| Heart Rate at 60% Intensity | 159±11 | 151±14 | 0.055 |
| Peak HR Achieved | 189±9 | 186±11 | 0.218 |
| Systolic Blood Pressure at 60% Intensity | 146±16 | 149±14 | 0.567 |
| Diastolic Blood Pressure at 60% Intensity | 81±10 | 77±7 | 0.139 |
| Peak Systolic Blood Pressure | 165±22 | 167±21 | 0.885 |
| Peak Diastolic Blood Pressure | 89±11 | 82±10 | **0.002** |
| Peak Pulse Pressure | 76±23 | 86±23 | 0.168 |
| **Pulmonary Function** | | | |
| Breaths per Minute at Peak VO2 | 44.3±6.7 | 47.4±11.8 | 0.188 |
| Ventilatory Reserve at Peak (%) | 23.3±17.8 | 21.2±18.3 | 0.513 |
| **Objective Measure of Physical Activity** | | | |
| MVPA (hours/week) | 14.2±5.8 | 14.9±6.1 | 0.633 |
| VPA (hours/week) | 0.9±0.9 | 1.2±1.2 | 0.146 |

Exercise characteristics presented as mean±SD. P values adjusted for sex. OUE, oxygen uptake efficiency; VE/VCO2, minute ventilation/carbon dioxide production; RER, respiratory exchange ratio; VAT, ventilator aerobic threshold; MVPA, moderate to vigorous physical activity; VPA, vigorous physical activity. P values < 0.05 depicted in bold.

#### 5.4.2.2 Change in Ejection Fraction During Exercise Predicts Exercise Capacity in Preterm-born Young Adults

We previously reported altered LV structure and In Study 1, I reported reduced EF (ejection fraction) during exercise as a central defect of the preterm-born cardiac stress response. This impaired systolic response to exercise was characterized by poor increase in EF during exercise with a significant decline in EF observed when going from 40% to 60% exercise intensity. We assessed the relationship between percent of predicted $VO_2$ utilisation rate achieved and increase in EF when going from rest to 40% and 60% exercise intensity by performing sex adjusted multivariable linear regression modelling in each study group. In the preterm-born group, increase in EF when going from rest to 40% and 60% exercise intensity were significant predictors of predicted $VO_2$ utilisation rate achieved ($\beta$=0.724, P=0.014, and $\beta$=0.795, P=0.007 respectively). The same sex corrected multivariable regression analyses in term-born controls at 40% and 60% intensity were not significant ($\beta$=-0.069, P=0.746, and $\beta$=-0.089, P=0.644 respectively). The relationships between increase in EF when going from rest to 40% and 60% intensity and exercise capacity are shown in **Figure 5.2**.

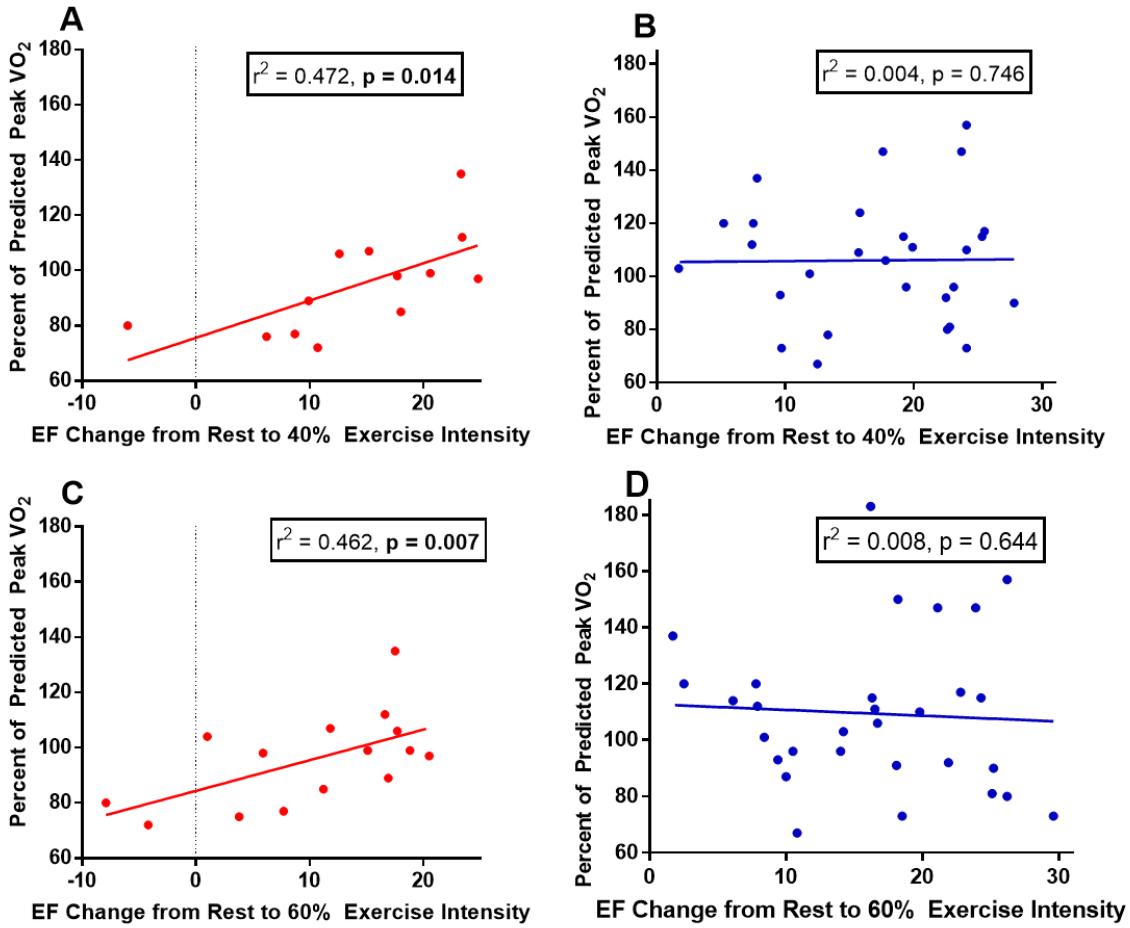



**Figure 5.2. Increase in EF from baseline to 40% and 60% Exercise Intensity Predicts Exercise Capacity in Preterm-born (panels A & C), but not Full-Term born (panels B & D) Young Adults**

Study 1 identified decreased exercise ejection fraction in preterm-born young adults with a significant decline in ejection fraction observed when going from 40% to 60% exercise intensity. In Study 2, change in EF from rest to 40% exercise intensity was predictive of percent of predicted peak $VO_2$ utilisation rate achieved in the preterm-born group (Panel A, $r^2$=0.472, N=13, P=0.014), but not in full-term born controls (Panel B, $r^2$=0.004, N=28, P=0.746). Change in EF from rest to 60% exercise intensity was similarly predictive of percent of predicted peak $VO_2$ utilisation rate achieved in the preterm-born group (Panel C, $r^2$=0.462, N=15, P=0.007), but not in full-term born controls (Panel D, $r^2$=0.008, N=30, P=0.644). Additionally, one preterm-born participant had a reduction in EF when going from rest to 40% exercise intensity and two had reductions in EF when going from rest to 60% intensity. In the control group, all subjects had an increase in EF when going from rest to 40% and also 60% exercise intensity. P and $r^2$ values and adjusted for sex.

#### 5.4.2.3 Sensitivity Analysis for Impacts of Pulmonary Capacity and Dynamic Function In Preterm-born Subjects

After analysing the relationship between exercise capacity and change in EF in response to exercise, I then performed sex-corrected multivariable linear regression analyses to determine if the relationships between change in EF and exercise capacity in preterm-born subjects were significantly influenced by pulmonary volume or dynamic pulmonary function. Percent of predicted FVC was therefore included as an independent variable in the regression model to assess the impact of pulmonary volume. Then, in two separate regression models, FEV1/FVC and then ventilatory reserve at peak $VO_2$ were each included as an independent variable to assess the impacts of dynamic lung function. When percent of predicted FVC was included as an independent variable, change in EF from rest to 40% workload remained a strong, significant predictor of exercise capacity (β=0.664, P=0.038) while percent of predicted FVC was not (β=0.147, P=0.588). Results for dynamic pulmonary function were similar, with change in EF from rest to 40% workload remaining a significant predictor of exercise capacity when FEV1/FVC, and ventilatory reserve at peak $VO_2$ were included in the regression (β=0.789, P=0.019 and β=0.664, P=0.037 respectively) while FEV1/FVC (β=-0.110, P=0.464) and ventilatory reserve at peak (β=-0.154, P=0.572) were not. Similarly, change in EF from rest to 60% workload also remained a significant predictor of exercise capacity when adjusted for percent of predicted FVC (β=1.072, P=0.018), FEV1/FVC (β=0.852, P=0.011), and ventilatory reserve at peak $VO_2$ (β=0.741, P=0.026) with all pulmonary function measures remaining non-significant (β=-0.313, P=0.369,

β=0.120, P=0.627 and β=-0.104, P=0.688) for percent of predicted FVC, FEV1/FVC, and ventilarory reserve at peak VO2 respectively.

## 5.5 Discussion

In the previous chapter, I identified and reported declining EF with increasing exercise intensity in young adults born preterm. In Chapter 5, I now report that otherwise healthy, normotensive, preterm-born young adults exhibited reduced aerobic exercise capacity, and that these reductions were significantly related to their level of impairment in LV EF increase when going from rest to 40% and 60% exercise intensity. Additionally, mechanical pulmonary function in this moderately preterm-born group (mean gestational age 32.8±3.22 weeks) was similar to controls, and upon performing sensitivity analysis for pulmonary volume and dynamic function, increase in LV EF from rest to 40 and 60% exercise load remained a significant predictor of exercise capacity in preterm-born adults.

### 5.5.1 Impaired Systolic Response to Exercise Underlies Reduced Exercise Capacity in Preterm-born young Adults

Aerobic exercise capacity is closely linked to cardiac performance and infrequently limited by pulmonary function in healthy populations (77,307). Several diseases including cardiac amyloidosis (308,309), diabetes (310,311), the metabolic syndrome (312,313), and heart failure of various etiologies (314-316) are associated with impaired exercise contractility, with overall exercise capacity subsequently reduced. However, the mechanisms responsible for diminished increases in exercise EF are understood to vary in each instance. In

the case of amyloidosis, a recent study by Clemmensen et al found that poor coronary blood flow resulting in subendocardial hypoperfusion with subsequent declines in longitudinal myocardial deformation is likely responsible for reduced systolic function and exercise capacity in these patients (309). Alternatively, in diabetes and the metabolic syndrome, dysfunctional myocardial energetics with reduced ATP production as a result of altered myocardial metabolism probably underlies systolic defects in these subjects (311-313). While we did not measure coronary flow reserve, coronary artery disease is rare in healthy, young, normotensive adults. However, due to the structural remodelling with thicker myocardial walls in the LV (7), those born preterm may have greater susceptibility to subtle, subclinical deficits in coronary and microvascular blood flow, though this would require further study to assess. In this study however, I did observe elevated LDL cholesterol, triglycerides, plasma glucose, plasma insulin, and insulin resistance in the preterm-born group vs controls. We have reported similar metabolic alterations in preterm-born adults in our previous work (7) and a 2013 meta-analysis of 27 studies found LDL cholesterol to be consistently elevated in preterm-born adults (7,282). Accordingly, while myocardial energetics were not assessed in this work, the presence of subclinical alterations in glucose and lipid metabolism suggests that myocardial energetics may indeed contribute to the impaired systolic response to exercise in preterm-born subjects. Future investigations that assess the baseline metabolic profile, myocardial energetics, myocardial perfusion index and exercise stress response could prove beneficial in elucidating the relationships between these areas.

### 5.5.2 Pulmonary Capacity does not Explain Reduced Exercise Capacity in the Typical Preterm-born Young Adult Population

In this study, I observed that impaired LV systolic response to exercise stress was strongly associated with diminished exercise capacity in preterm-born individuals. However, because lung development continues into the third trimester, pulmonary function is often considered a risk area for deficits and future vulnerability in preterm-born infants. In particular, the saccular and alveolar phases of development may be interrupted by premature delivery (317-319). In spite of this, spirometric values in our preterm-born group were similar to controls. Interestingly, this comports with a growing body of evidence that prematurity may be less impacting of pulmonary capacity later in life than once thought (320-322).

A recent longitudinal study of 4508 14-17 year old individuals reported on by Kotecha et al revealed minimal impacts in mechanical pulmonary function in those subjected to late preterm birth (321). Those born at 35 or more weeks' gestation who were tested at 8-9 years and also 14-17 years of age, had pulmonary function that was indistinguishable from term-born controls. In the groups born at 25-32 and also 33-34 weeks gestation, some aspects of pulmonary function were lower than controls when tested at 8-9 years of age, however, when tested at age 14-17, most deficits were reduced, with many, including FEV1 and FVC abolished entirely, indicating normalisation of mechanical pulmonary function into adolescence. However, new evidence suggests that some preterm-born groups may indeed have impaired pulmonary function despite typical pulmonary volumes. In a 2018 study, Goss et al reported

normal mechanical pulmonary function, but identified elevated pulmonary arterial pressure and reduced pulmonary gas diffusion rates in a group of young adults born at very low birth weight and an average gestational age of 28 weeks (323).

In Study 2, pulmonary volume and dynamic function was no different between study groups, and sensitivity analysis indicated that change in EF from rest to exercise, rather than mechanical ventilation, predicted exercise capacity. This suggests that mechanical ventilatory deficits do not underlie the observed reductions in exercise capacity. Rather, this reflects exercise physiology norms, where cardiac function is nominally a greater limiting factor of exercise capacity than pulmonary function (307). Correspondingly, adaptations to chronic aerobic training can be extensive in the cardiovascular system (77), but in the pulmonary system, they are comparatively minor and less impacting on aerobic capacity in the typical population (307,324,325).

Further indication that exercise capacity was limited by cardiac function but not pulmonary function in the preterm-born group comes from the $VE/VCO_2$ slope which was 28.1±3.3 in the preterm-born group vs 25.9±3.8 in controls (P=0.011). Elevations of the $VE/VCO_2$ slope commonly occur secondary to deficits in cardiac performance which drive a compensatory response of increased ventilation (294). This ventilation perfusion mismatch is common in heart failure, where exercise $VE/VCO_2$ slopes often exceed a value of 33, with rising values indicative of declining prognosis (326-328). Regarding the otherwise healthy, preterm-born young adults in this study, the presence of this compensatory increased ventilatory response relative to $CO_2$ production and $O_2$

utilisation, combined with typical ventilatory reserve margin (23.3%) are consistent with cardiac limited exercise performance (294).

### 5.5.3 Clinical Implications

Continued investigation will be necessary to more fully understand the clinical implications of these findings. Overall, the moderately premature gestational age of the preterm-born group in this study adds relevance, as it is representative of the majority of the preterm-born population (284). While study data indicates that reduced exercise capacity relates to increased risk, this generally reflects findings from older populations or those with pre-existing disease (329-332). Nevertheless, the fact that diminished exercise capacity was significantly associated with LV systolic impairments is additional evidence of decreased myocardial reserve in the preterm-born population (258). Considering the recently verified increased incidence of early heart failure in those born preterm (185), future longitudinal studies may identify prognostic benefits of exercise testing for risk stratification in preterm-born adults, particularly if other common risk factors such as hypertension or diabetes are present.

### 5.5.4 Study 2 Limitations

Study 2 shares most of the general limitations that were described for Study 1. Namely, the study is single centre, has a modest sample size, has relatively few subjects who were born very or extremely preterm, as well as a retrospective study design which could not facilitate complete early life medical

histories for all subjects. Additionally, the preterm-born group had a higher percentage of females, so P values were adjusted where appropriate.

A final limitation more specific to Study 2 is that pulmonary investigations primarily assessed mechanical ventilatory capacity and function but not pulmonary gas transfer function. While the declining systolic performance combined with typical ventilatory reserve and an increased $VE/VCO_2$ slope suggest that tailing cardiac performance is indeed the primary defect responsible for reduced exercise capacity, future investigation of pulmonary gas transfer function during exercise could add further insight.

## 5.6 Conclusions

Aerobic exercise capacity was significantly lower in preterm-born young adults than controls. This reduced exercise capacity was associated with the preterm-born functional phenotype of reduced LV systolic response to physical exercise. Further, there was no difference in pulmonary volume or dynamic mechanical ventilation between birth groups and pulmonary function did not explain reductions in exercise capacity.

Although below average, exercise capacity was within normal range and did not reflect acute clinical deficits. Two key strengths of this study are that the gestational age of the preterm-born subjects generally reflects the broader preterm-born population, and that the study included specific investigation of cardiac and pulmonary function both at rest and during exercise to facilitate mechanistic insights. Whether, and to what extent, reduced exercise capacity in

preterm-born young adults relates to long-term cardiovascular risk requires further study.

# Chapter 6 Study 3 Results Left Ventricular Remodelling and Physiological Stress Response in Young Adults with Modest Blood Pressure Elevation

## 6.1 Abstract

**Background -** Individual experience of exercise and willingness to comply with exercise prevention programs varies. We hypothesised early subclinical cardiovascular adaptations in young adults with modest blood pressure (BP) elevation may result in a more adverse response to low intensity exercise.

**Objectives -** To study whether young adults with elevated BP have an altered cardiac stress response and whether this relates to left ventricular structure.

**Methods -** We recruited 146 young adults (age 27±5 years) with a range of blood pressures for resting structural phenotyping with cardiac magnetic resonance. A sub-group (N=71) underwent cardiopulmonary exercise testing and exercise stress echocardiography while exercising at 40%, 60%, and 80% of peak exercise load. Myocardial stress response was assessed by changes in ejection fraction (EF) and post-exercise plasma stress biomarker (copeptin) release. Results were compared in those with systolic or diastolic BP≥120/80 mmHg (N=62, mean BP 129/77 mmHg) vs those with systolic and diastolic BP<120/80 mmHg (N=84, mean BP 110/66 mmHg).

**Results -** Resting EF was similar between groups. However, during physical exercise, elevated BP was associated with lower EF at 40% and 60% workload (73.9±3.25 vs 80.0±4.54%, P<0.001 and 75.2±6.59 vs 79.4±4.70%, P=0.026 respectively) as well as smaller increases in EF from resting levels (10.4±5.92 vs 19.0±6.90%, P<0.001 and 11.5±8.34 vs 17.5±7.31%, P=0.029 at

40% and 60% respectively). Furthermore, at 40% workload, perceived exertion was greater in those with higher BP (10.7±1.9 vs 9.6±1.9 on Borg scale, P=0.039). Finally, elevated post-exercise plasma copeptin levels were predicted by lower EF at 40% workload ($r^2$=0.115, P=0.043). The elevated BP group had greater LV mass index (0.68±0.13 vs 0.62±0.12 g/ml, P=0.007) and average wall thickness (7.21±1.31 vs 6.62±1.44 mm, P=0.05) but values were within clinically normal ranges and did not predict variation in myocardial stress response.

**Conclusions -** Young adults with modest blood pressure elevation experience greater fatigue and a physiologically significant systolic defect in response to submaximal exercise as well as lower overall enjoyment of physical activity. Whether intervention can halt functional declines, and how altered perceived exertion and enjoyment of physical activity may confound exercise prescription needs further study.

**Publication Status:** The results presented in Chapter 6 have been accepted as a primary research abstract for the American Heart Association Scientific Sessions, Nov 2018. A completed manuscript is in final preparation for journal submission.

## 6.2 Introduction

Blood pressure (BP) elevation is a leading cause of global morbidity and mortality, directly contributing to 9 million annual deaths (148) and the loss of more disability-adjusted life years (DALYs) lost than any other disease or factor (149). New estimates based on 8.69 million study participants across 154

countries show that global BP elevation is increasing and strikingly, that systolic BP in the 110-115 mmHg range accounted for 48% more DALYs lost from 1990-2015 than did systolic BP > 140 mmHg (149). In late 2017, the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines extensive evidentiary review resulted in lowering the clinical thresholds for Stage I hypertension from 140 to 130 mmHg and categorizing systolic BP of 120-129 as “elevated” (147). Extensive local and government efforts to reduce hypertension have been unable to curtail the growing prevalence of high BP (156). Exacerbating the issue is the mounting data showing that even modest increases in BP have substantial fiscal and human impacts (147,156,157,333).

Mature populations are typically targeted for clinical BP trials (147,232), however, BP elevation is rising in young adults with increased morbidity predicted to follow (334-337). In the United Kingdom, 25% of adults over 25 years old are estimated to be hypertensive (150). Currently, increased physical activity is the frontline prescription for BP reduction in young adults (147,338), however, adherence to exercise prescription is often poor and our review of exercise interventions found that BP reductions were abolished within 12 months of completing supervised exercise sessions (232).

Clinical studies indicate that irreversible cardiovascular damage and risk may onset prior to clinical intervention (156) but earlier intervention could be beneficial (339,340). BP reductions of 2 mmHg may reduce long term disease load and reduce stroke risk by 10% (341). Nevertheless, for younger adults, study data is insufficient to facilitate common 5-10 year risk calculations or alter

current guidance (147,338) and since most young hypertensives lack compound risk factors or significant signs of target organ damage (TOD) such as left ventricular hypertrophy (LVH), they are infrequently treated under current guidance.

Better risk stratification must identify risk markers in otherwise healthy young adults who are absent of resting deficits or traditional signs of TOD. Also, effective exercise intervention will require that experiential or physiological barriers to exercise be identified and countered. Combined assessment of the resting baseline and exercise stress response is optimally suited for investigating risk and exercise barriers in young adults with BP elevation. Stress echocardiography can unmask sub-acute cardiac dysfunction that is compensated at rest and has well-proven prognostic value for assessing risk in healthy and diseased populations alike (71). We therefore completed resting cardiac magnetic resonance imaging and physiological phenotyping on young adults with a range of blood pressures, and also accomplished assessment of physical activity enjoyment, cardiopulmonary exercise testing (CPET) and exercise stress echocardiography on a subgroup to test the hypothesis that early subclinical cardiovascular adaptations in young adults with modest blood pressure (BP) elevation may result in a more adverse response to physical exercise.

## 6.3 Methods

Study 3 uses participants and data from the YACHT and EVS studies. Methods are described in detail in Chapter 3 with a summary overview of study

measures provided in **Figure 3.1.** The central investigations of this chapter focus on baseline cardiac magnetic resonance imaging for detailed structural analysis as well as exercise stress testing in a subgroup.

### 6.3.1 Study Population

The study population for this chapter includes Group 3, N=62 (31 exercise tested), term-born young adults with elevated blood pressure, and Group 4, N=84 (40 exercise tested), normotensive term-born young adult controls. The groups are described in detail in Chapter 2, with a summary of the groups provided in **Figure 2.3**, page 81.

### 6.3.2 Study Visit

The study visit for Study 3 included all the measurements described and collected within the EVS study, with the exercise testing subgroup also completing all measures described for the YACHT study. Study visit details are described in detail in Chapter 3 with an overview of study visit procedures presented in **Figure 3.1,** page 85.

#### 6.3.2.1 Overview

Participants were instructed to fast overnight for 12 hours but to drink plenty of water and continue taking any prescribed medications prior to attending a study visit at the University of Oxford Centre for Clinical Magnetic Resonance (OCMR) and Oxford Cardiovascular Clinical Research Facility (CCRF) in the John Radcliffe Hospital in Oxford, UK. All measurements were completed by trained study investigators and clinical staff. General study visit procedures and measures are provided in greater detail in Chapter 3.

### 6.3.3 Assessment of Physical Activity Enjoyment

Enjoyment of physical activity was assessed by completion of the validated Physical Activity Enjoyment Scale (PACES) (342,343) following cardiopulmonary exercise testing at the end of the study visit. The questionnaire includes 18 questions which are scored from 1-7 by participants resulting in a possible summed composite score ranging from 18-126. Higher scores are indicative of greater overall enjoyment of physical activity, the composite score for the questionnaire was used for analysis.

### 6.3.4 Plasma Copeptin Measurement

Copeptin levels were measured using a commercially available sandwich immunoassay for C-terminal ProAVP (Copeptin) on the BRAHMS KRYPTOR Compact Plus platform (ThermoFisher Scientific, Paisley, UK) according to vendor guidelines by trained study investigators who were blinded to sample allocation to participants. Additional assay details are provided in Chapter 3.

### 6.3.5 Statistics

Statistical analysis was performed using SPSS Version 23. Shapiro-Wilk testing and visual inspection were used to assess normality of variable distribution. Direct between-group comparisons were performed using independent-samples Student t-tests for normally distributed data and Mann-Whitney and Kruskal-Wallis tests for non-normally distributed data. Linear regression modelling was completed using forced entry with missing data excluded pairwise. Standardised regression coefficients (β) are presented for multivariable linear regressions. Pearson correlations (*r*) were used for simple

and partial bivariate correlations. Where appropriate, exercise testing sub-group comparisons between elevated and normal blood pressure groups were adjusted for age and sex by multivariable linear regression modelling to account for differing distributions between groups. This includes demographic, anthropometric, biochemistry, blood pressure and LV echocardiography results.

## 6.4 Results

### 6.4.1 Group Characteristics

There were no differences in age, sex, height or weight between groups. Body mass index was 24.4±3.80 in the elevated BP group vs 23.0±2.93 kg/m$^2$ in controls P=0.014. Resting and awake average ambulatory systolic and diastolic blood pressure were higher in the elevated blood pressure group (133±14 vs 120±10 mmHg, P<0.001 and 79±10 vs 71±6 mmHg, P<0.001 respectively). Resting brachial systolic and diastolic BP was also greater in the elevated BP group (129±10 vs 110±6 mmHg, P<0.001 and 77±8 vs 66±6 mmHg, P<0.001 respectively). Fifteen out 62 (24%) of the elevated BP group were on antihypertensive medication vs 0% of the normal BP Group (P<0.001). Within the elevated BP group, those on antihypertensive medication had higher systolic and diastolic BP than those who were not medicated (134 vs 127 mmHg, P=0.005 and 83 vs 75 mmHg, P=0.006 respectively). Group characteristics are provided in **Table 6.1.**

**Table 6.1. Group Characteristics**

| | Elevated Blood Pressure (N=62) | Normal Blood Pressure (N=84) | P value |
|---|---|---|---|
| **Demographics and Anthropometry** | | | |
| Age | 26.8±5.51 | 26.5±4.51 | 0.681 |
| Male n (%) | 31 (50) | 42 (50) | >0.999 |
| Height (cm) | 173.6±9.86 | 174.0±9.92 | 0.807 |
| Weight (kg) | 73.9±14.04 | 70.2±13.35 | 0.113 |
| BMI (kg/m$^2$) | 24.4±3.80 | 23.0±2.93 | **0.014** |
| **Brachial Blood Pressure (mmHg)** | | | |
| Resting Systolic | 129±10 | 110±6 | **<0.001** |
| Resting Diastolic | 77±8 | 66±6 | **<0.001** |
| Awake Average Ambulatory Systolic | 133±14 | 120±10 | **<0.001** |
| Awake Average Ambulatory Diastolic | 79±10 | 71±6 | **<0.001** |
| Antihypertensive Medication n (%) | 15 (24) | 0 (0) | **<0.001** |
| ACEi or ARB | 10 (16) | 0 (0) | **<0.001** |
| Calcium Channel Blocker | 6 (10) | 0 (0) | **0.003** |
| Beta Blocker | 2 (3) | 0 (0) | 0.099 |
| Diuretic | 2 (3) | 0 (0) | 0.099 |
| Multi-Drug Therapy | 4 (6) | 0 (0) | **0.018** |
| **Biochemistry** | | | |
| Total Cholesterol mmol/L | 4.53±1.07 | 4.37±0.87 | 0.332 |
| HDL mmol/L | 1.44±0.35 | 1.48±0.36 | 0.496 |
| LDL mmol/L | 2.57±0.88 | 2.49±0.65 | 0.511 |
| Triglycerides mmol/L | 1.04±0.51 | .89±0.46 | 0.072 |
| High Sensitivity C-Reactive Protein | 1.53±2.92 | 1.53±3.72 | 0.990 |
| Glucose mmol/L | 4.90±0.41 | 4.79±0.45 | 0.154 |
| Insulin pmol/L | 47.6±37.9 | 39.1±31.9 | 0.154 |
| Insulin resistance | 0.83±0.52 | 0.66±0.67 | 0.270 |

Group Characteristics presented as mean±standard deviation. BMI, Body Mass Index; ACEi, Angiotensin Converting Enzyme inhibitor; ARB, Angiotensin II Receptor Blocker; HDL, High Density Lipoprotein; LDL, Low Density Lipoprotein. P values < 0.05 depicted in bold.

### 6.4.2 Resting Cardiac Profile

#### 6.4.2.1 Sub-clinical Left Ventricular Mass Increase in Young Adults with Elevated Blood Pressure

CMR showed that LV length was identical between groups and mean LV diameter was greater in the elevated BP group though this difference did not achieve significance (5.52±0.48 vs 5.48±0.46cm, P=0.056). The elevated blood pressure group had greater raw LV mass (104.8±22.7 vs 95.5±22.2g, P=0.041) which remained greater when indexed to body surface area (55.6±10.0 vs 52.0±9.6 g/$m^2$, P=0.041) and also when indexed to end diastolic volume (0.68±0.13 vs 0.62±0.12 g/ml, P=0.007). All participants were below standard mass thresholds for clinical LVH (102g/$m^2$ in males, 88g/$m^2$ females) (12,67,344). In the elevated BP group, LV wall thickness in the anterior, lateral, posterior, septal, and inferior septal segments was greater than controls (6.62±1.34 vs 6.01±1.62mm, P=0.023, 7.14±1.49 vs 6.24±1.38mm, P<0.001, 7.05±1.41 vs 6.51±1.64mm, P=0.048, 8.13±1.74 vs 7.52±1.79mm, P=0.050, and 7.61±1.81 vs 6.79±2.06mm, P=0.019 respectively). Average wall thickness was also greater in the elevated BP group than controls (7.21±1.31 vs 6.62±1.44 mm, P=0.015). There was no between-group difference in relative wall thickness, and relative wall thickness for all participants was below the clinical threshold for concentric remodelling of 0.42. CMR data are presented in **Table 6.2.**

**Table 6.2. Magnetic Resonance Imaging Results**

| | Elevated Blood Pressure (N=62) | Normal Blood Pressure (N=84) | P value |
|---|---|---|---|
| **Left Ventricle Function** | | | |
| Heart Rate (Beats/min) | 70.4±12.6 | 63.9±8.93 | **0.001** |
| Ejection Fraction (%) | 64.8±5.32 | 63.2±4.81 | 0.072 |
| **Left Ventricle Structure** | | | |
| Diameter (cm) | 5.52±0.48 | 5.48±0.46 | 0.056 |
| Length | 10.0±0.80 | 10.0±0.76 | 0.950 |
| Mass (g) | 104.8±22.7 | 95.5±22.24 | **0.020** |
| LV Mass/BSA ($g/m^2$) | 55.6±10.0 | 52.0±9.64 | **0.041** |
| LV Mass/EDV (g/ml) | 0.68±0.13 | 0.62±0.12 | **0.007** |
| Anterior Wall Thickness (mm) | 6.62±1.34 | 6.01±1.62 | **0.023** |
| Lateral Wall Thickness (mm) | 7.14±1.49 | 6.24±1.38 | **<0.001** |
| Posterior Wall Thickness (mm) | 7.05±1.41 | 6.51±1.64 | **0.048** |
| Inferior Wall Thickness (mm) | 6.76±1.72 | 6.64±1.71 | 0.694 |
| Septal Wall Thickness (mm) | 8.13±1.74 | 7.52±1.79 | **0.050** |
| Inferior Septal Wall Thickness(mm) | 7.61±1.81 | 6.79±2.06 | **0.019** |
| Average Wall Thickness | 7.21±1.31 | 6.62±1.44 | **0.015** |
| Relative Wall Thickness | 0.24±0.06 | 0.24±0.05 | 0.764 |
| **Clinical Indicators of LVH** | | | |
| LV Mass/BSA: >102(male) or >88(female), n (%) | 0 (0) | 0 (0) | - |
| RWT > 0.42, n (%) | 0 (0) | 0 (0) | - |
| Posterior Wall Thickness: >13 mm (male) >12 mm (female), n (%) | 0 (0) | 0 (0) | - |
| Septal Wall Thickness: >13 mm (male) >12 mm (female), n (%) | 0 (0) | 0 (0) | - |

Magnetic Resonance Imaging values presented as mean±standard deviation. LV, Left Ventricle; BSA, Body Surface Area; EDV, End Diastolic Volume; LVH, Left Ventricular Hypertrophy; RWT, Relative Wall Thickness. P values < 0.05 depicted in bold.

#### 6.4.2.2 Preserved Resting Cardiac Function in Young Adults with Elevated Blood Pressure

In the exercise testing subgroup, those with elevated BP had a higher resting heart rate than controls (65±12.3 vs 60±10.0 beats per minute, P=0.018). Resting echocardiography showed no difference in EF, with 63.2±5.8% in the elevated BP group vs 62.6±5.2% in controls, P=0.707. 2-Dimensional echocardiography strain analysis showed no between group differences in peak systolic circumferential or radial strain or strain rate. However, peak systolic longitudinal strain and strain rate were reduced in the higher BP group vs controls, -18.8±2.53% vs -20.8±2.98%, P=0.012, and -0.97±0.43 vs -1.15±0.21 %/s, P=0.0433 respectively. Exercise testing sub-group characteristics are presented in **Table 6.3.**

**Table 6.3. Exercise Testing Group Characteristics**

| | Elevated Blood Pressure (N=31) | Normal Blood Pressure (N=40) | P value |
|---|---|---|---|
| **Demographics** | | | |
| Age | 26.5±6.0 | 24.3±4.1 | 0.068 |
| Male, n (%) | 19 (61) | 20 (50) | 0.350 |
| Height, (cm) | 175±10.3 | 174.0±10.3 | 0.858 |
| Weight, (kg) | 77.8±15.6 | 70.0±12.8 | 0.710 |
| BMI ($kg/m^2$) | 25.3±3.7 | 22.9±2.6 | **0.140** |
| **Brachial Blood Pressure (mmHg)** | | | |
| Resting Systolic | 133±11 | 112±4 | **<0.001** |
| Resting Diastolic | 78±10 | 65±5 | **<0.001** |
| Antihypertensive Medication | 7 (23) | 0 (0) | **<0.001** |
| ACEi or ARB | 5 (16) | 0 (0) | **0.014** |
| Calcium Channel Blocker | 3 (10) | 0 (0) | 0.061 |
| Beta Blocker | 1 (3) | 0 (0) | 0.532 |
| Diuretic | 0 (0) | 0 (0) | - |
| Multi-Drug Therapy | 1 (3) | 0 (0) | 0.532 |
| **Resting Echocardiography** | | | |
| Heart Rate (Beats/min) | 65±12.3 | 60±10.0 | **0.018** |
| Ejection Fraction (%) | 63.2±5.8 | 62.6±5.2 | 0.707 |
| Diastolic Function | | | |
| E/A Ratio | 1.49±0.31 | 1.85±0.45 | **0.001** |
| E (cm/sec) | 80.5±14.6 | 87.8±15.7 | 0.088 |
| e' (cm/sec) | 12.7±2.4 | 15.2±2.6 | **0.002** |
| E/e' | 6.48±1.85 | 6.11±2.27 | 0.825 |
| Left Atrial Dimension (cm) | 3.05±0.37 | 3.04±0.36 | 0.751 |
| Peak Systolic Strain | | | |
| Longitudinal Strain (%) | -18.8±2.53 | -20.8±2.98 | **0.012** |
| Systolic Strain Rate (%/s) | -0.97±0.43 | -1.15±0.21 | **0.033** |
| Circumfurential Strain (%) | -26.5±4.2 | -27.1±3.79 | 0.423 |
| Systolic Strain Rate (%/s) | -1.59±0.30 | -1.55±0.29 | 0.586 |
| Radial Strain (%) | 49.9±19.8 | 44.9±14.2 | 0.278 |
| Systolic Strain Rate (%/s) | 2.47±0.85 | 2.24±0.711 | 0.183 |

Subgroup characteristics presented as mean±standard deviation. BMI, Body Mass Index; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; E, peak early diastolic velocity of transmitral flow; A, peak late velocity of transmitral flow; e', average peak early myocardial velocity. P values adjusted for age and sex.

### 6.4.3 Physiological Exercise Stress Response

#### 6.4.3.1 **Participants Completion of Stress Echocardiography**

Following the recovery period after peak exercise testing, all participants were encouraged to carry out cycle ergometry stress echocardiography at 40, 60 and 80% of their peak achieved exercise intensity. Consistent with study protocol presented at consent, participants understood that all assessments were voluntary. Of 31 participants with elevated blood pressure, 21 performed the stress echocardiography assessment while the remaining 10 elected not to attempt stress echocardiography due to existing fatigue. All 21 participants with elevated blood pressure who performed stress echocardiography completed the 40% intensity stage, one dropped out due to fatigue prior to the 60% exercise intensity stage and three more dropped out due to fatigue prior to the final stage at 80% intensity. Of 40 participants with normal blood pressure, 29 performed the stress echocardiography assessment while the remaining 11 elected not to attempt stress echocardiography due to existing fatigue. All 29 participants with normal blood pressure who performed stress echocardiography completed the 40 and 60% exercise intensity stages, but four did not attempt the final stage at 80% intensity due to fatigue.

#### 6.4.3.2 **Stress Echocardiography Image Quality**

Due to challenges with imaging quality, some stress echocardiography scans were not of sufficient quality for analysis. In the elevated blood pressure group, image quality allowed for determination of EF for 15 scans at 40% intensity, 15 scans at 60% intensity and 11 scans at 80% intensity. In the normal

blood pressure group, image quality allowed for determination of EF for 24 scans at 40% intensity, 25 scans at 60% intensity and 21 scans at 80% intensity.

#### 6.4.3.3 Adverse Cardiac Stress Response Profile At 40% of Peak Workload in Young Adults with Elevated Blood Pressure

##### *6.4.3.3.1 Impaired Early Systolic Response to Exercise in Young Adults with Elevated Blood Pressure*

When subjected to the physiological stress of physical exercise, the systolic response was less in the elevated BP group than in normotensive controls. At 40% and 60% exercise intensity, the EF was lower in the elevated BP group (73.9±3.25% vs 80.0±4.54%, P<0.001 and 75.2±6.59% vs 79.4±4.70%, P=0.026 respectively). The increase in EF from baseline to 40% and 60% exercise intensity was lower in the elevated BP group as well (10.4±5.92 vs 19.0±6.90%, P<0.001 and 11.5±8.34 vs 17.5±7.31%, P=0.029 respectively). Additionally, at 40% exercise intensity, the greatest individual EF achieved within the elevated BP group was 77.9% which was 2.1% below the mean EF for controls at the same workload. EF from rest through all stages of exercise is presented in **Figure 6.1.** Age and sex adjusted linear regression of resting systolic BP as a predictor of EF at 40% workload showed that BP strongly predicted EF at 40% of peak workload (β=-0.491, P=0.001), **Figure 6.2 Panel A**. Heart rate however was similar between groups at 40% workload with 127±13 beats per minute in the elevated BP group vs 130±12 beats per minute in controls, P=0.689.

#### *6.4.3.3.2 Reduced Myocardial Deformation During Early Exercise Response*

In 2-D speckle tracking echocardiography at 40% of peak exercise load, myocardial deformation was reduced in those with elevated BP vs controls (-20.23±2.67% vs -23.09±2.38%, P=0.005 respectively) **Figure 6.2 Panel B**.

#### *6.4.3.3.3 Elevated Blood Pressure is Associated with Increased Perceived Exertion During Submaximal Exercise Response*

At 40% of peak exercise workload, rate of perceived exertion (RPE) measured on a Borg scale was higher in those with elevated vs normotensive controls (10.7±1.86 vs 9.60±1.90, age and sex adjusted P=0.039) **Figure 6.2 Panel C**.

#### *6.4.3.3.4 Elevated Circulating Cardiac Stress Signal Follows Poor Systolic Response to Exercise*

In the sub-group that underwent stress-testing, age and sex adjusted circulating copeptin levels were similar between the elevated BP group and controls both at rest (P=0.283) and immediately following maximal CPET testing (post-exercise) (P=0.883). Post-exercise copeptin levels were then compared to systolic performance at 40% exercise intensity. Multivariable regression modelling correcting for age and sex revealed that across the entire group, those with lesser gains in EF when going from rest to 40% exercise load had greater post-exercise circulating copeptin levels (β=-0.329, P=0.043). The relationship

between submaximal change in EF from rest to 40% exercise intensity and post exercise plasma copeptin level is shown in **Figure 6.2, Panel D.**

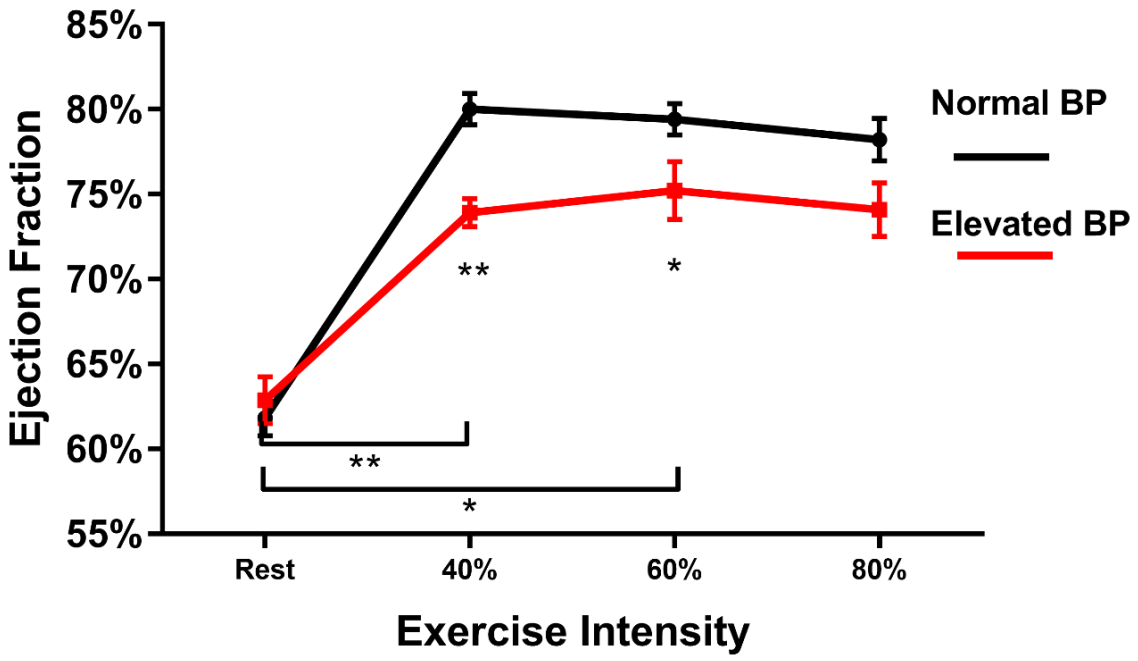



**Figure 6.1. 2-D echocardiography ejection fraction (EF) with increasing exercising intensity in young adults with elevated blood pressure vs controls.**

Resting EF was similar between groups. At 40% and 60% of peak exercise intensity, the elevated blood pressure group (red plot) had a lower EF than controls (73.9±3.25, N=15 vs 80.0±4.54%, N=24, P<0.001 and 75.2±6.59, N=15 vs 79.4±4.70%, N=25, P=0.026 respectively) and also a smaller increase in EF when going from baseline to 40 and 60% exercise intensity (10.4±5.92, N=15 vs 19.0±6.90%, N=24, P<0.001 and 11.5±8.34 vs 17.5±7.31%, P=0.029 respectively). Error bars represent SEM. **Denotes significantly lower than controls (P<0.001), * denotes significantly lower than controls (P<0.05). P values adjusted for age and sex.

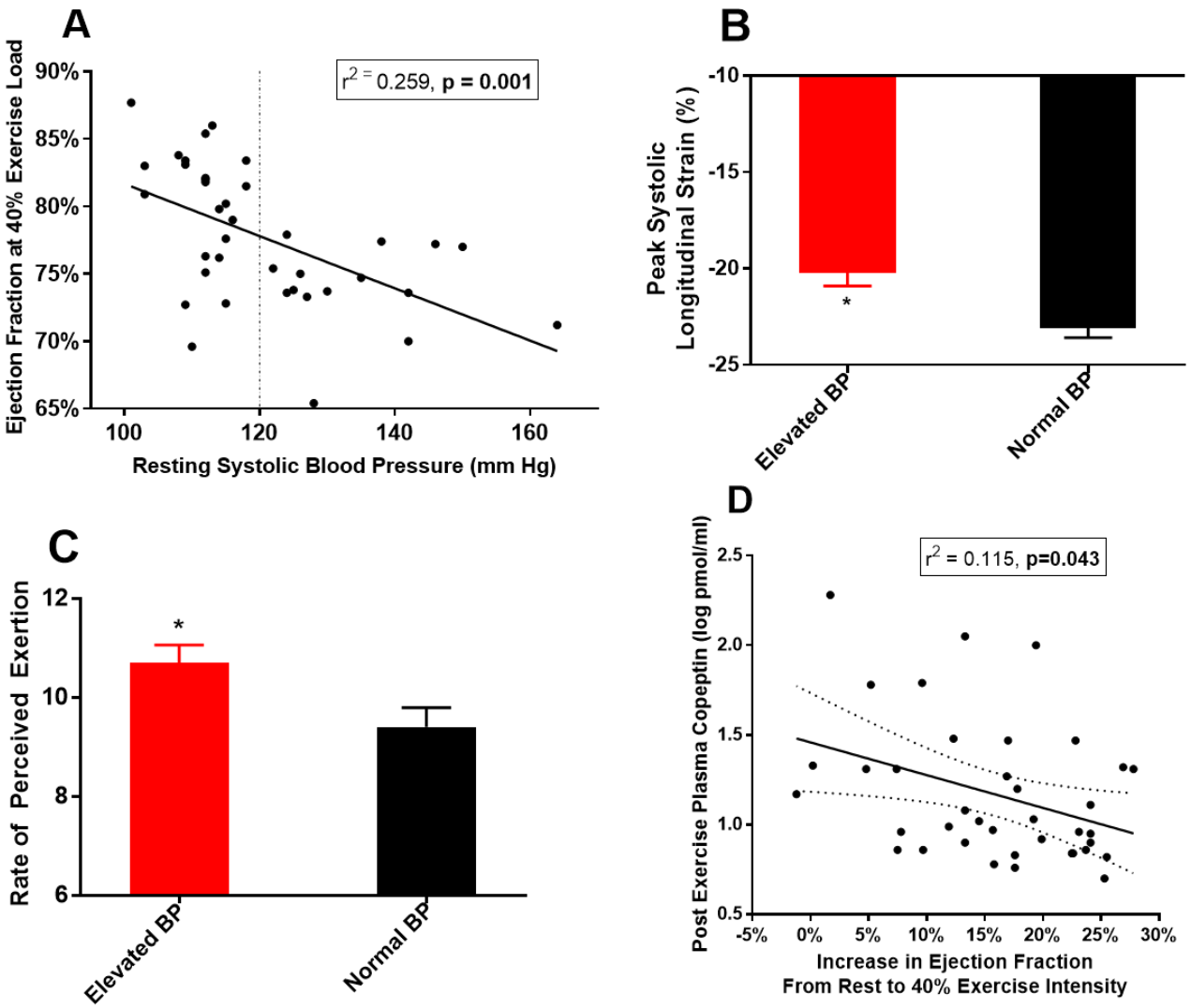



**Figure 6.2. Functional and Physiological Phenotyping of 40% Exercise Load Stress Response Profile**

The greatest functional declines in the elevated BP group occurred at 40% exercise load. Panels A-D present a broad, multivariable cardiac stress response profile of key phenotypic parameters that relate to cardiac function at 40% exercise load. All β, *r* and P values adjusted for age and sex.

Panel A: Resting systolic blood pressure predicted ejection fraction (EF) at 40% exercise load in young adults (β=-0.491, $r^2$=0.259, N=39, P=0.001). EF was significantly lower at 40% of peak exercise load in young adults with blood pressure ≥ 120/80 than those with blood pressure < 120/80 (73.9±3.25 vs 80.0±4.54%, P=0.001). At 40% exercise load, the greatest individual EF achieved within the higher BP group was 2.1% below the mean EF for controls. 95% confidence intervals depicted.

Panel B: 2-D echocardiography peak systolic longitudinal strain in young adults with elevated blood pressure vs controls at 40% exercise intensity. Peak systolic longitudinal strain was reduced in the elevated blood pressure group (red column) vs controls (-20.23±2.67%, N=15 vs 23.09±2.38%, N=22 P=0.005). Error bars represent SEM. *Denotes significantly lower than controls (P=0.005).

Panel C: Rate of perceived exertion (RPE) of elevated vs control BP group at 40% exercise load. At 40% intensity, RPE was greater in the elevated BP group (red column) (10.7±1.86 vs 9.6±1.90, age and sex adjusted P=0.039). Between-group RPE was similar at all other workloads. Error bars represent SEM. *Denotes significantly lower than controls (age and sex adjusted P=0.039).

Panel D: Relationship between the change in EF when going from rest to 40% exercise intensity and post-exercise plasma copeptin level. Low sub-maximal contractile reserve at 40% exercise intensity predicted increased post-exercise plasma copeptin levels (β=-0.331, $r^2$=0.115, N=39, P=0.043). 95% confidence intervals depicted.

#### 6.4.3.4 Resting Blood Pressure and Perceived Exertion at 40% Exercise Load Predict Overall Enjoyment of Physical Activity

##### *6.4.3.4.1 Lower Physical Activity Enjoyment in Young Adults with Elevated Blood Pressure*

Enjoyment of physical activity measured by the composite score of the Physical Activity Enjoyment Scale (PACES) questionnaire was 56.7±9.9 in young adults with elevated BP vs 63.1±10.2 in normotensive participants (age and sex adjusted P=0.017), indicating greater enjoyment of physical activity in the normotensive group **Figure 6.3, Panel A**.

##### *6.4.3.4.2 Physical Activity Enjoyment Declines with Increasing Blood Pressure*

In age and sex adjusted multivariable linear regression analysis, increased resting systolic BP was associated with lower physical activity

enjoyment (age and sex adjusted β=-0.392, $r^2$=0.147, P=0.002). **Figure 6.3, Panel B**.

#### *6.4.3.4.3 Young Adults with Elevated Blood Pressure Experienced Greater Perceived Exertion only at 40% of Peak Workload*

There was no difference in RPE between groups at the end of the warmup phase of exercise testing. At 40% of peak exercise workload, mean RPE on the Borg scale was 1.1 units higher in those with elevated BP vs normotensive controls (10.7±1.86 vs 9.60±1.90, age and sex adjusted P=0.039). Between group RPE was similar at 60% exercise load, and identical at 80% exercise load. The between group RPE difference for each stage of exercise is presented in **Figure 6.3**, **Panel C**.

#### *6.4.3.4.4 Perceived Exertion at 40% Load Strongly Predicts Physical Activity Enjoyment Level in Young Adults with Elevated Blood Pressure*

When assessed by BP group, greater perceived exertion at 40% exercise intensity was strongly associated with lower overall enjoyment of physical activity in participants with elevated BP (age and sex adjusted β=-0.561, $r^2$=0.324, P=0.004), but not in normotensive participants (age and sex adjusted β=0.070, $r^2$=0.005, P=0.690). The strength of correlation in the elevated BP group was sufficiently robust to produce a statistically significant relationship across the entire study group (age and sex adjusted β=-0.295, $r^2$=0.086, P=0.022). The group relationships between perceived exertion at 40% exercise load and enjoyment of physical activity (PACES composite score) are shown in **Figure 6.3, Panel D.**

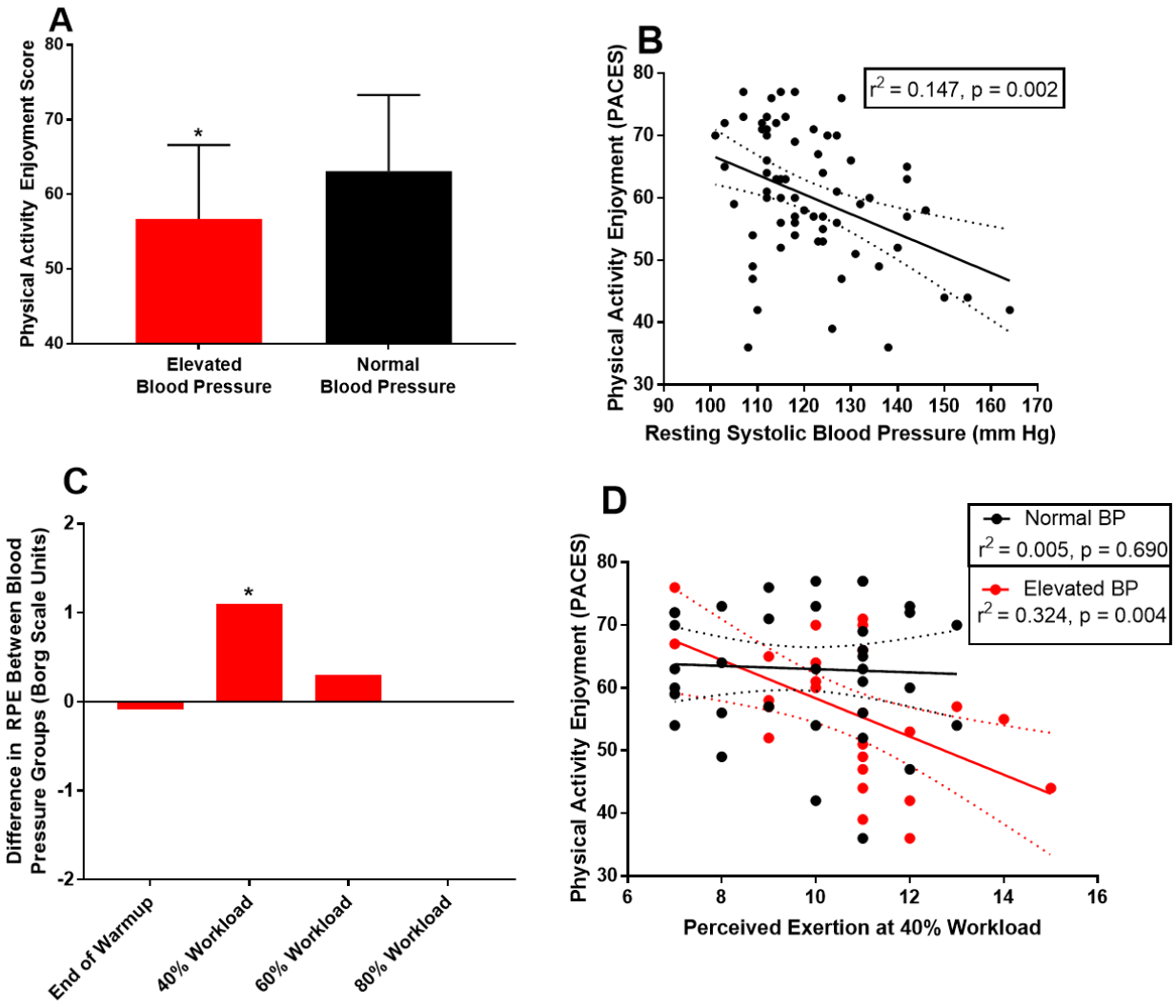



**Figure 6.3. Greater Resting Systolic Blood Pressure and Perceived Exertion during Low Intensity Exercise are Associated with Decreased Enjoyment of Physical Activity in Young Adults**

Greater resting systolic blood pressure as well as perceived exertion at 40% exercise load were associated with lower overall enjoyment of physical exercise assessed by the composite score on the Physical Activity Enjoyment Scale (PACES) questionnaire. Panels A-D present key relationships between blood pressure, physical activity enjoyment, and perceived exertion at low intensity exercise (40% workload). All β, *r* and P values adjusted for age and sex.

Panel A: Physical activity enjoyment measured by the PACES composite score was lower in young adults with elevated blood pressure than in normotensive young adults (56.7±9.9 vs 63.1±10.2, P=0.017).

Panel B: Across the entire study group, greater resting systolic BP was associated with lower physical activity enjoyment (β=-0.392, $r^2$=0.147, P=0.002).

Panel C: Difference in rate of perceived exertion (RPE) of elevated BP group vs controls across stages of exercise intensity. At 40% intensity, RPE was 1.1 unit

greater on the Borg scale in the elevated BP group (10.70±1.86 vs 9.60±1.90, age and sex adjusted P=0.039). RPE in the elevated BP group vs controls was similar at all other workloads: Post-warmup (7.31±1.05 vs 7.65±1.82, P=0.778), 60% workload (13.3±1.64 vs 13.03±1.35, P=0.355), 80% workload (15.9±1.60 vs 15.9±1.54, P=0.856). Because PE at 80% workload was identical between groups, the between group difference at this stage was "0.0" which is not visible on the graph. *Denotes significantly higher than controls (age and sex adjusted P=0.039).

Panel D: Perceived exertion at 40% workload was a strong and significant predictor of physical activity enjoyment in the elevated BP group (β=-0.561, $r^2$=0.324, P=0.004), but not in controls (β=-0.070, $r^2$=0.005, P=0.690). The correlation in the elevated BP group was sufficiently robust to produce a statistically significant relationship across the entire study group (β=-0.295, $r^2$=0.086, P=0.022).

#### *6.4.3.4.5 Impaired Submaximal Exercise Stress Response Relates to Blood Pressure but not LV Mass*

Sensitivity analysis was performed using a series of multivariable regression models to determine if the greater LV mass identified on CMR was associated with the adverse stress response in the elevated BP group. Because the greatest impairment was observed at 40% exercise intensity, EF at 40% workload was selected as the dependent variable for these analyses. Multivariable regression analysis in the stress testing sub-group with independent variables of age, sex, resting systolic BP, and LV mass indexed to BSA showed that resting systolic BP was a significant predictor of EF at 40% workload (β=-0.508, P=0.003) but LV mass indexed to BSA was not (β=0.126, P=0.410). The same model was run using LV mass indexed to EDV instead of BSA and yielded similar results with resting systolic BP remaining a significant predictor of EF at 40 % exercise intensity (β=-0.545, P=0.002,) but not LV mass indexed to EDV (β=0.208, P=0.237). An additional sensitivity analysis using similar multivariable regression models correcting for systemic vascular

resistance (SVR) instead of LV mass was also performed and found no relationship between SVR and EF at 40% load (β=0.059, P=0.745) however the relationship in between resting systolic BP and EF at 40% workload remained significant (β=-0.484, P=0.003).

## 6.5 Discussion

This study reports for the first time that young adults with modest blood pressure elevation have preserved resting ejection fraction but experience greater fatigue and a physiologically significant LV systolic defect in submaximal exercise stress response. Additionally, modest BP elevation in young adults was associated with lower enjoyment of physical activity and subclinical LV structural remodelling. However, the adverse stress response related to BP elevation but not variation in LV mass.

## 6.6 Reduced Systolic Performance during Sub-maximal Exercise may Indicate Risk – Is this Response Reversible?

The link between LVH and cardiovascular risk is well reported and integrated into clinical practice, but LVH is uncommon in young adults even though it may be present in more than half of hypertensives over the age of 50 (41,64,123-125). Framingham, CARDIA, and other longitudinal studies indicate that hypertensives medicated to treatment goals still have twice the risk for cardiovascular events as unmedicated normotensives. This is likely due to irreversible long term cardiovascular damage and associated risk realised during years of unmanaged BP elevation (339,340,345,346). However, impaired

systolic response to exercise is a strong independent predictor of morbidity and mortality in mature adult and diseased populations (71). These new findings further support the notion that sub-clinical TOD and associated increased risk trajectories may be established early in the etiology of hypertensive disease. Further study is still needed to understand whether lifestyle intervention or drug therapy may ameliorate the functional defects and any risk related to the impaired cardiac exercise stress response of these young adults. Nevertheless, this study provides new evidence that delaying active BP management in young adult populations may allow progression of disease processes that have already resulted in functional declines.

## 6.7 Elevated Post-exercise Circulating Stress Signal Follows Poor Systolic Response to Exercise

Circulating copeptin level is a useful measure of cardiac stress (347-350). In this young adult study, the observed cardiac remodelling and dysfunction was physiologically significant but remained subclinical. It is noteworthy however, that the elevated post-exercise cardiac stress signaling was associated with poor LV contractile performance in response to low intensity exercise (Figure 2, Panel D). This reflects a pathological profile, rather than a physiological profile where increased stress signaling could be a consequence of greater exercise performance. Additionally because this profile was observed across the entire study group, it further highlights that dysfunctional cardiovascular response to low intensity exercise is an important and viable diagnostic area for identifying

increased cardiovascular disease risk, particularly in asymptomatic individuals (351-354).

## 6.8 Possible Mechanism Underlying Impaired Left Ventricular Exercise Stress Response

Multiple mechanisms could contribute to the impaired left ventricular stress response in the elevated blood pressure group and may include elements of metabolic (312,313), autonomic (355-358) and endothelial dysfunction (359,360). The elevated blood pressure group had a mild increase in body mass index, which might have contributed to a metabolic defect, although there were no differences in fasting glucose, insulin and insulin resistance. Diminished endothelial responses are present early in cardiovascular disease including pre-hypertensive young adults (360-362) and endothelial dysfunction could lead to altered vasorelaxant responses that impact on exercise systolic response (358,359). This could manifest directly through limitation of myocardial microcirculation function or by increasing systemic hemodynamic loading and peripheral resistance. Similarly, autonomic variations often accompany hypertensive disease and can blunt nominal vasomotive exercise response (358), or may delay sympathetic ramping of systolic response to exercise due to chronic baseline over-activation (357,363,364). The difference in ejection fraction was less significant at higher exercise workload. The alterations at 40% workload may therefore reflect a slower adaptation of the cardiovascular system to physical stress rather than the declining performance observed in the preterm-born group in Study 1. Interestingly, early exercise fatigue has been reported in

a hypertensive rat model, although in this experiment it was attributed to changes in diastolic function (365). Cardiac mass and longitudinal strain at rest were different in this young adult study but the differences were subclinical and did not associate with differences in exercise response. However, it remains possible early changes in myocardial structure such as presence of fibrosis may influence systolic function on stress and further imaging studies will be of value.

## 6.9 Clinical Implications

These newly identified functional declines in exercise response warrant attention and further study to better understand possible impacts to daily life as well as progression toward acute sequelae of hypertensive disease such as heart failure (2,366-368). Additionally, three study findings in particular may reveal compounding physiological and experiential confounders of current exercise prescription in young adults with elevated BP:

- Higher blood pressure was significantly associated with diminished enjoyment of physical activity ($r^2$=0.147, P=0.002), **Figure 6.3, Panel B** which indicates a barrier to lifestyle change may exist in this population which is not addressed through current clinical norms.
- Perceived exertion between BP groups was similar at all exercise intensities except 40% of peak workload, where it was significantly higher in the elevated BP group (P=0.039). At this workload, the average heart rate was 127 BPM in the elevated BP group and 130 BPM in controls which falls between 60% and 70% of max heart rate calculated by 220-age. Because standard clinical guidance directs

young adults with elevated BP to perform sustained exercise activity at these heart rates (147,189,223,338), current exercise prescription targets the only exercise workload where exercise experience was disproportionately unfavorable in those with elevated BP.

- Across the group, perceived exertion at 40% workload was significantly associated with lower enjoyment of physical activity, ($r^2$=0.086, P=0.022), **Figure 3 Panel D**, an effect that resulted exclusively from a significant association in the elevated BP group ($r^2$=0.324, P=0.004) indicating that individual experience at 40% exercise intensity may be largely determinative of the overall perception of physical activity enjoyment in young adults with elevated BP.

These critical relationships between BP, physical activity enjoyment, and perceived exertion, combined with the systolic defect at 40% exercise workload reveal a combination of physiological and experiential states which may help explain why physical activity guidelines have been ineffective for BP management in young adults (232,369,370).

In light of this, crossover therapy starting with pharmaceutical measures, then subsequently adding increased physical activity, warrants investigation for BP intervention in young adults. This approach could improve the possibility of gaining additive benefits from both medication and lifestyle change. A clinical trial of mature African American men showed drug therapy with physical exercise produced additive benefit over drugs alone on blood pressure and LVH regression, despite reduced drug doses in the exercising group (371). Going

forward, new trials are needed to investigate if novel intervention strategies such as drug crossover therapy can reduce these physiological and experiential barriers to physical activity in young adults and thereby facilitate increased physical activity as well.

## 6.10 Study 3 Limitations

Study 3 has multiple limitations. Exercise stress testing was completed at the end of a full-day visit in order not to confound other measures and not all participants were willing to continue with the sub-maximal exercise testing after CPET testing. This introduces a risk of bias towards inclusion of more physically able individuals that could dilute differences between groups. As previously discussed in the Limitations for Study 1, some potentially confounding lifestyle and demographic factors were not analysed. Also, the stress imaging protocol for future work has been revised to collect stress imaging during CPET testing in order to remove any inherent selection bias from this measurement. Also, due to challenges with exercise imaging, not all scans were of sufficient quality for analysis. The sample size for the exercise testing subgroup was modest and this elevated BP group was slightly older and more male so P values were adjusted for age and sex where appropriate. Nevertheless, post hoc analysis showed this sample of 146 participants with cardiovascular magnetic resonance data provided 81% power with P=0.05 for the observed 0.47 SD between group difference for LV mass indexed to end diastolic volume and the sub-sample of 71 participants with cardiopulmonary exercise test data had an estimated power of 99% with P=0.05 to detect the observed 1.55 SD between group difference for EF at 40% workload. All scans were anonymized to avoid bias during analysis,

including image quality assessment. Also, as a cross-sectional study, pre-medication study datasets were not available for the 15 participants on antihypertensives which did not allow for analysis of the effect of medication on RPE and other elements of the stress response. As a single center study located in the Oxford area that included a mix of passive and purposive recruitment of young adults with elevated risk, this study population may not reflect the broader population in the local Oxford area. This strategy resulted in significant demographic homogeneity between groups and limited investigations to physiological parameters rather than socioeconomic factors.

## 6.11 Conclusions

Young adults with modest blood pressure elevation experience a physiologically significant systolic defect and greater fatigue in response to submaximal physical exercise. They also have lower overall enjoyment of physical activity which relates to their fatigue during early exercise response which may help explain lack of compliance with physical activity prescription in this population. BP elevation also associated with sub-clinical LV remodelling, however, the adverse stress response related to BP but not LV mass variation. Whether intervention can halt functional declines, and how perceived exertion and enjoyment of physical activity may confound exercise prescription needs further study.

# Chapter 7 Study 4 Results Exercise Capacity and Left Ventricular Function in Young Adults with modest Blood Pressure Elevation

## 7.1 Abstract

**Background –** Blood pressure elevation and reduced exercise capacity are strong predictors of future cardiovascular risk. Whether blood pressure elevation relates to exercise capacity in young adults is poorly studied and has yielded conflicting results.

**Objectives -** To study whether young adults with elevated BP have reduced aerobic exercise capacity and whether exercise capacity relates to blood pressure.

**Methods -** We recruited 71 young adults (age 25±5 years) with a range of blood pressures for detailed baseline cardiovascular phenotyping and also cardiopulmonary exercise stress testing. Results were compared in those with systolic or diastolic BP≥120/80 mmHg (N=31, mean BP 133/78 mmHg) vs those with systolic and diastolic BP<120/80 mmHg (N=40, mean BP 112/65 mmHg).

**Results –** Exercise capacity was significantly lower in the elevated blood pressure group (98±23% vs 112±23% of predicted peak $VO_2$, P=0.022). Additionally, multivariable modeling comparing systolic and diastolic brachial, 24 hour awake ambulatory, asleep and aortic blood pressure measures identified aortic diastolic blood pressure as the strongest correlate of exercise capacity (β=-0.527, $r^2$=0.249, P<0.001).

**Conclusions –** Blood pressure level, particularly aortic diastolic pressure, is inversely associated with exercise capacity in young adults. How exercise capacity in young adults relates to long-term risk, and whether elevated aortic

diastolic blood pressure may associate with compounding risk factors requires further study.

**Publication Status:** The results presented in Chapter 7 are currently being developed into a manuscript for future submission.

## 7.2 Introduction

Blood pressure elevation is a growing cardiovascular risk factor in the young adult population (150,336,372,373). A large body of clinical research data also shows that reduced exercise capacity is an independent predictor of cardiovascular risk (329-332). While many studies including our recent systematic review (232) show that increased physical activity has at least short term beneficial effects on blood pressure, the basic relationship between exercise capacity and blood pressure elevation remains poorly researched. Existing reports are inconclusive and usually assess older populations, often with comorbidities. A 2006 study on mature adults with elevated blood pressure by Kokinnos et al found that 24-hour, but not clinic blood pressure was higher in those who achieved “low” exercise capacity assessed by Bruce protocol (374). Alternatively, a 2017 report, also in mature adults, found no relationship between blood pressure elevation and exercise capacity (375). Data in young adults is minimal and focused on non-typical populations including competitive athletes (376) and pathology groups such as Tetralogy of Fallot (377). As a result, accepted relationships between blood pressure and exercise capacity in typical young adult populations are largely putative. A clinical consequence of this status quo is a poor understanding of whether and when, risk associated with

increased blood pressure in generally healthy young adults may be compounded by risks associated with reduced exercise capacity.

In Study 3, I reported physiologically significant systolic impairment during submaximal exercise response in young adults with elevated blood pressure. However, systolic deficits were reduced at higher intensity stages of exercise. In Study 4, I hypothesise that young adults with elevated BP will have reductions in exercise capacity that are proportional to their degree of blood pressure elevation. In addition to investigating the general relationship between blood pressure and exercise capacity, I also aim to compare differing measures of blood pressure (clinic, ambulatory and aortic, systolic and diastolic) to better understand which measures of blood pressure may best correlate to exercise capacity. Because differing measures of blood pressure have distinct relationships to cardiovascular function and risk (50,341,378-380), the analyses of Study 4 should produce novel insights into how baseline blood pressure characteristics in generally healthy young adults may relate to decreased exercise capacity, and correspondingly, cardiovascular risk.

## 7.3 Methods

Study 4 uses participants and data exclusively from the YACHT study. Methods are described in detail in Chapter 3 with a summary overview of study measures provided in **Figure 3.1.** The central investigations of this chapter focus on a combination of the blood pressure assessments and cardiopulmonary exercise testing for exercise capacity measurement, with the relationships in these areas investigated extensively.

### 7.3.1 Study Population

The study population for this chapter includes the exercise testing subgroups from Study 3: Group 3, N=31, term-born young adults with elevated blood pressure, and Group 4, N=40, normotensive term-born young adult controls. The groups are described in detail in Chapter 2, with a summary of the groups provided in **Figure 2.3**, page 81.

### 7.3.2 Study Visit

The study visit for Study 4 included all the measurements described and collected within the YACHT study. The study visit is described in detail in Chapter 3 with an overview of study visit procedures presented in **Figure 3.1,** page 85.

### 7.3.3 Statistics

General statistical methods are described in Chapter 3 page 98. The YACHT study was designed around an estimated 6-10% between group difference in peak $VO_2$ and was conservatively powered at 80% with P = 0.05 to detect a 6% difference in percent of predicted peak $VO_2$ utilisation rates between 44 normotensive preterm-born and 44 normotensive term-born adults with 15 participants per sub-group needed to achieve 80% power and P = 0.05 to detect a 10% difference in percent of predicted peak $VO_2$ utilisation rates. Though the difference was not significant, the elevated BP group was older and more male so P values and regression models were adjusted for age and sex where appropriate.

## 7.4 Results

### 7.4.1 Group Characteristics

The average age in the elevated blood pressure group was 26.5±6.0 years vs 24.3±4.1 years in the control group though the difference was not statistically significant (P=0.068). BMI was higher in the elevated BP group than controls (25.3±3.7 vs 22.9±2.6 kg/$m^2$ respectively, P=0.009). Resting systolic and diastolic blood pressures were 133±11 and 78±10 mmHg respectively in the elevated blood pressure group vs 112±4 and 65±5 mmHg in normotensive controls (P<0.001). 23% of the elevated BP group were on antihypertensive medication vs 0% of the normal BP Group (P<0.001). Baseline spirometric assessment showed similar pulmonary function in the two groups. Forced vital capacity (FVC) averaged 104±11% of predicted capacity in the elevated blood pressure group vs 105±12% in controls (P=0.805) and there was also no difference in FEV1/FVC between groups. Group characteristics are provided in **Table 7.1.**

**Table 7.1. Group Characteristics**

| | Elevated Blood Pressure (N=31) | Normal Blood Pressure (N=40) | P value |
|---|---|---|---|
| **Sub Group Characteristics** | | | |
| Age | 26.5±6.0 | 24.3±4.1 | 0.068 |
| Male, n (%) | 19 (61) | 20 (50) | 0.350 |
| Height, (cm) | 175±10.3 | 174.0±10.3 | 0.858 |
| Weight, (kg) | 77.8±15.6 | 70.0±12.8 | 0.071 |
| BMI ($kg/m^2$) | 25.3±3.7 | 22.9±2.6 | **0.014** |
| **Blood Pressure** | | | |
| Brachial Systolic (mmHg) | 133±11 | 112±4 | **<0.001** |
| Brachial Diastolic (mmHg) | 78±10 | 65±5 | **<0.001** |
| Aortic Systolic (mmHg) | 115±16 | 95±6 | **<0.001** |
| Aortic Diastolic (mmHg) | 79±13 | 66±5 | **<0.001** |
| Antihypertensive Medication | 7 (23) | 0 (0) | **<0.001** |
| ACEi or ARB | 5 (16) | 0 (0) | **0.014** |
| Calcium Channel Blocker | 3 (10) | 0 (0) | 0.061 |
| Beta Blocker | 1 (3) | 0 (0) | 0.532 |
| Diuretic | 0 (0) | 0 (0) | - |
| Multi-Drug Therapy | 1 (3) | 0 (0) | 0.532 |
| **Biochemistry** | | | |
| Total Cholesterol mmol/L | 4.31±0.73 | 4.26±0.74 | 0.749 |
| HDL Cholesterol mmol/L | 1.35±0.31 | 1.47±0.26 | 0.193 |
| LDL Cholesterol mmol/L | 2.40±0.56 | 2.38±0.60 | 0.728 |
| Triglycerides mmol/L | 1.00±0.42 | 0.88±0.36 | 0.246 |
| High Sensitivity C-Reactive Protein | 1.15±1.35 | 1.16±1.8 | 0.945 |
| Glucose mmol/L | 4.92±0.29 | 4.86±0.53 | 0.650 |
| Insulin pmol/L | 44.6±28.8 | 34.5±33.4 | 0.135 |
| **Pulmonary Function** | | | |
| Percent of Predicted FVC (%) | 104±11 | 105±12 | 0.805 |
| FEV1/FVC Ratio | 0.83±0.08 | 0.81±0.09 | 0.106 |

Group Characteristics presented as mean±standard deviation. P values adjusted for age and sex. BMI, body mass index; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; HDL, high density lipoprotein; LDL, low density lipoprotein; FVC, forced vital capacity; FEV1, forced expiratory velocity in one second. P values < 0.05 presented in bold. Weight, BMI, Blood Pressure, Biochemistry, and Fev1/FVC adjusted for age and sex. P values below 0.05 depicted in bold.

### 7.4.2 Baseline Left Ventricular Structure and Function

#### 7.4.2.1 Resting Cardiac Magnetic Resonance Structural Imaging

General LV structure between the two groups was similar with no significant differences identified. There was no difference in LV end diastolic volume indexed to body surface area (83.8±15.3 vs 87.9±10.5 ml/$m^2$, P=0.383). Mean LV mass indexed to end diastolic volume was 0.66±0.15 g/ml in the elevated BP group vs 0.59±0.15 g/ml but this did not reach statistical significance (P=0.062). Cardiac magnetic resonance structural characteristics are presented in **Table 7.2**.

#### 7.4.2.2 Resting Echocardiography Left Ventricular Function

Baseline echocardiography showed no difference in resting ejection fraction between groups with the elevated blood pressure group averaging 63.2±5.8% vs 62.6±5.8% in normotensive controls (P=0.811). Doppler echocardiography showed that peak early and late diastolic transmitral flow velocity varied between groups with an E to A ratio of 1.49±0.31 in the elevated blood pressure group vs 1.85±0.45 in normotensive controls (P=0.001). Peak systolic longitudinal strain was -18.8±2.53% in those with higher blood pressure vs -20.8±2.98% in the control group (P=0.012). Peak systolic longitudinal strain rate differed between the elevated blood pressure and control groups (-0.97±0.43 vs -1.15±0.21 %/s, P=.033). Short axis myocardial deformation characteristics were similar, with no between group differences in peak systolic circumferential or radial strain and strain rate. Resting echocardiography P

values were adjusted for age and sex. Resting echocardiography data is presented in **Table 7.2**.

**Table 7.2. Baseline Structure and Function Characteristics**

| | Elevated Blood Pressure (N=31) | Normal Blood Pressure (N= 40) | P value |
|---|---|---|---|
| **LV Structure (CMR)** | | | |
| Diameter (cm) | 5.30±0.42 | 5.31±0.39 | 0.856 |
| LV EDV/BSA (ml/m2) | 83.8±15.3 | 87.9±10.5 | 0.283 |
| Mass (g) | 105.5±18.1 | 94.8±19.3 | 0.099 |
| LV Mass/BSA (g/m$^2$) | 54.0±8.0 | 52.1±9.8 | 0.585 |
| LV Mass/EDV (g/ml) | 0.66±0.15 | 0.59±0.11 | 0.062 |
| Septal Wall Thickness (mm) | 7.08±1.71 | 6.33±1.39 | 0.134 |
| Average Wall Thickness | 6.43±1.10 | 5.82±1.03 | 0.098 |
| Relative Wall Thickness | 0.225±0.057 | 0.221±0.056 | 0.787 |
| **LV Function (Echocardiography)** | | | |
| Heart Rate (beats/min) | 65±12 | 60±10 | **0.037** |
| Ejection Fraction (%) | 63.2±5.8 | 62.6±5.2 | 0.811 |
| Diastolic Function | | | |
| E/A Ratio | 1.49±0.31 | 1.85±0.45 | **0.001** |
| E (cm/sec) | 80.5±14.6 | 87.8±15.7 | 0.088 |
| e' (cm/sec) | 12.7±2.4 | 15.2±2.6 | **0.002** |
| E/e' | 6.48±1.85 | 6.11±2.27 | 0.825 |
| Left Atrial Dimension (cm) | 3.05±0.37 | 3.04±0.36 | 0.751 |
| Peak Systolic Strain | | | |
| Longitudinal Strain (%) | -18.8±2.53 | -20.8±2.98 | **0.012** |
| Systolic Strain Rate (%/s) | -0.97±0.43 | -1.15±0.21 | **0.033** |
| Circumfurential Strain (%) | -26.5±4.2 | -27.1±3.79 | 0.423 |
| Systolic Strain Rate (%/s) | -1.59±0.30 | -1.55±0.29 | 0.586 |
| Radial Strain (%) | 49.9±19.8 | 44.9±14.2 | 0.278 |
| Systolic Strain Rate (%/s) | 2.47±0.85 | 2.24±0.711 | 0.183 |

Values presented as mean±SD. CMR; cardiac magnetic resonance, LV; Left Ventricular, EDV; end diastolic volume, BSA; body surface area, E; peak early diastolic transmitral flow velocity, A; peak late transmitral flow velocity; e', average peak early myocardial velocity. P values < 0.05 presented in bold. P values adjusted for age and sex.

### 7.4.3 Exercise Characteristics

#### 7.4.3.1 Reduced Aerobic Exercise Capacity in Young Adults with Elevated Blood Pressure

In peak upright cycle ergometry cardiopulmonary exercise testing, the peak respiratory exchange ratio (RER) of the elevated blood pressure group was 1.19±0.07 with identical values (1.19±0.06) in the normotensive controls, P=0.888. Aerobic exercise capacity differed significantly between groups however. Young adults with blood pressure elevation nominally achieved 98±23% of their predicted peak $VO_2$ utilisation rate vs 111±23% in controls, P=0.022. Peak $VO_2$ utilisation rate measured in ml/kg/min was lower in the elevated blood pressure group vs controls as well with 35.4±10.7 vs 41.0±8.8 ml/kg/min in subjects with elevated blood pressure vs controls respectively, P=0.004. Heart rate through all phases of active exercise was similar between groups as was ventilatory reserve at peak ventilation. Objective measures of physical activity from wrist-worn accelerometers showed no between group difference in physical activity levels. Exercise characteristics are presented in **Table 7.3**.

**Table 7.3. Exercise Characteristics**

| | Elevated Blood Pressure (N=31) | Normal Blood Pressure (N=40) | P value |
|---|---|---|---|
| **Exercise Performance** | | | |
| % of Predicted Peak $VO_2$ (%) | 98±23 | 112±23 | **0.022** |
| Peak $VO_2$ (ml/kg/min) | 35.4±10.7 | 41.0±8.8 | **0.004** |
| Peak Respiratory Exchange Ratio | 1.19±0.07 | 1.19±0.06 | 0.984 |
| Heart Rate at VAT | 122±20 | 124±35 | 0.776 |
| Heart Rate at 40% Load | 127±13 | 130±12 | 0.689 |
| Heart Rate at 60% Load | 144±14 | 152±14 | 0.224 |
| Heart Rate at 80% Load | 167±14 | 164±12 | 0.462 |
| Heart Rate at Peak $VO_2$ | 184±14 | 184±12 | 0.124 |
| Peak Heart Rate Achieved | 185±13 | 186±11 | 0.567 |
| Heart Rate Decay (BPM/min) | -19.6±8.2 | -21.9±5.8 | 0.147 |
| $VE/VCO_2$ Slope | 26.8±3.2 | 25.6±3.6 | 0.110 |
| Peak Exercise Systolic Pressure | 189±29 | 167±20 | **0.001** |
| Peak Exercise Diastolic Pressure | 95±14 | 79±11 | **0.001** |
| Change in Systolic Pressure | 45±22 | 41±21 | 0.626 |
| Peak Pulse Pressure | 94±26 | 88±22 | 0.405 |
| Ventilatory Reserve at Peak (% of MVV) | 20.7±15.4 | 20.3±19.7 | 0.615 |
| **Objective Measures of Physical Activity** | | | |
| VPA (hrs/wk) | 1.1±0.9 | 1.3±1.2 | 0.353 |
| MVPA (hrs/wk) | 14.3±5.0 | 15.2±6.3 | 0.473 |
| **Rate of Perceived Exertion** | | | |
| At VAT | 11.2±2.15 | 11.7±2.30 | 0.370 |
| At 40% Load | 10.7±1.86 | 9.60±1.90 | **0.039** |
| At 60% Load | 13.3±1.63 | 13.0±1.34 | 0.357 |
| At 80% Load | 15.9±1.58 | 15.9±1.51 | 0.856 |
| At Peak $VO_2$ | 17.8±1.70 | 18.1±1.79 | 0.561 |

Values presented as mean±SD. VAT, ventilatory aerobic threshold; $VE/VCO_2$, ratio of minute ventilation to $CO_2$ production; MVV, maximum ventilatory volume; VPA, vigorous physical activity; MVPA, moderate to vigorous physical activity; VAT, ventilatory anaerobic threshold. P values adjusted for age and sex except for % of Predicted Peak $VO_2$. P values below 0.05 depicted in bold.

#### 7.4.3.2 Aortic Diastolic Blood Pressure is a Highly Significant Predictor of Exercise Capacity in Young Adults of Various Blood Pressures

After completing between group comparisons of exercise capacity, I then performed a combination of stepwise and forced entry multivariable linear regression model analyses to assess the relationships between various blood pressure measures and aerobic exercise capacity across the entire study group. First, accepting limitations due to collinearity of blood pressure measures, I completed an age and sex adjusted stepwise linear regression model (F to enter 0.05, to remove 0.10) with percent of predicted $VO_2$ utilisation rate as the dependent variable and the following eight blood pressure measurements all included as independent variables:

- Brachial systolic and diastolic BP
- Aortic (central) systolic and diastolic BP
- 24-hour awake ambulatory systolic and diastolic BP
- Asleep systolic and diastolic BP

Stepwise regression modeling included only aortic diastolic pressure and excluded all other blood pressure values in the prediction model for percent of predicted peak $VO_2$ utilisation rate ($\beta$=-0.527, P<0.001). I then performed a series of seven separate age and sex corrected forced entry multivariable linear regression to directly compare the relative predictive effect of aortic diastolic blood pressure vs each of the seven other blood pressure variables listed above. Despite collinearity, aortic diastolic pressure was a significant predictor of exercise capacity in six out of seven models, with very high significance ($P \leq 0.001$) in five models. The single model in which aortic diastolic BP was not

significant was model 2, when it was forced in as an independent variable along with brachial diastolic blood pressure. In this instance, despite high collinearity, the p value for aortic diastolic approached, but did not achieve significance (P=0.058). In all seven models, every other measure of blood pressure was highly non-significant (P=0.324 to P=0.926) when forced into the model with aortic diastolic pressure. The results of the comparison models are presented in **Table 7.4**, a plot of the relationship between aortic diastolic blood pressure and exercise capacity is presented in **Figure 7.1**.

**Table 7.4. Multivariable Regression Model Results Comparing Alternate BP Measures vs Aortic Diastolic BP in Predicting Exercise Capacity**

| Model | Alternate BP Measure Independent Variable | Alternate BP Measure β | Alternate BP Measure P Value | Aortic Diastolic BP β | Aortic Diastolic BP P value |
|---|---|---|---|---|---|
| | Resting Brachial (mmHg) | | | | |
| 1 | Systolic | 0.171 | 0.324 | -0.659 | **<0.001** |
| 2 | Diastolic | -0.025 | 0.926 | -0.506 | 0.058 |
| | 24-hr Awake (mmHg) | | | | |
| 3 | Systolic | 0.104 | 0.432 | -0.575 | **<0.001** |
| 4 | Diastolic | -0.042 | 0.780 | -0.503 | **0.001** |
| | Asleep Average (mmHg) | | | | |
| 5 | Systolic | 0.196 | 0.151 | -0.564 | **<0.001** |
| 6 | Diastolic | 0.006 | 0.964 | -0.530 | **<0.001** |
| | Aortic (mmHg) | | | | |
| 7 | Systolic | 0.05 | 0.826 | -0.570 | **0.016** |

In seven separate age and sex adjusted multivariable regression models, each including forced entry of aortic diastolic BP and an alternate BP measure, aortic diastolic BP remained a significant predictor of exercise capacity (% of predicted VO2 utilisation rate) in six out of seven models. No other BP measure was a significant predictor of exercise capacity when included in the regression model along with aortic diastolic BP. BP; blood pressure. Bold denotes P value < 0.05.

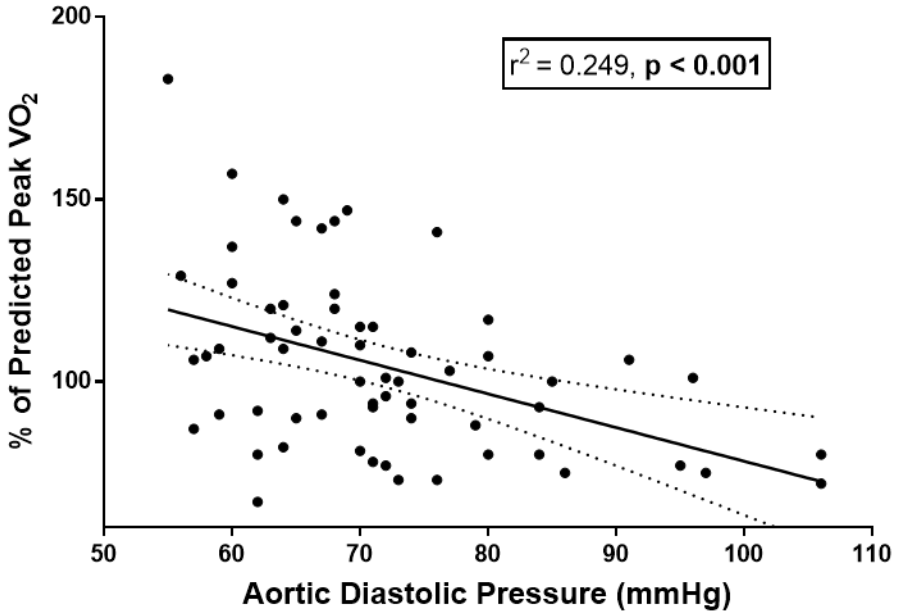



**Figure 7.1. Decreasing Exercise Capacity with Increasing Aortic Diastolic Pressure in Young Adults.**

Multivariable regression modeling of awake ambulatory, resting clinic, and aortic blood pressure measures revealed that aortic diastolic pressure was the strongest predictor of exercise capacity. Age and sex corrected regression analysis of aortic diastolic blood pressure and % of predicted peak $VO_2$ utilisation rate achieved indicated that aortic diastolic blood pressure was a strong correlate of exercise capacity in young adults of various blood pressures (age and sex adjusted $\beta$=-0.527, $r^2$=0.249, P<0.001).

#### 7.4.3.3 Comparing Aortic Diastolic Pressure to Other Correlates of Exercise Capacity

A series of separate, age and sex adjusted multivariable linear regression models with percent of predicted $VO_2$ utilisation rate as the dependent variable were completed for each of the following common correlates of exercise capacity:

- resting heart rate

- resting LV end diastolic volume indexed to body surface area (EDV/BSA)
- resting LV stroke volume indexed to body surface area
- hours per week of vigorous physical activity (VPA)

Resting heart rate was not a significant predictor of exercise capacity (P=0.123). LV EDV/BSA, resting stroke volume index, and VPA were all significantly correlated with exercise capacity (β=0.629, β=0.577 and β=0.366; P<0.001, P<0.001 and P=0.003 respectively).

An additional series of similar multivariable linear regression model analyses were performed to assess relationships between exercise capacity and the following selected study specific measures which were significantly different between blood pressure groups in Studies 3 and/or 4:

- Peak Systolic Longitudinal Strain
- E to A ratio
- LV mass indexed to end diastolic volume
- body mass index (BMI)
- EF (ejection fraction) at 40% workload

Peak systolic longitudinal strain, LV mass indexed to EDV, BMI and EF at 40% workload were not predictive of exercise capacity (P=0.236, P=0.120, P=0.365 and P=0.464 respectively). E to A ratio was a significant predictor of exercise capacity (β=0.256, P=0.046).

A final age and sex adjusted multivariable regression model with antihypertensive medication as the independent variable was run within the elevated BP group only. This model showed no significant relationship between antihypertensive medication and exercise capacity (P=0.123). Correlates of exercise capacity are presented in **Table 7.5**.

**Table 7.5. Multivariable Regression Models Assessing Correlates of Exercise Capacity**

| Model | Independent Variable | β | P Value |
|---|---|---|---|
| | **Common Correlates of Exercise Capacity** | | |
| 1 | Resting Heart Rate | -0.191 | 0.123 |
| 2 | Resting LV EDV/BSA | 0.629 | **<0.001** |
| 3 | Resting SVidx (ml/$m^2$) | 0.577 | **<0.001** |
| 4 | VPA (hrs/week) | 0.366 | **0.003** |
| | **Study Specific Measures** | | |
| 5 | Peak Systolic Longitudinal Strain (%) | -0.157 | 0.236 |
| 6 | E to A Ratio | 0.256 | **0.046** |
| 7 | LV mass/EDV (g/ml) | -0.228 | 0.120 |
| 8 | BMI (kg/$m^2$) | -0.119 | 0.365 |
| 9 | EF at 40% workload | -0.131 | 0.464 |
| 10 | Antihypertensive Medication | -0.366 | 0.123 |

Four common correlates of exercise capacity and six study specific measures were analysed in separate age and sex adjusted multivariable regression models to assess if they were predictive of exercise capacity (% of predicted peak $VO_2$ utilisation rate). Among common correlates of exercise capacity, LV EDV and SV indexed to BSA, as well as hours per week of vigorous physical activity significantly predicted exercise capacity (β=0.629, β=0.577 and β=0.366; P<0.001, P<0.001 and P=0.003 respectively). Among selected study specific measures, only E to A ratio significantly predicted exercise capacity (β 0.256, P=0.046). LV, left ventricle; EDV, end diastolic volume; BSA, body surface area; SV, stroke volume; idx, indexed; VPA, vigorous physical activity; E, peak early diastolic transmitral flow velocity; A, peak late diastolic transmitral flow velocity; BMI, body mass index; EF, ejection fraction. Bold denotes P value < 0.05.

## 7.5 Discussion

Study 4 showed that young adults had an inverse relationship between blood pressure and exercise capacity. Among blood pressure measures, aortic diastolic blood pressure was most strongly correlated with exercise capacity, approaching the predictive power of widely accepted strong correlates of exercise capacity including indexed LV end diastolic volume and stroke volume. E to A ratio, which was lower in the elevated BP group, was also a significant predictor of exercise capacity. Further longitudinal study is needed to more precisely reveal the relationships between blood pressure, exercise capacity and cardiovascular risk. However, the highly significant association between aortic diastolic blood pressure and exercise capacity suggests that those with substantially elevated diastolic blood pressure may be more likely to experience compounded risk factors from blood pressure elevation as well as reduced exercise capacity.

### 7.5.1 Possible Mechanisms Underlying Aortic Diastolic Pressure as a Highly SIgnificant Correlate of Exercise Capacity

#### 7.5.1.1 Aortic Blood Pressure Exceeds Brachial Measures in Predicting Cardiovascular Risk and Disease Progression

Commonly available devices can measure aortic blood pressure non-invasively with equivalent accuracy to typical clinical oscillometric measurements of brachial blood pressure (241,242,381,382). In several recent studies, aortic BP outperformed traditional brachial measurements for risk stratification and disease management (383-387), most likely because sensitive organs such as

the heart, brain and kidneys experience pressure much more closely approximating aortic rather than peripheral pressure (385). Furthermore, peripheral measurements are more confounded by normal vascular aging processes (379) and pharmaceutical interventions may differentially effect central and peripheral BP (388). This may compromise clinical interventions, as studies indicated with atenolol, where outcomes were inferior despite substantial brachial pressure reductions (385,389,390). Not surprisingly, aortic BP measurements have repeatedly been shown to relate more closely to signs of acute and also sub-clinical target organ damage than peripheral measures (380,391). Similarly, in Study 4, aortic BP, specifically diastolic pressure, decisively outperformed other BP measures in predicting exercise capacity.

#### 7.5.1.2 Diastolic Blood Pressure may Better Relate to a Range of Cardiovascular Risk and Performance Indicators

##### *7.5.1.2.1 Cardiovascular Risk Indicators that Relate to Diastolic Blood Pressure*

Systolic BP elevation is accepted as a greater overall independent risk factor than diastolic BP elevation (341,392,393). While this is well established, study data also indicates that some cardiovascular parameters and risk factors including diastolic function, systemic vascular resistance, and arterial stiffness more closely associate with aortic diastolic than other BP measures (385,394,395). In a study of healthy young adult male subjects, Nürnberger et al concluded that diastolic pressure was the strongest determinant of augmentation index (AIx) and pulse wave velocity (PWV) and the only determinant that remained significant in multiple regression analyses (50).

Further, a 2014 systematic review of 1627 exercise intervention subjects found that aerobic exercise reduced AIx and PWV (396). These data and the findings of Study 4 suggest that aortic diastolic pressure may relate to broad but specific aspects of cardiac and vascular phenotypes, some of which are mediated by chronic aerobic exercise (395,396). Finally, although reliable, aortic systolic measurements are subject to more confounding effects including height and gender (395), which may further contribute to aortic diastolic pressure being the blood pressure measure that most strongly correlated to exercise capacity in Study 4.

#### *7.5.1.2.2 Parameters which may Physiologically Impact Exercise Capacity*

SVR and AIx, two parameters related to aortic diastolic pressure (50,379,385,395,397) may have direct physiological implications on exercise capacity. While these two parameters are related and mathematically associated with diastolic pressure (241,385,396), they are mechanistically distinct and may uniquely relate to exercise capacity. AIx relates to the level of systolic pulse wave reflection, which occurs increasingly with greater PWV (due to arterial stiffness) allowing rapid propagation of the pulse wave down the arterial tree which then reflects off of the high resistance arteriolar bed and back toward the LV. While this effect is usually small in healthy young adults, AIx increases with higher blood pressure and vascular dysfunction. As AIx increases, the LV must pump against a greater reflected portion of it's own mechanical energy. This effect subsequently increases pulse pressure and decreases LV pumping efficiency (385,398). Such a loss of efficiency would be expected to reduce maximal exercise capacity. Additionally, increased SVR, which presents a greater preload to the LV also contributes to higher AIx due to higher resistance

in the arteriolar beds. However, greater SVR is also associated with a tissue level perfusion challenge, which in skeletal muscle may be mediated by variance in microvascular density and peripheral vasoconstriction (52,399,400). I hypothesise that collectively, these described mechanisms may in-part explain why aortic diastolic blood pressure so strongly related to exercise capacity in Study 4.

### 7.5.2 Clinical Implications

My working hypothesis explaining the findings of Study 4 links elevated diastolic pressure (particularly central/aortic) with multiple physiological mechanisms and states across the cardiovascular system which may combine to limit exercise capacity. These same states, namely increased arterial stiffness which indicates deterioration of the large conduit arteries, combined with increased systemic vascular resistance which indicates peripheral vascular defects (vasoconstriction and/or rarefaction) are also associated with increased risk. While further longitudinal data is needed, the findings of Study 4 further support the general advantages of aortic BP measurements over brachial measures. Additionally, young adults with elevated blood pressure may have compounding risk associated with reduced exercise capacity, particularly if aortic diastolic pressure is elevated.

### 7.5.3 Study 4 Limitations

Study 4 generally shares the limitations that were described for the exercise stress-testing sub-group in Study 3. Namely, the study had a modest sample size with the elevated BP group slightly older with more males so P

values were adjusted where appropriate, the purposive recruitment and the single centre study design make it unlikely that the study population reflects the larger general population, and finally, the cross-sectional study design limits inferences to causality.

## 7.6 Conclusions

In young adults, blood pressure level is inversely associated with aerobic exercise capacity. Amongst all common measures of blood pressure, aortic diastolic pressure was most strongly related to exercise capacity. Adverse physiological states such as increased peripheral resistance and augmentation index are generally associated with elevated aortic diastolic BP. Accordingly, young adults with elevated aortic diastolic BP may be more likely to experience compound risk factors. However, how exercise capacity in young adults relates to long-term risk, and whether elevated aortic diastolic blood pressure may be indicative of compounding risk factors in young adults requires further study.

# Chapter 8 CONCLUSIONS

In conclusion, this thesis aimed to provide extensive and novel insights into cardiovascular function and risk in two young adult populations with elevated risk for common cardiovascular pathologies including heart failure and stroke. Young adults born preterm and young adults with blood pressure elevation share some common cardiovascular phenotypic traits and were studied in detail to better understand each phenotype and their unique structure, function and stress response profiles.

My thesis focused on the left ventricle and employed deep cardiovascular phenotyping of left ventricular structure and function, circulating metabolic and cardiac stress biomarkers, and physiological and anthropometric measurements at baseline. Most importantly, I integrated cardiopulmonary exercise stress assessments employing a combination of echocardiographic, cardiopulmonary physiological, exercise performance, and circulating small molecule measurements. By analyzing and comparing the baseline states along with the developed exercise stress response profiles, I identified several key relationships and mechanisms which have substantially advanced our understanding of the consequences of prematurity and blood pressure elevation on left ventricular structure, function and stress response.

The first thesis objective was to gain an understanding of the impacts of the preterm-born left ventricular structure and function phenotype on the exercise stress response. In Chapter 4, I described the results of this investigation, which extensively employed echocardiographic imaging at the resting baseline and also during prescribed exercise intensities. The central finding was that preterm-born young adults have a compromised left ventricular systolic response to

exercise stress which is characterized by smaller gains in ejection fraction, which further decline with increasing exercise intensity. Submaximal cardiac output reserve was also lower in the preterm-born group. Additionally, the degree of systolic impairment significantly related to left ventricular length which is shortened in the preterm-born cardiac phenotype. The findings reported in this chapter provide evidence of reduced myocardial functional reserve in those born preterm which may in part explain their increased risk for early heart failure.

In Chapter 5, I reported on the consequences of the preterm-born cardiac structure and function phenotype on exercise capacity. The central methods were baseline cardiovascular phenotyping combined with cardiopulmonary exercise testing of aerobic capacity and cardiopulmonary function. In this chapter, I reported significantly lower exercise capacity in preterm-born young adults vs term-born controls. Of primary interest, I found that the impaired left ventricular systolic response to exercise (which I reported in Chapter 4) was highly predictive of exercise capacity reductions in preterm-born young adults. Further, pulmonary function was similar between groups and did not explain variation in exercise capacity. These findings indicate that the functional alterations in the preterm-born cardiac stress response phenotype most likely underlie reduced exercise capacity in most of the preterm-born population.

The last two results chapters switched investigative focus from prematurity to young adult blood pressure elevation. In Chapter 6, I reported on the relationships between modest blood pressure elevation and left ventricular structure and physiological exercise stress response. Because exercise prescription is rarely successful in young adults with elevated blood pressure, in

addition to cardiac magnetic resonance and physiological measurements, I also investigated perceptions related to physical activity and the exercise response. I found that young adults with modest blood pressure elevation have subclinical left ventricular mass increase, but notably, they have a physiologically significant systolic impairment in their sub-maximal exercise response. Interestingly, in young adults with elevated blood pressure, this alteration is associated with increased perceived exertion which strongly relates to their lower overall enjoyment of physical activity. The findings in this chapter suggest that risk may be already accumulating in otherwise healthy young adults with elevated blood pressure. And finally, the relationships between blood pressure elevation and adverse physiological and experiential response to exercise may confound exercise prescription, though this will require further study.

In Chapter 7, I reported my final investigation, where I tested my hypothesis that young adults with elevated blood pressure have reductions in aerobic exercise capacity proportional to their blood pressure elevation. I found that young adults with elevated blood pressure had lower aerobic capacity than normotensive controls. I also identified that among blood pressure measures, aortic diastolic pressure was uniquely robust in predicting exercise capacity, and speculated that this may be due to both the inherent precision of the measurement and also the range of central and peripheral cardiovascular parameters that it relates to. In light of these findings, young adults with elevated diastolic pressure could be more likely to suffer compounding risk associated with blood pressure elevation as well as reduced exercise capacity.

Overall, my thesis has advanced the collective understanding of the impacts of prematurity and blood pressure elevation on left ventricular structure, function and stress response. Though these groups have some similar cardiovascular traits and risk factors, I have been able to distinguish key elements that have specific clinical implications.

First, in those born preterm, my thesis findings indicate this population has an impaired systolic response to exercise stress. This appears to be a central functional alteration that contributes to reduced myocardial reserve. Although my investigations were cross-sectional, I suspect that this is an inherent characteristic of the phenotype. As such, this may exacerbate the risk, severity or progression of future pathologies such as heart failure in those born preterm. While further study is needed, earlier intervention may be needed in this population due the consequences of reduced myocardial reserve.

Second, in young adults with blood pressure elevation, though they share some common risk factors with their preterm-born peers, my thesis investigations suggest distinctly different disease etiologies as well as treatment and prevention challenges that may be relevant to this population. My findings of sub-clinical remodelling and impaired stress response supports previous studies indicating that risk has already begun to accumulate in this population. This risk and dysfunction however, is understood to stem from time of exposure to blood pressure elevation, rather than being an inherent phenotypic trait. Accordingly, early premordial intervention in this population may be able to permanently re-direct risk trajectories if lifecourse blood pressure management could be sustained. However, the newly identified adverse perceptions of physical

exercise in this population may require novel clinical strategies for such intervention to succeed. Longitudinal trials of exercise, drug, and crossover intervention strategies should be conducted to guide development of more effective approaches to managing risk in this population.

## 8.1 Future Questions

My doctoral research has helped produce a more complete understanding of cardiovascular form, function, and exercise stress response in young adults with a history of preterm birth and young adults with blood pressure elevation. However, the three following questions warrant specific future consideration and investigation.

1) **How do the dysfunctional left ventricular stress response profiles identified in this thesis relate to long term risk in these populations?**

In my study groups, I identified specific defects in the exercise stress response including impaired LV systolic response to exercise and lower aerobic capacity. While these are known risk factors for increased morbidity and mortality in older and diseased populations, very little is known about how these alterations relate to longitudinal risk in generally healthy young adults. Because my thesis analyses and results are based on cross-sectional data, longitudinal extrapolation of risk progression or functional declines could not be inferred.

Longitudinal data is challenging to collect, but allows for uniquely robust advances in our ability to understand and manage long-term risk. Ideally, large cohort studies with imagery based cardiac stress assessments would be

employed. With this approach, alterations such as impaired systolic response to exercise could be tracked over time to see if it indeed leads to inferior outcomes for future cardiovascular disease. In the preterm-born population, I would be primarily interested to track the change in ejection fraction in response to exercise to see if it predicted increased heart failure risk. In the young adults with elevated blood pressure, I would like to study the impaired systolic response to submaximal exercise to assess its prognostic value for adverse myocardial remodelling associated with hypertension such as left ventricular hypertrophy.

2) **Is exercise capacity an important predictor of future risk in the preterm-born population?**

Similar to my first question, reduced exercise capacity is an independent risk factor in older and diseased populations, but this has not been studied longitudinally in the preterm-born population. Additionally, because of the younger age of these study groups, it would be interesting and beneficial to gain a better understanding of how exercise may predict future intermediate risk factors such as left ventricular hypertrophy, coronary artery disease, heart failure, and hypertension.

I am particularly interested in further study of exercise capacity in the preterm-born population because their LV exercise stress response has such a strong relationship to exercise capacity. As such, there is a great deal of clinically important discovery to be done in this population. One benefit of exercise testing studies is that basic exercise assessments are more easily scalable to large

studies, while more detailed stress and exercise testing could perhaps be completed in a subgroup.

3) **Which of these defective stress responses can be beneficially modified by intervention?**

This is a broad and relatively open question but an important one. In the case of preterm-born young adults, there was a substantial relationship between altered left ventricular structure and cardiac stress response. This functional remodelling (altered stress response) was a significant predictor of exercise capacity. If future, longitudinal studies identify that these alterations are relatively fixed in the preterm-born cardiac phenotype, very early intervention may be needed in higher-risk preterm-born individuals.

Alternatively, in the case of blood pressure elevation, I would like to study how crossover therapies starting with antihypertensive medication and then adding increased physical activity may be effective in improving the adverse physiological and perceived exercise stress response and also aid in long-term adherence to exercise prescription.

## 8.2 Future Work

I was fortunate to join the research team at the University of Oxford Cardiovascular Clinical Research Facility (CCRF) at a time when I could work extensively on the operations and analysis of the YACHT study, while also playing a central role in developing, initiating, managing and conducting the follow-on TEPHRA trial. The TEPHRA trial will form the initial basis of my future

work. As indicated on **Figure 2.1,** the EVS and YACHT studies are part of an integrated protocol structure which has progressed into the TEPHRA trial.

TEPHRA maintains the standard core of measures including an exercise testing component, but adds a randomized control trial element with a 16 week supervised aerobic exercise intervention. The study will compare a supervised physical activity intervention consisting of 16 weeks structured exercise, physical activity self-monitoring and motivational coaching with a control group receiving usual care/minimal intervention. 200 young adults aged 18-35 years, including a subgroup of preterm-born participants will be recruited through open recruitment and direct invitation. Participants will be randomised in a ratio of 1:1 to either the exercise intervention arm or control arm. Primary outcome will be ambulatory blood pressure monitoring at 16 weeks with measure of sustained effect at 12 months. Study measures include multimodal cardiovascular assessments; peripheral vascular measures, blood sampling, microvascular assessment, baseline and exercise stress echocardiography, objective physical activity monitoring and a subgroup will complete multi-organ magnetic resonance imaging. A summary of TEPHRA is provided below at **Figure 8.1**.

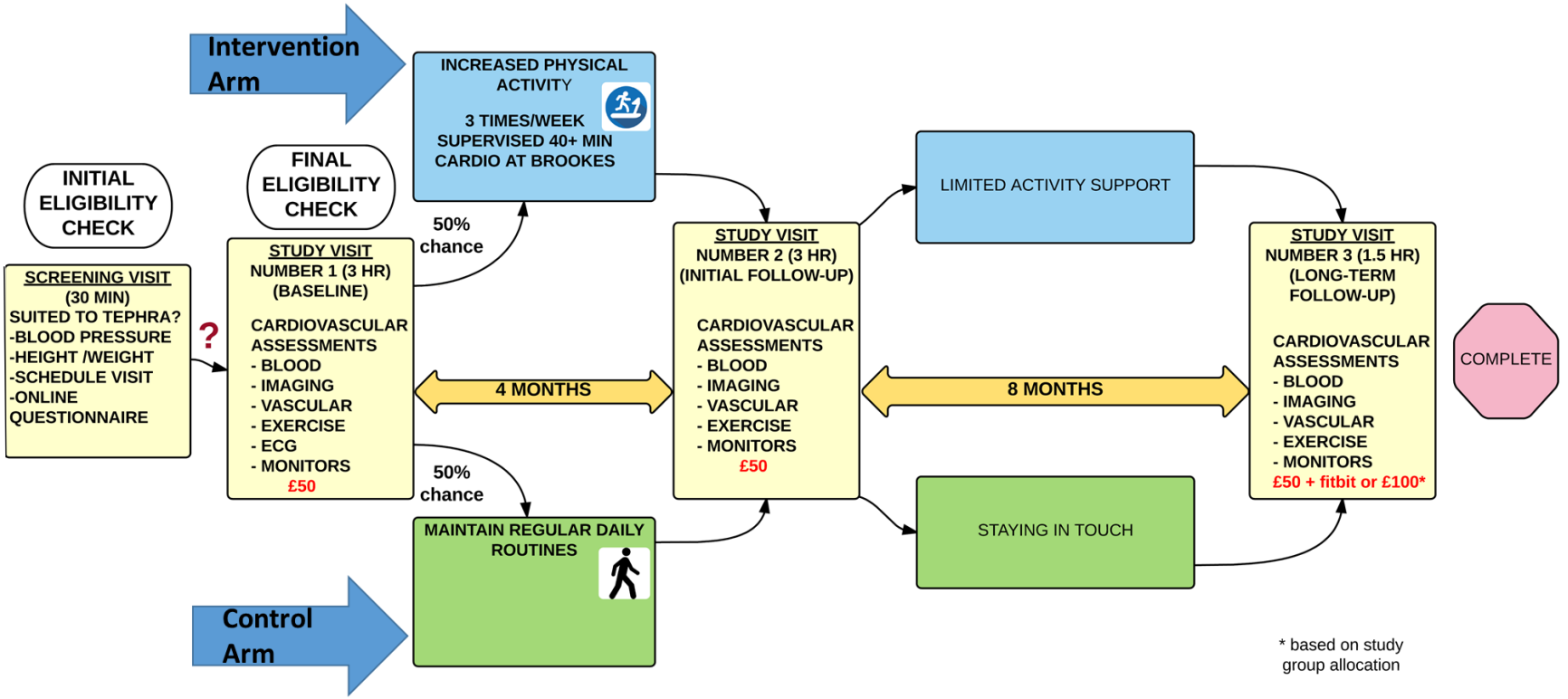



**Figure 8.1. Trial of Exercise to Prevent HypeRtension in Young Adults (TEPHRA) Overview**

The TEPHRA trial continues targeted investigation of young adults with the risk factors of blood pressure elevation and a history of preterm birth. Results of this trial will deliver novel, randomised control trial data that reports the effect of physical activity intervention on blood pressure integrated with detailed cardiovascular phenotyping in young adults. The data provided by TEPHRA will support the development the evidence-base for management of blood pressure and the broader cardiovascular risk profile in young adult population. Additionally, data from TEPHRA will provide the opportunity to investigate the effects of exercise training regimens on the stress response profiles described in this thesis.

At the time of drafting this dissertation, over 190 participants have been recruited, completed their baseline assessments, and subsequently randomized into TEPHRA. With the data collected from TEPHRA, I will be able to investigate the effects of increased physical activity on the exercise stress response in those with elevated blood pressure and those born preterm. Analyses of these future data will further elucidate elements of the cardiovascular stress response that were presented in this thesis.

Finally, as member of the United States Air Force, and active duty faculty member in the Biology Department at the United States Air Force Academy, I would like to conduct research on some of the extreme endurance events that are held at The Academy. The aim would be to identify whether lifestyle, birth history, and cardiovascular phenotypic factors may predict alterations in immediate post-event cardiac function and stress profile. Secondarily, I would like to study how these same factors relate to cardiac function and stress profile 48 hours post-event. I aim to develop a protocol for implementation on the "Falcon 50" race scheduled for Spring 2020.

## REFERENCES


1. Cook C, Cole G, Asaria P, Jabbour R, Francis DP. The annual global economic burden of heart failure. Int J Cardiol 2014;171:368-76.
2. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nature reviews Cardiology 2016;13:368-78.
3. van Riet EE, Hoes AW, Wagenaar KP, Limburg A, Landman MA, Rutten FH. Epidemiology of heart failure: the prevalence of heart failure and ventricular dysfunction in older adults over time. A systematic review. Eur J Heart Fail 2016;18:242-52.
4. Sengupta PP, Krishnamoorthy VK, Korinek J et al. Left ventricular form and function revisited: applied translational science to cardiovascular ultrasound imaging. J Am Soc Echocardiogr 2007;20:539-51.
5. Fernandez-Teran MA, Hurle JM. Myocardial fiber architecture of the human heart ventricles. The Anatomical record 1982;204:137-47.
6. Elzinga G, Toorop GP, Gross DR, Westerhof N. Geometry and pump function in cardiac ventricular hypertrophy. Am J Cardiol 1990;65:23g-29g.
7. Lewandowski AJ, Augustine D, Lamata P et al. Preterm heart in adult life: cardiovascular magnetic resonance reveals distinct differences in left ventricular mass, geometry, and function. Circulation 2013;127:197-206.
8. Cuspidi C, Meani S, Valerio C et al. Age and target organ damage in essential hypertension: role of the metabolic syndrome. American journal of hypertension 2007;20:296-303.
9. Materson BJ, Garcia-Estrada M, Degraff SB, Preston RA. Prehypertension is real and can be associated with target organ damage. Journal of the American Society of Hypertension : JASH 2017;11:704-708.
10. Strait JB, Lakatta EG. Aging-associated cardiovascular changes and their relationship to heart failure. Heart failure clinics 2012;8:143-164.
11. Kovacs G, Berghold A, Scheidl S, Olschewski H. Pulmonary arterial pressure during rest and exercise in healthy subjects: a systematic review. Eur Respir J 2009;34:888-94.
12. Lang RM, Badano LP, Mor-Avi V et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015;16:233-70.
13. Ho SY, Nihoyannopoulos P. Anatomy, echocardiography, and normal right ventricular dimensions. Heart 2006;92:i2-i13.
14. Algranati D, Kassab GS, Lanir Y. Why is the subendocardium more vulnerable to ischemia? A new paradigm. Am J Physiol Heart Circ Physiol 2011;300:H1090-100.
15. Hoffman JI, Baer RW, Hanley FL, Messina LM. Regulation of transmural myocardial blood flow. J Biomech Eng 1985;107:2-9.
16. Toyota E, Ogasawara Y, Hiramatsu O et al. Dynamics of flow velocities in endocardial and epicardial coronary arterioles. Am J Physiol Heart Circ Physiol 2005;288:H1598-603.
17. Greenbaum RA, Ho SY, Gibson DG, Becker AE, Anderson RH. Left ventricular fibre architecture in man. Br Heart J 1981;45:248-63.

18. Ingels NB, Jr., Hansen DE, Daughters GT, 2nd, Stinson EB, Alderman EL, Miller DC. Relation between longitudinal, circumferential, and oblique shortening and torsional deformation in the left ventricle of the transplanted human heart. Circ Res 1989;64:915-27.
19. Streeter DD, Jr., Spotnitz HM, Patel DP, Ross J, Jr., Sonnenblick EH. Fiber orientation in the canine left ventricle during diastole and systole. Circ Res 1969;24:339-47.
20. Buckberg GD, Fixler DE, Archie JP, Hoffman JI. Experimental subendocardial ischemia in dogs with normal coronary arteries. Circ Res 1972;30:67-81.
21. Hui L, Pemberton J, Hickey E et al. The contribution of left ventricular muscle bands to left ventricular rotation: assessment by a 2-dimensional speckle tracking method. J Am Soc Echocardiogr 2007;20:486-91.
22. Levy PT, Machefsky A, Sanchez AA et al. Reference Ranges of Left Ventricular Strain Measures by Two-Dimensional Speckle-Tracking Echocardiography in Children: A Systematic Review and Meta-Analysis. J Am Soc Echocardiogr 2016;29:209-225.e6.
23. Ellims AH, Shaw JA, Stub D et al. Diffuse myocardial fibrosis evaluated by post-contrast t1 mapping correlates with left ventricular stiffness. J Am Coll Cardiol 2014;63:1112-8.
24. Liu CY, Lai S, Kawel-Boehm N et al. Healthy aging of the left ventricle in relationship to cardiovascular risk factors: The Multi-Ethnic Study of Atherosclerosis (MESA). PloS one 2017;12:e0179947.
25. Maragiannis D, Alvarez PA, Ghosn MG et al. Left ventricular function in patients with hypertrophic cardiomyopathy and its relation to myocardial fibrosis and exercise tolerance. Int J Cardiovasc Imaging 2018;34:121-129.
26. Xiao Y, Liu Y, Liu J, Kang YJ. The Association Between Myocardial Fibrosis and Depressed Capillary Density in Rat Model of Left Ventricular Hypertrophy. Cardiovasc Toxicol 2018;18:304-311.
27. Maanja M, Wieslander B, Schlegel TT et al. Diffuse Myocardial Fibrosis Reduces Electrocardiographic Voltage Measures of Left Ventricular Hypertrophy Independent of Left Ventricular Mass. J Am Heart Assoc 2017;6.
28. Vergaro G, Prud'homme M, Fazal L et al. Inhibition of Galectin-3 Pathway Prevents Isoproterenol-Induced Left Ventricular Dysfunction and Fibrosis in Mice. Hypertension 2016;67:606-12.
29. Puntmann VO, Arroyo Ucar E, Hinojar Baydes R et al. Aortic stiffness and interstitial myocardial fibrosis by native T1 are independently associated with left ventricular remodeling in patients with dilated cardiomyopathy. Hypertension 2014;64:762-8.
30. Eroglu E. Left atrial fibrosis affects left ventricular systolic function in patients with atrial fibrillation. Turk Kardiyol Dern Ars 2014;42:20-1.
31. Shantsila E, Shantsila A, Blann AD, Lip GY. Left ventricular fibrosis in atrial fibrillation. Am J Cardiol 2013;111:996-1001.
32. Bertagnolli M, Dios A, Beland-Bonenfant S et al. Activation of the Cardiac Renin-Angiotensin System in High Oxygen-Exposed Newborn Rats: Angiotensin Receptor Blockade Prevents the Developmental Programming of Cardiac Dysfunction. Hypertension 2016;67:774-82.
33. Bertagnolli M, Huyard F, Cloutier A et al. Transient neonatal high oxygen exposure leads to early adult cardiac dysfunction, remodeling, and activation of the renin-angiotensin system. Hypertension 2014;63:143-50.

34. Mizuno T, Mickle DA, Kiani CG, Li RK. Overexpression of elastin fragments in infarcted myocardium attenuates scar expansion and heart dysfunction. Am J Physiol Heart Circ Physiol 2005;288:H2819-27.
35. Kovacs A, Papp Z, Nagy L. Causes and pathophysiology of heart failure with preserved ejection fraction. Heart Fail Clin 2014;10:389-98.
36. Meijs MF, Doevendans PA, Cramer MJ et al. Relation of common carotid intima-media thickness with left ventricular mass caused by shared risk factors for hypertrophy. J Am Soc Echocardiogr 2009;22:499-504.
37. Ibrahim el SH, Miller AB, White RD. The relationship between aortic stiffness and E/A filling ratio and myocardial strain in the context of left ventricular diastolic dysfunction in heart failure with normal ejection fraction: insights from magnetic resonance imaging. Magn Reson Imaging 2011;29:1222-34.
38. Baulmann J, Homsi R, Un S, Vetter H, Dusing R, Mengden T. [Arterial stiffness in arterial hypertension. A new risk factor for left ventricular hypertrophy and cardiac insufficiency?]. Dtsch Med Wochenschr 2004;129:447-52.
39. Moczulska B, Kubiak M, Bryczkowska A, Malinowska E. [Pulse wave velocity as an early marker of diastolic heart failure in patients with hypertension]. Pol Merkur Lekarski 2017;42:142-144.
40. Podgorski M, Grzelak P, Kaczmarska M, Polguj M, Lukaszewski M, Stefanczyk L. Feasibility of two-dimensional speckle tracking in evaluation of arterial stiffness: Comparison with pulse wave velocity and conventional sonographic markers of atherosclerosis. Vascular 2018;26:63-69.
41. Levy D, Anderson KM, Savage DD, Kannel WB, Christiansen JC, Castelli WP. Echocardiographically detected left ventricular hypertrophy: prevalence and risk factors. The Framingham Heart Study. Annals of internal medicine 1988;108:7-13.
42. Chantler PD, Lakatta EG, Najjar SS. Arterial-ventricular coupling: mechanistic insights into cardiovascular performance at rest and during exercise. Journal of applied physiology (Bethesda, Md : 1985) 2008;105:1342-51.
43. Dekleva M, Lazic JS, Soldatovic I et al. Improvement of Ventricular-Arterial Coupling in Elderly Patients with Heart Failure After Beta Blocker Therapy: Results from the CIBIS-ELD Trial. Cardiovasc Drugs Ther 2015;29:287-94.
44. Matsumoto K, Tanaka H, Ooka J et al. Significant prognostic impact of improvement in ventriculo-arterial coupling induced by dobutamine stress on cardiovascular outcome for patients with dilated cardiomyopathy. Eur Heart J Cardiovasc Imaging 2016;17:1296-1304.
45. Sunagawa K, Maughan WL, Sagawa K. Optimal arterial resistance for the maximal stroke work studied in isolated canine left ventricle. Circ Res 1985;56:586-95.
46. di Salvo G, Pacileo G, Limongelli G et al. Abnormal regional myocardial deformation properties and increased aortic stiffness in normotensive patients with aortic coarctation despite successful correction: an ABPM, standard echocardiography and strain rate imaging study. Clin Sci (Lond) 2007;113:259-66.
47. Kilicgedik A, S CE, Gurbuz AS et al. Left Atrial Mechanical Function and Aortic Stiffness in Middle-aged Patients with the First Episode of Atrial Fibrillation. Chin Med J (Engl) 2017;130:143-148.

48. Santilli SM, Fiegel VD, Knighton DR. Changes in the aortic wall oxygen tensions of hypertensive rabbits. Hypertension and aortic wall oxygen. Hypertension 1992;19:33-9.
49. Han WQ, Wu LY, Zhou HY et al. Changes in the composition of the thoracic aortic wall in spontaneously hypertensive rats treated with losartan or spironolactone. Clin Exp Pharmacol Physiol 2009;36:583-8.
50. Nürnberger J, Dammer S, Opazo Saez A, Philipp T, Schäfers RF. Diastolic blood pressure is an important determinant of augmentation index and pulse wave velocity in young, healthy males. Journal of human hypertension 2003;17:153.
51. Westerhof N, Westerhof BE. A review of methods to determine the functional arterial parameters stiffness and resistance. Journal of hypertension 2013;31:1769-75.
52. Wilenius M, Tikkakoski AJ, Tahvanainen AM et al. Central wave reflection is associated with peripheral arterial resistance in addition to arterial stiffness in subjects without antihypertensive medication. BMC Cardiovasc Disord 2016;16.
53. Bevilacqua M, Savonitto S, Bosisio E et al. Role of the Frank-Starling mechanism in maintaining cardiac output during increasing levels of treadmill exercise in beta-blocked normal men. Am J Cardiol 1989;63:853-7.
54. Katz AM. Ernest Henry Starling, his predecessors, and the "Law of the Heart". Circulation 2002;106:2986-92.
55. Borlaug BA, Reddy YNV. Some Laws Were Not Made to Be Broken: When Frank-Starling Reserve Is Lost in Heart Failure. JACC Cardiovasc Imaging 2017;10:1250-1252.
56. Nanbu T, Nakakoshi T, Yonezawa K, Kitabatake A. Myocardial high-energy phosphate metabolism in patients with stable chronic dilated cardiomyopathy under a dobutamine-induced prolonged mild workload. Am Heart J 1999;138:641-5.
57. Cimini CM, Weiss HR. Hypertension-induced cardiac hypertrophy. Oxygen supply and consumption with pacing. Hypertension 1990;16:35-42.
58. Savage DD. Overall risk of left ventricular hypertrophy secondary to systemic hypertension. Am J Cardiol 1987;60:8I-12I.
59. Rossi MA. Pathologic fibrosis and connective tissue matrix in left ventricular hypertrophy due to chronic arterial hypertension in humans. Journal of hypertension 1998;16:1031-41.
60. Frohlich ED, Gonzalez A, Diez J. Hypertensive left ventricular hypertrophy risk: beyond adaptive cardiomyocytic hypertrophy. Journal of hypertension 2011;29:17-26.
61. Koziolova NA, Shatunova IM, Lazarev IA. [Risk factors of development of left ventricular hypertrophy in patients with hypertensive disease with high compliance to treatment]. Kardiologiia 2012;52:25-30.
62. Okwuosa TM, Soliman EZ, Lopez F, Williams KA, Alonso A, Ferdinand KC. Left ventricular hypertrophy and cardiovascular disease risk prediction and reclassification in blacks and whites: the Atherosclerosis Risk in Communities Study. Am Heart J 2015;169:155-61 e5.
63. Rostoff P, Gackowski A, Nessler J, Piwowarska W. [Left ventricular hypertrophy - current views on the pathophysiology, association with cardiovascular risk, and therapeutic options]. Kardiologia polska 2010;68:815-23.
64. Kannel WB. Left ventricular hypertrophy as a risk factor: the Framingham experience. J Hypertens Suppl 1991;9:S3-8; discussion S8-9.

65. Wharton G, Steeds R, Allen J et al. A minimum dataset for a standard adult transthoracic echocardiogram: a guideline protocol from the British Society of Echocardiography. Echo research and practice 2015;2:G9-G24.
66. Stoylen A, Molmen HE, Dalen H. Importance of length and external diameter in left ventricular geometry. Normal values from the HUNT Study. Open heart 2016;3:e000465.
67. Bulow R, Ittermann T, Dorr M et al. Reference ranges of left ventricular structure and function assessed by contrast-enhanced cardiac MR and changes related to ageing and hypertension in a population-based study. Eur Radiol 2018;28:3996-4005.
68. Liu B, Dardeer AM, Moody WE et al. Reference ranges for three-dimensional feature tracking cardiac magnetic resonance: comparison with two-dimensional methodology and relevance of age and gender. Int J Cardiovasc Imaging 2018;34:761-775.
69. Zeng M, Qiao Y, Wen Z et al. The Association between Diffuse Myocardial Fibrosis on Cardiac Magnetic Resonance T1 Mapping and Myocardial Dysfunction in Diabetic Rabbits. Sci Rep 2017;7:44937.
70. Linz W, Schaper J, Wiemer G, Albus U, Scholkens BA. Ramipril prevents left ventricular hypertrophy with myocardial fibrosis without blood pressure reduction: a one year study in rats. Br J Pharmacol 1992;107:970-5.
71. Sicari R, Nihoyannopoulos P, Evangelista A et al. Stress echocardiography expert consensus statement: European Association of Echocardiography (EAE) (a registered branch of the ESC). European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology 2008;9:415-37.
72. Spampinato RA, Tasca M, Borger MA et al. Advanced symptoms are associated with myocardial damage in patients with severe aortic stenosis. J Cardiol 2017;70:41-47.
73. Glenney SS, Brockemer DP, Ng AC, Smolewski MA, Smolgovskiy VM, Lepley AS. Effect of Exercise Training on Cardiac Biomarkers in At-Risk Populations: A Systematic Review. J Phys Act Health 2017;14:968-989.
74. Graettinger WF. The cardiovascular response to chronic physical exertion and exercise training: an echocardiographic review. Am Heart J 1984;108:1014-8.
75. Cipryan L, Vala R. Cardiac autonomic regulation after continuous and intermittent maximal exercise interventions. J Sports Med Phys Fitness 2015;55:495-505.
76. Duncker DJ, Bache RJ. Regulation of coronary blood flow during exercise. Physiol Rev 2008;88:1009-86.
77. Hellsten Y, Nyberg M. Cardiovascular Adaptations to Exercise Training. Comprehensive Physiology 2015;6:1-32.
78. Agostoni P, Vignati C, Gentile P et al. Reference Values for Peak Exercise Cardiac Output in Healthy Individuals. Chest 2017;151:1329-1337.
79. Delp MD, Laughlin MH. Regulation of skeletal muscle perfusion during exercise. Acta physiologica Scandinavica 1998;162:411-9.
80. Feigl EO. Coronary physiology. Physiol Rev 1983;63:1-205.
81. Wang TJ, Wollert KC, Larson MG et al. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. Circulation 2012;126:1596-604.

82. Simovic AM, Stojkovic AK, Dejan JM, Savic D. Is it Possible to Predict Mortality in Preterm Neonates, Based on a Single Troponin I Value at 24 h? Indian journal of pediatrics 2016;83:466-7.
83. Kelly D, Squire IB, Khan SQ et al. C-terminal provasopressin (copeptin) is associated with left ventricular dysfunction, remodeling, and clinical heart failure in survivors of myocardial infarction. J Card Fail 2008;14:739-45.
84. Sanders-van Wijk S, van Empel V, Davarzani N et al. Circulating biomarkers of distinct pathophysiological pathways in heart failure with preserved vs. reduced left ventricular ejection fraction. European Journal of Heart Failure 2015;17:1006-1014.
85. Sinning C, Ojeda F, Zeller T et al. Cardiovascular Mortality in Chest Pain Patients: Comparison of Natriuretic Peptides With Novel Biomarkers of Cardiovascular Stress. Canadian Journal of Cardiology 2016;32:1470-1477.
86. Shehata AR, Ahlberg AW, Gillam LD et al. Direct comparison of arbutamine and dobutamine stress testing with myocardial perfusion imaging and echocardiography in patients with coronary artery disease. The American journal of cardiology 1997;80:716-20.
87. Sicari R, Cortigiani L. The clinical use of stress echocardiography in ischemic heart disease. Cardiovasc Ultrasound 2017;15:7.
88. Zoppellaro G, Venneri L, Khattar RS, Li W, Senior R. Simultaneous Assessment of Myocardial Perfusion, Wall Motion, and Deformation during Myocardial Contrast Echocardiography: A Feasibility Study. Echocardiography (Mount Kisco, NY) 2016;33:889-95.
89. Hoffmann R, Borges AC, Kasprzak JD et al. Analysis of myocardial perfusion or myocardial function for detection of regional myocardial abnormalities. An echocardiographic multicenter comparison study using myocardial contrast echocardiography and 2D echocardiography. European journal of echocardiography : the journal of the Working Group on Echocardiography of the European Society of Cardiology 2007;8:438-48.
90. Kokkinos P, Myers J, Doumas M et al. Exercise capacity and all-cause mortality in prehypertensive men. American journal of hypertension 2009;22:735-41.
91. McGraw S, Romano S, Jue J, Bauml MA, Chung J, Farzaneh-Far A. Impact of Stress Cardiac Magnetic Resonance Imaging on Clinical Care. The American Journal of Cardiology 2016;118:924-929.
92. AbouEzzeddine OF, McKie PM, Scott CG et al. Biomarker-based risk prediction in the community. European journal of heart failure 2016;18:1342-1350.
93. Januzzi JL, Felker GM. Surfing the Biomarker Tsunami at JACC: Heart Failure∗. JACC: Heart Failure 2013;1:213-215.
94. Omland T, White HD. State of the Art: Blood Biomarkers for Risk Stratification in Patients with Stable Ischemic Heart Disease. Clin Chem 2017;63:165-176.
95. Tehrani DM, Seto AH. Third universal definition of myocardial infarction: update, caveats, differential diagnoses. Cleveland Clinic journal of medicine 2013;80:777-86.
96. Lee G, Twerenbold R, Tanglay Y et al. Clinical benefit of high-sensitivity cardiac troponin I in the detection of exercise-induced myocardial ischemia. American Heart Journal 2016;173:8-17.
97. Ndumele CE, Coresh J, Lazo M et al. Obesity, subclinical myocardial injury, and incident heart failure. JACC Heart failure 2014;2:600-7.

98. McEvoy JW, Chen Y, Nambi V et al. High-Sensitivity Cardiac Troponin T and Risk of Hypertension. Circulation 2015;132:825-33.
99. Karadeniz L, Coban A, Ince Z, Turkoglu U, Can G. Cord blood cardiac troponin T and nonprotein-bound iron levels in newborns of mild pre-eclamptic mothers. Neonatology 2010;97:305-10.
100. Roberts E, Ludman AJ, Dworzynski K et al. The diagnostic accuracy of the natriuretic peptides in heart failure: systematic review and diagnostic meta-analysis in the acute care setting. BMJ (Clinical research ed) 2015;350:h910-h910.
101. Vargas KG, Kassem M, Mueller C, Wojta J, Huber K. Copeptin for the early rule-out of non-ST-elevation myocardial infarction. International Journal of Cardiology 2016;223:797-804.
102. Baumann G, Dingman JF. Distribution, blood transport, and degradation of antidiuretic hormone in man. J Clin Invest 1976;57:1109-16.
103. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem 2006;52:112-9.
104. Lewandowski AJ, Bradlow WM, Augustine D et al. Right ventricular systolic dysfunction in young adults born preterm. Circulation 2013;128:713-20.
105. Lewandowski AJ, Davis EF, Yu G et al. Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. Hypertension 2015;65:607-14.
106. Leipälä JA, Boldt T, Turpeinen U, Vuolteenaho O, Fellman V. Cardiac hypertrophy and altered hemodynamic adaptation in growth-restricted preterm infants. Pediatric research 2003;53:989-93.
107. Blencowe H, Cousens S, Oestergaard MZ et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012;379:2162-72.
108. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet (London, England) 2008;371:75-84.
109. Blencowe H, Cousens S, Chou D et al. Born too soon: the global epidemiology of 15 million preterm births. Reproductive health 2013;10 Suppl 1:S2-S2.
110. Usher R. Reduction of Mortality From Respiratory Distress Syndrome of Prematurity with Early Administration of Intravenous Glucose and Sodium Bicarbonate. Pediatrics 1963;32:966-75.
111. Obladen M. History of surfactant up to 1980. Biology of the neonate 2005;87:308-16.
112. Halliday HL. History of surfactant from 1980. Biology of the neonate 2005;87:317-22.
113. UK. Pregnancy and Ethnic Factors Influencing Births and Infant Mortality: 2013. UK Office for National Statistics, 2015:Statistical Bulletin.
114. Lawn JE, Kinney MV, Belizan JM et al. Born Too Soon: Accelerating actions for prevention and care of 15 million newborns born too soon. Reproductive Health 2013;10:S6-S6.
115. Kararigas G, Dworatzek E, Petrov G et al. Sex-dependent regulation of fibrosis and inflammation in human left ventricular remodelling under pressure overload. Eur J Heart Fail 2014;16:1160-7.

116. Ul Haq MA, Wong C, Hare DL. Heart failure with preserved ejection fraction: an insight into its prevalence, predictors, and implications of early detection. Rev Cardiovasc Med 2015;16:20-7.
117. de Simone G, Izzo R, Losi MA et al. Depressed myocardial energetic efficiency is associated with increased cardiovascular risk in hypertensive left ventricular hypertrophy. Journal of hypertension 2016;34:1846-53.
118. Nicoletti A, Heudes D, Hinglais N et al. Left ventricular fibrosis in renovascular hypertensive rats. Effect of losartan and spironolactone. Hypertension 1995;26:101-11.
119. Varo N, Etayo JC, Zalba G et al. Losartan inhibits the post-transcriptional synthesis of collagen type I and reverses left ventricular fibrosis in spontaneously hypertensive rats. Journal of hypertension 1999;17:107-14.
120. Levy D, Anderson KM, Savage DD, Balkus SA, Kannel WB, Castelli WP. Risk of ventricular arrhythmias in left ventricular hypertrophy: the Framingham Heart Study. Am J Cardiol 1987;60:560-5.
121. Kannel WB. Left ventricular hypertrophy as a risk factor in arterial hypertension. Eur Heart J 1992;13 Suppl D:82-8.
122. Zalawadiya SK, Gunasekaran PC, Bavishi CP, Veeranna V, Panaich S, Afonso L. Left ventricular hypertrophy and risk reclassification for coronary events in multi-ethnic adults. Eur J Prev Cardiol 2015;22:673-9.
123. Cuspidi C, Sala C, Negri F, Mancia G, Morganti A. Prevalence of left-ventricular hypertrophy in hypertension: an updated review of echocardiographic studies. Journal of human hypertension 2012;26:343-9.
124. Cuspidi C, Meani S, Sala C, Valerio C, Negri F, Mancia G. Age related prevalence of severe left ventricular hypertrophy in essential hypertension: echocardiographic findings from the ETODH study. Blood pressure 2012;21:139-45.
125. Kannel WB, Levy D, Cupples LA. Left ventricular hypertrophy and risk of cardiac failure: insights from the Framingham Study. Journal of cardiovascular pharmacology 1987;10 Suppl 6:S135-40.
126. Ciardullo AV, Azzolini L, Bevini M et al. A diagnosis of left ventricular hypertrophy on ECG is associated with a high cardiovascular risk: findings from a 40- to 69-year-old cohort in general practice. Fam Pract 2004;21:63-5.
127. Porthan K, Niiranen TJ, Varis J et al. ECG left ventricular hypertrophy is a stronger risk factor for incident cardiovascular events in women than in men in the general population. Journal of hypertension 2015;33:1284-90.
128. Okin PM, Hille DA, Kjeldsen SE, Devereux RB. Combining ECG Criteria for Left Ventricular Hypertrophy Improves Risk Prediction in Patients With Hypertension. J Am Heart Assoc 2017;6.
129. Kannel WB, Gordon T, Castelli WP, Margolis JR. Electrocardiographic left ventricular hypertrophy and risk of coronary heart disease. The Framingham study. Annals of internal medicine 1970;72:813-22.
130. Alkema M, Spitzer E, Soliman OII, Loewe C. Multimodality Imaging for Left Ventricular Hypertrophy Severity Grading: A Methodological Review. J Cardiovasc Ultrasound 2016;24:257-67.
131. Cuspidi C, Quarti F, Dell'Oro R et al. Long-term changes in left ventricular mass echocardiographic findings from a general population. Journal of hypertension 2017;35:2303-2309.

132. Toprak A, Wang H, Chen W, Paul T, Srinivasan S, Berenson G. Relation of childhood risk factors to left ventricular hypertrophy (eccentric or concentric) in relatively young adulthood (from the Bogalusa Heart Study). Am J Cardiol 2008;101:1621-5.
133. Bang CN, Gerdts E, Aurigemma GP et al. Four-group classification of left ventricular hypertrophy based on ventricular concentricity and dilatation identifies a low-risk subset of eccentric hypertrophy in hypertensive patients. Circ Cardiovasc Imaging 2014;7:422-9.
134. Papademetriou V, Kokkinos PF. Changes in structure and function of the left ventricle in the hypertension syndrome. Journal of cardiovascular risk 1997;4:261-5.
135. Rodrigues JCL, Amadu AM, Dastidar AG et al. Comprehensive characterisation of hypertensive heart disease left ventricular phenotypes. Heart 2016;102:1671-9.
136. de Gonzalo-Calvo D, Kenneweg F, Bang C et al. Circulating long-non coding RNAs as biomarkers of left ventricular diastolic function and remodelling in patients with well-controlled type 2 diabetes. Scientific reports 2016;6:37354-37354.
137. Hinojar R, Fernandez-Golfin C, Gonzalez-Gomez A et al. Prognostic implications of global myocardial mechanics in hypertrophic cardiomyopathy by cardiovascular magnetic resonance feature tracking. Relations to left ventricular hypertrophy and fibrosis. Int J Cardiol 2017;249:467-472.
138. Rubis P, Wisniowska-Smialek S, Biernacka-Fijalkowska B et al. Left ventricular reverse remodeling is not related to biopsy-detected extracellular matrix fibrosis and serum markers of fibrosis in dilated cardiomyopathy, regardless of the definition used for LVRR. Heart Vessels 2017;32:714-725.
139. Devereux RB, Roman MJ. Left ventricular hypertrophy in hypertension: stimuli, patterns, and consequences. Hypertens Res 1999;22:1-9.
140. Berger J, Ren X, Na B, Whooley MA, Schiller NB. Relation of concentric remodeling to adverse outcomes in patients with stable coronary artery disease (from the Heart and Soul Study). Am J Cardiol 2011;107:1579-84.
141. Mandry D, Eschalier R, Kearney-Schwartz A et al. Comprehensive MRI analysis of early cardiac and vascular remodeling in middle-aged patients with abdominal obesity. Journal of hypertension 2012;30:567-73.
142. Wang S, Xue H, Zou Y et al. Left ventricular hypertrophy, abnormal ventricular geometry and relative wall thickness are associated with increased risk of stroke in hypertensive patients among the Han Chinese. Hypertens Res 2014;37:870-4.
143. Hashem MS, Kalashyan H, Choy J et al. Left ventricular relative wall thickness versus left ventricular mass index in non-cardioembolic stroke patients. Medicine (Baltimore) 2015;94:e872.
144. Butenaerts D, Chrzanowska-Wasko J, Slowik A, Dziedzic T. Left ventricular geometry and white matter lesions in ischemic stroke patients. Blood pressure 2016;25:149-54.
145. Agabiti-Rosei E, Muiesan ML, Salvetti M. Evaluation of subclinical target organ damage for risk assessment and treatment in the hypertensive patients: left ventricular hypertrophy. J Am Soc Nephrol 2006;17:S104-8.
146. Wittekind SG, Edwards NM, Khoury PR et al. Association of Habitual Physical Activity With Cardiovascular Risk Factors and Target Organ

Damage in Adolescents and Young Adults. J Phys Act Health 2018;15:176-182.
147. Whelton PK, Carey RM, Aronow WS et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. J Am Coll Cardiol 2018;71:e127-e248.
148. World Health O. World Health Statistics 2012. 2012:109-109.
149. Forouzanfar MH, Liu P, Roth GA et al. Global Burden of Hypertension and Systolic Blood Pressure of at Least 110 to 115 mm Hg, 1990-2015. Jama-J Am Med Assoc 2017;317:165-182.
150. Scarborough P, Bhatnagar P, Wickramasinghe K, Smolina K, Mitchell C. Coronary heart disease statistics 2010 edition. British Heart Foundation 2010:21-21.
151. Nguyen QC, Tabor JW, Entzel PP et al. Discordance in national estimates of hypertension among young adults. Epidemiology (Cambridge, Mass) 2011;22:532-41.
152. Lazdam M, De La Horra A, Diesch J et al. Unique blood pressure characteristics in mother and offspring after early onset preeclampsia. Hypertension 2012;60:1338-1345.
153. Lewandowski AJ, Leeson P. Preeclampsia, prematurity and cardiovascular health in adult life. 2014:725-729.
154. Lewandowski AJ, Davis EF, Yu G et al. Elevated blood pressure in preterm-born offspring associates with a distinct antiangiogenic state and microvascular abnormalities in adult life. Hypertension 2015;65:607-614.
155. Yu GZ, Aye CY, Lewandowski AJ et al. Association of Maternal Antiangiogenic Profile at Birth With Early Postnatal Loss of Microvascular Density in Offspring of Hypertensive Pregnancies. Hypertension 2016;68:749-59.
156. O'Brien E. The Lancet Commission on hypertension: Addressing the global burden of raised blood pressure on current and future generations. J Clin Hypertens 2017;19:564-568.
157. Forouzanfar MH, Liu P, Roth GA. Global burden of hypertension and systolic blood pressure of at least 110 to 115 mm Hg, 1990-2015 (vol 3, pg 165, 2016). Jama-J Am Med Assoc 2017;317:648-648.
158. Williams B, Mancia G, Spiering W et al. 2018 ESC/ESH Guidelines for the management of arterial hypertension. Eur Heart J 2018;39:3021-3104.
159. Rich MW. Epidemiology, pathophysiology, and etiology of congestive heart failure in older adults. J Am Geriatr Soc 1997;45:968-74.
160. Linicus Y, Laufs U. [Epidemiology, etiology and pathophysiology of heart failure]. Med Monatsschr Pharm 2016;39:10-2.
161. Hunt SA, Abraham WT, Chin MH et al. 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the Diagnosis and Management of Heart Failure in Adults A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the International Society for Heart and Lung Transplantation. J Am Coll Cardiol 2009;53:e1-e90.
162. Ponikowski P, Voors AA, Anker SD et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the

special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016;37:2129-2200.
163. Mosterd A, Hoes AW. Clinical epidemiology of heart failure. Heart 2007;93:1137-46.
164. Bleumink GS, Knetsch AM, Sturkenboom MC et al. Quantifying the heart failure epidemic: prevalence, incidence rate, lifetime risk and prognosis of heart failure The Rotterdam Study. Eur Heart J 2004;25:1614-9.
165. Rossano JW, Kim JJ, Decker JA et al. Prevalence, morbidity, and mortality of heart failure-related hospitalizations in children in the United States: a population-based study. J Card Fail 2012;18:459-70.
166. Wong CM, Hawkins NM, Jhund PS et al. Clinical characteristics and outcomes of young and very young adults with heart failure: The CHARM programme (Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity). J Am Coll Cardiol 2013;62:1845-54.
167. Webster G, Zhang J, Rosenthal D. Comparison of the epidemiology and co-morbidities of heart failure in the pediatric and adult populations: a retrospective, cross-sectional study. BMC Cardiovasc Disord 2006;6:23.
168. Wong CM, Hawkins NM, Petrie MC et al. Heart failure in younger patients: the Meta-analysis Global Group in Chronic Heart Failure (MAGGIC). Eur Heart J 2014;35:2714-21.
169. Sliwa K, Mayosi BM. Recent advances in the epidemiology, pathogenesis and prognosis of acute heart failure and cardiomyopathy in Africa. Heart 2013;99:1317-22.
170. Filippatos G, Farmakis D, Bistola V et al. Temporal trends in epidemiology, clinical presentation and management of acute heart failure: results from the Greek cohorts of the Acute Heart Failure Global Registry of Standard Treatment and the European Society of Cardiology-Heart Failure pilot survey. Eur Heart J Acute Cardiovasc Care 2014;3.
171. Farmakis D, Parissis J, Lekakis J, Filippatos G. Acute heart failure: Epidemiology, risk factors, and prevention. Rev Esp Cardiol (Engl Ed) 2015;68:245-8.
172. Kaplinsky E. Sacubitril/valsartan in heart failure: latest evidence and place in therapy. Therapeutic advances in chronic disease 2016;7:278-290.
173. Heidenreich PA, Albert NM, Allen LA et al. Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association. Circulation Heart failure 2013;6:606-19.
174. Jessup M, Abraham WT, Casey DE et al. 2009 focused update: ACCF/AHA Guidelines for the Diagnosis and Management of Heart Failure in Adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed in collaboration with the International Society for Heart and Lung Transplantation. Circulation 2009;119:1977-2016.
175. Zacharias M, Joffe S, Konadu E et al. Clinical epidemiology of heart failure with preserved ejection fraction (HFpEF) in comparatively young hospitalized patients. Int J Cardiol 2016;202:918-21.
176. Tromp J, Voors AA, Lam CSP. Heart failure with mid-range ejection fraction: causes and consequences. Eur J Heart Fail 2018;20:660-662.
177. Rastogi A, Novak E, Platts AE, Mann DL. Epidemiology, pathophysiology and clinical outcomes for heart failure patients with a mid-range ejection fraction. Eur J Heart Fail 2017;19:1597-1605.

178. Tadic M, Cuspidi C, Frydas A, Grassi G. The role of arterial hypertension in development heart failure with preserved ejection fraction: just a risk factor or something more? Heart Fail Rev 2018;23:631-639.
179. Szymanski PZ, Badri M, Mayosi BM. Clinical characteristics and causes of heart failure, adherence to treatment guidelines, and mortality of patients with acute heart failure: Experience at Groote Schuur Hospital, Cape Town, South Africa. S Afr Med J 2018;108:94-98.
180. Wilson AJ, Wang VY, Sands GB, Young AA, Nash MP, LeGrice IJ. Increased cardiac work provides a link between systemic hypertension and heart failure. Physiol Rep 2017;5:e13104.
181. Tsioufis C, Georgiopoulos G, Oikonomou D et al. Hypertension and Heart Failure with Preserved Ejection Fraction: Connecting the Dots. Current vascular pharmacology 2017;16:15-22.
182. Damasceno A, Mayosi BM, Sani M et al. The causes, treatment, and outcome of acute heart failure in 1006 Africans from 9 countries. Arch Intern Med 2012;172:1386-94.
183. McMurray JJ, Stewart S. Epidemiology, aetiology, and prognosis of heart failure. Heart 2000;83:596-602.
184. Yancy CW, Jessup M, Bozkurt B et al. 2016 ACC/AHA/HFSA Focused Update on New Pharmacological Therapy for Heart Failure: An Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America (vol 68, pg 1476, 2016). Journal of the American College of Cardiology 2016;68:1495-1495.
185. Carr H, Cnattingius S, Granath F, Ludvigsson JF, Edstedt Bonamy AK. Preterm Birth and Risk of Heart Failure Up to Early Adulthood. J Am Coll Cardiol 2017;69:2634-2642.
186. Clifton EAD, Day FR, De Lucia Rolfe E et al. Associations between body mass index-related genetic variants and adult body composition: the Fenland cohort study. Int J Obes (Lond) 2017;41:613-9.
187. Napoli N, Rastelli A, Ma C, Colleluori G. Genetic Polymorphism at Val(80) (rs700518) of the CYP19A1 Gene is. 2015;25:377-81.
188. McMullan RC, Kelly SA, Hua K et al. Long-term exercise in mice has sex-dependent benefits on body composition and metabolism during aging. Physiol Rep 2016;4:e13011.
189. Garber CE, Blissmer B, Deschenes MR et al. American College of Sports Medicine position stand. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. Med Sci Sports Exerc 2011;43:1334-59.
190. Lu Y, Day FR, Gustafsson S et al. New loci for body fat percentage reveal link between adiposity and cardiometabolic disease risk. Nat Commun 2016;7:10495.
191. Dale CE. Causal Associations of Adiposity and Body Fat Distribution with Coronary Heart Disease, Stroke Subtypes and Type 2 Diabetes: A Mendelian Randomization Analysis. 2017;135:2373-88.
192. Munagala VK, Hart CY, Burnett JC, Jr., Meyer DM, Redfield MM. Ventricular structure and function in aged dogs with renal hypertension: a model of experimental diastolic heart failure. Circulation 2005;111:1128-35.

193. Thomas-Kvidera D. Heart failure from diastolic dysfunction related to hypertension: guidelines for management. J Am Acad Nurse Pract 2005;17:168-75.
194. Grant JE, Bradshaw AD, Schwacke JH, Baicu CF, Zile MR, Schey KL. Quantification of Protein Expression Changes in the Aging Left Ventricle of Rattus norvegicus. Journal of proteome research 2009;8:4252-63.
195. Meschiari C, Ero OK, Pan H, Finkel T, Lindsey ML. The Impact of Aging on Cardiac Extracellular Matrix. GeroScience 2017;39:7-18.
196. Cuspidi C, Meani S, Valerio C, Fusi V, Sala C, Zanchetti A. Left ventricular hypertrophy and cardiovascular risk stratification: impact and cost-effectiveness of echocardiography in recently diagnosed essential hypertensives. Journal of hypertension 2006;24:1671-7.
197. Kou S, Caballero L, Dulgheru R et al. Echocardiographic reference ranges for normal cardiac chamber size: results from the NORRE study. Eur Heart J Cardiovasc Imaging 2014;15:680-90.
198. Lang RM, Bierig M, Devereux RB et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005;18:1440-63.
199. Knutsen KM, Stugaard M, Michelsen S, Otterstad JE. M-mode echocardiographic findings in apparently healthy, non-athletic Norwegians aged 20-70 years. Influence of age, sex and body surface area. J Intern Med 1989;225:111-5.
200. Chantler PD, Melenovsky V, Schulman SP et al. The sex-specific impact of systolic hypertension and systolic blood pressure on arterial-ventricular coupling at rest and during exercise. Am J Physiol Heart Circ Physiol 2008;295:H145-53.
201. Fall T, Hägg S, Ploner A et al. Age- and Sex-Specific Causal Effects of Adiposity on Cardiovascular Risk Factors. Diabetes 2015;64:1841-52.
202. Mangion K, Clerfond G, McComb C et al. Myocardial strain in healthy adults across a broad age range as revealed by cardiac magnetic resonance imaging at 1.5 and 3.0T: Associations of myocardial strain with myocardial region, age, and sex. J Magn Reson Imaging 2016;44:1197-1205.
203. Gerdts E, Izzo R, Mancusi C et al. Left ventricular hypertrophy offsets the sex difference in cardiovascular risk (the Campania Salute Network). Int J Cardiol 2018;258:257-261.
204. Jakovljevic DG, Donovan G, Nunan D et al. The effect of aerobic versus resistance exercise training on peak cardiac power output and physical functional capacity in patients with chronic heart failure. Int J Cardiol 2010;145:526-8.
205. De Souza MR, Pimenta L, Pithon-Curi TC, Bucci M, Fontinele RG, De Souza RR. Effects of aerobic training, resistance training, or combined resistance-aerobic training on the left ventricular myocardium in a rat model. Microscopy research and technique 2014;77:727-34.
206. McAllister RM, Hirai T, Musch TI. Contribution of endothelium-derived nitric oxide (EDNO) to the skeletal muscle blood flow response to exercise. Med Sci Sports Exerc 1995;27:1145-51.
207. Lash JM. Regulation of skeletal muscle blood flow during contractions. Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine 1996;211:218-35.

208. Carlson RE, Kirby BS, Voyles WF, Dinenno FA. Evidence for impaired skeletal muscle contraction-induced rapid vasodilation in aging humans. Am J Physiol Heart Circ Physiol 2008;294:H1963-70.
209. Spence AL, Naylor LH, Carter HH et al. A prospective randomised longitudinal MRI study of left ventricular adaptation to endurance and resistance exercise training in humans. J Physiol 2011;589:5443-52.
210. Venckunas T, Simonavicius J, Marcinkeviciene JE. Cardiac size of high-volume resistance trained female athletes: shaping the body but not the heart. Physiology international 2016;103:105-11.
211. Adler Y, Fisman EZ, Koren-Morag N et al. Left ventricular diastolic function in trained male weight lifters at rest and during isometric exercise. Am J Cardiol 2008;102:97-101.
212. Nolte K, Schwarz S, Gelbrich G et al. Effects of long-term endurance and resistance training on diastolic function, exercise capacity, and quality of life in asymptomatic diastolic dysfunction vs. heart failure with preserved ejection fraction. ESC heart failure 2014;1:59-74.
213. Papademetriou V, Kokkinos PF. Exercise Training and Blood Pressure Control in Patients With Hypertension. Journal of clinical hypertension (Greenwich, Conn) 1999;1:95-105.
214. Cornelissen VA, Smart NA. Exercise training for blood pressure: a systematic review and meta-analysis. J Am Heart Assoc 2013;2:e004473.
215. MacDonald JR. Potential causes, mechanisms, and implications of post exercise hypotension. Journal of human hypertension 2002;16:225-36.
216. De Souza JC, Tibana RA, Cavaglieri CR et al. Resistance exercise leading to failure versus not to failure: effects on cardiovascular control. BMC Cardiovasc Disord 2013;13:105.
217. Figueiredo T, Willardson JM, Miranda H, Bentes CM, Reis VM, Simao R. Influence of Load Intensity on Postexercise Hypotension and Heart Rate Variability after a Strength Training Session. Journal of strength and conditioning research 2015;29:2941-8.
218. Wonisch M, Marko C, Niebauer J, Pokan R, Schmid P, Wiesinger E. [Resistance training for patients with cardiovascular diseases]. Wien Klin Wochenschr 2012;124:326-33.
219. Caruso FR, Arena R, Phillips SA et al. Resistance exercise training improves heart rate variability and muscle performance: a randomized controlled trial in coronary artery disease patients. European journal of physical and rehabilitation medicine 2015;51:281-9.
220. Currie KD, Bailey KJ, Jung ME, McKelvie RS, MacDonald MJ. Effects of resistance training combined with moderate-intensity endurance or low-volume high-intensity interval exercise on cardiovascular risk factors in patients with coronary artery disease. Journal of science and medicine in sport 2015;18:637-42.
221. Moreira-Goncalves D, Ferreira-Nogueira R, Santos M et al. Exercise Training in Pulmonary Hypertension and Right Heart Failure: Insights from Pre-clinical Studies. Adv Exp Med Biol 2017;999:307-324.
222. Pollock ML, Franklin BA, Balady GJ et al. AHA Science Advisory. Resistance exercise in individuals with and without cardiovascular disease: benefits, rationale, safety, and prescription: An advisory from the Committee on Exercise, Rehabilitation, and Prevention, Council on Clinical Cardiology, American Heart Association; Position paper endorsed by the American College of Sports Medicine. Circulation 2000;101:828-33.

223. Pescatello LS, Franklin BA, Fagard R, Farquhar WB, Kelley GA, Ray CA. American College of Sports Medicine position stand. Exercise and hypertension. Med Sci Sports Exerc 2004;36:533-53.
224. Yano T, Lian CS, Afroundeh R, Shirakawa K, Yunoki T. Muscle pump in the vastus lateralis in the supine position in light prolonged exercise. J Sports Med Phys Fitness 2015;55:37-42.
225. Hulkkonen J, Aatola H, Palve K et al. Determinants of exercise peak arterial blood pressure, circulatory power, and exercise cardiac power in a population based sample of Finnish male and female aged 30 to 47 years: the Cardiovascular Risk in Young Finns Study. BMC Cardiovasc Disord 2014;14:35.
226. Sharman JE, LaGerche A. Exercise blood pressure: clinical relevance and correct measurement. Journal of human hypertension 2015;29:351-8.
227. Pelliccia A, Culasso F, Di Paolo FM, Maron BJ. Physiologic left ventricular cavity dilatation in elite athletes. Annals of internal medicine 1999;130:23-31.
228. Henriksen E, Sundstedt M, Hedberg P. Left ventricular end-diastolic geometrical adjustments during exercise in endurance athletes. Clinical physiology and functional imaging 2008;28:76-80.
229. Batterham AM, George KP, Birch KM, Pennell DJ, Myerson SG. Growth of left ventricular mass with military basic training in army recruits. Med Sci Sports Exerc 2011;43:1295-300.
230. Arbab-Zadeh A, Perhonen M, Howden E et al. Cardiac remodeling in response to 1 year of intensive endurance training. Circulation 2014;130:2152-61.
231. Weiner RB, DeLuca JR, Wang F et al. Exercise-Induced Left Ventricular Remodeling Among Competitive Athletes: A Phasic Phenomenon. Circ Cardiovasc Imaging 2015;8.
232. Williamson W, Foster C, Reid H et al. Will Exercise Advice Be Sufficient for Treatment of Young Adults With Prehypertension and Hypertension? A Systematic Review and Meta-Analysis. Hypertension 2016;68:78-87.
233. Whelton SP, Chin A, Xin X, He J. Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials. Annals of internal medicine 2002;136:493-503.
234. Dimeo F, Pagonas N, Seibert F, Arndt R, Zidek W, Westhoff TH. Aerobic exercise reduces blood pressure in resistant hypertension. Hypertension 2012;60:653-8.
235. Higashino R, Miyaki A, Kumagai H et al. Effects of lifestyle modification on central blood pressure in overweight and obese men. Blood pressure monitoring 2013;18:311-5.
236. McDonnell BJ, Maki-Petaja KM, Munnery M et al. Habitual exercise and blood pressure: age dependency and underlying mechanisms. American journal of hypertension 2013;26:334-41.
237. Ronan G, Wolk MJ, Bailey SR et al. ACCF/AHA/ASE/ASNC/HFSA/HRS/SCAI/SCCT/SCMR/STS 2013 multimodality appropriate use criteria for the detection and risk assessment of stable ischemic heart disease: a report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force, American Heart Association, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, Society of Cardiovascular Computed

Tomography, Society for Cardiovascular Magnetic Resonance, and Society of Thoracic Surgeons. J Nucl Cardiol 2014;21:192-220.
238. Sharman JE, La Gerche A, Coombes JS. Exercise and cardiovascular risk in patients with hypertension. American journal of hypertension 2015;28:147-58.
239. Gordon A, Tyni-Lenne R, Jansson E, Jensen-Urstad M, Kaijser L. Beneficial effects of exercise training in heart failure patients with low cardiac output response to exercise - a comparison of two training models. J Intern Med 1999;246:175-82.
240. Dieberg G, Ismail H, Giallauria F, Smart NA. Clinical outcomes and cardiovascular responses to exercise training in heart failure patients with preserved ejection fraction: a systematic review and meta-analysis. Journal of applied physiology (Bethesda, Md : 1985) 2015;119:726-33.
241. Shih YT, Cheng HM, Sung SH, Hu WC, Chen CH. Quantification of the calibration error in the transfer function-derived central aortic blood pressures. American journal of hypertension 2011;24:1312-7.
242. Narayan O, Casan J, Szarski M, Dart AM, Meredith IT, Cameron JD. Estimation of central aortic blood pressure: a systematic meta-analysis of available techniques. Journal of hypertension 2014;32:1727-40.
243. Nagueh SF, Appleton CP, Gillebert TC et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. Eur J Echocardiogr 2009;10:165-93.
244. Nagueh SF, Appleton CP, Gillebert TC et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009;22:107-33.
245. Bush A, Cramer D. Guidelines for the Measurement of Respiratory-Function. Resp Med 1994;88:798-798.
246. Pikkemaat M, Melander O, Bengtsson Bostrom K. Association between copeptin and declining glomerular filtration rate in people with newly diagnosed diabetes. The Skaraborg Diabetes Register. J Diabetes Complications 2015;29:1062-5.
247. Chen MJ, Fan X, Moe ST. Criterion-related validity of the Borg ratings of perceived exertion scale in healthy individuals: a meta-analysis. Journal of sports sciences 2002;20:873-99.
248. Fletcher GF, Ades PA, Kligfield P et al. Exercise Standards for Testing and Training. Circulation 2013;128:873.
249. Balady GJ, Arena R, Sietsema K et al. Clinician's Guide to Cardiopulmonary Exercise Testing in Adults. Circulation 2010;122:191.
250. Hildebrand M, VT VANH, Hansen BH, Ekelund U. Age group comparability of raw accelerometer output from wrist- and hip-worn monitors. Med Sci Sports Exerc 2014;46:1816-24.
251. Blencowe H, Cousens S, Chou D et al. Born Too Soon: The global epidemiology of 15 million preterm births. Reproductive Health 2013;10:S2-S2.
252. Aye CYL, Lewandowski AJ, Lamata P et al. Disproportionate cardiac hypertrophy during early postnatal development in infants born preterm. Pediatr Res 2017;82:36-46.
253. Clemm HH, Vollsaeter M, Røksund OD, Markestad T, Halvorsen T. Adolescents who were born extremely preterm demonstrate modest decreases in exercise capacity. Acta Paediatrica 2015;104:1174-81.
254. Ueda P, Cnattingius S, Stephansson O, Ingelsson E, Ludvigsson JF, Bonamy AK. Cerebrovascular and ischemic heart disease in young adults born preterm: a population-based Swedish cohort study. Eur J Epidemiol 2014;29:253-60.

255. Sicari R, Nihoyannopoulos P, Evangelista A et al. Stress echocardiography expert consensus statement: European Association of Echocardiography (EAE) European Journal of Echocardiography 2008;9:415-37.
256. Goda A, Lang CC, Williams P, Jones M, Farr MJ, Mancini DM. Usefulness of non-invasive measurement of cardiac output during sub-maximal exercise to predict outcome in patients with chronic heart failure. Am J Cardiol 2009;104:1556-60.
257. Bertagnolli M, Casali KR, De Sousa FB et al. An orally active angiotensin-(1-7) inclusion compound and exercise training produce similar cardiovascular effects in spontaneously hypertensive rats. Peptides 2014;51:65-73.
258. Leeson P, Lewandowski AJ. A New Risk Factor for Early Heart Failure: Preterm Birth. J Am Coll Cardiol 2017;69:2643-2645.
259. Eiby YA, Lumbers ER, Headrick JP, Lingwood BE. Left ventricular output and aortic blood flow in response to changes in preload and afterload in the preterm piglet heart. Am J Physiol Regul Integr Comp Physiol 2012;303:R769-77.
260. Smolich JJ, Walker AM, Campbell GR, Adamson TM. Left and right ventricular myocardial morphometry in fetal, neonatal, and adult sheep. The American journal of physiology 1989;257:H1-9.
261. Rudolph AM. Myocardial growth before and after birth: clinical implications. Acta Paediatr 2000;89:129-33.
262. Burrell JH, Boyn AM, Kumarasamy V, Hsieh A, Head SI, Lumbers ER. Growth and maturation of cardiac myocytes in fetal sheep in the second half of gestation. The anatomical record Part A, Discoveries in molecular, cellular, and evolutionary biology 2003;274:952-61.
263. Bensley JG, Stacy VK, De Matteo R, Harding R, Black MJ. Cardiac remodelling as a result of pre-term birth: implications for future cardiovascular disease. Eur Heart J 2010;31:2058-66.
264. Levin I, Naegler T, Kromer B et al. Observations and modelling of the global distribution and long-term trend of atmospheric 14CO2. Tellus B: Chemical and Physical Meteorology 2010;62:26-46.
265. Lazar E, Sadek HA, Bergmann O. Cardiomyocyte renewal in the human heart: insights from the fall-out. Eur Heart J 2017;38:2333-2342.
266. Bergmann O, Bhardwaj RD, Bernard S et al. Evidence for cardiomyocyte renewal in humans. Science 2009;324:98-102.
267. Mollova M, Bersell K, Walsh S et al. Cardiomyocyte proliferation contributes to heart growth in young humans. Proc Natl Acad Sci U S A 2013;110:1446-51.
268. Uygur A, Lee RT. Mechanisms of Cardiac Regeneration. Dev Cell 2016;36:362-74.
269. Lewandowski AJ, Lamata P, Francis JM et al. Breast Milk Consumption in Preterm Neonates and Cardiac Shape in Adulthood. Pediatrics 2016;138.
270. Bertagnolli M, Luu TM, Lewandowski AJ, Leeson P, Nuyt AM. Preterm Birth and Hypertension: Is There a Link? Current Hypertension Reports 2016;18:28.
271. Davis EF, Lewandowski AJ, Aye C et al. Clinical cardiovascular risk during young adulthood in offspring of hypertensive pregnancies: insights from a 20-year prospective follow-up birth cohort. BMJ Open 2015;5:e008136.
272. Piepoli MF, Hoes AW, Agewall S et al. 2016 European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies

on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts): Developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). Eur J Prev Cardiol 2016;23:Np1-np96.
273. Vasan RS, Xanthakis V, Lyass A et al. Epidemiology of Left Ventricular Systolic Dysfunction and Heart Failure in the Framingham Study: An Echocardiographic Study Over 3 Decades. JACC Cardiovasc Imaging 2018;11:1-11.
274. Sipola-Leppänen M, Vääräsmäki M, Tikanmäki M et al. Cardiometabolic Risk Factors in Young Adults Who Were Born Preterm. Am J Epidemiol 2015;181:861-73.
275. Kajantie E, Hovi P. Is very preterm birth a risk factor for adult cardiometabolic disease? Seminars in fetal & neonatal medicine 2014;19:112-7.
276. Sutherland MR, Bertagnolli M, Lukaszewski MA et al. Preterm birth and hypertension risk: the oxidative stress paradigm. Hypertension 2014;63:12-8.
277. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-421.
278. Grundy SM. Metabolic syndrome update. Trends Cardiovasc Med 2016;26:364-73.
279. Zafar U, Khaliq S, Ahmad HU, Manzoor S, Lone KP. Metabolic syndrome: an update on diagnostic criteria, pathogenesis, and genetic links. Hormones (Athens, Greece) 2018;17:299-313.
280. de Jong F, Monuteaux MC, van Elburg RM, Gillman MW, Belfort MB. Systematic review and meta-analysis of preterm birth and later systolic blood pressure. Hypertension 2012;59:226-34.
281. Scuteri A, Laurent S, Cucca F et al. Metabolic syndrome across Europe: different clusters of risk factors. Eur J Prev Cardiol 2015;22:486-91.
282. Parkinson JR, Mj H, C G, S S, N M. Preterm birth and the metabolic syndrome in adult life: a systematic review and meta-analysis. Pediatrics 2013;131:e1240-e1240.
283. Kajantie E, Osmond C, Barker DJP, Eriksson JG. Preterm Birth—A Risk Factor for Type 2 Diabetes?: The Helsinki Birth Cohort Study. Diabetes Care 2010;33:2623-5.
284. Ananth CV, Ananth CV, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. The Journal of Maternal-Fetal & Neonatal Medicine 2006;19:773-782.
285. Andres RL, Day MC. Perinatal complications associated with maternal tobacco use. Seminars in neonatology : SN 2000;5:231-41.
286. Reitan T, Callinan S. Changes in Smoking Rates Among Pregnant Women and the General Female Population in Australia, Finland, Norway, and Sweden. Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco 2017;19:282-289.
287. Phung HN, Bauman AE, Young L, Tran MH, Hillman KM. Ecological and individual predictors of maternal smoking behaviour. Looking beyond individual socioeconomic predictors at the community setting. Addictive behaviors 2003;28:1333-42.
288. Wagijo MA, Sheikh A, Duijts L, Been JV. Reducing tobacco smoking and smoke exposure to prevent preterm birth and its complications. Paediatric respiratory reviews 2017;22:3-10.

289. Wang Y, Tuomilehto J, Jousilahti P et al. Lifestyle factors in relation to heart failure among Finnish men and women. Circulation Heart failure 2011;4:607-12.
290. Hoevenaar-Blom MP, Spijkerman AM, Kromhout D, Verschuren WM. Sufficient sleep duration contributes to lower cardiovascular disease risk in addition to four traditional lifestyle factors: the MORGEN study. Eur J Prev Cardiol 2014;21:1367-75.
291. Del Gobbo LC, Kalantarian S, Imamura F et al. Contribution of Major Lifestyle Risk Factors for Incident Heart Failure in Older Adults: The Cardiovascular Health Study. JACC Heart Fail 2015;3:520-528.
292. Winham SJ, de Andrade M, Miller VM. Genetics of cardiovascular disease: Importance of sex and ethnicity. Atherosclerosis 2015;241:219-28.
293. Goyal P, Paul T, Almarzooq ZI et al. Sex- and Race-Related Differences in Characteristics and Outcomes of Hospitalizations for Heart Failure With Preserved Ejection Fraction. J Am Heart Assoc 2017;6.
294. Balady GJ, Arena R, Sietsema K et al. Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. Circulation 2010;122:191-225.
295. Takahashi T, Hayano J, Okada A, Saitoh T, Kamiya A. Effects of the muscle pump and body posture on cardiovascular responses during recovery from cycle exercise. Eur J Appl Physiol 2005;94:576-83.
296. Edwards MO, Kotecha SJ, Lowe J, Watkins WJ, Henderson AJ, Kotecha S. Effect of preterm birth on exercise capacity: A systematic review and meta-analysis. Pediatr Pulmonol 2015;50:293-301.
297. Zysman-Colman Z, Tremblay GM, Bandeali S, Landry JS. Bronchopulmonary dysplasia – trends over three decades. Paediatrics & Child Health 2013;18:86-90.
298. Lovering AT, Elliott JE, Laurie SS et al. Ventilatory and sensory responses in adult survivors of preterm birth and bronchopulmonary dysplasia with reduced exercise capacity. Ann Am Thorac Soc 2014;11:1528-37.
299. Hestnes J, Hoel H, Risa OJ et al. Ventilatory Efficiency in Children and Adolescents Born Extremely Preterm. Front Physiol 2017;8:499.
300. St. Clair C, Norwitz ER, Woensdregt K et al. The Probability of Neonatal Respiratory Distress Syndrome as a Function of Gestational Age and Lecithin/Sphingomyelin Ratio. Am J Perinatol 2008;25:473-80.
301. Anadkat JS, Kuzniewicz MW, Chaudhari BP, Cole FS, Hamvas A. Increased risk for respiratory distress among white, male, late preterm and term infants. J Perinatol 2012;32:780-5.
302. Wang L, Chen L, Li R et al. Efficacy of surfactant at different gestational ages for infants with respiratory distress syndrome. Int J Clin Exp Med 2015;8:13783-9.
303. Tsopanoglou SP, Davidson J, Goulart AL, Barros MC, dos Santos AM. Functional capacity during exercise in very-low-birth-weight premature children. Pediatr Pulmonol 2014;49:91-8.
304. Clemm H, Roksund O, Thorsen E, Eide GE, Markestad T, Halvorsen T. Aerobic capacity and exercise performance in young people born extremely preterm. Pediatrics 2012;129:e97-e105.
305. Svedenkrans J, Henckel E, Kowalski J, Norman M, Bohlin K. Long-term impact of preterm birth on exercise capacity in healthy young men: a national population-based cohort study. PloS one 2013;8:e80869.

306. Huckstep OJ, Williamson W, Telles F et al. Physiological Stress Elicits Impaired Left Ventricular Function in Preterm-Born Adults. J Am Coll Cardiol 2018;71:1347-1356.
307. Bassett DR, Jr., Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc 2000;32:70-84.
308. Lo Q, Haluska B, Chia EM et al. Alterations in regional myocardial deformation assessed by strain imaging in cardiac amyloidosis. Echocardiography 2016;33:1844-1853.
309. Clemmensen TS, Eiskjaer H, Molgaard H et al. Abnormal Coronary Flow Velocity Reserve and Decreased Myocardial Contractile Reserve Are Main Factors in Relation to Physical Exercise Capacity in Cardiac Amyloidosis. J Am Soc Echocardiogr 2018;31:71-78.
310. Gusso S, Hofman P, Lalande S, Cutfield W, Robinson E, Baldi JC. Impaired stroke volume and aerobic capacity in female adolescents with type 1 and type 2 diabetes mellitus. Diabetologia 2008;51:1317-20.
311. Wahl MP, Scalzo RL, Regensteiner JG, Reusch JEB. Mechanisms of Aerobic Exercise Impairment in Diabetes: A Narrative Review. Frontiers in endocrinology 2018;9:181.
312. Moaref A, Faraji M, Tahamtan M. Subclinical left ventricular systolic dysfunction in patients with metabolic syndrome: A case-control study using two-dimensional speckle tracking echocardiography. ARYA Atheroscler 2016;12:254-258.
313. Fournier SB, Reger BL, Donley DA et al. Exercise reveals impairments in left ventricular systolic function in patients with metabolic syndrome. Experimental physiology 2014;99:149-63.
314. Martens P, Verbrugge FH, Nijst P, Dupont M, Mullens W. Limited contractile reserve contributes to poor peak exercise capacity in iron-deficient heart failure. Eur J Heart Fail 2018;20:806-808.
315. Ichinose M, Sala-Mercado JA, Coutsos M et al. Dynamic cardiac output regulation at rest, during exercise, and muscle metaboreflex activation: impact of congestive heart failure. Am J Physiol Regul Integr Comp Physiol 2012;303:R757-68.
316. Abudiab MM, Redfield MM, Melenovsky V et al. Cardiac output response to exercise in relation to metabolic demand in heart failure with preserved ejection fraction. Eur J Heart Fail 2013;15:776-85.
317. Langston C, Kida K, Reed M, Thurlbeck WM. Human lung growth in late gestation and in the neonate. Am Rev Respir Dis 1984;129:607-13.
318. Joshi S, Kotecha S. Lung growth and development. Early Hum Dev 2007;83:789-94.
319. Colin AA, McEvoy C, Castile RG. Respiratory morbidity and lung function in preterm infants of 32 to 36 weeks' gestational age. Pediatrics 2010;126:115-28.
320. Lovering AT, Laurie SS, Elliott JE et al. Normal pulmonary gas exchange efficiency and absence of exercise-induced arterial hypoxemia in adults with bronchopulmonary dysplasia. Journal of applied physiology (Bethesda, Md : 1985) 2013;115:1050-6.
321. Kotecha SJ, Watkins WJ, Paranjothy S, Dunstan FD, Henderson AJ, Kotecha S. Effect of late preterm birth on longitudinal lung spirometry in school age children and adolescents. Thorax 2012;67:54-61.
322. Kaczmarczyk K, Wiszomirska I, Szturmowicz M, Magiera A, Blazkiewicz M. Are preterm-born survivors at risk of long-term respiratory disease? Ther Adv Respir Dis 2017;11:277-287.

323. Goss KN, Beshish AG, Barton GP et al. Early Pulmonary Vascular Disease in Young Adults Born Preterm. Am J Respir Crit Care Med 2018.
324. Powers SK, Coombes J, Demirel H. Exercise training-induced changes in respiratory muscles. Sports Med 1997;24:120-31.
325. Dempsey J, Hanson P, Pegelow D, Claremont A, Rankin J. Limitations to exercise capacity and endurance: pulmonary system. Canadian journal of applied sport sciences Journal canadien des sciences appliquees au sport 1982;7:4-13.
326. Shen Y, Zhang X, Ma W et al. VE/VCO(2) slope and its prognostic value in patients with chronic heart failure. Exp Ther Med 2015;9:1407-12.
327. Arena R, Myers J, Aslam SS, Varughese EB, Peberdy MA. Peak VO2 and VE/VCO2 slope in patients with heart failure: a prognostic comparison. Am Heart J 2004;147:354-60.
328. Arena R, Myers J, Hsu L et al. The minute ventilation/carbon dioxide production slope is prognostically superior to the oxygen uptake efficiency slope. J Card Fail 2007;13:462-9.
329. Kokkinos P, Doumas M, Myers J et al. A graded association of exercise capacity and all-cause mortality in males with high-normal blood pressure. Blood pressure 2009;18:261-7.
330. Kokkinos P, Manolis A, Pittaras A et al. Exercise capacity and mortality in hypertensive men with and without additional risk factors. Hypertension 2009;53:494-9.
331. Kokkinos P, Faselis C, Myers J et al. Statin therapy, fitness, and mortality risk in middle-aged hypertensive male veterans. American journal of hypertension 2014;27:422-30.
332. Shaya GE, Al-Mallah MH, Hung RK et al. High Exercise Capacity Attenuates the Risk of Early Mortality After a First Myocardial Infarction: The Henry Ford Exercise Testing (FIT) Project. Mayo Clin Proc 2016;91:129-39.
333. A Randomized Trial of Intensive versus Standard Blood-Pressure Control. New England Journal of Medicine 2015;373:2103-2116.
334. Johnson HM, Thorpe CT, Bartels CM et al. Antihypertensive Medication Initiation Among Young Adults with Regular Primary Care Use. J Gen Intern Med 2014;29:723-31.
335. Johnson HM, Thorpe CT, Bartels CM et al. Undiagnosed hypertension among young adults with regular primary care use. Journal of hypertension 2014;32:65-74.
336. Battistoni A, Canichella F, Pignatelli G, Ferrucci A, Tocci G, Volpe M. Hypertension in Young People: Epidemiology, Diagnostic Assessment and Therapeutic Approach. High Blood Press Cardiovasc Prev 2015;22:381-8.
337. Pletcher MJ, Bibbins-Domingo K, Lewis CE et al. Prehypertension During Young Adulthood and Coronary Calcium Later in Life: The Coronary Artery Risk Development in Young Adults Study. Annals of internal medicine 2008;149:91-9.
338. Mancia G, Fagard R, Narkiewicz K et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension 2013;31:1281-357.
339. Liu K, Colangelo LA, Daviglus ML et al. Can Antihypertensive Treatment Restore the Risk of Cardiovascular Disease to Ideal Levels?: The Coronary Artery Risk Development in Young Adults

(CARDIA) Study and the Multi-Ethnic Study of Atherosclerosis (MESA). J Am Heart Assoc 2015;4:e002275.
340. Vasan RS, Massaro JM, Wilson PW et al. Antecedent blood pressure and risk of cardiovascular disease: the Framingham Heart Study. Circulation 2002;105:48-53.
341. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;360:1903-13.
342. Mullen SP, Olson EA, Phillips SM et al. Measuring enjoyment of physical activity in older adults: invariance of the physical activity enjoyment scale (paces) across groups and time. Int J Behav Nutr Phys Act 2011;8:103.
343. Latorre Roman PA, Garcia Pinillos F, Navarro Martinez AV, Izquierdo Rus T. Validity and reliability of Physical Activity Enjoyment Scale questionnaire (PACES) in children with asthma. The Journal of asthma : official journal of the Association for the Care of Asthma 2014;51:633-8.
344. Kawel-Boehm N, Maceira A, Valsangiacomo-Buechel ER et al. Normal values for cardiovascular magnetic resonance in adults and children. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 2015;17:29.
345. Thomopoulos C, Parati G, Zanchetti A. Effects of blood pressure lowering on outcome incidence in hypertension: 3. Effects in patients at different levels of cardiovascular risk--overview and meta-analyses of randomized trials. Journal of hypertension 2014;32:2305-14.
346. Lieb W, Enserro DM, Sullivan LM, Vasan RS. Residual Cardiovascular Risk in Individuals on Blood Pressure-Lowering Treatment. J Am Heart Assoc 2015;4.
347. Morawiec B, Kawecki D, Przywara-Chowaniec B et al. Copeptin as a Prognostic Marker in Acute Chest Pain and Suspected Acute Coronary Syndrome. Dis Markers 2018;2018:6597387.
348. Alquezar A, Santalo M, Rizzi M et al. [Combined high-sensitivity copeptin and troponin T evaluation for the diagnosis of non-ST elevation acute coronary syndrome in the emergency department]. Emergencias 2017;29:237-244.
349. Dungen HD, Tscholl V, Obradovic D et al. Prognostic performance of serial in-hospital measurements of copeptin and multiple novel biomarkers among patients with worsening heart failure: results from the MOLITOR study. ESC heart failure 2018;5:288-296.
350. Wang J, Tan GJ, Han LN, Bai YY, He M, Liu HB. Novel biomarkers for cardiovascular risk prediction. J Geriatr Cardiol 2017;14:135-150.
351. Cohn JN. Identifying the risk and preventing the consequences of cardiovascular disease. Heart Lung Circ 2013;22:512-6.
352. Tanaka S, Masuda T, Kamada Y et al. Excessive SBP elevation during moderate exercise discriminates patients at high risk of developing left ventricular hypertrophy from hypertensive patients. Journal of hypertension 2018.
353. Duprez DA, Cohn JN. Identifying early cardiovascular disease to target candidates for treatment. Journal of clinical hypertension (Greenwich, Conn) 2008;10:226-31.
354. Cohn JN, Hoke L, Whitwam W et al. Screening for early detection of cardiovascular disease in asymptomatic individuals. Am Heart J 2003;146:679-85.

355. Zhao W, Swanson SA, Ye J et al. Reactive oxygen species impair sympathetic vasoregulation in skeletal muscle in angiotensin II-dependent hypertension. Hypertension 2006;48:637-43.
356. Bhati P, Shenoy S, Hussain ME. Exercise training and cardiac autonomic function in type 2 diabetes mellitus: A systematic review. Diabetes Metab Syndr 2018;12:69-78.
357. Julius S, Valentini M. Consequences of the increased autonomic nervous drive in hypertension, heart failure and diabetes. Blood Press Suppl 1998;3:5-13.
358. Amaral JF, Borsato DMA, Freitas IMG, Toschi-Dias E, Martinez DG, Laterza MC. Autonomic and Vascular Control in Prehypertensive Subjects with a Family History of Arterial Hypertension. Arq Bras Cardiol 2018;110:166-174.
359. Olson KM, Augeri AL, Seip RL, Tsongalis GJ, Thompson PD, Pescatello LS. Correlates of endothelial function and the peak systolic blood pressure response to a graded maximal exercise test. Atherosclerosis 2012;222:202-7.
360. Beck DT, Martin JS, Casey DP, Braith RW. Exercise training improves endothelial function in resistance arteries of young prehypertensives. Journal of human hypertension 2014;28:303-9.
361. Boulanger CM. Secondary endothelial dysfunction: hypertension and heart failure. J Mol Cell Cardiol 1999;31:39-49.
362. Ashor AW, Lara J, Siervo M et al. Exercise modalities and endothelial function: a systematic review and dose-response meta-analysis of randomized controlled trials. Sports Med 2015;45:279-96.
363. Julius S, Nesbitt S. Clinical consequences of the autonomic imbalance in hypertension and congestive heart failure. Scand Cardiovasc J Suppl 1998;47:23-30.
364. Moinuddin A, Gupta R, Saxena Y. Autonomic Function Tests in Prehypertensive Young Adult Males of Uttarakhand Region. Indian journal of physiology and pharmacology 2016;60:45-51.
365. Guazzi M, Brenner DA, Apstein CS, Saupe KW. Exercise intolerance in rats with hypertensive heart disease is associated with impaired diastolic relaxation. Hypertension 2001;37:204-8.
366. Kannan A, Janardhanan R. Hypertension as a risk factor for heart failure. Curr Hypertens Rep 2014;16:447.
367. Diez J. Arterial hypertension in patients with heart failure. Heart Fail Clin 2014;10:233-42.
368. Karaye KM, Saidu H, Bala MS, Yahaya IA. Prevalence, clinical characteristics and outcome of pulmonary hypertension among admitted heart failure patients. Ann Afr Med 2013;12:197-204.
369. Williamson W, Reid H, Boardman H et al. The Effectiveness of Physical Activity Interventions to Reduce Blood Pressure in Young Adults with Increased Cardiovascular Risk: A Systematic Review and Meta-Analysis. Heart 2015;101:A89-A89.
370. Zhang Y, Moran AE. Trends in the Prevalence, Awareness, Treatment, and Control of Hypertension Among Young Adults in the United States, 1999 to 2014. Hypertension 2017;70:736-742.
371. Kokkinos PF, Narayan P, Colleran JA et al. Effects of regular exercise on blood pressure and left ventricular hypertrophy in African-American men with severe hypertension. The New England journal of medicine 1995;333:1462-7.
372. Olsen MH, Angell SY, Asma S et al. A call to action and a lifecourse strategy to address the global burden of raised blood pressure on

current and future generations: the Lancet Commission on hypertension. Lancet 2016;388:2665-2712.
373. Dores H, Santos P, Salvador F et al. Blood pressure in young adults. Rev Port Cardiol 2010;29:1495-508.
374. Kokkinos P, Pittaras A, Manolis A et al. Exercise capacity and 24-h blood pressure in prehypertensive men and women. American journal of hypertension 2006;19:251-8.
375. Jung MH, Ihm SH, Lee DH, Chung WB, Jung HO, Youn HJ. Prehypertension is associated with early complications of atherosclerosis but not with exercise capacity. Int J Cardiol 2017;227:387-392.
376. Mazic S, Suzic Lazic J, Dekleva M et al. The impact of elevated blood pressure on exercise capacity in elite athletes. Int J Cardiol 2015;180:171-7.
377. Yap J, Tan JL, Le TT et al. Assessment of left ventricular preload by cardiac magnetic resonance imaging predicts exercise capacity in adult operated tetralogy of Fallot: a retrospective study. BMC Cardiovasc Disord 2014;14:122.
378. Wang KL, Cheng HM, Chuang SY et al. CENTRAL OR PERIPHERAL SYSTOLIC OR PULSE PRESSURE: WHICH BEST RELATES TO TARGET-ORGANS AND FUTURE MORTALITY? Journal of hypertension 2009;27:461-7.
379. Hodson B, Norton GR, Booysen HL et al. Brachial Pressure Control Fails to Account for Most Distending Pressure-Independent, Age-Related Aortic Hemodynamic Changes in Adults. American journal of hypertension 2016;29:605-13.
380. Zuo J, Chu S, Tan I, Butlin M, Zhao J, Avolio A. Association of Haemodynamic Indices of Central and Peripheral Pressure with Subclinical Target Organ Damage. Pulse (Basel) 2018;5:133-143.
381. O'Rourke MF, Adji A. Noninvasive studies of central aortic pressure. Curr Hypertens Rep 2012;14:8-20.
382. Papaioannou TG, Karageorgopoulou TD, Sergentanis TN et al. Accuracy of commercial devices and methods for noninvasive estimation of aortic systolic blood pressure a systematic review and meta-analysis of invasive validation studies. Journal of hypertension 2016;34:1237-48.
383. Avolio A. Central Aortic Blood Pressure and Cardiovascular Risk. A Paradigm Shift? 2008;51:1470-1471.
384. Ram CV. Central aortic blood pressure assessment and cardiovascular risk. Indian Heart J 2010;62:13-6.
385. McEniery CM, Cockcroft JR, Roman MJ, Franklin SS, Wilkinson IB. Central blood pressure: current evidence and clinical importance. Eur Heart J 2014;35:1719-25.
386. Bulas J, Potocarova M, Murin J, Kozlikova K, Luha J, Caprnda M. Central Systolic Hypertension in Patients with Well-Controlled Hypertension. Biomed Res Int 2017;2017:8158974.
387. Hashimoto J. Central Hemodynamics for Management of Arteriosclerotic Diseases. J Atheroscler Thromb 2017;24:765-778.
388. McGaughey TJ, Fletcher EA, Shah SA. Impact of Antihypertensive Agents on Central Systolic Blood Pressure and Augmentation Index: A Meta-Analysis. American journal of hypertension 2016;29:448-57.
389. Dahlof B, Devereux RB, Kjeldsen SE et al. Cardiovascular morbidity and mortality in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. Lancet 2002;359:995-1003.

390. Carlberg B, Samuelsson O, Lindholm LH. Atenolol in hypertension: is it a wise choice? Lancet 2004;364:1684-9.
391. Piskorz D, Bongarzoni L, Citta L et al. Functional Organ Damage in Cardiovascular Low Risk Patients with High Central Aortic Pressure. High Blood Press Cardiovasc Prev 2015;22:281-7.
392. Kannel WB. Elevated systolic blood pressure as a cardiovascular risk factor. The American Journal of Cardiology 2000;85:251-255.
393. Lin YP, Han CL, Lin GM. Reverse epidemiology of systolic blood pressure levels on admission with the mortality risk in heart failure: Is it a matter of obesity paradox? J Cardiol 2017;69:596.
394. Subherwal S, de las Fuentes L, Waggoner AD, Heuerman S, Spence KE, Davila-Roman VG. Central Aortic Pressure is Independently Associated With Diastolic Function. Am Heart J 2010;159:1081-8.
395. Rosenwasser RF, Shah NK, Smith SM et al. Baseline predictors of central aortic blood pressure: a PEAR substudy. Journal of the American Society of Hypertension : JASH 2014;8:152-8.
396. Ashor AW, Lara J, Siervo M, Celis-Morales C, Mathers JC. Effects of exercise modalities on arterial stiffness and wave reflection: a systematic review and meta-analysis of randomized controlled trials. PloS one 2014;9:e110034.
397. Stergiopulos N, Meister JJ, Westerhof N. Determinants of stroke volume and systolic and diastolic aortic pressure. The American journal of physiology 1996;270:H2050-9.
398. Pierce DR, Doma K, Leicht AS. Acute Effects of Exercise Mode on Arterial Stiffness and Wave Reflection in Healthy Young Adults: A Systematic Review and Meta-Analysis. Front Physiol 2018;9.
399. Diaz A. Reference Intervals of Central Aortic Blood Pressure and Augmentation Index Assessed with an Oscillometric Device in Healthy Children, Adolescents, and Young Adults from Argentina. 2018;2018.
400. Lee SW, Youm Y, Kim CO et al. Association between skeletal muscle mass and radial augmentation index in an elderly Korean population. Archives of gerontology and geriatrics 2014;59:49-55.

# APPENDICES

YACHT Study Participant Information Leaflet

Physical Activity Enjoyment Scale (PACES) Questionnaire

**DIVISION OF CARDIOVASCULAR MEDICINE**
**RADCLIFFE DEPARTMENT OF MEDICINE**
**UNIVERSITY OF OXFORD**

Tel:. +44(0) 1865 572832
e-mail : yacht@cardiov.ox.ac.uk

Oxford University Hospitals NHS
NHS Trust

# Young Adult Cardiovascular Health Trial 2 (YACHT2)

## Participant Study Information Leaflet

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish.

- Part 1 tells you the purpose of this study and what will happen to you if you take part.
- Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information, contact details are provided at the bottom of Part 2. Thank you for reading this.

## PART 1

**What is the purpose of the study?**

There is preliminary evidence that early life factors and events from the time of birth may be associated with differences in blood pressure and exercise capacity as adults. Recently, we have reported that specific events around birth, such as being born prematurely or having a mother who had high blood pressure while pregnant may affect heart and blood vessel structure and function. We are now conducting a study on young people to investigate if any of these features have an influence on exercise capacity, and whether this relates to alterations in heart, brain, liver and blood vessel structure and function, including variations in blood pressure. This will help us understand whether these changes are relevant to the development of high blood pressure. High blood pressure is associated with increased risk of cardiovascular diseases, such as heart attacks and stroke in later life. High blood pressure is a common problem, affecting 1 in 4 of the adult population in the United Kingdom. The information from this study will help us understand more about young adult cardiovascular health and the effects of prematurity and events around the time of birth on adult health.

**Why have I been invited?**

We are inviting you to take part because we want to recruit volunteers with a range of birth histories and different blood pressures. We would like to include participants who were born preterm (delivered less than 37 weeks gestation) and those who were born full term (delivered after 37 weeks gestations). We also wish to include participants with a range of different blood pressures, including people who know they have high blood pressure. We will be including people who are attending the John Radcliffe Hospital, as well as members of the local community, and participants from previous studies who have had details on birth history and early life collected.

**Do I have to take part?**

Participation in this study is entirely voluntary and you are free to refuse to take part or to withdraw from the study at any time without giving a reason, and without this affecting your future medical care. If you decide to take part, a copy of this information leaflet and consent form will be given to you to keep.

**What would happen to me if I took part?**

If you are interested in taking part in the study you will first be contacted by the study team to arrange a telephone conversation. This will be to answer any questions you might have and to discuss the study further with you. If you are willing, we will seek and record your verbal consent to discuss your participation in the study. We will then ask you to tell us about your current medical history and recall your birth history to make sure there are no reasons you should not take part in the study. Provided you are happy to continue we will then arrange a convenient time for you to meet the researchers for your study visits. You will be asked to attend the Cardiovascular Clinical Research Facility at the John Radcliffe Hospital in Oxford.

We are planning a baseline assessment on all of our participants which will take a total time commitment of between 4-5 hours. The first visit is to profile your cardiovascular system and measure your exercise capacity on a bicycle. For the initial baseline assessment it is possible to complete the study measures on a single morning visit, but if you prefer, we can split the study over two days. If the measures are completed over two visits we would complete the MRI imaging during one visit which would last approximately 2 hours. The other study visit would cover the other cardiovascular measures and exercise testing described below. This visit would last approximately 3 hours. We will provide the option of having MRI imaging of the brain. This can be completed during the MRI visit or as a separate visit, depending on the availability of the scanner and your preferences. We cannot guarantee that this visit can be completed during the MRI visit, but please let us know your preference. You will need to be fasted for 6 hours prior to attending for all study visits. Light refreshments will be offered after each visit. Travel expenses will be reimbursed by filling a claim form and providing receipts.

**MRI Study Visit**

Below is a description of the Magnetic Resonance Imaging study visit. **Start of Visit (15 minutes)**

- We will ask you to come to the Oxford Centre for Clinical Magnetic Resonance Research, having fasted for 6 hours beforehand, where you will be met by the study investigator.
- The study investigator will explain the study visit and you will first give your consent in writing to take part in the study.

- We will insert a needle connected to a tube, called a cannula, into a vein on the back of your hand or forearm to allow us to deliver contrast dye into your system and take blood without having to perform multiple injections. Please let us know if you would like to lie down while this is done.
- During the study visits we will ask you to donate a total of up to 80mLs (or 16 teaspoons) of blood throughout the course of the study. If you prefer to complete the study measures in a single visit, the first sample of blood, approximately 20mLs will be taken before the MRI scanning. If the study measures are being completed over two visits, the first sample of blood, approximately 20mLs will be taken before the Cardiopulmonary Exercise Testing. The blood samples will be stored and analysed for substances and genes involved in blood vessel function and cardiovascular disease development.

  To ensure the cannula remains unblocked, in order to allow collection of further blood samples, the cannula may need to be flushed with 5mls of salty water called normal saline after each blood sample is taken. This would equate to up to a total of 20mls of normal saline being used to keep the the cannula from becoming blocked. This is a safe method used in clinical practice. The cannula is intended to allow delivery of contrast dye, and for collection of blood without the need for multiple needle pricks. It is also possible for participants to have conventional blood sampling, involving a number of needle pricks, if your prefer.

**MRI Scanning of the Liver and Heart (80 minutes)**

You will have an MRI scan of your liver and heart. This will take place in the Oxford Centre for Clinical Magnetic Resonance Research which is also in the John Radcliffe Hospital. MRI scans are safe, non-invasive and do not involve any ionizing radiation (x-rays). However, they do involve the use of a strong magnetic field, so if you have any of the following, you would not be suitable for a scan, and would not be able to take part in this study:

- a permanent pacemaker
- metal clips in blood vessels of the brain
- an injury to the eye involving fragments of metal
- shrapnel injuries
- other metal or electronic implants affected by the magnetic field

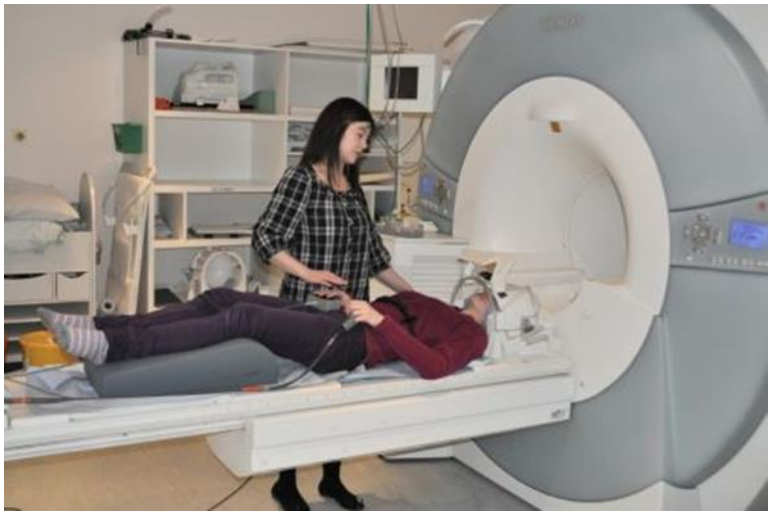


We will ask you to complete a safety questionnaire to confirm there are no reasons we should not do the scan. The MRI scanner is shaped like a polo mint, the hole inside measuring about 60 centimetres wide. You will be asked to lie still on your back while your liver and heart are scanned. You will be asked to breathe in and out and hold your breath for several seconds for some of the scans. The MRI machine has a tunnel called its"bore" that you will be positioned in while the scans are obtained. While you lie still on the MRI couch we will slide you into the bore of the scanner so you are in the right position to capture the images. The scanner can be noisy so you will be provided with correctly fitted ear phones and will be able to listen to music.

- In order to evaluate fully the blood circulation and the heart muscle, we shall inject some contrast dye, called Gadolinium or GAD, through the cannula in your arm. This is generally considered safe.
- During the MRI scan you will hear some loud noises (earplugs provided). The radiographer and researcher will be able to see you throughout the scan and will provide you with a call button which you can press at any time.

**Optional Brain MRI Scan (30 minutes)**

In addition to the heart and liver MRI we are inviting our participants to have an optional brain MRI scan. There will be no obligation to have the brain scan



- For the brain MRI scan while lying still on your back, with your head resting on a pillow a small frame will be positioned around your head. The frame is called a coil which optimises the MRI images. To optimise the MRI image it is important that you keep your head in the same position.

  If you are completing the study in one visit, we will then bring you back to the Oxford Cardiovascular Clinical Research Facility for the rest of the study visit. The remainder of the visit is described below.If you are completing the study in more than one visit, once we have finished scanning you, we will provide you with refreshments, and this will be the end of the study visit. In some cases, dependent on the availability of the MRI scanner and operator support, the MRI of heart and liver may need to be done at a separate time to the brain scan. The MRI of the brain would then be completed as a separate visit.

**Cardiovascular Assessment and Exercise Visit**

**Start of Visit**

- If you are attending as a separate visit, we will ask you to come to the Oxford Cardiovascular Clinical Research Facility, having fasted for 6 hours beforehand, where you will be met by the study investigator. The study investigator will explain the study visit and ensure you have provided consent in writing to take part in the study.

**Questionnaire and Anthropometric Measures (45 minutes)**

- We will give you the study questionnaire at the start of the visit, it takes approximately 35 minutes to complete. You will be encouraged to complete the questionnaire in the time provide. However, if

required, there will be an option to complete the questionnaire at home. We will give you a stamped addressed envelope to return the questionnaire.

- You will then have some simple measures made of your body composition, so this means measurement of your weight, height, and an estimate of your body fat using skinfold measurements.
- We will ask you to give a urine sample so that we can test for the presence of micro-albuminuria, an indicator of cardiovascular and blood vessel function related to blood pressure.

**Vascular Measures (40minutes)**

- We will measure the small blood vessels on the back of your middle finger using a microscope. We will ask you to place your finger under a microscope lens. Light will be shone on your finger and with the microscope we will record the number of small blood vessels visible on your finger. It is a painless, non-invasive procedure.
- We will also measure how stiff your blood vessels are using a device connected to 4 blood pressure cuffs on each limb. You will be asked to lie flat on a bed for this test and we will put the blood pressure cuffs on your arms and legs to take the measurements.

**Echocardiogram (30 minutes)**

We will perform an ultrasound (Echocardiogram or ECHO) of your heart. You will be asked to take your clothing off down to your waist and put on a hospital gown that is untied at the front to allow the study investigators access to place the ultrasound probe on your chest. You will be asked to lie on a couch on your left side. The probe is placed on your chest and lubricating jelly is used so the probe makes good contact with the skin. Ultrasound waves then create images of your heart on the scanner monitor. In addition to ultrasounding the heart the study team will take ultrasound images of the arteries in your neck. This will involve lying still on your back while the ultrasound probe is placed over the side of your neck. The study team are trained to perform ultrasound and echocardiograms, and will ensure that your privacy is maintained throughout the visit. Women can request that a female echocardiographer can perform this part of the study.

**Cardiopulmonary Exercise Testing (55minutes)**

- Exercise testing will allow assessment of your heart and lung function during exercise and is the same test used in young people and athletes to see how well they respond to exercise. The exercise test will be performed on a stationary bike. You will be wearing a mask over your mouth and nose whilst you are exercising; which will allow us to measure your oxygen use and fitness. You will be

taught to communicate with the mask on, and you may stop the test at any time for any reason. As you cycle on the bike, various pieces of equipment will be used to monitor your body's response such as your heart rate and blood pressure. You will thus have a blood pressure cuff on your arm and small sticky pads will be placed on your chest and connected to a machine which will make a tracing of your heart rhythm. We will also attach two sticky sensors to the thigh muscle on one of your legs so we can measure the oxygen used by the muscle during exercise. You will need to wear appropriate clothing to exercise. We will need to be able to access the skin on your chest and torso and your upper thigh to be able to attach the monitoring equipment.

- The exercise testing will be in two parts. First you will perform a test lasting approximately 10-15 minutes where the resistance you pedal against gradually increases. The test is designed to be increasingly difficult as it progresses and, at the end, you will exercise at the hardest intensity for about 3-4 minutes. This is likely to make you feel quite exhausted, breathe heavily and your muscles may ache, particularly in those unaccustomed to exercise.
- Before, during and after the first exercise test we will use the cannula in the back of your hand to take a sample of blood. This will allow the study team to measure changes in your blood associated with exercising, such as changes in your blood sugars and fats and markers relating to the function of your blood vessels. If you prefer, we do not need to use the cannula for this part of the study, and can use conventional methods of blood sampling instead. This would involve multiple needle pricks.

- At the end of the first bout of exercise you will be able to get off the bicycle and take the mask off your face. You will then have a rest for 20 minutes before exercising again. This time you will not need to wear the mask. You will be asked to exercise for 3 short intervals up to 3 minutes each. The resistance pedalled against will be increased at the start of each interval. During each interval we will take measures of your blood pressure and a scan of your heart using the ultrasound machine. The ultrasound of your heart is similar to the procedure described above, this time the study investigator will take images of your heart while you exercise. The ultrasound probe will be placed on your chest and lubricating jelly is used so the probe makes good contact with the skin. Ultrasound waves then create images of your heart on the scanner monitor.

**Fitting of 24 hour and 9 day monitoring equipment (10minutes)**

- At the end of the visit, we will ask you to wear an activity monitor which is similar to a wrist watch which will measure your physical activity for 9 days.
- We will also ask you to wear a blood pressure monitor for 24 hours. It consists of a blood pressure cuff worn on the upper arm that easily fits under clothing. The cuff is attached by a lead to a small

box which is similar in size to a Walkman. This can be placed in a pocket or attached to a belt which the study team will provide you. This portable device will measure blood pressures half hourly during the day and hourly at night. This will mean the cuff inflates and deflates every 30 minutes during the day and every 60 minutes at night. It is unlikely that it will disturb your sleep. We ask that you do not drive with the device turned on and we will show you how to turn the device on and off.

- We will also ask you to wear a heart rhythm monitor for 24 hours. This will measure how the speed of your heart varies over the course of the day. It is the size of a mobile phone and connected via wires to 3 stickers that will be placed on your torso.
- We will provide a stamped addressed envelope to return the three devices to us after this time. If we do not receive the devices back after 2 weeks we will give you a telephone call to remind you to return the devices.

**Expenses**

Travel expenses will be reimbursed if you provide receipts and complete a claim form. Light refreshments will be available. As a thank for participation we would like to provide you with a £20 Amazon Voucher following each of your study visits.

**What will I have to do?**

- Attend 1 to 3 visits at the Oxford Cardiovascular Clinical Research Facility and the Oxford Centre for Clinical Magnetic Resonance Research at the John Radcliffe Hospital
- Consent to taking part in this study by signing a form
- We would ask for you to fast for a minimum of 6 hours prior to your visit. If you are attending in the morning this would mean fasting overnight and avoiding having any breakfast on the morning of the study (ie eat normally the night before and avoid any breakfast on the morning of the scan). If you are attending an afternoon visit you can have a light breakfast before 8am in the morning.
- Undergo the procedures as described above.
- Complete the study questionnaire and if you take it home to complete we will ask you to return the questionnaire in the stamped addressed envelope when returning the study devices.
- There is no training required for the exercise test, but you are asked to not do any strenuous exercise 48 hours prior to the test. During the test you will need to wear comfortable, loose-fitting clothing and comfortable shoes. We will need to be able to access the skin on your chest to attach the monitoring and the skin on the upper thigh of one of your legs.
- We will ask you to wear the study monitors and return to the research team with the envelope provided.

**Are there any side effects of participating?**

- Some people find having a cannula in their arm uncomfortable and there can be bruising at the site of needle entry. Our staff are highly trained in cannula insertion and we will make sure you are as comfortable as possible;
- The magnetic resonance and ultrasound scans have no known adverse effects. However, the use of magnets means that the scan procedure is not suitable for people with magnetic pacemakers, mechanical heart valves, other implants such as an aneurysm clip, hip replacement, or if you carry other pieces of metal that have accidentally entered your body (for instance during a car accident). Also, if you have a history of seizures or are currently claustrophobic you should not take part. As some scans are noisy, we would give you earplugs to make this quieter for you. It is important that these are fitted correctly as they are designed to protect your ears. Some people may experience a mild dizzy sensation as they are moved into the scanner. This is normal and the sensation starts to go away as soon as you are in the scanner. Although we believe this is a very safe study, appropriate resuscitation facilities will be in place.
- The gadolinium contrast agent has occasionally been thought to cause headache and mild rash.
- You will experience an increase in your breathing and heart rate during the exercise testing which is a normal response. Occasionally, some people may feel discomfort in their legs following the exertion of the exercise. You may also sweat and it is important to wear appropriate clothing to exercise in.

**Are there any possible risks from taking part?**

It is not felt that there are any significant disadvantages or risks for you in taking part.

- While there is no evidence to suggest that MRI is harmful during pregnancy, the Department of Health advises against scanning pregnant women. We do not test for pregnancy for this study and if you think you may be pregnant you should not take part in this study.
- The gadolinium contrast agent very rarely (less than one in a thousand times) can cause a severe allergic reaction. In people with healthy kidneys, gadolinium is removed from the bloodstream within a few hours. If we have any concerns we will take a sample of blood to test your kidney function to make sure you are able to process the dye. We will not give you the dye if we have any concerns.
- Cardiopulmonary exercise testing is very safe and is even used in people with heart failure. However, as can occur with all forms of hard exercise, very occasionally some people have significant changes in their heart rate and rhythm that requires medical attention. Although the risk of this happening is small, the test is carried out in a room equipped with emergency

monitoring, emergency medications and resuscitation equipment. Medical personnel will be available throughout the testing. Your heart rate and electrocardiography are monitored throughout the exercise testing and if there are any concerns you would be asked to stop exercising.

**What are the possible benefits of taking part?**

There will be no specific benefits for you of taking part but we hope the findings will identify new ways to prevent cardiovascular disease in the future.

**What happens when the research study stops?**

You will officially end participation in this study. Copies of any publications connected to this study will be available from our research team on request.

**Will my taking part in the study be kept confidential?**

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. Detailed information is given in Part 2.

***If the information in Part 1 has interested you and you are considering participation, please read the additional information in Part 2 before making any decision.***

## PART 2

**Unexpected findings on your tests**

In the unlikely event the tests we do during the study show any significant abnormalities, a designated clinical specialist will discuss the implications with you and may arrange for further investigations as necessary. However, it is important to note that we do not carry out tests for diagnostic purposes, and therefore these tests are not a substitute for a clinical appointment. Rather, our tests are intended for research purposes only. So if we find anything unusual, it would be appropriate for the study team to contact your GP so that they can arrange ongoing clinical care for you. But we would only do this after we and the specialist had discussed your options and gained your permission.

**What will happen if I don't want to carry on with the study?**

You are free to withdraw from the study at any time. If you wish, we can then just make use of the information we already have about you. We will ensure that, if your samples and information are used for future research, they are anonymous, or we can destroy any identifiable samples or information we hold about you**.**

**What if something goes wrong or I have a complaint?**

If you wish to complain about any aspect of the way in which you have been approached or treated during the course of this study, you should contact Professor Paul Leeson, 01865572846 paul.leeson@cardiov.ox.ac.uk or you may contact the University of Oxford Clinical Trials and Research Governance (CTRG) office on 01865 572224 or the head of CTRG, email CTRG@admin.ox.ac.uk.

*Harm: The University has arrangements in place to provide for harm arising from participation in the study for which the University is the Research Sponsor.*

**Will my taking part in the study be kept confidential?**

i) With your consent we will inform your GP of your participation in the study and place a copy of your signed informed consent form in your hospital notes if they are accessible.

ii) All information which is collected about you during the course of the research will be kept strictly confidential. Information and samples that relate to you will be stored with a unique study code number that is not immediately personally identifiable but will allow the study team to link together the different types of information.

iii) We will keep your unique study code number in a separate secure database within the Division of Cardiovascular Medicine at the University of Oxford that will allow the study team to link the code number to your personal details so that we can contact you in the future about any matters relating to the study.

iv) Responsible members of the University of Oxford may be given access to data for monitoring and/or audit of the study to ensure we are complying with regulations. All study investigators and responsible members of the University of Oxford have a duty of confidentiality to you as a research participant.

**Participation in future research**

We will ask if we can contact you about future studies. If you consent, we will keep your contact details separately from research data you have provided. Both your details and data will carry the same unique ID. This means your data is anonymised but that we can 'link' details to data. In this way we can approach subjects about studies relevant to their particular history. You can withdraw your consent for future contact at any time.

**Involvement of the general practitioner**

At the start of the visit we will ask for your consent to inform your GP of your participation in the study. No extra visit to your GP will be required. In the unlikely event of us finding anything unusual in the tests during the study we will ask for your consent to inform your GP.

**What will happen to any samples I give?**

All samples will be retained in a secure environment for future analysis and will be stored in an anonymous format at the John Radcliffe Hospital and Wellcome Trust Centre for Human Genetics, University of Oxford under the custodianship of the Division of Cardiovascular Medicine. The samples of serum and DNA will be stored for at least 10 years and may be used in future ethically approved studies as our understanding of blood vessel function grows. Future research may include genetic research (see below).

**Will any genetic tests be done?**

It is possible that some samples will be used for genetic research. This research may be conducted by the study research team or collaborating research teams. The samples will be stored in an anonymous format but a record of who donated the samples will be kept so that we can relate any findings to your medical history. Keeping these records ensures that if you decide to withdraw your consent for us to keep your data, we will be able to destroy your samples. The genetic tests would involve looking at common variations in genes that affect how blood vessels work. We do not propose to test for inherited genetic diseases, or for conditions that will involve any other members of your family. There is no evidence to suggest that the results of these genetic studies are likely to have significant implications for you personally.

**What will happen to the results of the research study?**

We anticipate that the results will be published in a scientific journal for the benefit of the wider medical community. However, individual patients will not be identified in any publication and your personal and clinical details will remain strictly confidential. Any scientific publications arising from the study will be available on request to all participants as well as a summary of the main findings. You would have no legal right to a share of any profits that may arise from the research. The information generated from the study may be used by research team members working towards higher postgraduate degree qualifications and doctoral thesis.

**Who is organising and funding the research?**

The investigators are Professor Paul Leeson, Dr Wilby Williamson and Dr Adam Lewandowski. If you wish to know more about any aspect of the study, please contact Dr Wilby Williamson on 01865 572832 or via the study email yacht@cardiov.ox.ac.uk. The research is funded by the British Heart Foundation HSR00020 (BHF Ref: PG/13/58/30397). It has been organised by researchers at the University of Oxford, and the University of Oxford will act as research sponsor for this study.

**Who has reviewed the study?**

This study was given a favourable ethical opinion for conduct by the South Central: Berkshire Research Ethics Committee (Ref: 14/SC/0275).

**Where can I find independent information about taking part in research?**

You can visit the website for INVOLVE at http://www.invo.org.uk/, which gives information about public involvement in research studies.

**Further information and contact details**

Please feel free to contact our research team in Oxford if you require any further information about the study. If you ask to know more about the research, we will then contact you in the next few weeks.

Contact details for further information:

Dr Wilby Williamson, Clinical Research Fellow and Doctoral Student (Division of Cardiovascular Medicine)
wilby.williamson@cardiov.ox.ac.uk Tel 01865 572832 or 01865 572831

Dr Adam Lewandowski, Postdoctoral Research Fellow (Division of Cardiovascular Medicine)
adam.lewandowski@cardiov.ox.ac.uk Tel 01865 572832 or 01865 572831

**Thank you for taking time to read this information sheet and considering taking part in the study**

| | |
|---|---|
| Study Full Title | Young Adult Cardiovascular Health Trial 2 |
| Participant ID | |
| Researchers | Professor Paul Leeson, Dr Wilby Williamson, Dr Henry Boardman, Dr Kenny McCormick, Ms Yvonne Kenworthy, Dr Brenda Kelly, Dr Lucy Mackillop, Dr Adam Lewandowski, Dr Charlie Foster, Dr Aiden Doherty |

**Please initial box**

| | |
|---|---|
| 1. I confirm that I have read and understand the information sheet (version 2.4, October 2014) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | |
| 2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. | |
| 3. I understand that relevant sections of my medical notes (including birth & neonatal records) and data collected during the study may be looked at by authorized individuals from the University of Oxford, from regulatory authorities, or from the Oxford University Hospitals NHS Trust where it is relevant to my taking part in this research. I permit these individuals access to my records. | |
| 4. I understand why blood samples are being taken, how the samples will be collected, that giving samples for this research is voluntary and that I am free to withdraw my approval for use of the sample at any time without my legal rights being affected. I understand that data collected from the samples will be used in the results of the research study. | |
| 5. I understand my blood samples may be used by the study group or shared with research collaborators for the purposes of genetic research aimed at understanding the genetic influence on blood vessel function, but the results of these investigations are unlikely to have any implications for me personally. | |
| 6. I agree to gift blood samples taken for the purpose of the research study to the University of Oxford. If a commercial product were developed as a result of this study, I will not profit financially from such a product. | |
| 7. I understand that anonymised images of my body, taken as part of magnetic resonance/ultrasound scans, may be used in research publications. I give consent for future use of images for any publications that may arise from the research. | |
| 8. I understand that my samples will be stored in anonymised format as outlined in the information sheet and may also be used in future ethically approved research and research publications | |
| 9. I understand that I will be informed if the results of any medical tests done as part of the research are important for my health and that my GP will also (with my permission) be informed of the results of medical tests performed as part of the research which are important for my healthcare. | |
| 10. I agree to take part in the above study. | |
| 11. (Optional). I agree to have brain imaging during the Magnetic Resonance Imaging study measures. | |
| 12. (Optional). I agree for my GP to be informed of my participation in the study. | |
| 13. (Optional). I agree to being contacted in the future to ask if I am interested in future related studies. | |

| | | |
|---|---|---|
| | | |
| Name of participant | Signature | Date |
| | | |
| Name of person taking consent | Signature | Date |

*For researchers: original kept(tick) ( ): copy given to participant (tick) ( )*

# PHYSICAL ACTIVITY ENJOYMENT SCALE

**41. Please rate how you feel at the moment about the physical activity you have been doing. Below is a list of feelings with respect to physical activity. For each feeling, please circle the number that best describes you.**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| a. | I enjoy it | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I hate it |
| b. | I feel bored | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I feel interested |
| c. | I dislike it | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I like it |
| d. | I find it pleasurable | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I find it unpleasurable |
| e. | I am very absorbed in this activity | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I am not at all absorbed in this activity |
| f. | It's no fun at all | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's a lot of fun |
| g. | I find it energizing | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I find it tiring |
| h. | It makes me depressed | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It makes me happy |
| i. | It's very pleasant | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's very unpleasant |
| j. | I feel good physically while doing it | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I feel bad physically while doing it |
| k. | It's very invigorating | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's not at all invigorating |
| l. | I am very frustrated by it | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I am not at all frustrated by it |
| m. | It's very gratifying | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's not at all gratifying |
| n. | It's very exhilarating | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's not at all exhilarating |
| o. | It's not at all stimulating | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's very stimulating |
| p. | It gives me a strong sense of accomplishment | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It does not give me any sense of accomplishment |
| q. | It's very refreshing | 1 | 2 | 3 | 4 | 5 | 6 | 7 | It's not at all refreshing |
| r. | I felt as though I would rather be doing something else | 1 | 2 | 3 | 4 | 5 | 6 | 7 | I felt as though there was nothing else I would rather be doing |