Exercise tolerance in ageing and hypertrophic cardiomyopathy: from pathophysiology to a lifestyle intervention

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Declaration

This thesis is submitted for the degree of Doctor of Philosophy at Newcastle University. I, Amy Susan Fuller declare that the data and all other information in the present thesis is my own original research, unless stated otherwise. I certify that no material in the present thesis has been submitted for any other degree or qualification, and that all published material was the result of research carried out during the present doctoral study.

Dedication

This thesis is dedicated to my mother, Miss Susan P. Smith.

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List of abbreviations

ABPR	Abnormal blood pressure response
ACEi	Angiotensin-converting enzyme inhibitor
ACSM	American College of Sports Medicine
ACTC1	Actin alpha cardiac muscle 1
ADP	Adenosine diphosphate
AG	Antigen retrieval
AIC	Aaike information criterion
AT	Anaerobic threshold
ATP	Adenosine triphosphate
AF	Atrial fibrillation
ALT	Alanine aminotransferase
ARB	Angiotensin receptor blocker
BID	Twice daily
BMI	Body mass index
CI	Complex I
CII	Complex II
CIII	Complex III
CIV	Complex IV
CV	Complex V
CO	Cardiac output
CoA	Acyl-coenzyme A

CPET	Cardiopulmonary exercise testing
CRP	C-reactive protein
CV	Cardiovascular
CVP	Central venous pressure
DNA	Deoxyribonucleic acid
e'	Mitral annular early diastolic velocity
E/A	Early/ late transmitral diastolic velocity
ECG	Electrocardiogram
EDTA	Ethylenediamine tetra-acetic acid
EDVLV	End diastolic volume of the left ventricle
eGFR	Estimated glomerular filtration rate
ETC	Electron transport chain
$FADH_2$	Flavin adenine dinucleotide
FDA	Food and Drug Administration
HADC1	Histone deactylase 1
HADS	Hospital Anxiety and Depression Scale
HbA1C	Haemoglobin A1c
HCM	Hypertrophic Cardiomyopathy
HDL-C	High density lipoprotein cholesterol
HF	Heart Failure
HFpEF	Heat failure and preserved ejection fraction
HMG-CoA	β-Hydroxy β-methylglutaryl-CoA

HR	Heart Rate
GTP	guanosine triphosphate-binding proteins
ICD	Implantable cardioverter-defibrillator
IFM	Interfibrillar mitochondria
IMS	Intermembrane space
INR	International normalised ratio
LAVi	Left atrial volume index
LDL-C	Low density lipoprotein cholesterol
LME	Linear mixed effect
LV	Left ventricular
LVEF	Left ventricular ejection fraction
LVH	Left ventricular hypertrophy
LVIDd	LV internal diameter end-diastole
LVOT	Left ventricular outflow tract
LVOTO	Left ventricular outflow tract obstruction
MAP	Mean arterial pressure
MLHF	Minnesota living with heart failure questionnaire
MTCO1	Cytochrome c oxidase subunit I
MtDNA	Mitochondrial deoxyribonucleic acid
MYBPC3	Myosin binding protein C
MYH7	B-myosin heavy chain 7
MYL2	Myosin regulatory light chain 2

MYL3	Myosin regulatory light chain 3
NAD+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide plus hydrogen
NDUFB8	NADH: ubiquinone oxidoreductase subunit B
NF-kB	Nuclear factor-kB
NGS	Normal goat Serum
NICOM	Non-invasive cardiac output monitoring
NLME	Nonlinear mixed effect
NO_2^-	Nitrite
NO_3^-	Dietary nitrate
NO	Nitric oxide
NOS	Nitric oxide synthase
NPC	No Primary Control
NSVT	Non-sustained ventricular tachycardia
NT-proBNP	N-terminal pro-brain natriuretic peptide
NUTH	Newcastle Upon Tyne Hospitals
NYHA	New York Heart Association
OXPHOS	Oxidative phosphorylation
PGC	Peroxisome proliferator-activated receptor gamma coactivator
PLWd	Posterior wall thickness at end-diastole
Q	Coenzyme Q
Qd	Once a day

QoL	Quality of Life
REML	Residual maximum likelihood
RER	Respiratory exchange ratio
ROS	Reactive oxygen species
SCD	Sudden cardiac death
SF-36	Short Form-36 Health Survey
SILICOFCM	In Silico trials for drug tracing the effects of sarcomeric protein mutations leading to familial cardiomyopathy
SSM	Sarcolemmal membrane
SVT	Sustained ventricular tachycardia
TBST	Tris buffered saline plus tween
TC	Total cholesterol
TEE	Transoesophageal echocardiography
TGF-ß	Transforming growth factor-beta
TNFα	Tumor necrosis factor alpha
TNNT2	Cardiac troponin T
TNNI3	Cardiac troponin I
TP	Total protein
TPM1	Tropomyosin 1
TPR	Total peripheral resistance
TTE	Transthoracic echocardiography
VDAC	Voltage-dependant ion channel
VE	Pulmonary ventilation

VE/CO_2	Ventilation equivalents for carbon dioxide
VE/VO_2	Ventilation equivalents for oxygen
$\dot{V}O_2max$	Maximal oxygen consumption
$\dot{V}O_2peak$	Peak oxygen consumption
WHO	World Health Organisation
WT	Wild-type

List of Publications and Presentations from Thesis

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Fuller, AS, Charman, SJ., Alyahya, AI., Eggett, C., Bailey, K., Okwose, NC., MacGowan, GA., & Jakovljevic, DG. A preliminary evaluation of a novel lifestyle intervention in patients with hypertrophic cardiomyopathy. *NUTCRI Live 2022, Newcastle-Upon-Tyne, 27 May 2022, Oral Presentation.*

Abstract

Ageing and hypertrophic cardiomyopathy (HCM) are characterised by reduced functional capacity and quality of life (QoL); predictors of long-term outcomes. Chronic inflammation is a driver of pathophysiological cardiovascular hallmarks in ageing and HCM, including left ventricular (LV) hypertrophy and diastolic dysfunction. Exercise intolerance in ageing and HCM is suggested to be aetiological of central and/or peripheral factors. Excess decline in functional capacity with age can be potentially attenuated with physical activity and exercise. In addition, lifestyle interventions including exercise training and dietary nitrate (NO_3^-) supplementation can safely improve exercise tolerance in individuals with heart failure, but few studies report effects in HCM. The mitochondria play a central role in the initiation and development of inflammation and may be a driver of pathological cardiac alterations in ageing and HCM.

The present thesis firstly, investigated the effect of age and sex on mechanisms of exercise tolerance in healthy individuals, with physical activity level (low, medium, or high) as a confounding factor. Secondly, it explored determinants of exercise tolerance in HCM compared to healthy controls. Thirdly, it evaluated the effects of a novel 16-week lifestyle intervention incorporating increased physical activity (step count) and NO_3^- supplementation on cardiovascular function and QoL in HCM. Finally, in an animal model of cardiac hypertrophy and inflammageing, the thesis assessed the association between mitochondrial function and pathological phenotype in an ageing and HCM.

The major findings of the present thesis suggest that i, Part A), the ability of skeletal muscles to extract delivered oxygen (represented by reduced arteriovenous O_2 difference) at peak oxygen consumption ($\dot{V}O_2peak$) is the key determinant of exercise tolerance in healthy older individuals when physical activity level is controlled for. However, no interaction between age and sex was found for $\dot{V}O_2peak$ and determinants (i.e., cardiac output and arteriovenous O_2 difference). In contrast, females present with accelerated age-related vascular change in comparison to males. i, Part B), in comparison to healthy individuals, arteriovenous O_2 difference at $\dot{V}O_2peak$ is the key determinant of exercise tolerance in individuals with HCM. ii) A 16-week novel lifestyle intervention may significantly improve vasodilation (i.e., blood pressure), which is related to cardiac remodelling, and QoL. However, no change in exercise tolerance or cardiac function in individuals with HCM was observed. iii) reduced mitochondrial complex I (CI) activity and mitochondrial biogenesis may be associated with the cardiac pathological alterations in ageing and HCM.

In conclusion, the findings of the present thesis add knowledge about the deficiencies in cardiovascular function caused by primary ageing with the novel inclusion of physical activity category as a confounding factor in final analysis. Providing key information for improvement of clinical interventions to help prevent a state of dependence in our ageing population. Next, the present thesis helped to further define the underlying mechanisms of exercise intolerance in individuals with HCM by the novel addition of comparing the clinical population to healthy individuals, which is imperative to help define therapeutic targets to lessen the burden to those individuals affected. A novel lifestyle intervention in individuals with HCM combining increased physical activity and NO_3^- supplementation may positively impact long-term outcomes in this clinical population. Finally, the work provides a platform for further investigation into the contribution of mitochondrial function in pathological cardiac hypertrophy in ageing and HCM after the novel assessment of CI and CIV function in an aged and HCM mouse cardiac phenotype model.

In individuals with HCM, the present thesis suggests that lifestyle interventions incorporating NO_3^- supplementation and increased physical activity may improve factors associated with long-term outcomes such as QoL and blood pressure. Following the completion of a clinical trial including a larger study population and dissecting of the combined intervention, lifestyle behaviours may be implemented into clinical care after the completion of a clinical trial with a larger study population.

Chapter 1. Introduction

1.1 Exercise tolerance

Exercise tolerance is defined as the level of physical exertion an individual may achieve prior to reaching a state of exhaustion (Bisi;Stagni and Gnudi, 2011; Riebe *et al.*, 2018). It is commonly represented by maximal or peak oxygen consumption ($\dot{V}O_2$max or $\dot{V}O_2$peak, respectively) and is a measure of maximal/peak aerobic capacity. Exercise tolerance is also known as cardiorespiratory fitness or functional capacity, and assessed using cardiopulmonary exercise testing (CPET) (Koutlianos *et al.*, 2013). $\dot{V}O_2$max is the gold standard measure of exercise tolerance, defined as an ability to transport and consume oxygen during strenuous exercise (Bisi;Stagni and Gnudi, 2011). It represents the point during exercise at which an individuals' oxygen uptake no longer increases, despite further increase in the exercise intensity (Bisi;Stagni and Gnudi, 2011). $\dot{V}O_2$peak is defined as the highest value of oxygen uptake an individual has reached during exercise testing following which the test is terminated (Whipp and Ward, 1990). The American College of Sports Medicine (ACSM) provides formulas for estimating an individuals' $\dot{V}O_2$max indirectly during various modes of exercise (i.e. walking, running, stepping, leg and arm ergometers) (Glass and Gregory, 2007). The accuracy of indirectly estimated $\dot{V}O_2$max has been questioned due to potential over-overestimation of values obtained from CPET. (Glass and Gregory, 2007; Koutlianos *et al.*, 2013).

CPET quantifies exercise (functional) capacity objectively, and can determine aetiology of exercise intolerance (Wasserman, 2012). It allows an estimate of cardiovascular risk and can be used as a predictor of morbidity and mortality in healthy and clinical populations (Wasserman and Whipp, 1975; Gibbons *et al.*, 2000; Aktas *et al.*, 2004). In the clinical setting, CPET is routinely used in the evaluation of cardiac and pulmonary disorders (Dennis, 1992). However, studies have also used CPET to assess the physiological response to increased workload in healthy populations (Herdy *et al.*, 2016).

Asides from $\dot{V}O_2$max and $\dot{V}O_2$peak, commonly evaluated and reported measurements from CPET include pulmonary ventilation (VE), respiratory coefficient or respiratory exchange ratio (RER), ventilation equivalents for oxygen (VE/VO_2) or carbon dioxide (VE/CO_2), anaerobic threshold (AT) and heart rate (HR) (Herdy *et al.*, 2016). Oxygen consumption is calculated using the Fick equation, and is limited by the ability of the cardiorespiratory system to deliver oxygen during exercise in healthy individuals (Fick, 1870; Bassett and Howley, 2000):

Oxygen consumption (ml/kg/min) = Cardiac Output (L/min) x Arteriovenous O_2 difference (mL/100mL of blood)

Arteriovenous O_2 difference is defined as the ability of skeletal muscle to extract and metabolise delivered oxygen, whereas cardiac output is the amount of blood ejected from the left ventricle per minute and is calculated as the product of heart rate and stroke volume (Fick, 1870).

$$\textit{Cardiac Output (L/min)} = \textit{Heart Rate (beats/min)} \times \textit{Stroke Volume (mL/beat)}$$

1.2 Exercise tolerance in healthy individuals

Exercise is generally well tolerated in healthy individuals however, factors such as age, sex, genetics, and overall health status can influence exercise capacity in this group (Wasserman and Whipp, 1975). Results from the Fitness Registry and the Importance of Exercise National Database (FRIEND) of 7783 maximal treadmill tests (males and females, 20-79 years of age) has produced cardiorespiratory fitness reference data in the general, healthy population (Kaminsky;Arena and Myers, 2015). Average values for $\dot{V}O_2$max for males and females, at different ages is presented in Table 1.1.

Table 1.1 $\dot{V}O_2$max (mL/kg/min^{-1}) results for males and females of different ages, adapted from FRIEND registry ***(****Kaminsky;Arena and Myers, 2015)*

	V̇O2max (mL/kg/min^{-1})	
Age group (years)	Males (n=4611)	Females (n=3172)
20-29 (n = 513 males/ 410 females)	47.6±11.3	37.6±10.2
30-39 (n = 963 males/ 608 females)	43.0±9.9	30.9±8.0
40-49 (n = 1372 males/ 843 females)	38.8±9.6	27.9±7.7
50-59 (n = 1078 males/ 805 females)	33.8±9.1	24.2±6.1
60-69 (n = 593 males/ 408 females)	29.4±7.9	20.7±5.0
70-79 (n = 137 males/ 98 females)	25.8±7.1	18.3±3.6
All (n = 4611 males/ 3172 females)	37.9±11.1	27.6±9.1

If a healthy individual achieves $\dot{V}O_2$max and AT of < 85% and 40% of age- and sex-predicted values respectively, their overall exercise tolerance and ability to perform physical exercise is reduced (i.e., exercise intolerance) (Wasserman and Whipp, 1975; Lavie *et al.*, 2019). It is an important clinical finding associated with reduced functional independence, quality of life (QoL), and increased incidence of cardiovascular and all-cause morbidity and mortality in general and clinical populations (Hawkins and Wiswell, 2003; Byberg *et al.*, 2009; Shephard, 2009; Shiroma and Lee, 2010; Lavie *et al.*, 2019). Additionally, low cardiorespiratory fitness in adolescence is associated with a higher burden of disability in later life (Henriksson *et al.*, 2019). Four major limiting factors for $\dot{V}O_2$max have been defined: pulmonary diffusing capacity, maximum cardiac output, O_2 carrying capacity of blood and skeletal muscle characteristics e.g., mitochondrial enzyme levels (Bassett and Howley, 2000).

1.2.1 Ageing and exercise tolerance

Many studies confirm that $\dot{V}O_2$max declines non-linearly, by around 10% per decade after 25 years of age, ~15% per decade between the ages of 50 and 70 years old and accelerates to over 20% after 70 years of age (Fleg *et al.*, 2005; Jackson *et al.*, 2009). However, such excess decline in functional capacity with age can be potentially attenuated with physical activity and exercise (Malbut;Dinan and Young, 2002; Hawkins and Wiswell, 2003; Huang *et al.*, 2005).

In older but otherwise healthy individuals, reports suggest that the ability of skeletal muscles to extract delivered O_2 (represented by arteriovenous O_2 difference) is the key determinant of exercise tolerance (Rivera *et al.*, 1989; McGuire *et al.*, 2001; Fuller *et al.*, 2021).

1.2.1.1 Central and peripheral response to exercise with ageing

It has been suggested that maximum cardiac output accounts for 70-85% of $\dot{V}O_2$max (Bassett and Howley, 2000; Fleg *et al.*, 2005). Maximum cardiac output declines with age as a result of a reduction in heart rate and/or stroke volume, as previously demonstrated in old individuals ($\geq$ 60 years of age) by a peak cardiac index one-third of younger individuals ($\leq$ 32 years of age) (Stratton *et al.*, 1994; Fleg *et al.*, 2005). The major cause of decline in maximum cardiac output with ageing has been observed to be heart rate, whilst maximum stroke volume is preserved throughout the life (Fleg *et al.*, 1995).

Heart rate declines with age due to a decline in sinoatrial myocyte activity, fibrosis, cellular loss in the bundle of His and reduced *B*-adrenergic responsiveness (Olivetti *et al.*, 1991; Cheitlin, 2003; Strait and Lakatta, 2012). It has been suggested that 50-70% of atrial pacemaker cells undergo apoptosis before reaching 50 years of age (Cheitlin, 2003). The decrease in quantity of cardiomyocytes is also accompanied with progressive hypertrophy of the remaining cells (Dai *et al.*, 2015). Such hypertrophy leads to an asymmetrical increase in myocardial and interventricular septum thickness, resulting in myocardial shape change from elliptical to spheroid (Olivetti *et al.*, 1991).

In the Baltimore longitudinal study of ageing in healthy sedentary males and females, older individuals presented with reduced maximal heart rate, cardiac index and left ventricular ejection fraction (LVEF) (Vaitkevicius *et al.*, 1993). In contrast, there was an increase in left ventricular (LV) end diastolic and end systolic volume. In agreement with the Baltimore longitudinal study , other studies confirmed that the heart maintains, and in some cases increases, maximal stroke volume and myocardial contractility in older age through the age-associated

increase in end diastolic volume and the Frank-Starling mechanism (Rodeheffer *et al.*, 1984a; Fleg *et al.*, 1994; Jakovljevic *et al.*, 2015; Houghton *et al.*, 2016). Such age-related increase in stroke volume allows cardiac output to be maintained with ageing, despite a decrease in heart rate.

Pathophysiological and molecular changes occur within the myocardium with ageing contributing to the reduction in $\dot{V}O_2max$ (Strait and Lakatta, 2012). For example, LV wall thickness increases progressively with age in both males and females, and is associated with age-related diastolic dysfunction, heart failure and mortality (Lakatta and Levy, 2003). Increase in wall thickness is a result of extracellular matrix deposition and cardiomyocyte growth (Gerstenblith *et al.*, 1977; Levy *et al.*, 1988). An increase in collagen deposition is related to a decrease in exercise tolerance, and is further associated with increased risk of arrhythmias, disrupted communication between myocytes, diastolic and systolic dysfunction (Kwak, 2013). Both the change in cardiac thickness and overall myocardium shape to spheroid from elliptical impair contractile efficiency and lead to cardiac wall stress (Strait and Lakatta, 2012). Biomechanical strain on the remaining cardiomyocytes and chronically activated neurohormonal systems lead to stimulation of a number of inflammatory pathways and lead to further cardiac hypertrophy and fibrosis, as described by Heineke and Molkentin (Heineke and Molkentin, 2006). Furthermore, cardiac function is greatly compromised with a sedentary lifestyle, particularly due to cardiac remodelling of the left ventricle via fibrosis and localised apoptosis (Kwak, 2013).

In addition, there is a significant age-related increase in reactive oxygen species production by the mitochondria in both cardiac tissue and the vasculature (Judge *et al.*, 2005; Ungvari *et al.*, 2007). When the mitochondria is dysfunctional there is a cascade of cellular physiological function impairments. Hallmarks of mitochondrial ageing include but are not limited to: reduced number and volume, fragmented reticulum, oxidative damage and impairment of cellular respiration of cardio myocytes (Dai;Rabinovitch and Ungvari, 2012; Nilsson and Tarnopolsky, 2019). The heart's tolerance to oxidative stress decreases with age because antioxidant enzymes known as glutathione peroxidase and superoxide dismutase decrease in concentration (Abete *et al.*, 1999). Although the mitochondrial respiratory complexes II, III and V are less affected by ageing, there is diminished electron transport activity by complexes I and IV (critical components are encoded by mitochondrial deoxyribonucleic acid (mtDNA)) (Navarro and Boveris, 2007). Declined complex I and IV activity leads to a decline in mitochondrial state 3 respiration (i.e., the state of oxidative phosphorylation) and increased state 4 (i.e., resting respiratory state; oxygen consumption in the absence of adenosine diphosphate)

through age-related reduction in mitochondrial oxidative phosphorylation (Chance and Williams, 1955b; Chance and Williams, 1955a; Navarro and Boveris, 2007). Electron leakage and generation of mitochondrial reactive oxygen species may have a direct relationship with impaired electron transport (Dai;Rabinovitch and Ungvari, 2012).

Cardiac tissue is rich in mitochondria (~30% of cellular volume) to meet high metabolic energy demand (Chen;Liu and Dorn, 2011). The richness in mitochondria makes the heart particularly susceptible to oxidative damage due to reactive oxygen species being produced by the mitochondria as a by-product of oxidative phosphorylation (Dai;Rabinovitch and Ungvari, 2012).

Arteriovenous O_2 difference indicates how much oxygen is extracted from the blood by skeletal muscle. A decrease in muscle mass and oxidative capacity are suggested to be the main mechanism for age-related decline in arteriovenous O_2 difference (Hawkins *et al.*, 2001; Short *et al.*, 2005). The age-related decline in the number and function of mitochondria is related to atrophy of skeletal muscle (i.e. sarcopenia) in elderly individuals, which reduces oxygen extraction capability during exercise (Houghton *et al.*, 2016). In agreement, a previous study concluded that 35% of age-related decline in $\dot{V}O_2max$ occurs as a result of sarcopenia (Rosen *et al.*, 1998).

Sarcopenia leads to a loss of strength and power, especially in the lower limb muscle groups (Sakuma and Yamaguchi, 2012). The cellular processes that characterize sarcopenia include mitochondrial dysfunction, denervation, inflammatory and hormonal changes (Sakuma and Yamaguchi, 2012). Myofibrillar and mitochondrial protein synthesis reduction is the result of lower levels of insulin-like growth factor 1, a potent regulator of muscle mass, and other anabolic cytokine production in muscle tissue (Goldspink and Harridge, 2004). It has been suggested that mitochondrial enzyme levels are a limiting factor for maximum oxygen uptake, because the mitochondria are the final destination of oxygen consumption in the electron transport chain within muscle fibres (Bassett and Howley, 2000). Furthermore, with ageing there is a promotion of electron leakage, increased reactive oxygen species production, and reduced cellular adenosine triphosphate production (Ungvari *et al.*, 2018).

Alterations in mitochondrial number allow slight increase in oxygen extraction, thus suggesting its small contribution to $\dot{V}O_2max$ i.e., a 2.2-fold increase in mitochondrial enzymes leads to a very modest increase in $\dot{V}O_2max$ (20-40%) (Holloszy and Coyle, 1984; Saltin and Strange, 1992). It has also been shown that the mitochondrial oxidative capacity, represented by

succinate dehydrogenase activity (i.e., catalyser of the oxidation of succinate into fumarate in the krebs cycle) is related to $\dot{V}O_2$max (van der Zwaard *et al.*, 2016). Further research also highlights that older individuals (>60 years) demonstrate ~50% lower oxidative capacity per volume of muscle than in healthy and younger adults (Conley;Jubrias and Esselman, 2000). In summary, reduced $\dot{V}O_2$max is related to a reduction in oxidative capacity of the mitochondria and mitochondrial content within the muscle (Conley;Jubrias and Esselman, 2000).

There is a relationship between lower functional capacity and skeletal muscle capillarisation/ metabolic enzyme activities as well as sarcopenia in older individuals (Nicklas *et al.*, 2008; Prior *et al.*, 2016). In addition, there is an age-related reduction in blood flow to skeletal muscle and muscle perfusion capacity (Prior *et al.*, 2016). This is as a result of decline in macrovascular function (i.e., impaired endothelium-dependent vasodilation and increase in arterial stiffening), and microvascular dysfunction (i.e., alterations in muscle capillarisation and capillary recruitment) (Prior *et al.*, 2016). The result of such physiological changes is muscle atrophy through a reduction in uptake and delivery of essential nutrient, amino acids, oxygen, hormone, and substrates (Prior *et al.*, 2016). A decrease in muscle fibre size may be preceded by a reduction in capillaries; the pulmonary oxygen uptake kinetics that are reduced in older compared to younger individuals, contributing to diminished ability of older individuals to increase oxygen delivery capacity to skeletal muscles during exercise (DeLorey;Kowalchuk and Paterson, 2004). The decline in oxygen extraction ability may be augmented through increased capillary density and mitochondrial enzyme levels as a result of regular exercise training (Spina *et al.*, 1993; Liu and Latham, 2009).

1.2.2 Sex and exercise tolerance with ageing

Age-related exercise intolerance occurs in both males and females and is associated with reduced arteriovenous O_2 difference (Doherty, 2003). Reduced arteriovenous O_2 difference is caused by a decline in muscle mass and strength due to lower levels of testosterone in males, and oestrogen and progesterone in females after the age of 50 (Doherty, 2003). Besides the decline of hormonal synthesis, an increase of catabolic factors such as interleukin-1B leads to decrease in muscle mass (Deschenes, 2004). Furthermore, oxidative capacity is reduced via loss of mitochondrial content and function (Conley;Jubrias and Esselman, 2000; Murias;Kowalchuk and Paterson, 2010a; Murias;Kowalchuk and Paterson, 2010b). There is a greater age-related decline in $\dot{V}O_2$max, cardiac output and heart rate in males than females (Hossack and Bruce, 1982; Hawkins and Wiswell, 2003; Fleg *et al.*, 2005). This is due to increased cardiomyocyte loss and hypertrophy occurring more in males than females (Olivetti *et al.*, 1995). Goldspink

and colleagues (Goldspink *et al.*, 2009) demonstrated that males have a significantly higher age-related decline in maximum cardiac power output and cardiac reserve compared to females, of which correlated with a 21% reduction in LV mass. In contrast, females of similar age showed preservation of LV mass, maximum cardiac power output and cardiac reserve (Goldspink *et al.*, 2009). Such study findings may be resultant of females preserving most of their cardiomyocytes when compared to males, who progressively lose 30-35%, through the cardio-protective effects of oestrogen and higher levels of IGF-1, which stimulates anti-apoptotic signals (Olivetti *et al.*, 1995; Patten *et al.*, 2004). Furthermore, there is an increase in pulsatile afterload and workload on the left ventricle after changes in arterial compliance, which is greater in females due to a greater age-related increase in systolic and pulse pressures (Franklin *et al.*, 1997a; Najjar *et al.*, 2004). The increase in pulsatile afterload and workload on the left ventricle add to LV mass preservation in females (Franklin *et al.*, 1997a; Najjar *et al.*, 2004).

Physical activity and exercise play an important role in preserving exercise tolerance with ageing. Evidence suggests however that males and females respond differently to exercise training, particularly in later life (Spina *et al.*, 1993). Firstly, physical activity may have protective effects against sarcopenia in males, but not females (Rivera *et al.*, 2016). Secondly, in response to an endurance exercise training, older males increase maximum stroke volume, whereas females demonstrate significant increases in arteriovenous O_2 difference (Spina *et al.*, 1993; Murias;Kowalchuk and Paterson, 2010a). The mechanisms behind increased stroke volume in older males include LV volume-overload hypertrophy and training-induced LV hypertrophy (Ehsani *et al.*, 1991; Spina *et al.*, 1993).

1.3 Introduction to Hypertrophic Cardiomyopathy

1.3.1 Definition and epidemiology

Hypertrophic cardiomyopathy (HCM) is a disease in which the heart muscle becomes abnormally thick (hypertrophied) (Geske;Ommen and Gersh, 2018). Many individuals with HCM live a normal lifespan without signs and symptoms of disease. In symptomatic cases, individuals with HCM present with either a non-obstructive or obstructive left ventricular outflow tract (LVOT). Non-obstructive LVOT individuals have a gradient of <30 mmHg at rest and in response to exercise (Geske;Ommen and Gersh, 2018). In contrast, when an individual is diagnosed with obstruction, a LVOT gradient ≥30 mmHg occurs at rest and/or in response to exercise/pharmacological stress testing (Geske;Ommen and Gersh, 2018). LVOT obstruction

is commonly associated with advanced age due to progression of disease and leads to breathlessness, chest pain and syncope (Maron *et al.*, 2006). Further classification of HCM is detailed in Section 1.4.

According to clinical guidelines, HCM is characterised by an increased left ventricular (LV) wall thickness (i.e. ≥15 mm) in one or more myocardial segments that is not solely explained by abnormal loading conditions (Gersh *et al.*, 2011a; Geske;Ommen and Gersh, 2018). It has been recognised that HCM is an important cause of morbidity and mortality in individuals of all ages. The recently published average age of diagnosis documented in the Sarcomeric Human Cardiomyopathy Registry suggests disease onset occurs in affected individuals in their early 50's (Canepa *et al.*, 2020). Additionally, population-based cohort studies in Germany and Korea, as well as the International Sarcomeric Human Cardiomyopathy Registry (SHaRe) suggest that prevalence increases gradually with age, with disease prevalence and incidence peaking between ~70 and 80 years of age (Husser *et al.*, 2018; Canepa *et al.*, 2020; Moon *et al.*, 2020). Research has also demonstrated an association between age and increased expression of mutations (Marian and Braunwald, 2017).

HCM is the most inherited cardiac disease. Over 50% of diagnosed individuals present with familial HCM, and due to recent advancements in technology more than 1,400 sarcomere mutations that are encoded by >11 genes have been discovered (Maron;Maron and Semsarian, 2012). Sarcomeric genes account for 40-60% (dependent on the study population) of clinical HCM cases, with MYH7 and MYBPC3 mutations accounting for the majority (70%) of sarcomeric variant-positive cases (Maron;Maron and Semsarian, 2012; Ommen *et al.*, 2020). The genetics (i.e., aetiology) of HCM is specifically explained in section 1.5.

There is global variation in prevalence. A review of population studies suggest a global prevalence of one in 200 people when familial inheritance and diagnostic imaging are considered, and one in 500 people when disease is confirmed using echocardiography (0.2%) (Maron *et al.*, 1995; Semsarian *et al.*, 2015). In the largest HCM prevalence study based on imaging in the United Kingdom, a prevalence percentage of 0.11% to 0.22% is reported (Lopes *et al.*, 2021). In Germany, the United States and China, a prevalence of HCM is reported as between 0.03-0.07% (Maron *et al.*, 2016b; Husser *et al.*, 2018; Bai *et al.*, 2022).

Since 2010, the mean international diagnosis age has been ~51 years, with females presenting with a higher diagnosis age than men by ~five years (Canepa *et al.*, 2020). HCM is more prevalent in males than in females, with a ratio estimated at 3:2 (Canepa *et al.*, 2020) until the

age of 70, when the difference in prevalence between males and females disappear (Preveden *et al.*, 2022). The consistent male predominance is unexpected, due to the autosomal dominant pattern of inheritance of HCM (Ho *et al.*, 2018).

Diagnosis may be delayed due to late onset and day-to-day variability of signs and symptoms associated with cardiac hypertrophy (Niimura *et al.*, 2002; Geske;Ommen and Gersh, 2018). Delayed diagnosis has been reported in females compared to males (~six years later), and females present with more severe symptoms and clinical presentation at the time of diagnosis (Rowin *et al.*, 2019; Preveden *et al.*, 2022). In contrast, over-diagnosis may occur in children, where hypertrophy may occur because of other conditions such as Anderson-Fabry disease (Seo *et al.*, 2016).

1.3.2 Mortality and hospitalisation

The cumulative burden of HCM to the diagnosed individual is considerable and dominated by heart failure (HF) and atrial fibrillation in later stages of the disease (Ho *et al.*, 2018). The average time from diagnosis to the onset of HF is 11 years, and once HF has been confirmed in HCM, mean time to mortality or heart transplantation is four years (Melacini *et al.*, 2010). The most common rhythm disorder (atrial fibrillation), has similar prevalence in males and females (Preveden *et al.*, 2022). However, females are more likely to develop advanced drug-refractory HF when compared to men (i.e., ~55% vs. ~35%, respectively) and present with more severe symptoms (i.e., New York Heart Association Class (NYHA) III/IV ~70% vs ~50%) (Rowin *et al.*, 2019; Preveden *et al.*, 2022). In coherence, females with HCM have an increased all-cause mortality in comparison to males (i.e., ~9% vs ~5% in 4 years follow-up) (Siontis;Ommen and Geske, 2019; Trongtorsak *et al.*, 2021).

The 1, 3, 5 and 10-year survival rate of individuals with obstructive and non-obstructive HCM has been observed as 98%, 94%, 82% and 75% after meta-analysis (Liu *et al.*, 2017b). Such survival rate was observed to improve when individuals with HCM have access to modern clinical strategies (i.e., implantable cardioverter-defibrillators (ICDs)) were assessed; 100%, 97%, 96%, and 87%, respectively (Mielczarek *et al.*, 2022). There is an estimated HCM-related mortality rate of 0.5%/year globally (Maron;Rowin and Maron, 2022). In Europe, an excess mortality rate is observed in individuals with HCM when compared to the general population (i.e., standardised mortality ratio 2.0) (Lorenzini *et al.*, 2020). Such all-cause mortality is observed to have an increased trend with age in individuals with HCM (Lorenzini *et al.*, 2020). In comparison to historical cohorts, although still in excess to the general European population,

the annual mortality rate has reduced from ~5%/year in the 1980s, to 0.5-1% within the last decade (McKenna and Deanfield, 1984; Maron *et al.*, 2016a; Lorenzini *et al.*, 2020). The reduction in HCM-related mortality is likely owed to improved therapeutic intervention (i.e., leading to aborted and non-fatal events) as well as older average age of recent cohorts, and therefore lessened symptomatic status of individuals with HCM in recent studies.

Mortality rate in HCM has also been suggested to be age and sex specific in children (Ostman-Smith *et al.*, 2008). In childhood HCM, females have a risk of sudden cardiac death (SCD) that peaks earlier (10-11 years of age) in comparison to males (15 years of age) (Ostman-Smith *et al.*, 2008). Diagnosis in childhood is associated with worse prognosis, and individuals with a family history of HCM have a significantly higher mortality rate (Ho *et al.*, 2018; Norrish *et al.*, 2019).

Global and United Kingdom studies suggest that the main cause of death in young individuals with HCM is SCD, whereas in elderly, it is often a diagnosis of HF (Lorenzini *et al.*, 2020; Abdelfattah *et al.*, 2022). Improved treatment has resulted in a lowered global HCM-related SCD rate from 0.73% to 0.32% per year, two-fold less than the pre-treatment era (Abdelfattah *et al.*, 2022).

Hospitalisation has been reported to be required in approximately 35-40% of individuals with HCM, irrespective of age or sex (Ciabatti *et al.*, 2020; Choi *et al.*, 2022). The average length of hospital stay is ~5.9 days (Tripathi *et al.*, 2019). Of the described hospitalised individuals, mortality rate is observed to be ~7% within 90 days of discharge (Choi *et al.*, 2022). The described study also found that males had a significantly higher all-cause mortality than women within 90 days of discharge, which is contrasting to other studies where HCM-related mortality did not differ by sex (Choi *et al.*, 2019; Rowin *et al.*, 2019; Siontis;Ommen and Geske, 2019). Reasons for differing mortality rates based on sex may be a result of a higher age of enrolled individuals with HCM in the study by Choi and colleagues and therefore greater prevalence of comorbidities.

Cardiovascular hospitalisations are the most common (~85%;) when compared to non-cardiovascular admissions (15%) (Ciabatti *et al.*, 2020). Findings suggest that symptomatic status, higher prevalence of comorbidities, age, larger left atrial volume and reduced indexed right ventricular end-diastolic volume at baseline were associated with increased hospital admissions (Ciabatti *et al.*, 2020). Length of hospital stay is significantly increased with presence of arrhythmia than in the absence of (i.e., 6.5 days vs 5.5 days, respectively) (Tripathi

et al., 2019; Ciabatti *et al.*, 2020). In addition, the presence of arrhythmia increases in hospital mortality significantly when compared to without (i.e., 4.6% vs 2.9%) (Tripathi *et al.*, 2019).

1.3.3 Symptom prevalence and clinical phenotype

There is high variability in clinical manifestation but a common HCM phenotype (i.e., hypertrophied and non-dilated LV) is usually manifested in young adulthood (Maron *et al.*, 1986). Two thirds of individuals with HCM have a normal lifespan, are asymptomatic or minimally symptomatic with similar features of mild HF (Marian and Braunwald, 2017). Those affected with HCM may also be taking part in various sporting activities at the time of diagnosis (Brosnan and Rakhit, 2018). Asymptomatic individuals have low-risk profiles, however they are not void of fatal events (Maron *et al.*, 2009). In some individuals SCD is the first clinical manifestation of HCM, particularly in young undiagnosed individuals (Maron *et al.*, 2009).

Development of signs and symptoms such as dyspnoea, syncope, fatigue, arrhythmias, and reduced exercise tolerance commonly occur throughout the life in individuals with HCM (Sharma *et al.*, 2000; Le *et al.*, 2009; Finocchiaro *et al.*, 2015; Marian and Braunwald, 2017; Smith *et al.*, 2018).

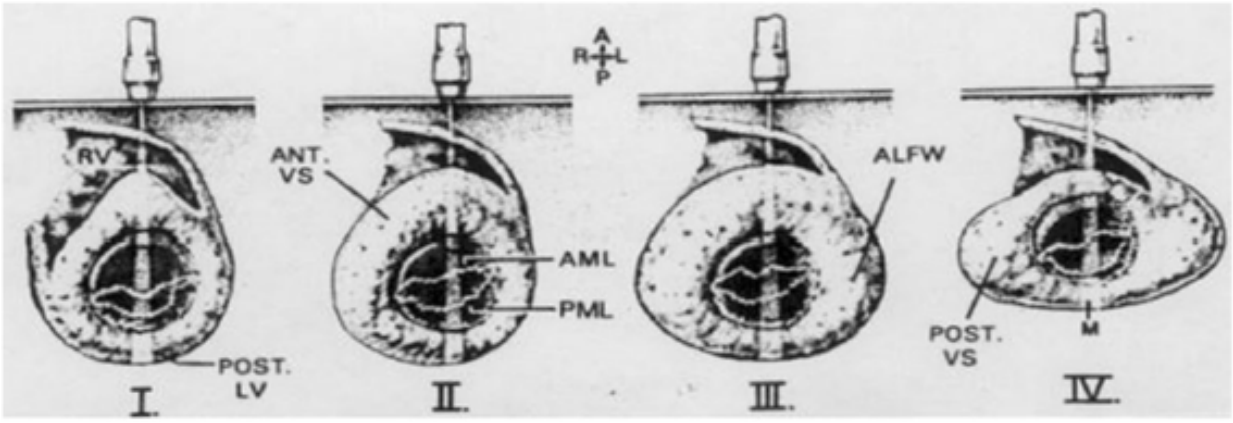
The major pathophysiological conditions leading to symptoms include diastolic dysfunction, LVOT obstruction, myocardial oxygen supply-demand imbalance, cardiac arrhythmias, and chronotropic incompetence (Efthimiadis *et al.*, 2006). It has been suggested that the majority (~70%) of individuals with obstructive HCM are symptomatic and display a higher New York Heart Association (NYHA) Class (defined in section 1.4.1) than non-obstructive groups (Lafitte *et al.*, 2013). However, the relationship between the severity of LVOT obstruction or degree of LV hypertrophy do not necessarily correlate with symptomatic status (Gersh *et al.*, 2011b; Smith *et al.*, 2018). Thus, NYHA Class cannot be used as an independent predictor of functional capacity in HCM because symptoms tend to underestimate the severity of exercise intolerance (Gersh *et al.*, 2011b; Smith *et al.*, 2018).

Individuals with non-obstructive HCM may also present with signs and symptoms including but not limited to chest pain during and after exercise, syncope, exertional dyspnoea, palpitations, and a progression to HF with age (Melacini *et al.*, 2010). Such signs and symptoms are resultant of diminished diastolic function (secondary to reduced LV chamber size) and presence of microvascular ischaemia (Cecchi *et al.*, 2003; Bravo *et al.*, 2012).

1.4 Classification of Hypertrophic Cardiomyopathy

HCM is mainly characterised as either obstructive (more common) or non-obstructive (less common) (Shapiro and McKenna, 1983). Since the World Health Organisation classification of cardiomyopathy (WHO/ISFC, 1980), extensive research has been completed to explore the heterogeneity of HCM and clinical phenotypes presented.

Classification based on distribution of hypertrophy was first demonstrated by Maron (1981) using two dimensional echocardiogram (i.e. I, basal septum hypertrophy; II, whole septum hypertrophy; III, hypertrophy of the septum, anterior and anterolateral walls; IV, apical hypertrophy) (Maron;Gottdiener and Epstein, 1981). The original figure is presented in Figure 1.1.



Antero-septum	All the septum	Septum and at least a part of the LV free wall (posterior, anterior or lateral wall)	Other localisations (posterior, apex or lateral wall)

Figure 1.1. Phenotypic classification of hypertrophic cardiomyopathy according to Maron (1981) (Adopted from (Maron;Gottdiener and Epstein, 1981)). LV, left ventricle.

Following such findings, three functional phenotypes were designed known as subaortic obstruction, midventricular obstruction, and cavity obliteration (i.e., obstruction due to systolic anterior motion of the mitral valve with ventricular septal contact, asymmetric left ventricular hypertrophy causing obstruction and contact of opposite myocardium walls in the apical view, respectively) (Wigle *et al.*, 1985). Anatomy of HCM was then assessed to define HCM classes that included the level and location of hypertrophy (i.e. sigmoid septum, neutral septum, reverse curvature septum, mid-ventricular and apical HCM) (Syed *et al.*, 2008). Most individuals with HCM are diagnosed with asymmetric septal hypertrophy with or without LVOT obstruction,

which falls into the reverse curvature and sigmoid septum phenotypes (Lewis and Maron, 1991; Syed *et al.*, 2008).

In the most recent update of HCM phenotypic classification, echocardiographic data analysis has been completed to present four-patterns of classification (i.e. pattern 1, only septal hypertrophy; pattern 2, septum and other segments other than the apex; pattern 3, apical with other LV segment hypertrophy; pattern 4, only apical hypertrophy). Patterns 1 and 2 are associated with a less symptomatic phenotype, as presented in Figure 1.2A-2D (Helmy *et al.*, 2011).

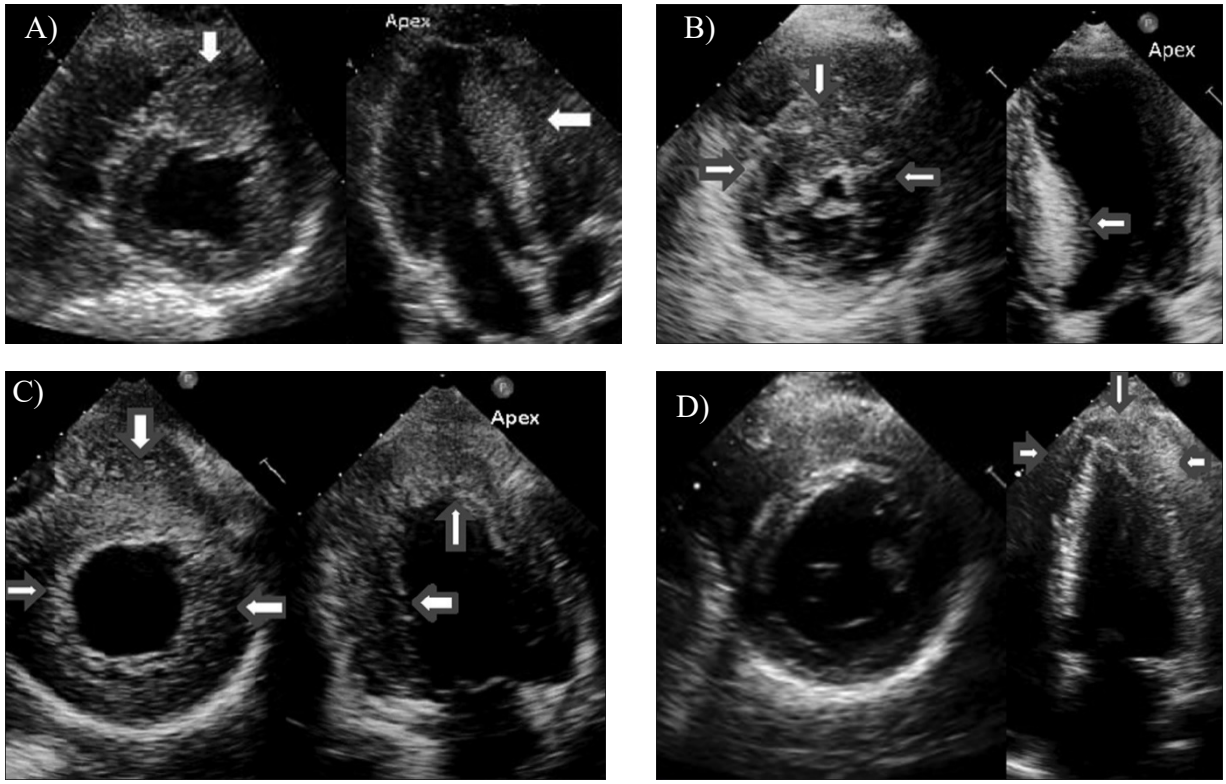


Figure 1.2. Phenotypic classification of hypertrophic cardiomyopathy Helmy and colleagues' (2011). All images show parasternal short axis, and four chamber views, with arrows pointing to hypertrophy. (A) Pattern 1 i.e., septum hypertrophy alone; (B) Pattern 2 i.e., hypertrophy in more than one segment but not apex; (C) Pattern 3 hypertrophy affecting more than one segment including apex; (D) Pattern 4 i.e., no hypertrophy of the basal segments and hypertrophy affecting only the apex.

Four clinical stages of HCM were defined, i.e., non-hypertrophic HCM, classic phenotype, adverse remodelling, and overt dysfunction (Olivotto *et al.*, 2012). The four stages were recently discussed and highlighted in literature with the aim to provide guidance for clinicians (Michels *et al.*, 2017). Literature further confirms that disease progression includes life-long LV remodelling and progressive dysfunction until end-stage HCM which occurs in 5-7% of individuals with HCM) (Olivotto *et al.*, 2012). However, natural evolution of HCM is slow and therefore identification of individuals with highest risk of advanced LV dysfunction and HF at an early stage is imperative to allow timely interventions (Olivotto *et al.*, 2012). As individuals

progress through the stages, symptoms, physical limitation, and LV filling pattern worsen. For example, LVOT obstruction is most common in Stage II: classic phenotype (Olivotto *et al.*, 2012). The stages of HCM with prevalence and defined ejection fraction are presented in Figure 1.3.

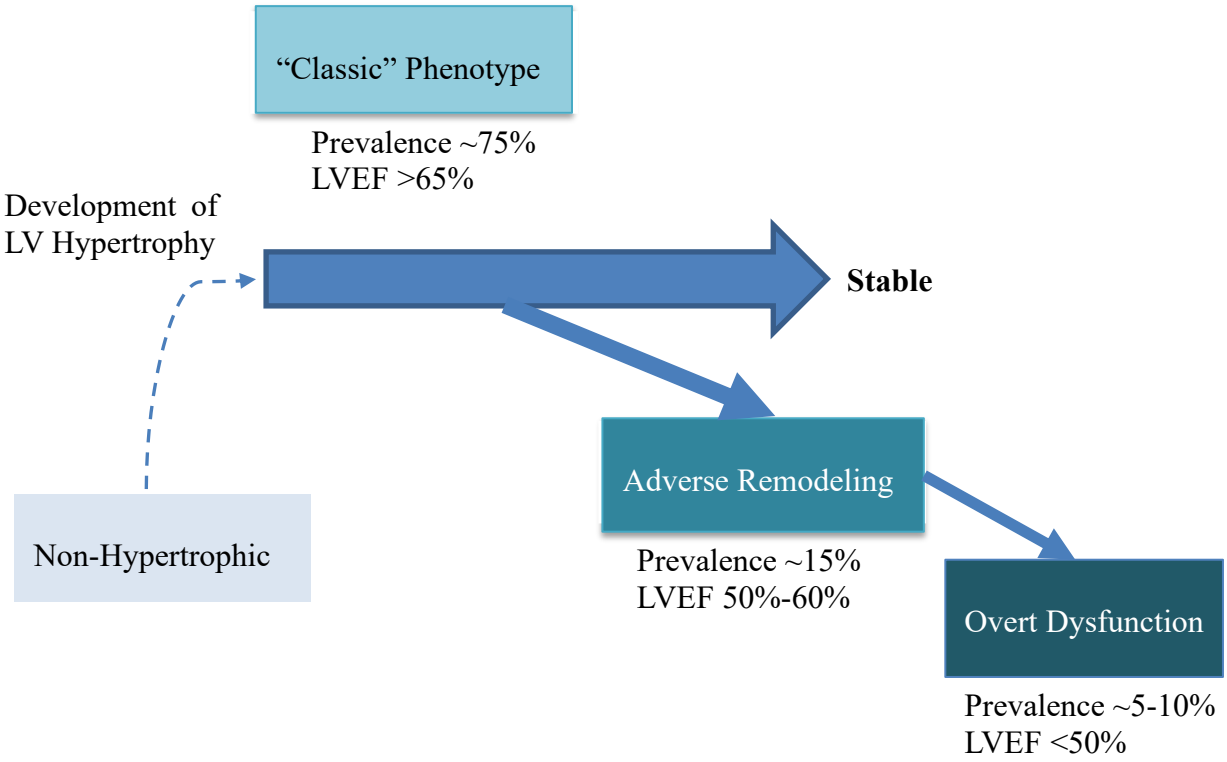


Figure 1.3. Stages of hypertrophic cardiomyopathy (HCM). Adapted from (Olivotto *et al.*, 2012). Thickness of the dark blue lines are associated with the prevalence of each stage from HCM cohorts. LV, left ventricular; LVEF, left ventricular ejection fraction.

It must be acknowledged that measurements of left ventricular ejection fraction (LVEF), which is the most frequently used measure of LV systolic function remain normal (i.e. 50-70%) throughout the initial stages of disease, and may remain so until the final stages of disease progression (Almaas *et al.*, 2013). The pathophysiological alterations that occur within the first stage of disease progression lead to the progressive reduction of LVEF over time, making reduced LVEF a characteristic hallmark of stage III disease (Marstrand *et al.*, 2020). However, as LVEF is often normal it should be considered with caution in management of HCM (Ommen *et al.*, 2020).

1.4.1 Classification of hypertrophic cardiomyopathy according to functional capacity

The New York Heart Association (NYHA) Class is routinely used for individuals with HCM and is a functional classification system for HF based on patient symptom severity and ability

to perform habitual physical activity (Scrutinio *et al.*, 1994). A patient progression rate from NYHA functional class I or II to III/IV has been observed in clinical studies of 1-2% annually, with faster progression for individuals >65 years old (Pokorski, 2002). The four NYHA categories are based on the individuals' functional limitation during exercise and is presented in Table 1.2.

Table 1.2: New York Heart Association and Weber classification of heart failure (1994). Adopted from (Dolgin, 1994).

Class	Patient Symptoms	Peak oxygen consumption (ml/kg/min)
Class I (Mild)	No limitation of physical activity.	>20
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, or dyspnoea.	16-20
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnoea.	10-15
Class IV (Severe)	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases	<10

1.5 Aetiology of hypertrophic cardiomyopathy

HCM is the most common predominantly monogenic heart muscle disease presented with different expressions (Richard *et al.*, 2006; Marian and Braunwald, 2017). The disease is inherited in an autosomal dominant pattern, except for clinical cases with an origin of mitochondrial deoxyribonucleic acid (mtDNA) mutation (Marian and Roberts, 2001). Out of those individuals clinically diagnosed with HCM, the underlying genetic cause is only confirmed in 34% of individuals (Bos *et al.*, 2014). Missense mutations are the most common cause (90%) of pathology via nonsynonymous amino acid substitution, leading to altered functional properties of proteins (Maron;Maron and Semsarian, 2012).

The strongest evidence of HCM causal genetic mutations are B-myosin heavy chain 7 (MYH7), myosin binding protein C (MYBPC3), myosin regulatory light chain 2 (MYL2), myosin regulatory light chain 3 (MYL3), cardiac troponin I (TNNI3), cardiac troponin T (TNNT2), tropomyosin 1 (TPM1) and actin alpha cardiac muscle 1 (ACTC1) (Ommen *et al.*, 2020). The MYH7 and MYBPC3 account for 70% of sarcomeric variant-positive cases (Ommen *et al.*,

2020). Accessory proteins such as MYL2 contribute to the prevalence because of their relationship with the sarcomere (i.e., stabilises the lever arm of the myosin head) and hypertrophy risk (Manivannan *et al.*, 2020).

Approximately 3% of pathogenic variants account for phenocopy conditions and the remaining clinical population have an aetiology void of sarcomeric mutations, no detectable mutation, or an unknown origin (Elliott *et al.*, 2014; Alfares *et al.*, 2015). Regarding non-sarcomeric HCM, it is suggested that sporadic HCM and phenocopies (0.1%-1% HCM >35 years old) account for the majority of such cases (Jacoby and McKenna, 2012). Sporadic mutations are *de novo* genetic alterations that lead to HCM, these mutations are not present in the parents and follow the usual progression stages of HCM (Roma-Rodrigues and Fernandes, 2014). Figure 1.4 demonstrates the prevalence of the known causal HCM genetic variants. "Other" sarcomeric genes include troponin I, α-Actinin2, α-Actin, myozenin2 and myosin light chains and "Other genetic and non-genetic causes" include but are not limited to phenocopies and digenic/ oligogenic causes of disease (Marian and Braunwald, 2017).

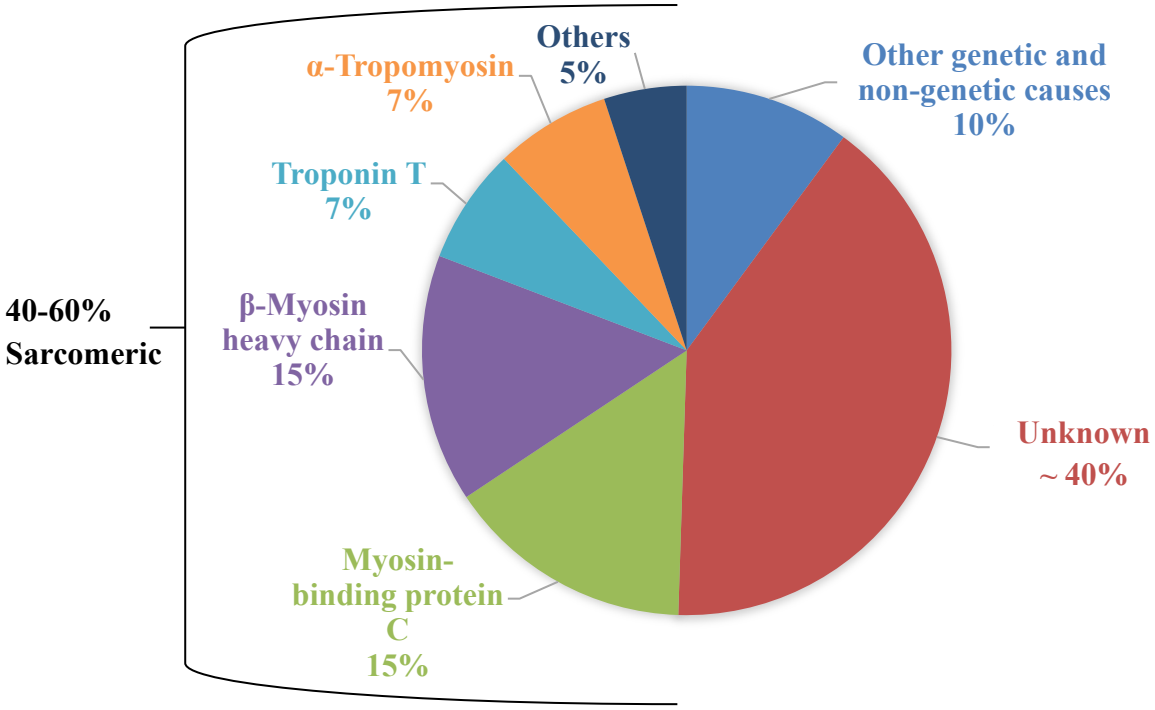


Figure 1.4. Distribution of HCM-causing genetic mutations. Adopted from (Marian and Braunwald, 2017)

Compound mutations (5% familial HCM cases) may lead to worsened phenotype in otherwise milder sarcomere mutants (Viswanathan *et al.*, 2017). Two (digenic) or more (oligogenic) causal mutations within the same or different genes are aetiological of a more severe ventricular hypertrophy and poorer prognosis (Richard *et al.*, 2003; Hodatsu *et al.*, 2014; Marian and

Braunwald, 2017). Examples of such mutations include MYBPC3 and cis mutants (Blair *et al.*, 2001; Lekanne Deprez *et al.*, 2006; Hodatsu *et al.*, 2014). A definition of the variable phenotypic expressions of HCM produced by double mutations on one single gene and one mutation on two casual genes is unachievable because of limited data (Marian and Braunwald, 2017). Nonetheless, it is now evident that this subset of individuals does not fall into the "single gene disorder" category of HCM (Marian and Braunwald, 2017).

Lifestyle and environmental/epigenetic factors (e.g., nutrients, toxins, and stress) may also play a role in HCM aetiology, suggested by great phenotypic heterogeneity between and within families with HCM (Finocchiaro *et al.*, 2017). For example, it has been demonstrated that obesity leads to an increase in myocardial thickness and faster disease progression of HCM (Olivotto *et al.*, 2013; Finocchiaro *et al.*, 2017).

1.5.1 Sarcomeric gene mutations

Research has demonstrated that individuals carrying sarcomeric mutations exhibit clinical disease and functional limitation at an earlier age, present with greater burden and an earlier onset of HCM-related complications (Ho *et al.*, 2009; Ho *et al.*, 2018). However, some sarcomere gene carriers present with incomplete penetrance (i.e. genotype positive-phenotype negative) (Maurizi *et al.*, 2019). Incomplete penetrance is associated with benign progression of disease and low risk of pathophenotype development, with ~10% of cases translating into clinical HCM during the lifespan (Maurizi *et al.*, 2019).

Sarcomeric gene mutations lead to an abnormal reduction in myocardium contractility (Fatkin *et al.*, 2000; Cohn *et al.*, 2018). MYH7 mutants are associated with a more severe phenotype, complete penetrance and younger age of onset which leads to poorer prognosis and more severe diastolic dysfunction than MYBPC3 (Michels *et al.*, 2009; Velicki *et al.*, 2020). Unlike other genes, the majority of MYBPC3 pathological mutations are nonsense mutations causing a frame shift, leading to a truncated protein, premature stop codon and haploinsufficiency of cardiac myosin binding protein-C (van Dijk *et al.*, 2009).

Disease phenotype and the extent that myocardium contraction is affected depends on the mutant gene, the locus and abundance of mutated protein (Roma-Rodrigues and Fernandes, 2014). Individuals with disease affecting the thin-filament typically present with milder and age-dependent LV hypertrophy and fibrosis (Varnava *et al.*, 2001a; Pasquale *et al.*, 2012). However, progression to an advanced LV dysfunction, HF and greater NYHA class is significantly more likely than in thick-filament disease (Coppini *et al.*, 2014). Mutations in

TNNT2 are associated with high prevalence of premature SCD due to severe myocyte disarray (Varnava *et al.*, 2001b; Pasquale *et al.*, 2012) . In clinic, an abnormal blood pressure response to exercise is the most common risk predictor for SCD in such individuals, and arrhythmic events correlate with extent of LV hypertrophy (Varnava *et al.*, 2001b; Pasquale *et al.*, 2012).

Other discovered but not as prevalent causal sarcomeric genetic mutations with extensive evidence are in thin-filament genes i.e. TNNT2, cardiac troponin I (*TNNI3*) and α-tropomyosin (*TPM1*) (10%) as well as the rare *MYL2,* myosin essential light chain (*MYL3*) and actin alpha cardiac muscle I (*ACTC1*) (Marian and Braunwald, 2017).

Screening has diagnostic value in HCM, however several sarcomeric mutations are not disease specific. For example, sarcomeric mutations may also cause familial cardiovascular diseases such as dilated cardiomyopathy and restrictive cardiomyopathy (Sen-Chowdhry *et al.*, 2016).

1.6 Pathophysiology of hypertrophic cardiomyopathy

HCM can present at any point in the life, and progression to end-stage disease is challenging to predict (Ho *et al.*, 2018). Pathogenesis of HCM is unique, complex, and not completely understood. Research suggests that genetic mutations relate to cardiomyocyte stress (i.e., inducing stress-responsive signalling kinases and trophic factors that activate a transcriptional cascade), causing the pathological characteristics of HCM (Marian and Braunwald, 2017). Recent technological advancements have resulted in improvements in molecular understanding of disease pathophysiology since the time when electrocardiogram was used to screen and diagnose HCM, and echocardiogram to aid specificity (Maron *et al.*, 1995; Ryan *et al.*, 1995). The characteristic pathological hallmarks of HCM interconnect and include, but are not limited to; diastolic dysfunction, LVOT obstruction, myocardial ischemia and mitral regurgitation (Albakri, 2018). At the molecular level in individuals with pathological B-myosin mutations, it has been demonstrated through functional analysis that alteration of actomyosin function is responsible for disease pathogenesis (Witjas-Paalberends *et al.*, 2013).

HCM disease progression demonstrates a reduction in overall cardiac function due to diastolic dysfunction, cardiac fibrosis and hypertrophy (Marian and Braunwald, 2017). The listed pathological findings are worsened because of increased intracavitary pressure gradient resultant of LVOT obstruction, which leads to an inability to increase stroke volume through impaired LV contractile reserve (Wigle *et al.*, 1985; Raj;Ranka and Goyal, 2022). In agreement with this finding, research has demonstrated that individuals with HCM present with significantly reduced LV end-diastolic and LV end-systolic volumes (Huang *et al.*, 2018).

Some investigators suggest that HCM represents a model of diastolic dysfunction due to marked impairment in diastolic filling rate, impaired LV relaxation and increased stiffness of the LV (Huang *et al.*, 2018). The characteristic pathophysiological manifestation of enlarged cardiomyocytes leads to LV hypertrophy, resulting in intraventricular blood flow obstruction due to LVOT narrowing, and in turn is responsible for diastolic dysfunction (Williams *et al.*, 2015). Under resting condition, most measures of diastolic function including mitral flow velocity, left atrial volume, early (E) and late (A) transmitral diastolic velocities do not differ between healthy control and individuals with HCM (Nihoyannopoulos *et al.*, 1992; Finocchiaro *et al.*, 2015). However, individuals with HCM have a smaller E wave, higher A wave, larger E/A ratio, longer isovolumetric relaxation time, early wave duration and slower deceleration rate of the early wave during activity when compared to healthy controls (Nihoyannopoulos *et al.*, 1992). LV hypertrophy also leads to oxygen demand and supply mismatch because of increased myocardial oxygen consumption. The mismatch leads to myocardial ischemia (with or without infarction) and angina, mostly caused by deranged coronary artery blood flow throughout the hypertrophic tissue (Raphael *et al.*, 2016).

At the cellular level, it has been highlighted that cardiac pathophysiology has an association with dysfunction and structural alterations of the mitochondria (Chistiakov *et al.*, 2018). Studies investigating cardiac energetics report mitochondrial dysfunction as a common pathogenic mechanism in HCM (Ranjbarvaziri *et al.*, 2021), The mitochondria's function is important as it is responsible for supply of 90% of energy (ATP) consumed by the myocardium (Kargaran *et al.*, 2020). To meet the increased energy demand of the myocardium, cardiomyocytes also have an increased mitochondrial content, achieved through higher mtDNA copy number (Kargaran *et al.*, 2020). Present research in individuals with mtDNA mutations demonstrates presentation of similar cardiac phenotype to individuals with HCM sarcomeric mutations (Elliott and McKenna, 2004). It is not clear whether such mitochondrial abnormalities in HCM are a primary or secondary event, but findings do suggest that mitochondrial dysfunction can be associated with HCM clinical phenotype (Unno *et al.*, 2009; Ranjbarvaziri *et al.*, 2021). Despite evidence available so far, it has been suggested that better understanding of the role of mitochondria and its association with HCM is warranted (Lucas *et al.*, 2003; Unno *et al.*, 2009; Kargaran *et al.*, 2020; Ranjbarvaziri *et al.*, 2021). Chapter 6 of this thesis provides an insight into the association between the mitochondria and HCM.

Histopathological features of HCM individuals with HF include hypertrophied myocytes (20μm), myocyte disarray, increased interstitial fibrosis, abnormal intramural arterioles with thickened walls and narrowed lumen (Moravsky *et al.*, 2013). The presence of hypertrophied

cardiomyocytes with nuclear abnormalities is a clinical hallmark predominant in HCM (Tejado and Jou, 2018). Such changes are a consequence of HCM and due to chamber enlargement and increased pressures and afterload (Rosca;Tandler and Hoppel, 2013; Tejado and Jou, 2018).

Another histopathological feature of HCM is myocyte disarray within myocardium tissue, which is most apparent in individuals who experience SCD (Varnava *et al.*, 2001a). Such findings are documented in both sarcomeric and all non-sarcomeric variants of HCM (Varnava *et al.*, 2001a). However, despite confirmation of myocyte disarray in HCM, there is currently no method to assess the presence or extent of myocyte disarray in individuals with HCM (*in vivo*) (Sen-Chowdhry *et al.*, 2016). Figure 1.5A-D demonstrates the key differences between a healthy and HCM myocardial section.

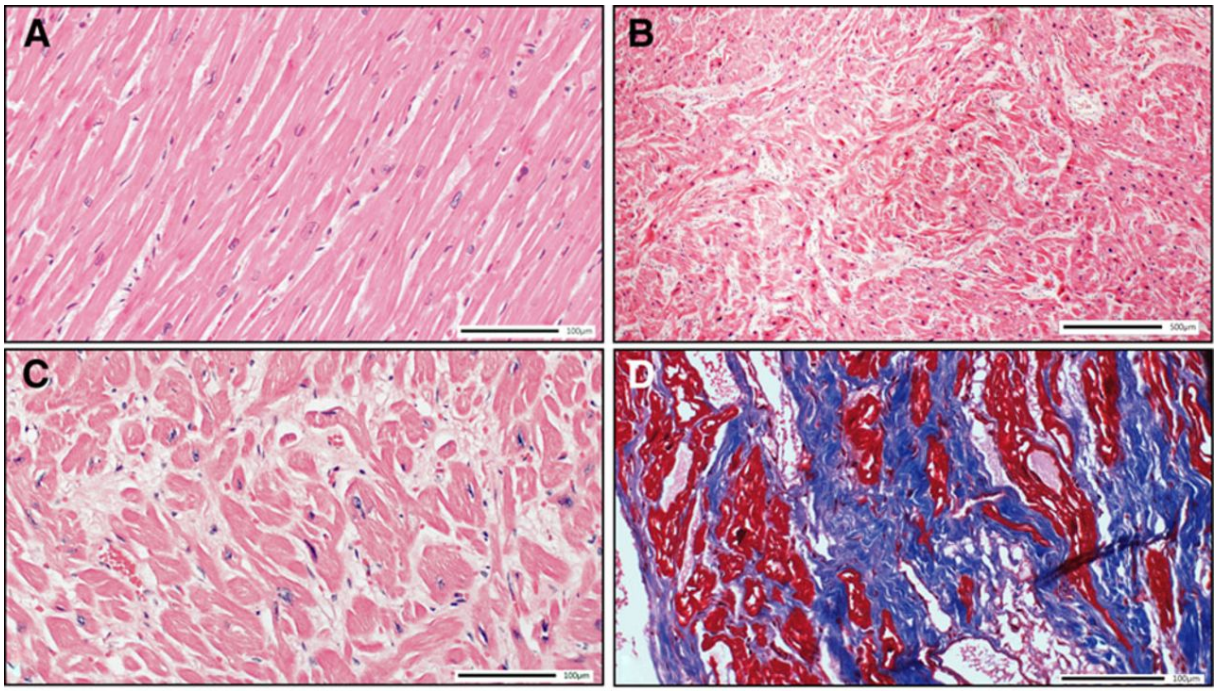


Figure 1.5. Histology of the healthy myocardium and HCM myocardium. **A)** Normal thin myocardial section stained with hematoxylin and eosin. **B)** Thin myocardial section from an individual with HCM's heart at Low-magnification (x4) and stained with hematoxylin and eosin, showing myocardial architecture disorganisation. **C)** Higher magnification (x20) hematoxylin and eosin-stained myocardial section with myocyte disarray. **D)** Thin myocardial section at higher magnification (x20) showing areas of interstitial fibrosis, stained with Masson trichrome in blue. Adopted from Marian and colleagues (2017) (Marian and Braunwald, 2017).

1.7 Diagnosis

An accurate and efficient diagnosis of HCM is important to reduce morbidity and mortality risk (Ommen *et al.*, 2020). The approach to HCM diagnosis as per the most recent American Heart Association guidelines (Ommen *et al.*, 2020) is presented in Figure 1.6. Factors that lead to HCM consideration and appear in primary care include unexplained symptoms, a family history of premature cardiac disease or echocardiographic abnormalities (Jacoby;DePasquale and McKenna, 2013). Regarding diagnosis in prenatal cases, ambiguous cases and high-risk patient identification, genetic testing is a valuable tool (Ommen *et al.*, 2020). Additionally, when individuals with a family variant or when this is unknown, screenings at intervals of 1-2 years in children or adolescents with genotype positive family and/or family with early onset HCM, 2-3 years in all other children and adolescents, and every 3-5 years in adults following diagnosis in another family member is recommended (Ommen *et al.*, 2020).

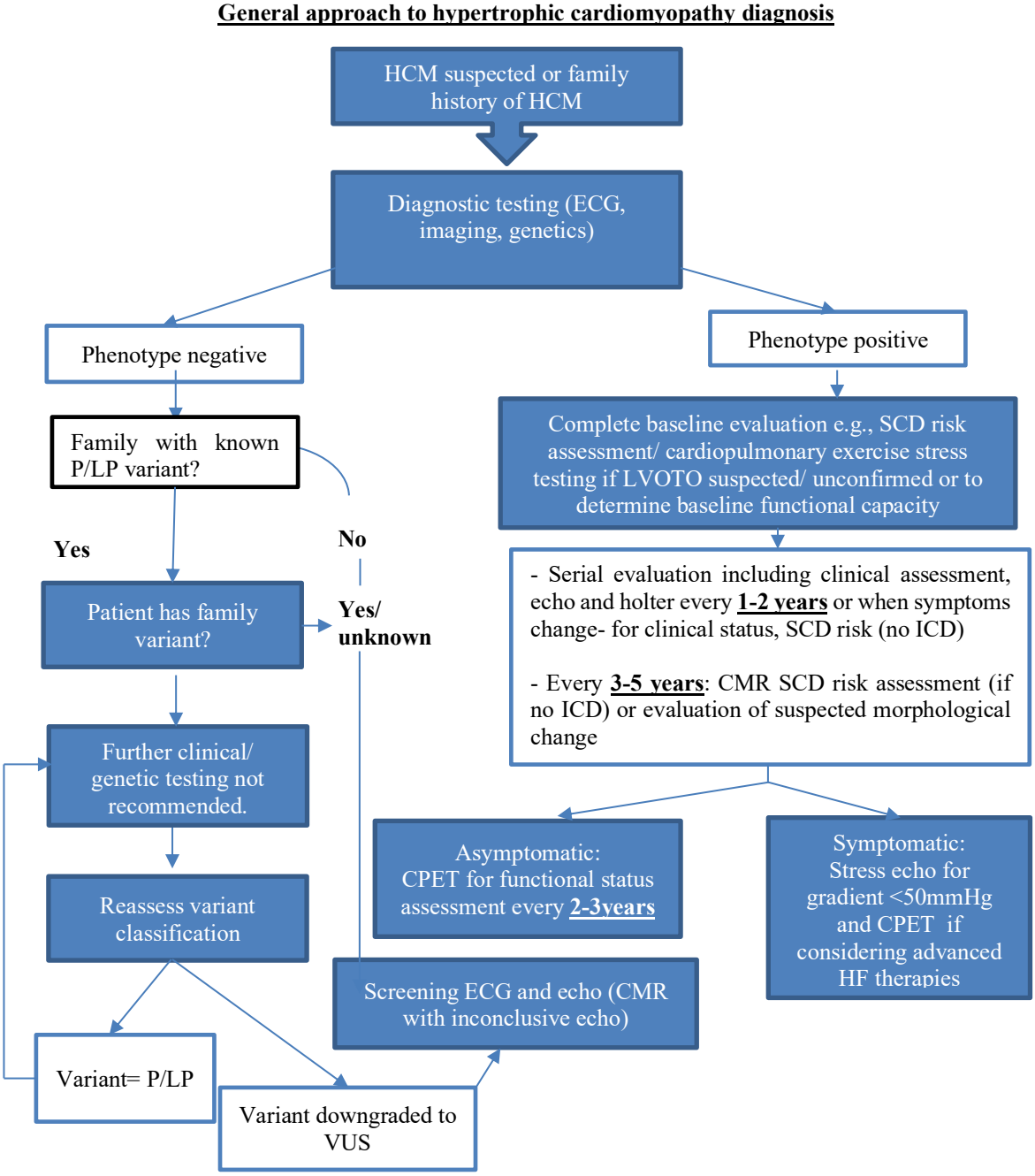


Figure 1.6. General approach to hypertrophic cardiomyopathy diagnosis. Adapted from (Ommen *et al.*, 2020). CMR, cardiovascular magnetic resonance; CPET, cardiopulmonary exercise test; ECG, electrocardiogram; HCM, hypertrophic cardiomyopathy; HF, heart failure; ICD, implantable cardioverter-defibrillator; LVOTO, left ventricular outflow tract obstruction; P/LP, pathogenic or likely pathogenic variant; SCD, sudden cardiac death; and VUS, variance of unknown significance.

An increased LV wall thickness in children as a body surface area adjusted z-score (i.e., number of standard deviations greater than the population mean) of more than two standard deviations, and a wall thickness of ≥15mm unexplained by loading conditions in adults fulfils HCM diagnostic criteria (Geske;Ommen and Gersh, 2018). In adults with first-degree family

members with HCM, a LV hypertrophy of ≥15mm is sufficient for diagnosis (Geske;Ommen and Gersh, 2018). In the latter instance, a combination of diagnostic tests that assess family history, echocardiographic and electrocardiographic abnormalities, multi-modal cardiac magnetic resonance imaging and non-cardiac symptoms should be completed (Ommen *et al.*, 2020). The basis of a HCM diagnosis is associated with LV wall thickness (Albakri, 2018). However, the disease pathological phenotype also includes ECG abnormalities, myocardial fibrosis, morphological abnormalities of the mitral valve leading to diminished function and abnormal coronary microcirculatory function (Albakri, 2018).

1.7.1 Non-invasive cardiac imaging: Echocardiography and cardiac magnetic resonance imaging

Echocardiographic examination is the most important diagnostic test in HCM and allows determination of structural and functional abnormalities of the heart (Wigle, 2001). Increased wall thickness can be found at any location, including the right ventricle, but is mostly associated with the interventricular septum in the basal LV (Klues;Schiffers and Maron, 1995). The extent of hypertrophy using cardiac imaging is recorded at end-diastole in short-axis views with correct beam alignment and orientation (Haland and Edvardsen, 2020). Such technique allows for avoidance of septal wall thickness over-estimation (Haland and Edvardsen, 2020). Chapter 3.10 provides details for echocardiography measurements in HCM.

In cases where echocardiogram cannot sufficiently document the site and extent of hypertrophy (i.e., particularly in apical HCM), cardiac magnetic resonance imaging (MRI) may be needed to confirm diagnosis (Ommen *et al.*, 2020). Cardiac MRI provides additional information, including the identification of non-contiguous regions of hypertrophy, scar tissue and infiltrative processes (Jacoby;DePasquale and McKenna, 2013).

1.7.2 Electrocardiography: Resting and ambulatory

Observation of an abnormal 12-lead ECG in combination with a normal echocardiogram is not uncommon in young individuals with HCM (Wigle, 2001). For example, the ECG trace will normally show a combination of LVH, ST-wave and T-wave abnormalities (McLeod *et al.*, 2009). For instance, apical HCM is referred to as giant T negativity syndrome, and most commonly diagnosed through ECG (Wigle, 2001). In addition, pathological Q waves that are large in the lateral and sometimes inferior leads may also be present (McLeod *et al.*, 2009).

The ECG is a sensitive and non-specific early marker of disease in first-degree relatives and allows visualisation of disease progression, therefore it is imperative that an ECG is performed each time there is a change in symptoms or wherever HCM is suspected (Ommen *et al.*, 2020) . Due to non-specificity, ECG recordings are commonly used in conjunction with other diagnostic methods (i.e., echocardiography and MRI) (Charron *et al.*, 2010). The combination of methods allows for assessment of hypertrophy distribution and identification of potential underlying causes of LV hypertrophy (Charron *et al.*, 2010). Reassessment should be offered to first-degree family relatives because a primary clinical evaluation presenting normal findings does not equal exclude disease development in the future (Charron *et al.*, 2010).

The abnormal clinical finding of asymptomatic non-sustained tachycardia is a presentation in around 30% of adults with HCM (Marian and Braunwald, 2017). Arrhythmias are reported to be a poorly tolerated, common manifestation in individuals with HCM, with a prevalence dependent on age (Wilke *et al.*, 2016; Rowin *et al.*, 2017). Atrial fibrillation is observed in ~25% of individuals with HCM and obstructive pathophysiology at an annual incidence of ~3% (Marian and Braunwald, 2017). Therefore, ambulatory monitoring of individuals with HCM is important to assess the risk of SCD or stroke risk (Ommen *et al.*, 2020).

1.8 Hypertrophic cardiomyopathy and exercise tolerance

The majority of individuals with a confirmed diagnosis of HCM demonstrate a lower level of oxygen consumption during exercise when compared to age- and sex-matched healthy controls (Sharma;Firoozi and McKenna, 2001). Individuals with HCM presenting with symptoms, presence of LVOT obstruction, and $\dot{V}O_2$max of <18 ml/kg/min have a significantly lower survival than those with a greater $\dot{V}O_2$max (Sorajja *et al.*, 2009). Therefore, understanding the physiological and molecular mechanisms underlying exercise intolerance in individuals with HCM may lead to improved patient management and clinical outcomes. The major pathophysiological mechanisms leading to functional limitation and exercise intolerance in individuals with HCM are outlined in Figure 1.7 and include peripheral deconditioning, reduced stroke volume (i.e. systolic dysfunction), diastolic dysfunction, reduced cardiac output, LVOT obstruction, mitral regurgitation, chronotropic incompetence and abnormal peripheral vasodilatory response to exercise (Jones *et al.*, 1998; Le *et al.*, 2009; Critoph *et al.*, 2014). Additionally, major predictors of exercise tolerance in individuals with HCM include demographic details (i.e., age and sex), comorbidities (i.e., diabetes), biomarkers (i.e., haemoglobin and N-terminal pro-brain natriuretic peptide) and echocardiographic indices of diastolic function (Smith *et al.*, 2018).

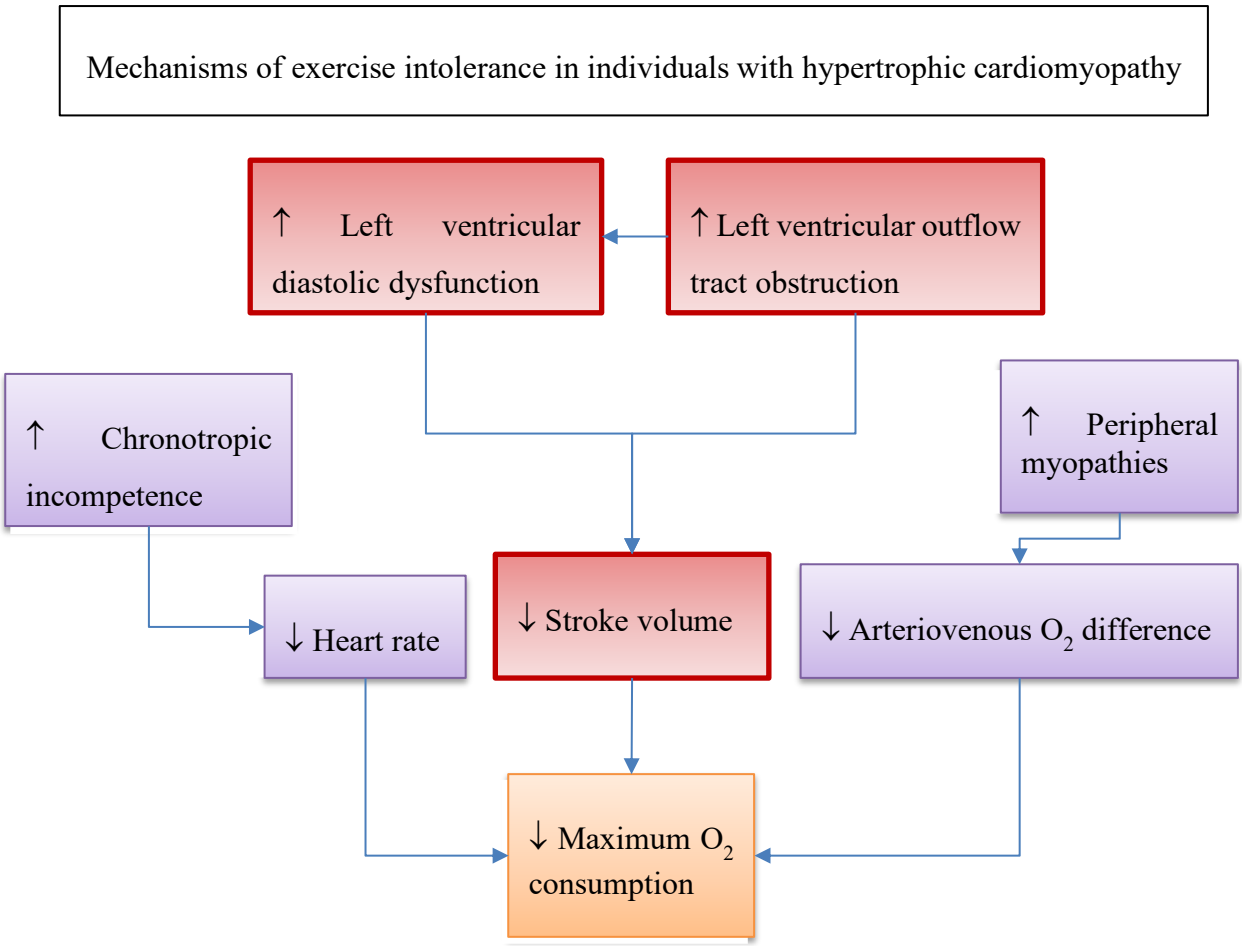


Figure 1.7. Mechanisms of exercise intolerance in hypertrophic cardiomyopathy. Adapted from (Magri and Santolamazza, 2017). ↑, increased; ↓, decreased. O_2, oxygen.

Myocardial performance is important during exercise, as stroke volume contributes significantly to $\dot{V}O_2$max (Higginbotham *et al.*, 1986; Lele *et al.*, 1995). In comparison to healthy individuals where stroke volume increases linearly with increased workload, inability to increase stroke volume through impaired diastolic function is a major determinant of exercise tolerance in individuals with HCM (Lele *et al.*, 1995). It has also been reported that in response to exercise, the ratio between $\dot{V}O_2$max and work rate becomes non-linear in individuals with HCM due to a reduction in cardiac output, impaired peripheral muscle perfusion and exercise-induced myocardial ischaemia when heart failure is present (Belardinelli *et al.*, 2003; Wasserman, 2012). Additionally, advanced diastolic dysfunction is associated with LV hypertrophy, interstitial fibrosis and myocardial disarray, and leads to a lowered exercise

tolerance in individuals with HCM when compared to normal diastolic function in the general population (Rakowski and Carasso, 2007; AlJaroudi *et al.*, 2012).

There is great patient-variability in functional capacity across the HCM clinical population, which may be explained by both genetic and non-genetic factors. For example, numerous studies have confirmed the association between abnormal sarcomeric and cellular function, and clinical phenotype (Marian and Braunwald, 2017).

1.8.1 Hypertrophic cardiomyopathy progression and exercise Intolerance

Exercise capacity predicts long-term outcomes in individuals with HCM, including progression to heart failure which may occur >10 years after diagnosis (Desai *et al.*, 2014; Magri and Santolamazza, 2017). There is an adverse remodelling of the cardiac tissue throughout life, and most individuals with HCM do not demonstrate functional capacity expected for their age (Desai *et al.*, 2014). Once the advanced HCM stage is reached, there is presence of heart failure, a reduction in cardiac output, and reduced oxygen delivery to organs and tissues (Arena *et al.*, 2007). Data from 4591 HCM individuals in the Genotype and Lifetime Burden of Disease in Hypertrophic Cardiomyopathy: Insights from the SHaRe study, confirmed that the cumulative burden of HCM is dominated by heart failure and atrial fibrillation, which occur several years after diagnosis (Ho *et al.*, 2018). A combination of the presence of sacomere defects and diagnosis at a young age are predictors of detrimental outcomes in the future. Out of those that were diagnosed at <40 years of age, 32% of individuals had ventricular arrhythmias compared with 1% of those diagnosed >60 years old (Ho *et al.*, 2018).

Individuals with HCM that achieve the expected level of functional capacity are typically younger and therefore at an early stage of disease progression (Desai *et al.*, 2014). In contrast, when later stages of disease progression are observed in combination with abnormal blood pressure (ABPR) to exercise, a higher incidence of heart failure hospitalisation is reported (Smith *et al.*, 2019). In addition, studies show that an increased ventilatory efficiency slope and left atrial diameter are associated with reduced functional capacity and heart failure disease progression in individuals with HCM (Coats *et al.*, 2015; Finocchiaro *et al.*, 2015; Magri and Santolamazza, 2017).

1.9 Aetiology of exercise intolerance in hypertrophic cardiomyopathy

1.9.1 Diastolic dysfunction

HCM represents a model of diastolic dysfunction, of which is highlighted and described in section 1.6.

During exercise, individuals with HCM demonstrate more pronounced diastolic dysfunction through doppler echocardiography (Nihoyannopoulos *et al.*, 1992). In both non-obstructive and latent-obstructive HCM, latent diastolic dysfunction (i.e. evidence of diastolic dysfunction during exercise or recovery) is a mechanism which significantly determines exercise intolerance (Re *et al.*, 2017). In such individuals, there is greater prevalence of $\dot{V}O_2$max <75% of maximum predicted value, which is demonstrated to be more significant in individuals without a gradient (i.e., LVOT obstruction) (Chikamori *et al.*, 1992; Re *et al.*, 2017).

Studies demonstrate that diastolic dysfunction through altered LV filling pressures i.e., ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e') has an independent association with $\dot{V}O_2$max (Nihoyannopoulos *et al.*, 1992). In addition, both E/e' and left atrial volume index, are the most strongly associated variables with ventilatory efficiency (VE/VCO_2) (Finocchiaro *et al.*, 2013).

1.9.1.1 Left ventricular outflow tract obstruction

LVOT obstruction (defined, with measurements in section 1.3) causes a reduction in overall cardiac function. Resting LVOT (Maron *et al.*, 2003) is strongly correlated with decreased exercise tolerance (Wigle *et al.*, 1985; Shah *et al.*, 2008) although, some HCM individuals with raised LVOT gradient at rest may have preserved functional capacity (Lafitte *et al.*, 2013). Whilst symptomatic individuals with LVOT obstruction are less physically active, their exercise tolerance may be like that of asymptomatic individuals (Sheikh *et al.*, 2015). The described preservation of exercise tolerance is proposed to be via decreased intraventricular gradient during exercise (Lafitte *et al.*, 2013).

In individuals with HCM without a LVOT gradient, only peak diastolic blood flow across the mitral valve, left ventricular and atrial volume indexes are predictive of exercise capacity (AlJaroudi *et al.*, 2012; Finocchiaro *et al.*, 2015; Luo *et al.*, 2015). The evidence suggests that LVOT obstruction and indexed left atrial volume are main measures of cardiac structure abnormality which determine exercise tolerance in individuals with HCM, although there is no significant relationship between exercise tolerance and LV wall thickness and LVOT gradient

(Maron *et al.*, 2006; Shah *et al.*, 2008; Le *et al.*, 2009; Smith *et al.*, 2019). Luo and colleagues confirmed previous findings whilst further demonstrating that individuals with HCM with presence of chronotropic incompetence demonstrate greater diastolic dysfunction and functional impairment (Luo *et al.*, 2015).

1.9.2 Mitral valve abnormalities

Mitral regurgitation is a secondary pathophysiological hallmark in individuals with HCM, as mentioned in section 1.6. Mitral filling patterns culminate in systolic anterior motion of mitral leaflets with presentation of an abnormal or restrictive pattern during exercise (de la Morena *et al.*, 2013). When various levels of mitral regurgitation occur during exercise, significant obstruction will be present (de la Morena *et al.*, 2013). A moderate association between mitral regurgitation and exercise intolerance has been reported and is suggested to be due to dynamic changes in mitral regurgitation severity affecting the increase in stroke volume and cardiac output (de la Morena *et al.*, 2013).

1.9.3 Haemodynamic response to exercise and chronotropic incompetence

It is suggested that when the blood pressure response to exercise is abnormal, impaired stroke volume and/or a vagal-driven peripheral vasodilation is often present, along with exercise-induced systolic dysfunction (Ciampi *et al.*, 2002; Magri and Santolamazza, 2017). Such individuals also present with a greater LVOT gradient and lower maximum cardiac output (Ciampi *et al.*, 2002; Campbell *et al.*, 2003; Smith *et al.*, 2019). Thus, ABPR leads to a significantly lower percentage of predicted and absolute $\dot{V}O_2$max (Ciampi *et al.*, 2007; Smith *et al.*, 2019).

Lower values of cardiopulmonary exercise stress parameters, including exercise tolerance in individuals with HCM occur because of chronotropic incompetence (i.e., inability to increase heart rate adequately during exercise), suggesting a correlation with symptomatic status (Sharma *et al.*, 2000; Luo *et al.*, 2015). Furthermore, heart rate reserve below 62 beats per minute is a reliable surrogate of a <80% predicted $\dot{V}O_2$max, thus indicating that CI and reduced heart rate reserve are strong determinants of functional capacity in individuals with HCM (Magri and Santolamazza, 2017). These secondary pathophysiological hallmarks of HCM are a part of the aggregation of mechanisms that lead to further deterioration of exercise tolerance (Efthimiadis *et al.*, 2011; Magri and Santolamazza, 2017).

1.9.4 Sex and exercise tolerance in hypertrophic cardiomyopathy

The risk of complications and mortality associated with HCM have been found to be greater in females (Geske *et al.*, 2017; Ghiselli *et al.*, 2020; Wasserstrum *et al.*, 2020; Trongtorsak *et al.*, 2021). In contrast, in an assessment of 2123 individuals with HCM, no disparity in survival rate between males and females with HCM was observed (Lele *et al.*, 1995). The lack of significant differences in age-adjusted all-cause mortality rate in male and female individuals with HCM is suggested to be owed to improved therapeutic strategies (Rowin *et al.*, 2019). In addition, females demonstrate significant age-related reduction in exercise tolerance when compared to male counterparts with HCM (Ghiselli *et al.*, 2020). Such findings were most apparent in post-menopausal individuals, although the predictive value of exercise tolerance on outcome in the HCM is only significant in males (Ghiselli *et al.*, 2020).

Genetic and endocrine factors are potential mechanisms leading to delayed phenotypic expression and disease onset in females (Hagège *et al.*, 2007). For example, oestrogens may have a protective effect on the cardiovascular system until menopause by delaying cardiomyocytes senescence (Goldspink *et al.*, 2009). In contrast, oestrogen deficiency is associated with myocardial hypertrophy, fibrosis, and impaired ventricular relaxation (Zhao *et al.*, 2014; Maslov *et al.*, 2019). In support of such hypothesis, a systematic literature review and meta-analysis of 9427 individuals found that females are diagnosed around the age of menopause (Trongtorsak *et al.*, 2021). In agreement of the link between oestrogen and cardiac diastolic dysfunction, research demonstrates that females are more likely to develop severe heart failure (NYHA class III/IV) symptoms, suggested to be resultant from higher E/e' ratios and greater obstructive physiology, despite similar degrees of hypertrophy as males (Rowin *et al.*, 2019). It is also hypothesised that a sedentary lifestyle in older females may also play a role when compared to males (Saberi *et al.*, 2017).

Trongtorsak and colleagues reported worsened outcome in females with HCM but this was contrasted by Meghji et al who reported no difference in outcome following surgical myectomy, potentially suggesting that such conclusion may be resultant of delayed diagnosis (Meghji *et al.*, 2019; Trongtorsak *et al.*, 2021). The findings suggest a pathophysiological role for greater age-related reduction in functional capacity in females and a need for increased sex-specific research and management in HCM.

1.9.5 Comorbidities as a determinant of exercise tolerance in hypertrophic cardiomyopathy

Approximately 75% of individuals with HCM present with one or more comorbidities (Sridharan *et al.*, 2022). Morbidities such as diabetes and obesity present with a decreased cardiovascular function (Smith *et al.*, 2018). There is independent association of diabetes with cardiac hypertrophy (Smith *et al.*, 2018). Diabetes occurs in approximately 10% of individuals with HCM, leads to both systolic and diastolic dysfunction, and increases risk of progression to heart failure. History of diabetes is the second strongest predictor of reduced exercise tolerance in HCM (Smith *et al.*, 2018). Prevalence of diabetes in individuals with HCM is greatest in older individuals (Wasserstrum *et al.*, 2018). In this patient population, diastolic dysfunction causes lowered functional capacity and an increase in heart failure symptoms as a result of giant filament titin property alterations, a clinical hallmark aetiological of increased cardiomyocyte stiffening (Wasserstrum *et al.*, 2018).

In addition, impaired collagen degradation and an increase in glucose cross-links induced by hyperglycaemia leads to myocardial stiffness (Miyazato *et al.*, 2005; Smith *et al.*, 2018). Affected individuals also present with interrelated pathophysiological mechanisms including increased mitochondrial dysfunction, fibrosis, and oxidative stress. Such factors contribute to reduced exercise tolerance and worsened NYHA class (Smith *et al.*, 2018; Wasserstrum *et al.*, 2018). Coexistence of diabetes and HCM leads to a more severe cardiac phenotype, further suggesting that the presence of diabetes may be harmful for the myocardium and thus limit cardiac efficiency to deliver oxygen during exercise (Wasserstrum *et al.*, 2018).

The acknowledgement of the burden of respiratory disorders in HCM are also important. There is an increasing number of individuals with HCM presenting with chronic obstructive lung disease (COPD), of which leads to a greater risk of in-hospital mortality than individuals without (Horoub *et al.*, 2022). Dyspnoea already present in individuals with HCM will be heightened with COPD present as a comorbidity, leading to reduced exercise tolerance (O'Donnell and Webb, 2008).

Hypertension (i.e., high blood pressure: ≥140/90mmHg) is a common comorbidity in HCM (i.e., >50%), and leads to exacerbated pathological cardiac remodelling, a worsened symptom prevalence, and is independently associated with atrial fibrillation and higher cardiovascular death risk (Dimitrow *et al.*, 1998; Guttmann *et al.*, 2017; Mace *et al.*, 2023). Therefore, disease progression and exercise tolerance is compromised when hypertension is present as a comorbidity in HCM.

1.10 Management of hypertrophic cardiomyopathy

1.10.1 Current pharmacological and non-pharmacological therapies

HCM pathophysiology is patient-specific, meaning that prescribed treatment is based on disease expression and underlying physiology on an individual basis (Jacoby;DePasquale and McKenna, 2013). Individuals should only receive treatment when there is presentation of obstruction-related symptoms (Jacoby;DePasquale and McKenna, 2013). Common pharmacological options are detailed in Table 1.3.

Table 1.3. Commonly used drugs for HCM (Ammirati *et al.*, 2016; Ommen *et al.*, 2020).

Drug	Indication	Starting dose/maximum dose
Beta-Blockers		
Propranolol	Angina/dyspnoea reduction in individuals with or without LVOTO; ventricular response control in individuals with AF; control of ventricular ectopic beats.	40 mg bid/ 80 mg bid (dose that has symptom benefit)
Atenolol	See propranolol.	25 mg qd/ 150 mg qd
Nadolol	See propranolol. NSVT incidence reduction, SCD prevention (especially when associated with amiodarone).	40 mg qd/ 80 mg bid
Metoprolol	See propranolol.	50 mg qd/ 100 mg bid
Bisoprolol	Treatment of systolic dysfunction and HF in end-stage individuals.	1.25 mg qd/ 15 mg qd
Calcium channel blockers		
Verapamil	Vasodilating properties. HR reduction; control of ventricular rate in individuals with AF; enhancement of diastolic filling	40 mg bid/ 240 mg bid
Diltiazem	See Verapamil	60 mg bid/ 180 mg bid
Felodipine	Refractory angina in HCM	5 mg qd
Antiarrhythmic agents		
Disopyramide	Relied of dynamic obstruction, in association with beta-blockers	125 mg bid/ 250 mg tid
Amiodarone	AF prevention, control of SVT/NSVT/Ventricular ectopic beats, reduction of appropriate ICD interventions	200 mg qd/ 200 mg qd
Sotalol	AF prevention	40 mg bid/ 80 mg bid
Oral anticoagulants		
Vitamin K inhibitors (warfarin; acenocoumarol)	Prevention of embolism and ischemic stroke in individuals with paroxysmal or permanent AF	INR target of 2-3 for warfarin and acenocoumarol
Direct thrombin (dabigatran) and direct activated factor X inhibitors (rivaroxaban, apixaban and edoxaban)	Prevention of embolism and ischemic stroke in individuals with paroxysmal or permanent AF	Recommended regimen doses based on individual molecule and patient characteristics

Abbreviations: AF, atrial fibrillation; BID, twice daily; HF, heart failure; HCM, hypertrophic cardiomyopathy; ICD, implantable cardioverter-defibrillator; INR, international normalised ratio; LVOTO, left ventricular outflow tract obstruction; NSVT, non-sustained ventricular tachycardia; qd, once a day; SCD, sudden cardiac death; SVT, sustained ventricular tachycardia.

Beta-blockers and calcium channel blockers are effective first-line treatment methods in symptomatic, obstructive HCM (Ommen *et al.*, 2020). However, combination therapy of the two is not preferential in individuals with HCM as such mode of therapy is unsupported by evidence (Ommen *et al.*, 2020). In contrast, antiarrhythmics, such as amiodarone, sotolol and disopyramide are safe when administered in combination with beta-blockers (Ommen *et al.*, 2020). The combination of certain antiarrhythmics and beta-blockers are preferential in individuals with refractory symptoms, leading to improved prognostic value. Oral diuretics (e.g., HCTZ, dyazide or Lasix) can also be of benefit to individuals with symptomatic HCM if the first-line therapy is not effective at controlling symptoms (Ommen *et al.*, 2020). It is when individuals that do not respond to beta-blockers or calcium channel blockers that they are trialled with more advanced therapy, such as with disopyramide or septal reduction therapy (Ommen *et al.*, 2020).

Current therapeutic strategies have been efficient at reducing morbidity in the HCM population, with a focus on reducing symptoms associated with HF and treatment of malignant arrhythmias (Tuohy *et al.*, 2020). However, such therapies are not targeted at prevention or reversal of the HCM phenotype. Thus, therapeutic research is targeted at assessing drugs with potential disease-modifying roles (i.e., targeting myocardial fibrosis and hypertrophy). Pharmacological therapies with potential long-term benefits include transforming growth factor-beta (TGF-ß) inhibitors, statins, angiotensin-II-receptor antagonists, aldosterone antagonists and mavacamten (i.e., myosin inhibitor) (Maltês and Lopes, 2020).

The current drug with the most promising outlook is mavacamten, the closest treatment to gene therapy available for individuals with obstructive HCM. Mavacamten specifically inhibits cardiac myosin to reduce the LVOT obstruction caused by LVH (Heitner *et al.*, 2019; Olivotto *et al.*, 2020). Thus, abnormal relaxation of myosin associated with HCM is stabilized following mavacamten intervention, which leads to diastolic function improvement (Heitner *et al.*, 2019; Olivotto *et al.*, 2020). Trials additionally suggest that individuals with more advanced disease may be most responsive to mavacamten administration (Ho *et al.*, 2020). Furthermore, mavacamten is not related to blood pressure or heart rate alterations like other drugs such as beta-blockers. The Food and Drug Administration (FDA) has approved treatment of symptomatic obstructive HCM with Camzyos (mavacamten) capsules as of 29/04/2022.

Surgical intervention may also be indicated, with surgical myectomy being common in individuals with HCM to relieve LVOT obstruction symptoms and reduce mitral regurgitation

(Gersh *et al.*, 2011a; Ommen *et al.*, 2020). Indications and non-pharmacological interventions in HCM are presented in Table 1.4.

Table 1.4. Current non-pharmacological interventions in HCM, information from 2020 AHA/ACC Guidelines for diagnosis and treatment of individuals with HCM (Ommen *et al.*, 2020)

Therapy	Indication
Alcohol-septal ablation	Release of obstruction whilst being a non-surgical procedure; for high-risk, elderly individuals. LVOT gradient/ symptom reduction.
Surgical septal myectomy	Young individuals with low-risk surgical profiles. Preferred in individuals with high levels of mitral regurgitation; LVOTO symptoms; reduction of HF symptoms and mitral regurgitation.
Dual chamber pacing	End-stage HCM and when alcohol-septal ablation cannot be performed; LVOTO, refractory symptoms and SRT indication; alleviation of the subaortic gradient and HF symptoms
Implantable cardioverter-defibrillator	Individuals at the highest risk for SCD e.g., family history of SCD, heart disease or ventricular arrhythmia/ cardiac arrest history.

HF, heart failure; HCM, hypertrophic cardiomyopathy; LVOT, left ventricular outflow tract; LVOTO, left ventricular outflow tract obstruction; SCD, sudden cardiac death; SRT, septal reduction therapy.

Referral to heart transplant care occur when disease progression reaches end-stage HF (~3.5% of HCM individuals) accompanied by congestive symptoms and refractory exertional limitation (Maron *et al.*, 1999; Torres and Perez-Villa, 2018). Whilst waiting for transplantation, pharmacological treatment is shifted to that used in individuals with HF. This includes standard care of diuretics, angiotensin-converting-enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARB) and beta-blockers (Ommen *et al.*, 2020). Figure 1.8 provides the algorithm for treatment of HF in HCM.

Algorithm for the treatment of heart failure in hypertrophic

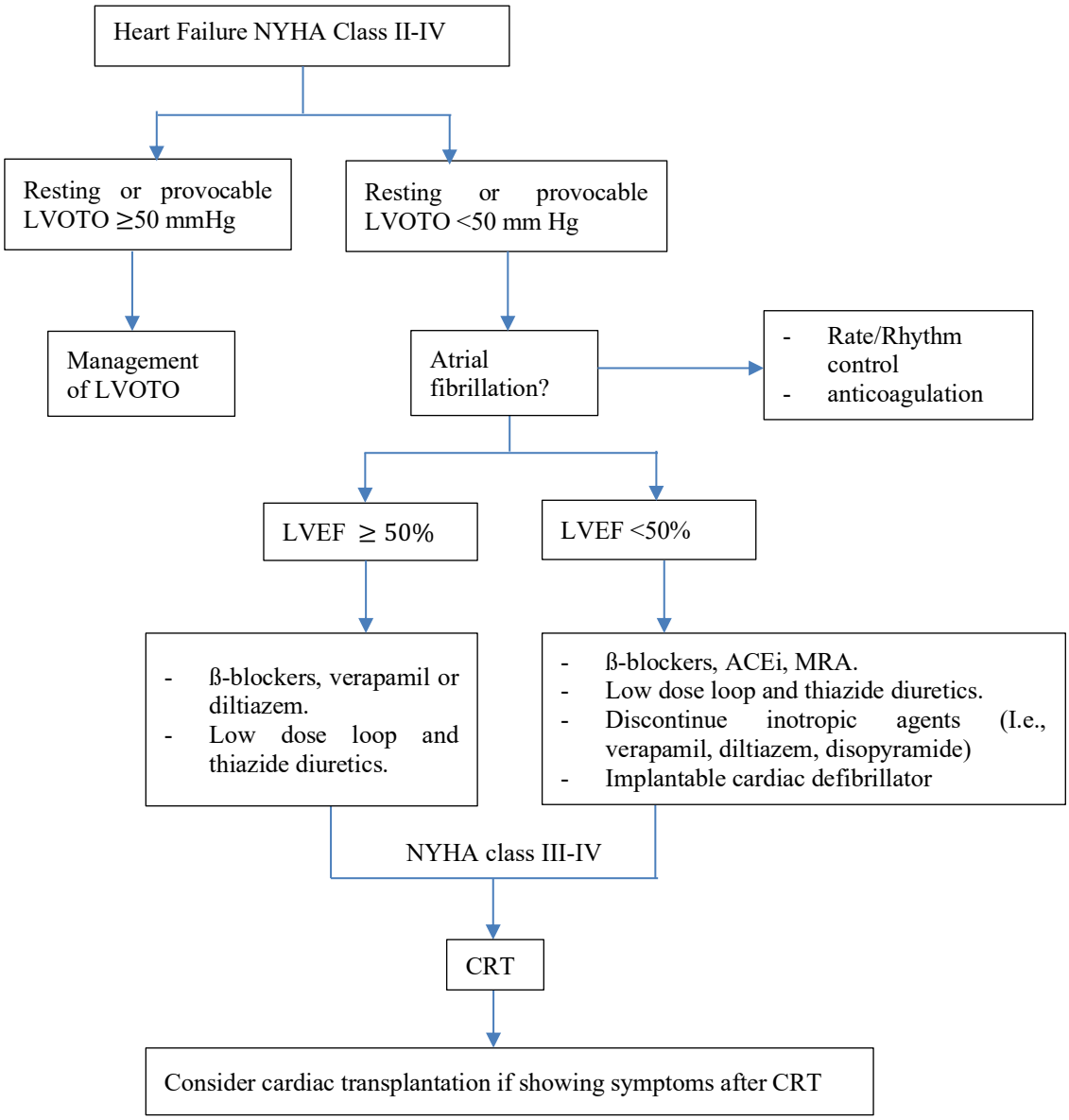


Figure 1.8. Algorithm for the treatment of heart failure in hypertrophic cardiomyopathy, adopted from Omen and colleagues, 2020 (Ommen *et al.*, 2020). ACEi, angiotensin-converting enzyme inhibitors; CRT, cardiac resynchronisation therapy; LVEF, left ventricular ejection fraction; LVOTO, left ventricular outflow tract obstruction; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association.

1.10.2 Lifestyle interventions and hypertrophic cardiomyopathy

1.10.2.1 Exercise Training

Lifestyle changes such as an increase in physical activity may prove beneficial to individuals diagnosed with HCM. An active lifestyle reduces all-cause and cardiovascular mortality risk in general and in clinical populations (Lee *et al.*, 2014). In individuals with HCM, reduced habitual physical activity is associated with worsened prognosis (Sweeting *et al.*, 2018).

The World Health Organisation (WHO) guidelines recommend that all adults complete 150-300 minutes of moderate-intensity, 75-150 minute vigorous-intensity activity, or an equivalent combination of aerobic physical activity weekly (Bull *et al.*, 2020). The American Heart Association guidelines and European Society of Cardiology guidelines on physical activity in individuals with HCM and cardiovascular disease recommend that most individuals with HCM recreationally partake in mild- to moderate-intensity exercise within WHO guidelines for the general population (Pelliccia *et al.*, 2019; Bull *et al.*, 2020; Ommen *et al.*, 2020; Pelliccia *et al.*, 2021). Previous guidelines suggested that individuals with HCM avoid high-intensity exercise training and restriction from competitive sport to avoid exercise-induced arrhythmia and worsening of pathological phenotype, regardless of symptomatic status or pathology (Elliott *et al.*, 2014; Maron *et al.*, 2015).

The benefits of increased physical activity in improving cardiovascular, metabolic, and psychological health have been documented in individuals with HCM (Saberi *et al.*, 2017; Dejgaard *et al.*, 2018). In the cited research, mild-moderate exercise training was reported as safe and associated with improved functional capacity and QoL (Klempfner *et al.*, 2015; Saberi *et al.*, 2017; Kwon *et al.*, 2021). Despite the described benefits, approximately 55% of the HCM population fail to meet the minimum exercise recommendations for the general population (Sweeting *et al.*, 2016). In a large cohort study comprising of 3283 individuals with HCM, ~70% of participants were pre-obese or obese (Fumagalli *et al.*, 2020). Obesity in individuals with HCM exacerbates clinical phenotype (i.e., is associated with increased LV mass), and leads to a more obstructive physiology, greater HF symptom burden, and SCD risk (Olivotto *et al.*, 2013; Fumagalli *et al.*, 2020). In coherence, there is a greater prevalence of atrial fibrillation, poorer health-related QoL, increased anxiety, and worsened social functioning in this clinical population (Ingles *et al.*, 2013; Reineck *et al.*, 2013).

In individuals with HCM, only one lifestyle intervention incorporating 16-weeks moderate-intensity exercise training has been completed (Saberi *et al.*, 2017). There was a modest

improvement in V̇o2peak, but no improvement in cardiac morphology observed. Although there is limited research in individuals with HCM, improvements in $\dot{V}o_2$peak have been well-documented in individuals with HF with preserved ejection fraction (HFpEF) after exercise intervention. Observed findings in HFpEF can be hypothesised to be similar in individuals with HCM because of phenotypic similarities such as systolic function preservation and LV dysfunction presentation (Henning, 2020). Table 1.5 details findings from studies that implemented moderate-intensity exercise training in individuals with HCM and HFpEF. In addition to the studies defined in table 1.6, Long and colleages completed a Cochrane meta-analysis on exercise-based cardiac rehabilitation for adults with heart failure. Findings suggest that exercise rehabilitation may improve all-cause mortality in the long-term (>12 months follow-up), improvement in disease-specific health-related quality of life, and exercise tolerance (Long *et al.*, 2019). Results are not included in the table due to the six trials including HFpEF individuals having an undefined number/ combining HFpEF and HFrEF results (Long *et al.*, 2019).

Table 1.5 The effect of moderate-intensity exercise training on exercise capacity in individuals with hypertrophic cardiomyopathy and heart failure with preserved ejection fraction

Author(s) and year	**Title**	**Design**	**Participant N/ (female %)** **Age (years)**	**Intervention**	**Findings**
Hypertrophic cardiomyopathy					
Saberi *et al.,* 2017	One Week of Daily Dosing with Beetroot Juice Improves Submaximal Endurance and Blood Pressure in Older Patients with Heart Failure and Preserved Ejection Fraction	Randomised clinical trial.	136 (42), 50±13 Usual activity 69 (41), 50±14 ET 67 (43), 51±13	16-weeks moderate-intensity exercise training	↑ V̇o2peak
Heart Failure with Preserved Ejection Fraction					
Kitzman *et al.,* 2010	Exercise Training in Older Patients With Heart Failure and Preserved Ejection Fraction	Randomised, controlled, single-blind trial.	53 (46) 70±6	16-weeks ET- 3 days pw exercise training	↑ V̇o2peak, ↑ peak CPO, ↑ tlim, ↑ 6MWT, ↑ Physical QOL.
Edelmann *et al.,* 2011	Exercise Training Improves Exercise Capacity and Diastolic Function in Patients With Heart Failure With Preserved Ejection Fraction Results of the Ex-DHF (Exercise training in	Prospective, multicentre, Randomised controlled trial.	64 (56), 65±7 ET: 44 (55), 65±7 Usual care only: 20 (60), 65±7	32 sessions. Weeks 1-4: x2 pw 50%-60% V̇o2peak Weeks 5 onwards: x3 pw 70% V̇o2peak, with resistance	↑ V̇o2peak, , ↓ E/e′ , ↓ LAVi.

	Diastolic Heart Failure) Pilot Study			training x2 pw	
Smart *et al.,* 2012	Exercise Training in Heart Failure With Preserved Systolic Function: A Randomized Controlled Trial of the Effects on Cardiac Function and Functional Capacity	Randomised controlled trial.	30 (47), 64±8 ET completed: 12 (42), 67±6 Control completed: 13 (54), 62±7	16 weeks ET	↑ V̇o2peak , ↔ diastolic and systolic function
Kitzman *et al.,* 2013	Effect of Endurance Exercise Training on Endothelial Function and Arterial Stiffness in Older Patients With Heart Failure and Preserved Ejection Fraction	Randomised, controlled, single-blind trial.	63 (76), 70±7 Exercise training: 32 (72), 70±7 Control: 31 (80), 70±7	16 weeks aerobic ET, 1h x3 pw. 40-50% gradually rising to 70% V̇o2peak.	↑ V̇o2peak, ↔ arterial stiffness, ↔ diastolic and systolic function.
Maldonado-Martin *et al*., 2017	Association Between 6-Minute Walk Test Distance and Objective Variables of Functional Capacity After Exercise Training in Elderly Heart Failure Patients With Preserved Ejection Fraction: A Randomized Exercise Trial.	Randomised, single-blinded controlled trial.	47 (87) ≥ 65 years old (average age not provided). ET: 23, Control: 24 (no other information provided).	Aerobic ET 1h, x3 days pw at 50-70% V̇o2peak.	↑ V̇o2peak, ↑ VT, ↔ 6-MWT.
Brubaker *et al.,* 2020	Effects on the Relationship of Physical Function and	Randomised, single-blinded	116 (81),70±7	16 weeks aerobic ET x3 pw.	↑ V̇o2peak, ↑ 6-MWT, ↑ SF-36, ↔ MLHF.

	Health-Related Quality of Life Among Older Heart Failure Patients With Preserved Ejection Fraction.	controlled trial.	ET: 58 (76), 70±7 Control: 58 (86), 70±6		
BP; blood pressure (mean arterial pressure and/or systolic blood pressure); BPr, blood pressure at rest (mean arterial pressure and/or systolic blood pressure) Tlim, time to exhaustion; TPR, Total Peripheral Resistance; $\dot{V}o_2peak$, peak oxygen uptake; VT, ventilatory threshold; WR_{max}, maximal power output.					

Controversial to previous guidelines, greater benefits to end-diastolic volume and diastolic filling characteristics may be achieved through adhering to regular high intensity exercise when compared to the modest effect of moderate intensity exercise training in individuals with HCM (Sheikh *et al.*, 2015; Dejgaard *et al.*, 2018). The benefits of exercise are highlighted in HCM athletes, where athletic remodelling of the heart is demonstrated through significantly lowered left ventricular filling pressure and lower systolic anterior motion of the mitral valve (Sheikh *et al.*, 2015). Thus, it is possible that exercise may lead to changes from a pathological to physiological hypertrophy in the heart at a molecular level (Konhilas *et al.*, 2006). Suggested alterations include a reduction in myocyte disarray and HCM hypertrophic markers such as the nuclear factor of activated T-cells (Konhilas *et al.*, 2006).

More studies are needed to confirm the physiological benefit associated with regular exercise training in HCM, particularly regarding cardiac remodelling, with an aim to halt disease progression and adverse events.

1.10.2.2 Dietary nitrate supplementation in Hypertrophic Cardiomyopathy

A healthy diet is linked to an improvement of heart health, as demonstrated in HF individuals (Lu and Jiang, 2014). Previous studies in individuals with HF have suggested that nutritional management in HCM may be beneficial due to the presence of nutritional deficiencies, which include cardiac cachexia and sarcopenia (Nishikido *et al.*, 2019). Significant loss of body fat, muscle and bone in combination with decreased food intake, increased energy requirements, increased production of inflammatory cytokines, tumour necrotic factor-α and a chronic low cardiac output state is prevalent in individuals with HF, and may be relevant to HCM (Finn *et al.*, 2010). However, there are no studies assessing nutritional management as a therapy for HCM.

The endothelium forms the inner cellular lining of blood vessels and regulates processes such as vascular tone and inflammation (Kruger-Genge *et al.*, 2019). Endothelial nitric oxide (NO) bioavailability is essential for vascular homeostasis and is important for cardiovascular health and prevents onset and progression of cardiovascular disease (Naseem, 2005). NO regulates blood flow and vascular tone by activating the soluble guanylate cyclase- cyclic guanosine monophosphate-protein kinase G pathway after diffusing into platelets and smooth muscle cells, and controlling mitochondrial respiration through inhibition of cytochrome c oxidase (Murad, 2006; Poderoso;Helfenberger and Poderoso, 2019). In addition, the signalling molecule mobilises stem cells and progenitor cells to ensure vascular repair, reduces inflammation, and modulates cardiac function after diffusion into cardiomyocytes (Aicher *et al.*, 2003; Seddon;Shah and Casadei, 2007; Tousoulis *et al.*, 2012).

Benefits of the signalling molecule are observed in the diseased or infarcted heart, where NO infiltration is enhanced and plays a pivotal cardio-protective and repairing role due to the ischemic microenvironment (Zhu *et al.*, 2021). The described benefits of NO can be hypothesised to reverse adverse cardiac remodelling in HCM.

Consumption of dietary nitrate (NO_3^-) (i.e., from beetroot and green leafy vegetables) increases circulating NO bioavailability through the NO_3^--nitrite (NO_2^-)-NO pathway, which is enhanced in hypoxic conditions (Lundberg;Weitzberg and Gladwin, 2008). The entero-salivary circulation facilitates the process. Following a nitrate-rich meal, NO_3^- is absorbed in the small intestine, with a bioavailability of >90% (Mensinga;Speijers and Meulenbelt, 2003). Once in circulation, the salivary glands take up ~25% of the NO_3^-, whereby facultative anaerobic bacteria reduce NO_3^- to NO_2^- via NO_3^- reductase enzymes (Lundberg;Weitzberg and Gladwin, 2008). The remaining ~75% NO_3^- is excreted by the kidneys. Once swallowed, NO_2^- may be chemically (low pH) further reduced to NO in the stomach. NO_3^- and remaining NO_2^- is also absorbed from the gastrointestinal tract and absorbed in to the blood, where NO_2^- is reduced directly to NO via molecules such as deoxygenated myoglobin, deoxygenated haemoglobin, mitochondrial electron transport chain enzymes, and xanthine oxidoreductase (Lundberg;Weitzberg and Gladwin, 2008).

Regarding the toxicity of NO_3^-, meta-analysis it has been confirmed that it is a NO_3^- dose over medium- and short-term intervention periods above the acceptable daily intake (ADI) defined by the World Health Organisation (WHO) (3.7mg/kg bw/day) that results in optimised vascular health and exercise performance in healthy individuals (Weitzberg and Lundberg, 2013; Senefeld *et al.*, 2020). Furthermore, claims that excessive NO_3^- supplementation (50mg/L) in

drinking water leads to methemoglobinemia in infants and cancer in healthy individuals have been unsupported (Fewtrell, 2004; Shannon *et al.*, 2021). However, more studies are required to confirm the lack of relationship between prolonged increase in NO_3^- above the WHO ADI and adverse effects.

Further reason to revaluate the recommended ADI of NO_3^- includes the dietary approach to stop hypertension plan. Such plan according to Hord and colleagues would exceed the WHO daily intake for NO_3^- by ~550% for a 60kg adult (Hord;Tang and Bryan, 2009).

In individuals with HFpEF, NO_3^- supplementation results in improvements in cardiorespiratory fitness and may be associated with therapeutic cardiac remodelling (Zamani *et al.*, 2015; Eggebeen *et al.*, 2016). Significantly improved cardiac output, reduced systemic vascular resistance and LV pulsatile afterload have been observed as mechanisms leading to increased $\dot{V}o2peak$ and time to exhaustion (Zamani *et al.*, 2015; Eggebeen *et al.*, 2016). Table 1.6 outlines the nitrate-nitrite-NO pathway and benefits of NO_3^- to cardiovascular health in individuals with HFpEF.

Table 1.6 The effect of dietary nitrate supplementation on exercise capacity and factors related to cardiac remodelling in individuals with heart failure with preserved ejection fraction

Author(s) and year	**Title**	**Design**	**Participant N/ (female %)** **Age (years)**	**Intervention**	**Findings**
Zamani *et al.,* 2015	Effect of Inorganic Nitrate on Exercise Capacity in Heart Failure with Preserved Ejection Fraction	Acute-Randomised, double-blind, placebo-controlled.	17 (12) 65.5±8.9	Single dose 12.9mmol NO_3^- (BRJ) vs placebo	↑V̇o2peak, ↑ CO, ↑WRmax, ↑tlim, ↔BP, ↓ arterial wave reflections, ↓ vascular resistance, and ↓ aortic augmentation index.
Eggebeen *et al.,* 2016	One Week of Daily Dosing with Beetroot Juice Improves Submaximal Endurance and Blood Pressure in Older Patients with Heart Failure and Preserved Ejection Fraction	i)Acute-Randomised, double-blind, placebo-controlled, crossover. ii)Chronic-Randomised, double-blind, placebo-controlled.	18 (85) 69±7	i)Single acute dose of BRJ, 6.1mmol nitrate vs. Placebo ii)One-week daily dosing BRJ, 6.1mmol nitrate vs. Placebo	i) ↔V̇o2peak, ↔tlim, ↔BP, ↓ BPr. ii) ↔V̇o2peak, ↑tlim, ↔BP, ↓ BPr.
Shaltout *et al.,* 2017 (Extension of Eggebeen Eggebeen *et al.,* 2016 study)	Effects of supervised exercise and dietary nitrate in older adults with controlled hypertension and/or heart failure with preserved ejection fraction	Chronic-Randomised, double-blind, placebo-controlled.	Placebo and exercise training: 9 (89%), 71 ± 8 Beetroot juice and exercise training: 10 (80%), 68 ± 6	6.1mmmol nitrate BRJ 3x pw vs Placebo.	↔V̇o2peak, ↔WRmax, ↔tlim, ↔BP (No added effect of BRJ)

BP; blood pressure (mean arterial pressure and/or systolic blood pressure); BPr, blood pressure at rest (mean arterial pressure and/or systolic blood pressure); CO, cardiac output; Tlim, time to exhaustion; TPR, Total Peripheral Resistance; $\dot{V}o_2peak$, peak oxygen uptake; WR_{max}, maximal power output.

Improvements associated with NO_3^- in cardiovascular disease are hypothesised to be via targeting of disease-related pathological alterations such as increased reactive oxygen species (ROS), endothelial dysfunction, mitochondrial muscle dysfunction, and reduced tissue perfusion and is less prevalent in healthy individuals (Omar *et al.*, 2016). Examples of therapeutic and beneficial effects of increased NO bioavailability include the indirect effect of β-Hydroxy β-methylglutaryl-CoA (HMG-CoA) reductase inhibitors in individuals with cardiovascular and metabolic disease, and the cardio-protective effects associated with the Mediterranean diet to cardiovascular health (Nadtochiy and Redman, 2011; Omar *et al.*, 2016). In the Mediterranean diet, the consumption of NO_3^- due to a three-fold higher consumption of vegetables than the average UK diet is around 400mg (i.e., ~four-fold) greater than the levels in the average European diet (Meah;Harrison and Davies, 1994; Trichopoulou *et al.*, 2003). NO_3^- intervention has not been evaluated in individuals with HCM.

1.11 Conclusion

Exercise tolerance is an important clinical determinant of mortality in both healthy and clinical populations, which declines with age and disease development. There are important sex-related differences which explain different levels of exercise tolerance between males and females, in both health and disease. In healthy older males, reduced exercise tolerance is mainly explained by reduced cardiac output. In older females, however, the main determinant of exercise tolerance is reduced arteriovenous O_2 difference.

Exercise intolerance is the most common symptom in individuals with HCM, and is associated with increased morbidity and mortality, and decreased QoL. Strong predictors of exercise intolerance include sex and a history of diabetes. The main mechanisms of exercise intolerance in HCM are diastolic dysfunction, LVOT obstruction, mitral regurgitation, ABPR to exercise and CI. Furthermore, a severe clinical phenotype presentation in females at the time of HCM diagnosis suggests the need for enhanced screening and early intervention which may slow down disease progression. Further research is needed to increase our understanding of the physiological and molecular mechanisms contributing to exercise intolerance in individuals with HCM.

Following the first characteristic description of HCM in 1958 (Teare, 1958), diagnosis and management of HCM has improved greatly due to technological advancements in the way of genetic testing, diagnostic techniques, and adverse event prevention. HCM results in reduced cardiac function and increased SCD risk. The common sarcomeric protein mutations

aetiological of HCM are MYH7 (more severe phenotype and younger age of onset), and MYBPC3. Individuals with HCM present with either obstructive (~2/3 cases) or non-obstructive pathophysiology, with high variability in symptomatic burden observed across the clinical population.

The onset to HF following diagnosis occurs after approximately 11 years, and disease progression has been defined through 4 stages: non-hypertrophic HCM, classical phenotype, adverse remodelling, and overt dysfunction. Molecular and physiological HCM pathology is not yet completely understood. Pathophysiological hallmarks of HCM include cardiac fibrosis, diastolic dysfunction, LVOT obstruction, myocardial ischemia, and mitral regurgitation. Molecular and histopathological features such as hypertrophied cardiomyocytes with nuclear abnormalities, myocyte disarray and alterations in actomyosin function are also observed in individuals with HCM.

Despite the increase in knowledge since initial discovery, research is still ongoing to find effective pharmacological treatment for individuals with HCM. Currently, pharmacological treatment is prescribed in the presence of obstruction-related symptoms and is currently limited to non-specific drugs such as beta-blockers. Mavacamten is currently the closest treatment to gene therapy in HCM, and specifically inhibits cardiac myosin to reduce the LVOT obstruction.

Lifestyle interventions incorporating improved diet and physical activity may also be beneficial to HCM disease progression, due to the association with improved CV health. Exercise training has been proven to improve exercise tolerance in individuals with HCM but has limited impact on cardiac morphology. In comparison, NO_3^- is associated with improvement in mechanisms related to LV function such as reduced arterial wave reflections and LV filling pressures in individuals with HFpEF. A finding that may translate to individuals with HCM and result in reversal of cardiac pathology over time.

This thesis is structured into eight chapters and details my PhD research programme. **Chapter 1** provides an introduction of the thesis and highlights the current knowledge on the pathophysiology of exercise intolerance in healthy individuals and hypertrophic cardiomyopathy (HCM). The chapter additionally reviews literature of the epidemiology, aetiology, diagnosis, and treatment of HCM. **Chapter 2** describes the rationale, aims, objectives and hypothesis of the thesis, and **Chapter 3** details the methodology used in the research programme. **Chapter 4 Part A** investigates the effect of age and sex on physiological determinants of exercise tolerance in healthy individuals when physical activity category (i.e.,

low, medium, and high) are taken into consideration and in **Part B** investigates the mechanisms of exercise intolerance in individuals with HCM. **Chapter 5** presents the feasibility of a novel lifestyle intervention and its effect on functional capacity and quality of life in individuals with HCM. **Chapter 6** investigates the mitochondrial function within the myocardium in experimental (mouse) model of HCM, with focus on mitochondrial complex I and complex IV (i.e., NDUFB8 and MTCO1 protein levels). **Chapter 7** demonstrates a general discussion of the main findings from the thesis along with implications of the research on patients and clinical practice, and recommendations for future research.

Chapter 2. Aims, hypotheses, and objectives

2.1 Introduction

As described in Chapter 1, exercise tolerance decreases with age in healthy individuals. In comparison to their healthy counterparts, individuals with hypertrophic cardiomyopathy (HCM) have altered cardiovascular function, and are associated with poor prognosis, increased risk of sudden cardiac death (SCD), reduced quality of life (QoL), and reduced exercise tolerance. In some cases, severe symptoms present under physical stress. In subsequent chapters discussing data, peak oxygen consumption ($\dot{V}O_2$peak) will be used in place of maximal oxygen consumption ($\dot{V}O_2$max) as we did not measure $\dot{V}O_2$max. The ability to measure peak oxygen consumption ($\dot{V}O_2$peak) in individuals with HCM has important prognostic utility, and exercise training has been shown to significantly increase exercise tolerance in this patient population (Saberi *et al.*, 2017). Presently, therapeutic options in HCM are limited. There is evidence to suggest that increased physical activity and dietary i.e., inorganic nitrate (NO_3^-) supplementation may increase exercise tolerance in heart failure but their combined effect in HCM remains to be determined. To determine this, we must first fully understand the mechanisms of reduced exercise tolerance in the healthy and ageing population. In HCM, an understanding of molecular mechanisms and pathological pathway of disease is also warranted. Molecularly, the present thesis explored role of mitochondrial function in HCM pathophysiology.

2.2 Study 1

2.2.1 Aim

To understand A) the effects of age and sex, as well as physical activity level on determinants of exercise capacity in healthy individuals, and B) the mechanisms of exercise intolerance in individuals with HCM.

2.2.2 Hypotheses

A) When physical activity level is controlled for, older people will demonstrate significantly lower exercise tolerance ($\dot{V}O_2$peak) and cardiac function (peak cardiac output) than younger people, males will demonstrate significantly higher absolute but not relative values of exercise tolerance and cardiac function. Cardiac output will be the major determinant of exercise tolerance in older age. B) Individuals with HCM will demonstrate significantly lower exercise tolerance ($\dot{V}O_2$peak) and cardiac function (peak cardiac output) compared to the general population when medication is controlled for.

2.2.3 Objectives

To achieve the described aim and evaluate the hypotheses, exercise tolerance ($\dot{V}O_2$peak) was assessed using graded cardiopulmonary exercise stress testing (CPET) on a semi recumbent cycle ergometer with simultaneous gas exchange measurements and haemodynamic (i.e., A) bioimpedance, and B) bioreactance) assessment.

Therefore, the primary outcome was the assessment of $\dot{V}O_2$peak, and the secondary outcome the differences in peak and resting haemodynamic measurements (i.e., cardiac output, stroke volume, heart rate, systolic, diastolic, and mean arterial blood pressure) between assessed groups.

2.3 Study 2

2.3.1 Aim

To assess the effect of a lifestyle intervention incorporating increased physical activity and NO_3^-on exercise tolerance, clinical phenotype, and quality of life in individuals with HCM.

2.3.2 Hypotheses

A 16-week lifestyle intervention incorporating daily NO_3^- supplementation in the form of a beetroot juice shot and increase in step count by at least 2000 steps/day on average will result in significant improvements in exercise tolerance, clinical phenotype, and quality of life when compared to control group in individuals with HCM.

2.3.3 Objectives

To achieve the described aim, the primary outcome for study 2 was change in $\dot{V}O_2$peak, with change in echocardiography markers of diastolic function (i.e., left atrial diameter, left atrial volume, left atrial volume index, E/e' ratio, left ventricular (LV) end diastolic volume, LV internal diameter end-diastole, and posterior wall thickness at end-diastole, change in quality of life, change in anxiety and depression, and the secondary outcome was change in biochemical markers of cardiac, liver, and kidney function.

2.4 Study 3

2.4.1 Aim

To define the association between mitochondrial function and hypertrophied clinical phenotype in a mouse model of ageing and HCM (*nfkb* -/- mice (p50)).

2.4.2 Hypotheses

There will be a significantly compromised mitochondrial structure and function, defined by key mitochondrial protein levels (i.e., Porin (mitochondrial mass); NDUFB8 (CI); and MTCO1 (CIV)) in older WT and p50 mice. In addition, there will be a significantly compromised mitochondrial structure and function, defined by key mitochondrial protein levels (i.e., Porin (mitochondrial mass); NDUFB8 (CI); and MTCO1 (CIV)) in p50 mice when compared to WT.

2.4.3 Objectives

To achieve the described aim, immunofluorescence staining, and confocal imaging was completed to quantify mitochondrial mass i.e., porin/ voltage dependant anion Channel 1 levels, complex I (CI) i.e., NDUFB8 protein levels, and complex IV (CIV) i.e., MTCO1 protein levels in p50 and wild-type (WT) mice. Each group also contained young (3mo) and old (12mo) mice. The primary outcome was to assess the effect of age and genotype on respiratory CI and CIV function. The secondary outcome was to assess the effect of age and genotype on mitochondrial mass.

Chapter 3: General Methodology

3.1 Introduction

This chapter describes the main clinical techniques and equipment used to complete the research studies that the present thesis is based upon. The methods described allowed for assessment of the effect of age and sex on mechanisms of exercise tolerance, the mechanisms of exercise intolerance in hypertrophic cardiomyopathy (HCM), and the effect of a lifestyle intervention on functional capacity, clinical phenotypic characteristics, and quality of life (QoL) in individuals with HCM. In addition, a systematic optimisation process and basic science methods allowed for an assessment of mitochondrial function in a cardiac mouse model of ageing and HCM phenotype.

The relevant methodologies for the present thesis are outlined in this Chapter, with further information provided in Chapters 4-6.

All clinical data was recorded in the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle Upon Tyne.

Data collection commenced January 2018 and was finalised February 2022.

3.2 Recruitment strategy

3.2.1 The effect of age and sex on mechanisms of exercise tolerance and the mechanisms of exercise intolerance in individuals with HCM

Recruitment strategies were as described below for A) the effect of age and sex on mechanisms of exercise tolerance, and B) the mechanisms of exercise intolerance in individuals with HCM:

A) Participants were first contacted by myself or Dr Nduka Okwose via email, telephone or in person to discuss the study so that they understood the study procedures and requirements.

B) Healthy individual's CPET and haemodynamic data was gifted from Professor Djordje Jakovljevic, with recruitment methods as detailed above.

Regarding HCM participants, potential and eligible participants were identified by a consultant cardiologist via the Cardiology clinics and North of England Cardiac Family History Service of Newcastle Upon Tyne Hospitals (NUTH). Once defined as potentially eligible, I contacted individuals by telephone call, and mailed a patient information sheet (Appendix 4) to those interested in participation.

3.2.2 Lifestyle intervention in Hypertrophic Cardiomyopathy study

I recruited eligible individuals with HCM by telephone as described above. I also attended and presented to the Northeast Cardiomyopathy UK support group to aid in recruitment.

3.3 Informed consent process

Chapter 4i was originally a validation study, with the aim of comparing Bluetooth, non-invasive haemodynamic device known as 'Smart-Cardia' to non-invasive bioimpedance (TaskForce, CNSystems, Graz, Austria). However, due to unforeseen complications when coded data was returned to the developers, the collected data was used to assess the effects of age and sex on mechanisms of exercise tolerance. Therefore, the consent form (Appendix 2) reflects the original study plan. Participating individuals provided informed consent following initial contact by email and telephone call to confirm willingness to participate after discussing validation study information.

Healthy individuals' data collected in Chapter 4ii was gifted from Professor Djordje Jakovljevic. The recruitment process involved individuals being initially contacted by email and telephone call and provided written informed consent (Houghton *et al.*, 2016).

Potentially eligible HCM participants (i.e., Chapter 4ii and Chapter 5) were given the relevant study information (i.e., Appendix 4), and the opportunity to ask any questions regarding study participation before providing written informed consent (Appendix 2). The consent form was countersigned by a member of the study team during the first research visit. The participant's consultant cardiologist was informed that their patient was participating in the clinical trial (Appendix 3).

3.4 Medical history and physical examination

I completed a medical history form and physical examination (Appendix 5 and 6) of participants upon arrival to the Clinical Research Facility. I measured participant body weight (kg) to the closest 100g and standing height (cm) to the closest mm using a weighing scale and stadiometer (Seca 769 electric column scale with telescopic measuring rod Seca 220, Seca, Hamburg, Germany).

When body weight and height were performed, shoes were removed. Furthermore, prior to body weight measurements, pockets were emptied of contents, heavy jewellery such as watches were

removed, and layers and outdoor wear taken off. To further standardise body weight, participants were asked to wear similar clothing for follow-up clinical visits (i.e., Chapter 5).

Body weight (kg)/(height(m))^2 was the equation that I used to calculate body mass index (BMI). Participants were not required to be fasted on arrival to study visit. For participants attending the lifestyle intervention, blood was drawn >2 hours after the last consumption of food (i.e., ensured by the study protocol procedure, 4.3.3.1).

Participants in the HCM lifestyle intervention attended study visits at the same time of day for follow-up (i.e., between 10am and 4pm and CPET between 1pm and 2.30pm). Healthy data (i.e., Chapter 4, A and B) was collected between 9.30am and 4.30pm.

Resting heart rate and blood pressure minutes was measured from the brachial artery in triplicate after participants had been seated, at rest, for at least five minutes. Legs of participants were uncrossed for the blood pressure measurement, and a validated Welch Allyn Spot Vital Signs oscillometric non-invasive blood pressure monitoring device was used minutes (Welch Allyn, NY, USA) (Davis;Dennis and Railton, 2005).

3.5 Electrocardiogram

As part of the physical examination process in Chapter 4 and 5, resting 12-lead electrocardiogram (ECG) in supine position was recorded. ECG was also recorded during CPET. The electrocardiogram provided a representation of the heart's electrical activity through analysis of the movement of ions across cardiomyocyte membranes which generate electrical charges (Fye, 1994). Such electrical changes were recorded on ECG graph paper at a speed of 25mm/s, with duration and voltage measured horizontally and vertically, respectively (Klabunde, 2017).

In the present thesis, I completed ECG to assess participants for cardiac abnormalities. Any contraindications were checked by a consultant cardiologist. During CPET involving individuals with HCM, a consultant cardiologist was present and observing along with myself. During exercise stress testing in healthy individuals myself (with ACSM exercise physiologist qualification) and Dr Nduka Okwose (ACSM Clinical exercise physiologist qualification) were always present during CPET. Therefore, ACSM contraindication to exercise regarding abnormal ECG was based on ACSM guidelines (Riebe *et al.*, 2018).

The positioning of 10 ECG electrodes consisting of limb leads (i.e., right arm, left arm, right leg, left leg) and chest leads (i.e. V1, V2, V3, V4, V5, V6) were attached as demonstrated in Figure 3.1.

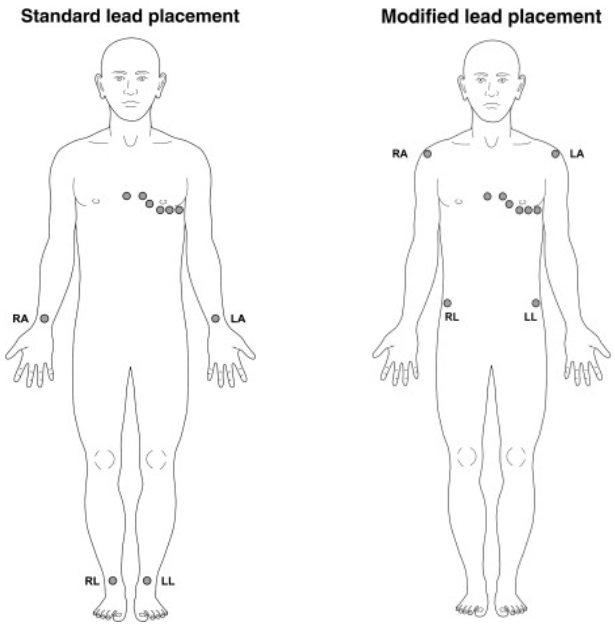


Figure 3.1. Standard electrocardiogram lead placement in supine and modified lead placement during cardiopulmonary exercise testing, adopted from (Sheppard *et al.*, 2011). LA, left arm; LL, left leg; RA, right arm; RL, left leg.

3.6 Unsuccessful screening protocol

Participants were referred to their general physician (GP) with a report of findings in the case of cardiac abnormality presentation at rest/ during exercise or contraindication to exercise by a consultant cardiologist. In the present thesis and at follow-up, two individuals with HCM presented with new onset atrial fibrillation on resting ECG and did therefore not complete CPET and their GP was notified of findings by a consultant cardiologist.

3.7 Non-invasive haemodynamics

3.7.1 Bioimpedance

The non-invasive methods integrated into the Task Force device (CNSystems, Graz, Austria) monitored haemodynamics at rest and during exercise (i.e., beat-to-beat heart rate via ECG and beat-to-beat stroke volume through transthoracic impedance cardiography to give the cardiac output measurement) in study i, Chapter 4 (Gratze *et al.*, 1998; Beitzke *et al.*, 2002; Fortin *et al.*, 2006; Jakovljevic;Trenell and MacGowan, 2014). I acquired measurements at rest in supine position for 30 minutes, and during exercise stress testing.

To record the described haemodynamics, an electrical current was applied through electrodes that I positioned at either side of the neck, and four on the lower thorax. The impedance (i.e., resistance) transmitted a current throughout the chest area (thoracic bioimpedance) (Jakovljevic;Trenell and MacGowan, 2014).

Cardiac output was calculated by the device through the impedance waveform and is proportional to the mean pressure drop of the systemic arterial-venous circuit (mean arterial pressure (MAP) - central venous pressure (CVP) (Stevanovic *et al.*, 2008). It is inversely proportional to the total peripheral resistance (TPR) of the circuit. Presently, there is no completely accurate way of measuring cardiac output and so measurements are based on assumptions. The equation for cardiac output (CO) is shown below (Stevanovic *et al.*, 2008):

$$CO \approx (MAP\text{-}CVP) / TPR$$

The taskforce device has acceptable performance and with good reproducibility (Brittain;Busk and Moller, 2018).

3.7.2 Bioreactance

Bioreactance non-invasively analysed blood-flow dependent changes in phase shifts of electrical currents occurring across the thorax in Chapter 4 (study B), and Chapter 5 at baseline and follow-up (Squara and Burkhoff, 2012). The NICOM system was used (Cheetah Medical Inc., Indianapolis USA). To record measurements, I applied 4 dual electrodes on the back of the thorax. One end introduced high frequency (75 kHz) current to the body and the other was a voltage input amplifier (Figure 3.2) (Keren;Burkhoff and Squara, 2007).

Haemodynamic measurements (i.e. cardiac output, stroke volume, blood pressure and HR) at rest in supine position for five minutes, and during CPET (Fortin *et al.*, 2006; Jakovljevic;Trenell and MacGowan, 2014). Calculation of a precise cardiac output was

complete using an average of signals received over one minute (Keren;Burkhoff and Squara, 2007).

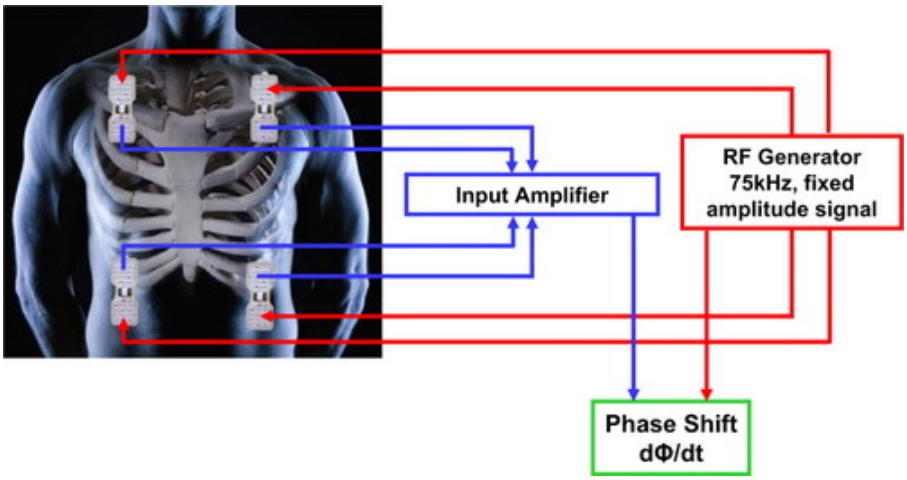


Figure 3.2. Non-invasive cardiac output monitoring (NICOM) system and body connection points. Adopted from (Keren;Burkhoff and Squara, 2007).

Validation and reliability studies confirm that bioreactance produces more similar estimates to those measured from oxygen consumption during exercise than bioimpedance (Jakovljevic *et al.*, 2012). When compared to thermodilution, the precision of bioreactance to determine haemodynamic changes is superior (Raval *et al.*, 2008).

3.8 Cardiopulmonary exercise stress testing

All participants completed a progressive exercise test using a semi recumbent cycle ergometer (Corival, Lode, Groningen, Netherlands) (figure 3.3). Dr Nduka Okwose, Dr Jadine Scragg and I led the exercise testing for healthy individuals in part A. Professor Djordje Jakovljevic kindly gifted healthy individual data used to assess the mechanisms of exercise intolerance in individuals with HCM in part B. Regarding data from HCM individuals, I led the data collection for this study and Miss Alaa Alyahya assisted following training after joining the research team just prior to and following the coronavirus pandemic. A consultant cardiologist supervised all HCM exercise stress testing.

For healthy individuals (Chapter 4, Part A), at least three minutes of resting (baseline) gas exchange and haemodynamic measurements were taken before exercising. A ramp protocol was followed until maximal exertion with participants maintaining a pedal frequency of 60-70 revolutions per minute. A warm-up (cycling at 10 watts for 2 min) was included in the exercise

test. Following this period, the workload increased at the rate of 10 watts per minute until volitional exhaustion.

For healthy individuals and individuals with HCM (Chapter 4, Part B), at least two minutes of resting (baseline) gas exchange and haemodynamic measurements were taken before exercising. A ramp protocol was followed until maximal exertion with participants maintaining a pedal frequency of 60-70 revolutions per minute. A warm-up of unloaded cycling for 1 min was included in the exercise test. Following this period, the workload increased at the rate of 10 watts per minute from a starting point of 30 watts until volitional exhaustion. For the lifestyle intervention in HCM (Chapter 5), the described protocol was complete at baseline and follow-up.

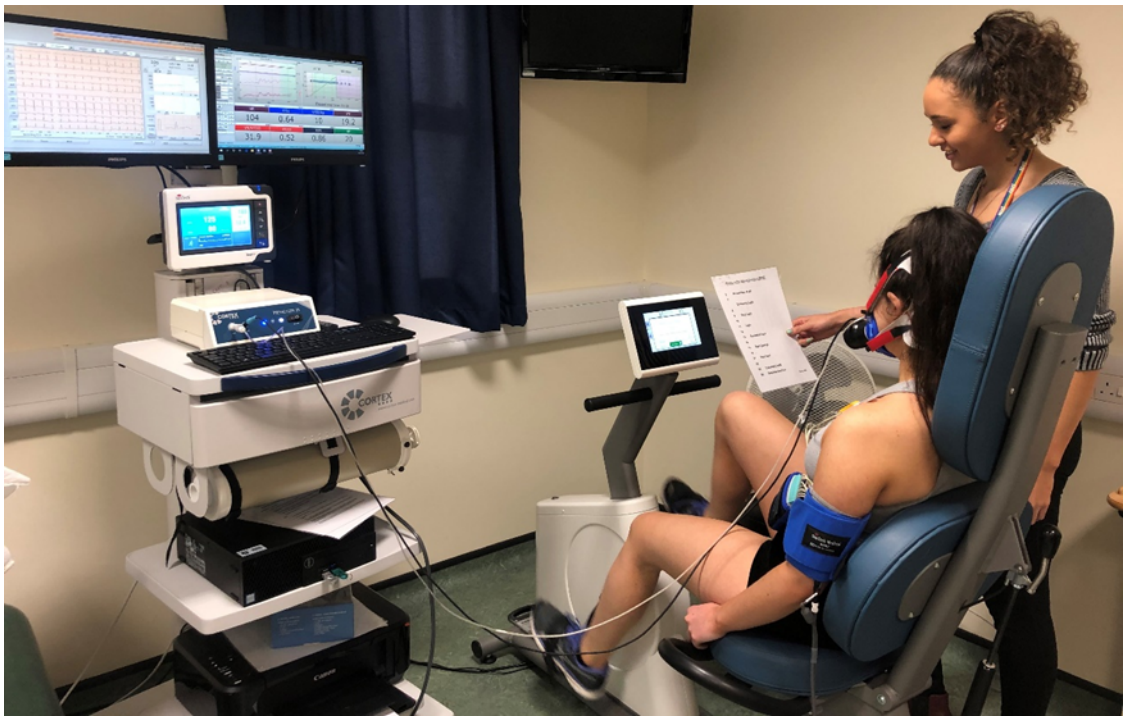


Figure 3.3. Cardiopulmonary exercise testing on semi recumbent cycle erg.

Termination of the CPET is described in the relevant chapters.

3.8.1 Gas analysis

In Chapter 4 and 5, CPET was complete with simultaneous gas exchange measurements. Hans Rudolph 7450 Series V2 facemask captured expired gas and Metalyzer® 3B-R3 (Cortex Metalyzer 3B, Cortex, Leipzig, Germany) CPET online gas analyser immediately quantified it.

Prior to calibration, I turned on the equipment for a minimum of 30 minutes to allow oxygen and carbon dioxide sensors to stabilise. An ambient air and gas cylinder at a known

concentration of 17.1% O2, 5.0% CO2 (BOC, Industrial Gases, Linde AG, Munich, Germany) and 3 litre syringe (Series 5530, Hans-Rudolph Inc, USA) used to check gas volume. The Metasoft software (Cortex Biophysik GmbH, Germany) calculated breath by breath volume of oxygen and carbon dioxide using standard metabolic algorithms employing the Haldane transformation (Haugen;Chan and Li, 2007). Such data was collected in 10 second averages.

3.8.2 Determining metabolic parameters at peak exercise

Oxygen consumption and cardiac output at peak exercise were defined as the average oxygen uptake and cardiac output during the last 30 seconds of exercise. Anaerobic threshold was automatically calculated using the v-slope method (Tan, 1986). Based on measured oxygen consumption and cardiac output, arteriovenous O_2 difference was calculated using the Fick equation:

$$O_2 \text{ consumption (ml/kg/min)} = \text{Cardiac Output (L/min)} \times \text{Arteriovenous } O_2 \text{ difference (mL/100mL of blood)}$$

The Fick equation has been previously demonstrated to yield equivalent measurements of oxygen consumption, cardiac output and arteriovenous O_2 difference when compared to direct measurement (Rubin *et al.*, 1982).

Stroke volume was calculated using cardiac output and heart rate measurements using the following equation:

$$Stroke\ volume\ (mL/beat) = \frac{Cardiac\ output\ (\text{L/min}) x 1000}{Heart\ rate\ (bpm)}$$

Cardiac power output (Watts), as a measure of overall function and pumping capability of the heart, was calculated as the product of cardiac output and mean arterial blood pressure, using the formulae (Tan, 1986; Williams *et al.*, 2001):

$$CPO(W) = \frac{MAP(mmHg)\ x\ CO(L/min)}{451}$$

Where CPO= cardiac power output; MAP= mean arterial pressure; CO= cardiac output.

3.8.3 Relative perceived effort

Borg Rating of Perceived Exertion (RPE) Scale (Appendix 8) was described to participants prior exercise testing, with an aim of reaching an effort level >16 (Borg, 1982). The Borg RPE scale is a valid tool that is routinely used in clinical studies and the workplace to assess exertion

(Williams, 2017). Throughout the exercise test, I presented the Borg scale to assess how the participant was perceptually feeling. This also allowed to observe any symptom presentation in response to exercise stress.

3.9 Blood analysis

Blood analysis is an important diagnostic test for pathological conditions such as heart disease, and allows for assessment of factors including but not limited to cardiac scarring, inflammation, heart attack risk, and the presence of diabetes (Cambronero et al., 2009; Korngold et al., 2009; McLean and Huang, 2012; Nguyen et al., 2015; Wettersten and Maisel, 2015; Ma et al., 2018). A blood sample was drawn from the antecubital vein in the arm by a trained phlebotomist of the Clinical Research Facility or qualified member of the research team, i.e., Dr Nduka Okwose, for Chapter 5. Aseptic non touch technique was used, and a butterfly needle (BD Vacutainer® Safety-LokTM blood collection system) was inserted to collect blood. The samples were temporarily stored in vacutainers at 2-8 degrees Celsius for <7 days until processing and analysis took place in a Clinical Biochemistry Accredited laboratory in the Newcastle upon Tyne Hospitals NHS Foundation trust.

The analysis of blood biomarkers allowed for assessment of the effects of the lifestyle intervention implemented in Chapter 5 on alterations in disease progression in HCM. Blood markers assessed included: biomarkers of liver and renal function (i.e., urea, creatinine, estimated glomerular filtration rate (eGFR)); lipid profile (total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), non-HDL-C, and T/HDL-C ratio, triglycerides); haemoglobin A1c; inflammation (i.e., albumin, bilirubin, alkaline phosphatase (ALP). C- reactive protein (CRP), total protein, and tumour necrosis factor alpha (TNFα)) and cardiac function (N-terminal-pro hormone BNP (NT-proBNP) and troponin-t).

3.9.1 Cardiac function (N-terminal-pro hormone BNP and Troponin-T)

A 5ml SST vacutainer® of whole blood was drawn at the baseline and follow-up. Troponin-T and NT-proBNP levels were quantified using the Roche Cobas c702 automated chemistry analyser (Roche Diagnostics, Basel, Switzerland) (Kaplan;Pesce and Kazmierczak, 2003). NT-proBNP predicts adverse prognosis in HCM, with abnormal levels associated with increased risk of death or transplantation (Coats *et al.*, 2013). Elevated cardiac troponin-T is associated with and useful for predicting the progression of adverse cardiac remodelling, and was therefore assessed in Chapter 5 (Kubo *et al.*, 2020).

3.9.2 Liver and renal function

The effects of the lifestyle intervention in HCM on renal function was assessed due to the kidneys playing an important role in cardiovascular disease development (Nowak and Arnlov, 2020). Liver function is also altered in HCM. HCM is also associated with decreased lipid uptake in the heart, leading to increased plasma lipids, lipid storage and gluconeogenesis in in liver and raised blood glucose which then exacerbates disease (Baskin;Bookout and Olson, 2014). Assessment of liver and kidney function also allows to assess adverse health changes in response to dietary supplementation.

3.9.2.1 Serum Urea

A 5ml SST vacutainer® of whole blood was drawn at the baseline and follow-up. Urea levels were quantified using the Roche Cobas c702 automated chemistry analyser (Roche Diagnostics, Basel, Switzerland) (Kaplan;Pesce and Kazmierczak, 2003). Serum urea allowed for assessment of liver and renal function because it is a nitrogenous end-product of metabolism (Hosten, 1990). In HCM, both high and low serum uric acid is correlated with increased risk of all-cause mortality (Wang *et al.*, 2020). Serum uric acid is significantly correlated with serum urea (Chen *et al.*, 2009).

3.9.2.2 Creatinine

A 5ml SST vacutainer® of whole blood was drawn at the baseline and follow-up. Creatinine levels were quantified using the Roche Cobas c702 automated chemistry analyser (Kaplan;Pesce and Kazmierczak, 2003). Creatinine allowed for assessment of renal function because it is a nitrogenous end-product of metabolism (Hosten, 1990). Creatinine is also related to hypertension, which promotes adverse cardiac remodelling in HCM (Dimitrow *et al.*, 1998; Coresh *et al.*, 2001; Guttmann *et al.*, 2017; Mace *et al.*, 2023).

3.9.2.3 Estimated glomerular filtration rate

In HCM participants, eGFR is a measure of renal function and independently associated with all-cause mortality, cardiovascular mortality, and adverse outcomes in HCM (Huang *et al.*, 2021). A 5ml SST vacutainer® of whole blood was drawn at the baseline and follow-up. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (Levey *et al.*, 2009). Where Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1:

$$eGFR = 141 \times \min(S_{Cr} \times 0.0113/k, 1)^{\alpha} \times \max(S_{Cr} \times 0.0113/k, 1)^{-1.209} \times 0.993^{Age}$$

[x 1.018 if female]

3.9.3 Lipid Profile

Alterations to lipid profile is related to adverse cardiac remodelling, particularly of the left ventricle. Hypercholesterolemia is associated with cardiac hypertrophy and fibrosis, and therefore a potential contributor to worsening HCM pathology (Kang *et al.*, 2009). In HCM phenotype animal models, the inhibition of cholesterol biosynthesis induces regression of HCM phenotype (i.e., improved left ventricular filling pressure, hypertrophy, and fibrosis) (Patel *et al.*, 2001). For each measure of lipid profile, A 5ml SST vacutainer® of whole blood was drawn at the baseline and follow-up. Levels of TC, HDL-C, and triglycerides were quantified using the Roche Cobas c702 automated chemistry analyser (Kaplan;Pesce and Kazmierczak, 2003). Non-HDL-C was calculated by subtracting HDL-C from TC. TC/HDL-C ratio was calculated by dividing TC by HDL-C.

3.9.4 Inflammation

Inflammation is a risk marker for cardiovascular disease and associated with adverse cardiac remodelling in HCM disease progression such as diastolic dysfunction, left ventricular hypertrophy, and myocardial fibrosis (Fang *et al.*, 2017; Rosca *et al.*, 2020). Inflammation was assessed by the quantification of:

i) Albumin
ii) ALP
iii) Bilirubin
iv) CRP
v) Total protein (measure of the two major proteins in the blood (i.e., albumin and globulin)
vi) TNFα

To quantify serum levels of the listed inflammatory biomarkers a 5ml SST vacutainer® of whole blood was drawn at the baseline and follow-up visit. A Roche Cobas c702 automated chemistry analyser was used (Kaplan;Pesce and Kazmierczak, 2003).

3.9.5 Haemoglobin A1c

A 4ml Ethylenediamine tetra-acetic acid (EDTA) vacutainer® of whole blood was drawn at the baseline and follow-up visit for the assessment of glycosylated haemoglobin (HbA1c). The Tosoh Haemoglobin A1c method was used and utilised the principles of ion exchange high

performance liquid chromatography for the automatic separation of HbA1c (NUTH, 2002). HbA1c is an indicator of blood glucose levels over the past 3 months and associated with overall cardiovascular mortality in the general (healthy) population, particularly when > 38.8 mmol/mol (Sinning *et al.*, 2021).

3.10 Transthoracic echocardiography (ultrasound)

The echocardiogram is a non-invasive tool that uses ultrasound to analyse myocardium physiology (Edler and Hertz, 2004). Methods of analysis include transthoracic (TTE), transoesophageal (TEE), stress and contrast echocardiography (Chambers;Monaghan and Jackson, 1988; Armstrong and Ryan, 2008). Each method has its own limitations. In the present thesis, TTE is the used method and therefore the focus.

In the 1980s, echocardiography became a useful and accurate method that could be applied to study clinical HCM (Doi *et al.*, 1980). 2D transthoracic echocardiography (TTE) is the first-line tool to measure real-time left ventricular (LV) hypertrophy, LV ejection fraction, systolic anterior motion of the mitral valve, and systolic and diastolic function in HCM (Mandes *et al.*, 2020). TTE is non-invasive, safe, inexpensive, and portable (Lang *et al.*, 2015). It provides a detailed depiction of cardiac anatomy and physiology.

Measurements in the present thesis (i.e., left atrial diameter, left atrial volume, left atrial volume index, E/e' ratio, LV end diastolic volume, LV internal diameter end-diastole, and posterior wall thickness at end-diastole) were acquired by a British Society of Echocardiography accredited echocardiographer (Professor Christopher Eggett and Mr Peter Luke). The same echocardiographer completed follow-up visits for the patient that they completed measurements for at baseline.

During assessment, a probe was placed on the chest with lubricating jelly, which allowed for good contact between the probe and skin. The transthoracic probe released a mechanical vibration towards the heart, whereby the velocity (i.e., frequency multiplied by wavelength) of the ultrasound was determined by the medium of the heart (the time taken for high-frequency sound waves to reach the probe again equalled the depth of the heart) (Ludwig, 1950). The intensity (i.e., amplitude) of the signal equalled size and density of the heart.

Colour and tissue Doppler allowed for real-time analysis of the direction and speed of blood through myocardial structures such as valves (tissue Doppler imaging) (Meimoun and Tribouilloy, 2008). Real-time images were acquired in the standard parasternal (long-axis) and

apical (apical 4, apical 2, and apical long) views, for which 3 cardiac cycles were recorded. Parasternal long-axis views were acquired at 3 levels: basal (at mitral valve level), midpapillary, and apical (minimum cavity distal to papillary muscle level). Peak velocity of the left ventricular outflow tract was recorded from the apical 5 chamber view by pulse Doppler, and pressure gradient was calculated.

In HCM participants, accurate measurements through the use of the correct technique is imperative due to the echocardiogram playing a pivotal role in diagnosis and assessment (Turvey *et al.*, 2021). The parasternal long-axis view at end-diastole was used in the present thesis because it is most accurate for LV wall thickness measurement (Williams;Frenneaux and Steeds, 2009). Determination of whether an individual with HCM was obstructive (i.e., gradient ≥30 mmHg) or non-obstructive (definitions are available in section 1.3.1) was performed using the instantaneous (i.e., peak) LV outflow tract (LVOT) gradient (Maron *et al.*, 2003).

3.11 Quality of Life (Minnesota living with heart failure and short form 36 health survey questionnaire) and Hospital anxiety and depression scale

HCM individuals in Chapter 5 completed a self-reported disease-related QoL questionnaire (i.e., Minnesota living with heart failure 21-item questionnaire (MLHFQ)), general health related quality of life questionnaire (i.e., Short Form-36 Health Survey (SF-36)), and hospital and anxiety questionnaire (i.e., hospital anxiety and depression scale (HADS)) at baseline and follow-up visits (please refer to Appendix 9 for questionnaires). I escorted all participants to a pleasant and quiet room in the Clinical Research Facility of the Royal Victoria Infirmary for lunch and to fill out the described hand-written, self-reported questionnaires.

3.11.1 Minnesota living with heart failure questionnaire and short form 36 health survey

The MLHFQ assessed physical, socioeconomic, and psychological impairment in HCM before and after the interventional period in the present thesis (Rector, 1987). The original purpose was to measure disease-related QoL in individuals with chronic HF individuals, and is presently the most used questionnaire with same purpose, due to its ability to measure and detect change over a period of time (Rector, 1987; Garin *et al.*, 2009). The MLHFQ is reliable and valid, displaying significant association with clinical outcome and disease severity in primary care (Morcillo *et al.*, 2007; Garin *et al.*, 2009; Naveiro-Rilo *et al.*, 2010).

Questions asked regard the extent that illness has prevented the individual from living how they would have wanted in the last month (i.e., specific to signs and symptoms, social relations, physical activity, and work topics). Such items split in to three dimensions: Physical dimension,

8 items; emotional dimension, 5 items; and overall dimension, 21 items. The participants ranked each item on a common scale of 0-5 (i.e., no effect on QoL to highest impact on QoL) which gave an end score and allowed assessment of how HCM influenced the individuals' QoL. Scores range from 0 to 105 points with a change in 5 points associated with a clinically relevant alteration in an individual's QoL. Lower scores indicated a better QoL through indication of a lesser effect of symptoms.

Another frequently used, self-administered, and generic measure of health related QoL is the Short Form-36 Health Survey (SF-36) (Ware, 2000). The questionnaire was the second QoL questionnaire used in the present lifestyle intervention in individuals with HCM. The questionnaire was developed for individual's 14-years of age or older and is observed to have good reproducibility and validity (Ware, 2000; Saris-Baglama *et al.*, 2007).

The eight scales (i.e., physical functioning (10 items); physical role limitations (four items); bodily pain (two items); general health perceptions (five items); energy/ vitality (four items); social functioning (two items); emotional role limitations (three items); and mental health (five items)) of the SF-36 is produced from the 40 scales used in the medical outcomes study (MOS) in 1989, which was a 2-year observational study in individuals with chronic conditions (Tarlov *et al.*, 1989). Score range is 0-100, where 100 represents best possible health.

3.11.2 Hospital anxiety and depression scale

The hospital anxiety and depression scale (HADS) is the most commonly used questionnaire to assess depression and anxiety in outpatient clinic, and was designed to help identify such disorders in those with physical illness (Zigmond and Snaith, 1983; Bjelland *et al.*, 2002). In both clinical and medically healthy individuals, anxiety has demonstrated association with cardiac mortality (i.e., 46% and 55% increased risk of cardiovascular disease and cardiac death) (Eng *et al.*, 2011). Therefore, screening of depression and anxiety in clinic may improve prognosis of individuals with cardiovascular disease. HADS demonstrates high level of performance in assessment of severity and presence of anxiety disorders and depression in individuals with pathology as well as the general population (Bjelland *et al.*, 2002).

The self-reported questionnaire allowed for assessment of anxiety and depression in the present thesis. Subscales measured emotional disorder severity and assessed how the participant felt in the last week.

The questionnaire comprises of 14 items, with no inclusion of symptoms relating to the body (i.e., physical symptoms including fatigue, appetite, or insomnia). The system focuses on anhedonic depressive symptoms (i.e., inability to feel pleasure). Seven items relate to anxiety, and seven to depression (i.e., 0-7, normal; 8-10, borderline abnormal/ mild; 11-14, moderately abnormal; 15-21, abnormal/ severe). Questions relating to anxiety and depression include statements such as "I feel tense or wound up" and "I still enjoy things I used to enjoy", respectively. Recent meta-analysis combining sensitivity and specificity of HADS-depression in 57 studies and concluded that a score of seven or more, eight or more and 11 or more had sensitivity and specificity of 82% and 78%; 74% and 84%; 44% and 95%, respectively (Wu *et al.*, 2021).

3.13 Step count monitoring

In Chapter 5, step count was objectively measured using a pedometer (Omron Health care, Model no: HJ-321-E, Japan). The use of the Omron HJ-321-E pedometer has been confirmed an accurate method to measure step-count and a feasible way to record physical activity level in individuals with cardiovascular disease and HFrEF(Giannakidou *et al.*, 2012; Okwose *et al.*, 2019).

3.14 Methods relevant to evaluation of mitochondrial function in ageing and hypertrophic cardiomyopathy

3.14.1 Mouse models

Animals used were C57BL/6 (WT) or genetically modified P50ko i.e., C57BL/6 *nfkb1*−/− mice (Jurk *et al.*, 2014; Bianchi *et al.*, 2021). The mice were bred in-house as homozygous lines and housed in groups of four to six. Mice were housed in pathogen-free conditions. Age-matched 3 month (3mo/ young) and 12-month (12mo/ old) male C57BL/6 *nfkb1*−/− mice and WT i.e., control mice were euthanised by cervical dislocation before the hearts were removed. The hearts were fixed in 4% formalin, processed and paraffin wax embedded. Following the described process, hearts were gifted by Professor Fiona Oakley.

3.14.2 Section preparation

After trimming blocks to full face tissue exposure, 4µm sections were cut using a HM325 rotary microtome (Thermo Scientific Microm) by Dr Anna Smith. Dr Anna smith also incubated sections prior to immunofluorescence staining for 1 hour at 60°C.

For the final assay, I deparaffinised sections in two changes of Histoclear (HS-200, National Diagnostics), and rehydrated them through an ethanol series (100% EtOH, 95% EtOH, and 70% EtOH) for 5 minutes in each. The sections were then washed in tap water. Antigen (Ag) retrieval was carried out using 10mM Tris Base, 1mM EDTA (Tris EDTA) pH8 in a pressurised antigen retrieval unit (2100 Antigen Retriever, Aptum Biologics Ltd.) for 40 minutes before slides were transferred promptly to a distilled water trough.

I then used a hydrophobic PAP pen was used to draw rings around sections prior to staining before being covered in TBST to prevent drying. Incubation for one hour at room temperature in 10% normal goat serum (NGS) was then completed to block non-specific binding sites before being rinsed using TBST. Endogenous biotin sites were blocked using an Avidin/Biotin Blocking Kit (Vector Laboratories) where each solution was added for 15 minutes and rinsed between and after using TBST. Sections were subsequently washed in TBST for 2x5 minutes on a rocking platform.

The immunofluorescence protocol was completed by both myself and Dr Anna Smith during optimisation (described in section 3.14.3). Dr Anna Smith guided me through the optimisation process due to the length and intensity of optimisation. For immunofluorescence training, I first shadowed Dr Anna Smith staining tissue sections that were not my cardiac sections, next, Dr Anna Smith overlooked as I stained tissue sections during optimisation before I confidently completed staining independently.

3.14.3 Immunofluorescence

Immunofluorescence plays an important role in biological research and the clinical setting. The technique uses a combination of primary (direct) and secondary (indirect) antibodies to detect antigens in tissues, organisms, cultured cells, or cell suspensions (Im *et al.*, 2019).

The immunofluorescence protocol followed was described by Rocha et al (Rocha *et al.*, 2015). Fixation was the preliminary step of immunofluorescence and prevented autolysis/ maintained antigenicity without altering cellular architecture. Deparaffination was completed using Histoclear and rehydrated (ethanol and distilled water) after being mounted on the glass slide, as described above (Harlow and Lane, 1999). Thirdly, antigen retrieval was performed (ag) to reinstate epitope-antibody reactivity after the alteration caused by fixation. In the present thesis, Heat-Induced Epitope Retrieval in a buffer solution was used to restore antigenicity of secondary and tertiary protein structure by reversal of cross-links. Next, the indirect (higher sensitivity and allowed detection of several targets in the same sample) immunofluorescence method was employed (figure 3.4A) and composed of two incubation steps where the primary antibody first bound the target epitope, and a fluorophore-tagged secondary antibody then bound the primary.

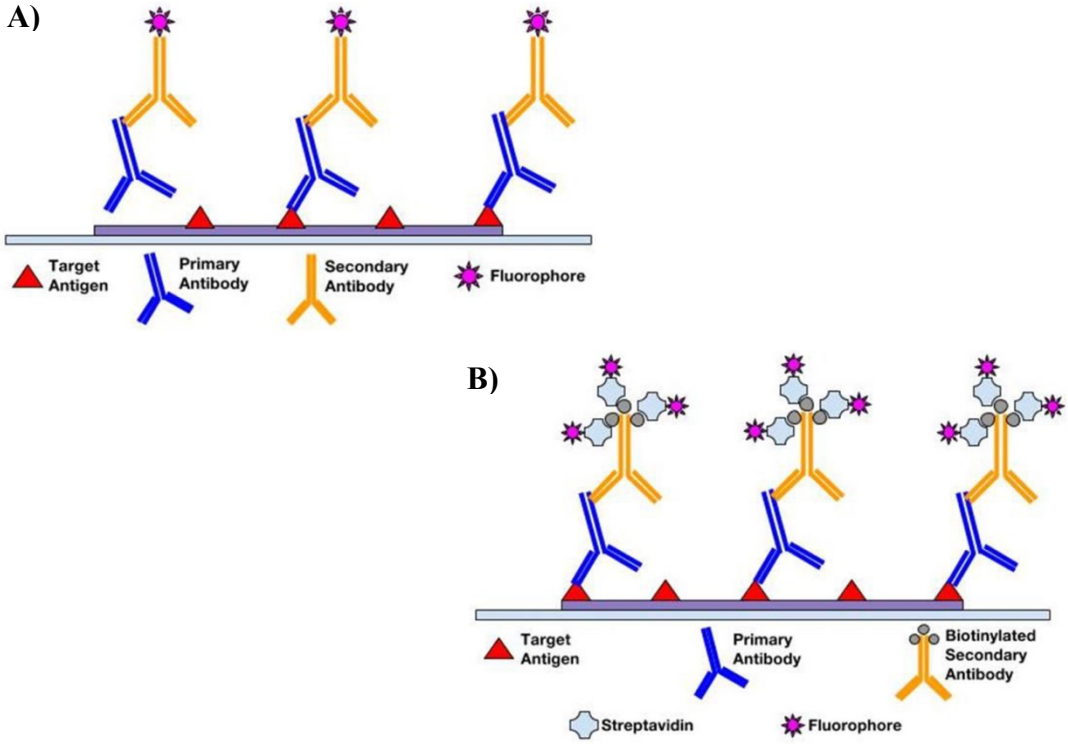


Figure 3.4 A) Indirect immunofluorescence; B) Use of a secondary antibody conjugated with multiple fluorophore-protein complexes to amplify the signal. Adopted from (Eggebeen *et al.*, 2016; Im *et al.*, 2019).

3.14.4 Oxidative phosphorylation immunofluorescence optimisation

Sections were incubated in the primary antibody cocktail (table 3.1) overnight at 4℃. No primary control (NPC) slides were only incubated with TBST. All sections were subsequently washed in TBST (3x5 minutes) on a rocking platform after returning to room temperature and

before being incubated with the secondary antibody (table 3.1) for two hours in the dark at room temperature. After incubation, sections were washed in TBST (3x5 minutes) on the rocking platform. Sections were then incubated with the tertiary antibody cocktail (table 3.1) for 2 hours in the dark at room temperature. After incubation, sections were washed in TBST (3x5 minutes) on the rocking platform. Finally, sections were mounted using Prolong Gold (P36930, Invitrogen) before being left to dry at room temperature and stored at 4℃ where fluorescent signal would be better retained. Slides were then viewed by fluorescence microscopy (Zeiss LSM800 Airyscan).

Table 3.1 Primary and secondary antibodies for immunofluorescent detection of OXPHOS complexes (CI and CIV), mitochondrial mass (porin) and cardiomyocyte boundary marker (laminin) in initial optimisation process.

Antibody	**Host**	**Dilution**	**Manufacturer**
Primary antibodies			
Laminin	Rabbit	1/50	L9393 Sigma Aldrich
Porin	Mouse	1/100	ab14734 Abcam
MTCO1	Mouse	1/50	ab14705 Abcam
NDUFB8	Mouse	1/50	ab110242 Abcam
Secondary antibody			
Biotinylated IgG1	Goat	1/200	A10519 Invitrogen
Tertiary antibodies			
Anti-rabbit IgG Alexa Fluor 405	Goat	1/100	A31556 Invitrogen
Anti-mouse IgG2b Alexa fluor 488	Goat	1/200	A11008 Invitrogen
Anti-mouse IgG2a Alexa Fluor 546	Goat	1/200	A21143 Invitrogen
Anti-mouse Strep Alexa Fluor 647	Goat	1/200	S32357 Invitrogen

The initial immunofluorescent staining method produced sections in which tissue had lifted. This was visible as spots throughout the section (Figure 3.5).

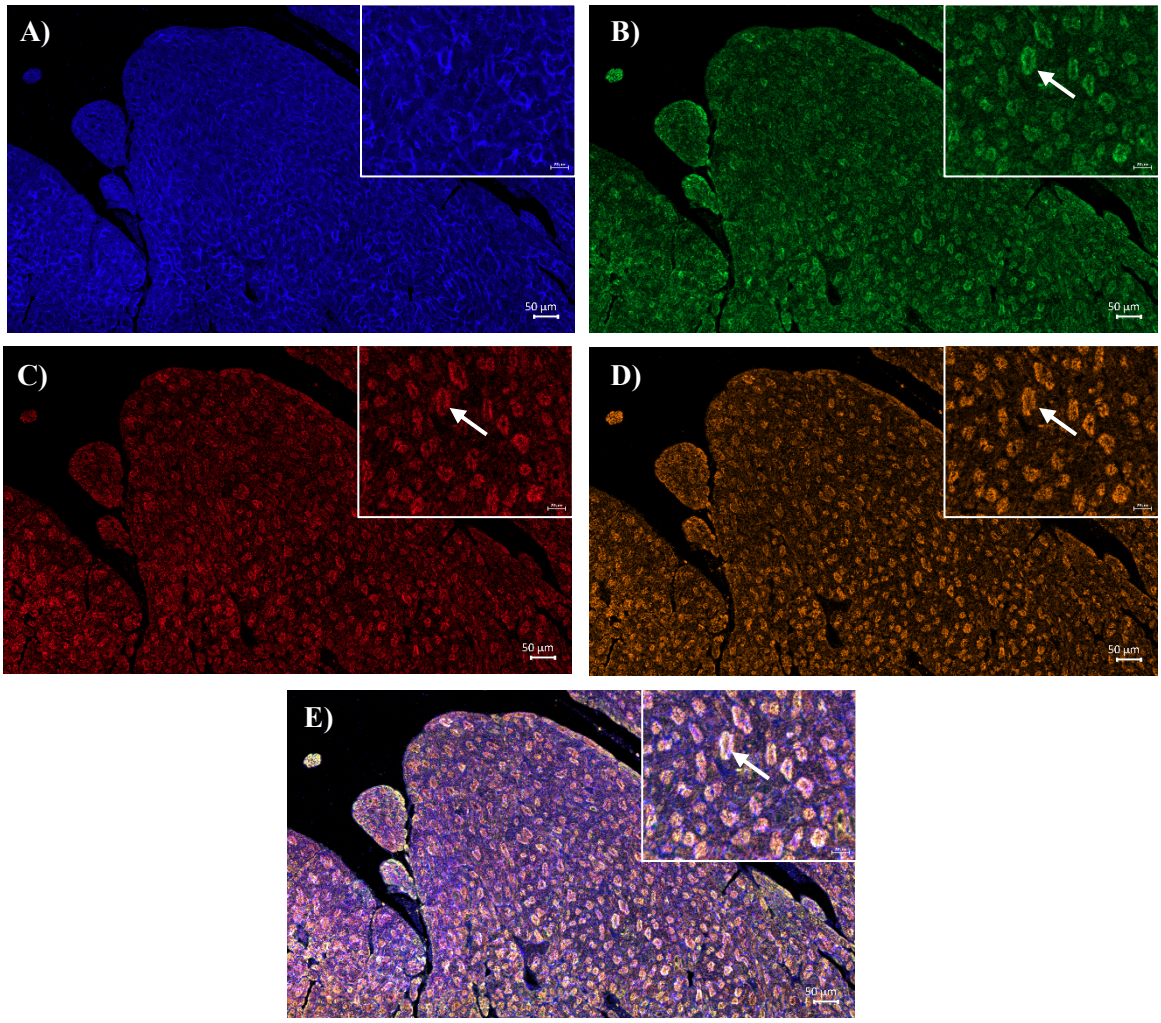


Figure 3.5. Representative image of initial heart tissue optimisation using 1mM EDTA at pH8 as antigen retrieval buffer. A) AlexaFluor 405 labels anti-laminin primary antibody (cellular membrane marker); B) AlexaFluor 488 labels anti-porin primary antibody (mitochondrial mass); C) AlexaFluor 647 labels anti-NDUFB8 primary antibody (mitochondrial complex I); D) AlexaFluor 546 labels anti-MTCO1 primary antibody (mitochondrial complex IV); E) merged image representing fluorescence from all channels combined. Magnification x20. Scale bars= 50um; 20um magnified inserts. Arrows denote lifted regions.

It was hypothesised that the high temperature and pressure of the antigen retrieval unit may have contributed to the lifted tissue artefact observed, therefore a 10-minute microwave incubation was used in place of the antigen retrieval unit whilst using the same immunofluorescent labelling protocol. Following microwave antigen retrieval spotted, lifted tissue was still visible and laminin was not labelled at all (figure 3.6).

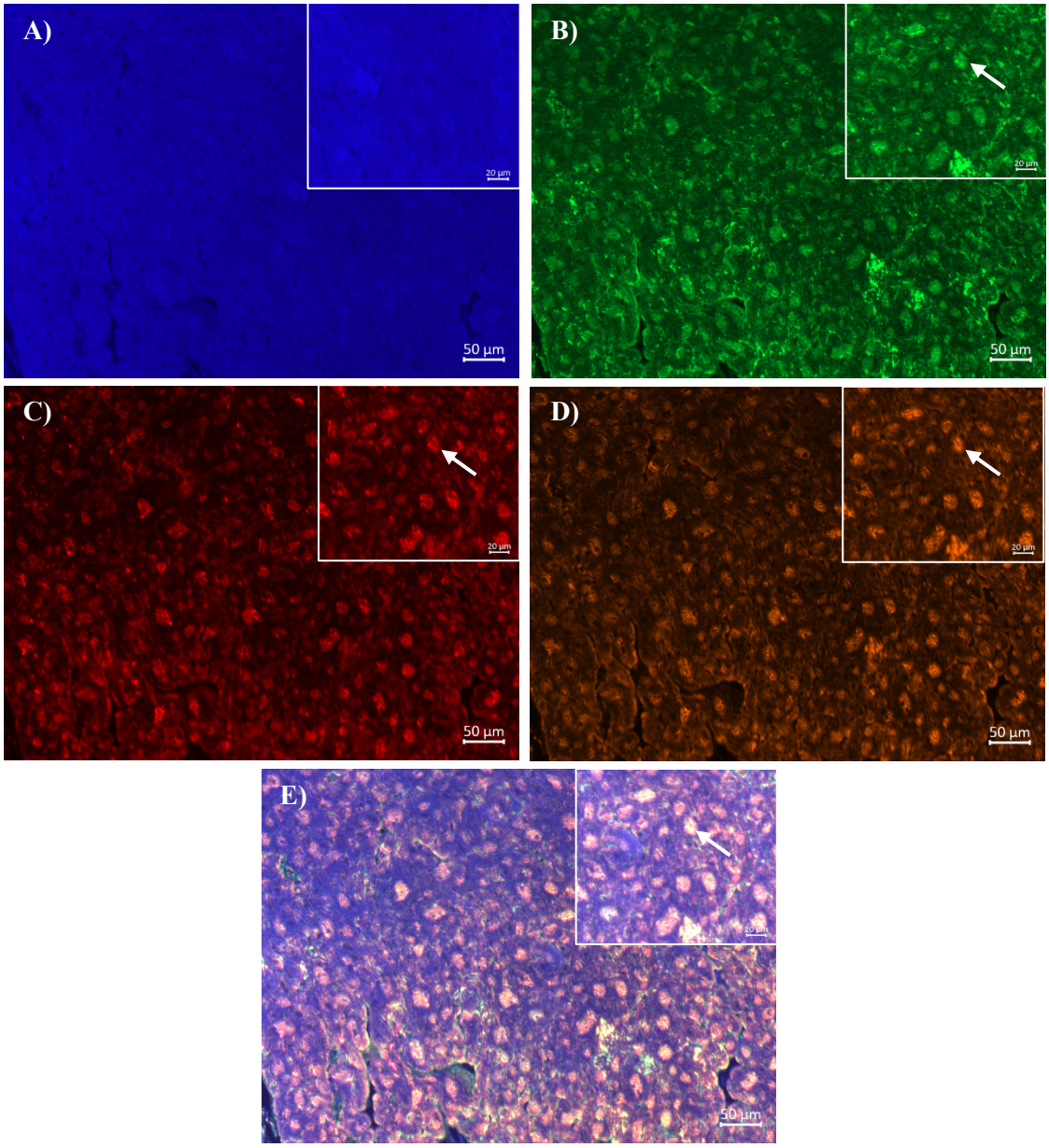


Figure 3.6. Representative images of heart tissue optimisation using the microwave for antigen retrieval for 10 minutes, and 1mM EDTA at pH8 as antigen retrieval buffer. A) AlexaFluor 405 labels anti-laminin primary antibody (cellular membrane marker); B) AlexaFluor 488 labels anti-porin primary antibody (mitochondrial mass); C) AlexaFluor 647 labels anti-NDUFB8 primary antibody (mitochondrial complex I); D) AlexaFluor 546

1mM EDTA buffer was now hypothesised to be the cause of the damage occurring to tissue. To test this hypothesised problem, 0.1 mM sodium citrate buffer at pH6 was used in place of the 1mM EDTA buffer. Sections were placed in both the antigen retrieval unit and microwave. In the antigen retrieval unit using citrate buffer, lifted spots became less apparent however laminin labelling failed and was not specific to the cell membrane. For this assay, NDUFB8 was omitted to reduce experimental time. When citrate buffer and microwave antigen retrieval

were used together, laminin labelling also failed (figure 3.7). Given that laminin labelling is required for subsequent cell segmentation analysis, and that laminin labelling was successful with EDTA based antigen retrieval, altering the pH of the EDTA buffer was proposed as a next optimisation step.

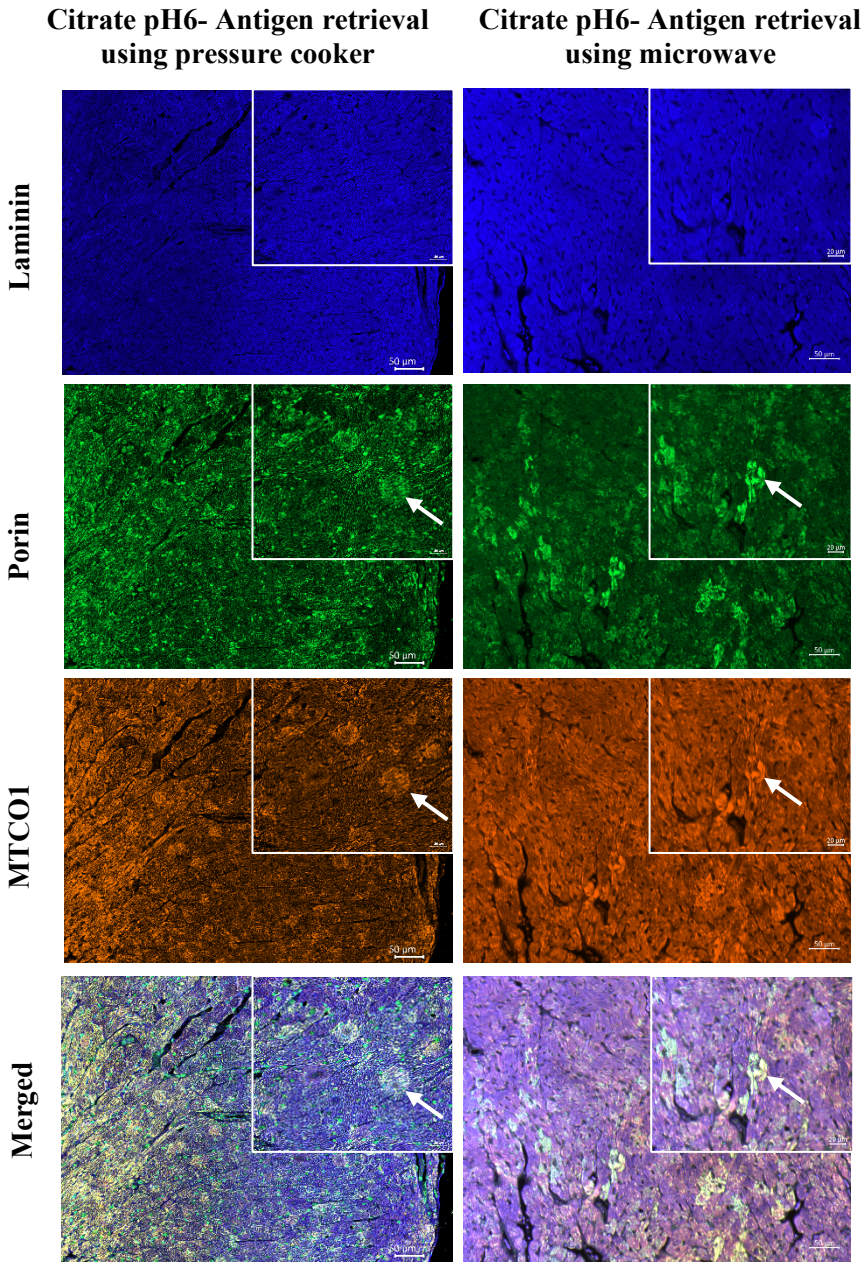


Figure 3.7. Representative images of heart tissue labelling using 0.1mM sodium citrate pH6 and left column- pressure cooker; right column- microwave for antigen retrieval. From top to bottom: AlexaFluor 405 labels anti-laminin primary antibody (cellular membrane marker); AlexaFluor 488 labels anti-porin primary antibody (mitochondrial mass); AlexaFluor 647 labels anti-NDUFB8 primary antibody (mitochondrial complex I); AlexaFluor 546 labels anti-MTCO1 primary antibody (mitochondrial complex IV); merged image representing fluorescence from all channels combined. Magnification x20. Scale bars= 50um; 20um magnified inserts. Arrows denote lifted regions.

To test whether the pH of EDTA buffer was damaging the tissue, 1mM EDTA buffer at pH7 and pH7.5 were trialled in the immunofluorescent labelling process. NDUFB8 was not included in this trial due to the additional time it takes experimentally. To attempt to improve laminin labelling, anti-laminin primary antibody was labelled with AlexaFluor 488 in place of AlexaFluor 405 on one section (1mM EDTA pH7.5). Anti-porin primary antibody was therefore labelled with AlexaFluor 405 in place of AlexaFluor 488 in this case. Tissue lifting artefact was still observed however laminin labelling appeared to improve with the use of AlexaFluor 488 (figure 3.8).

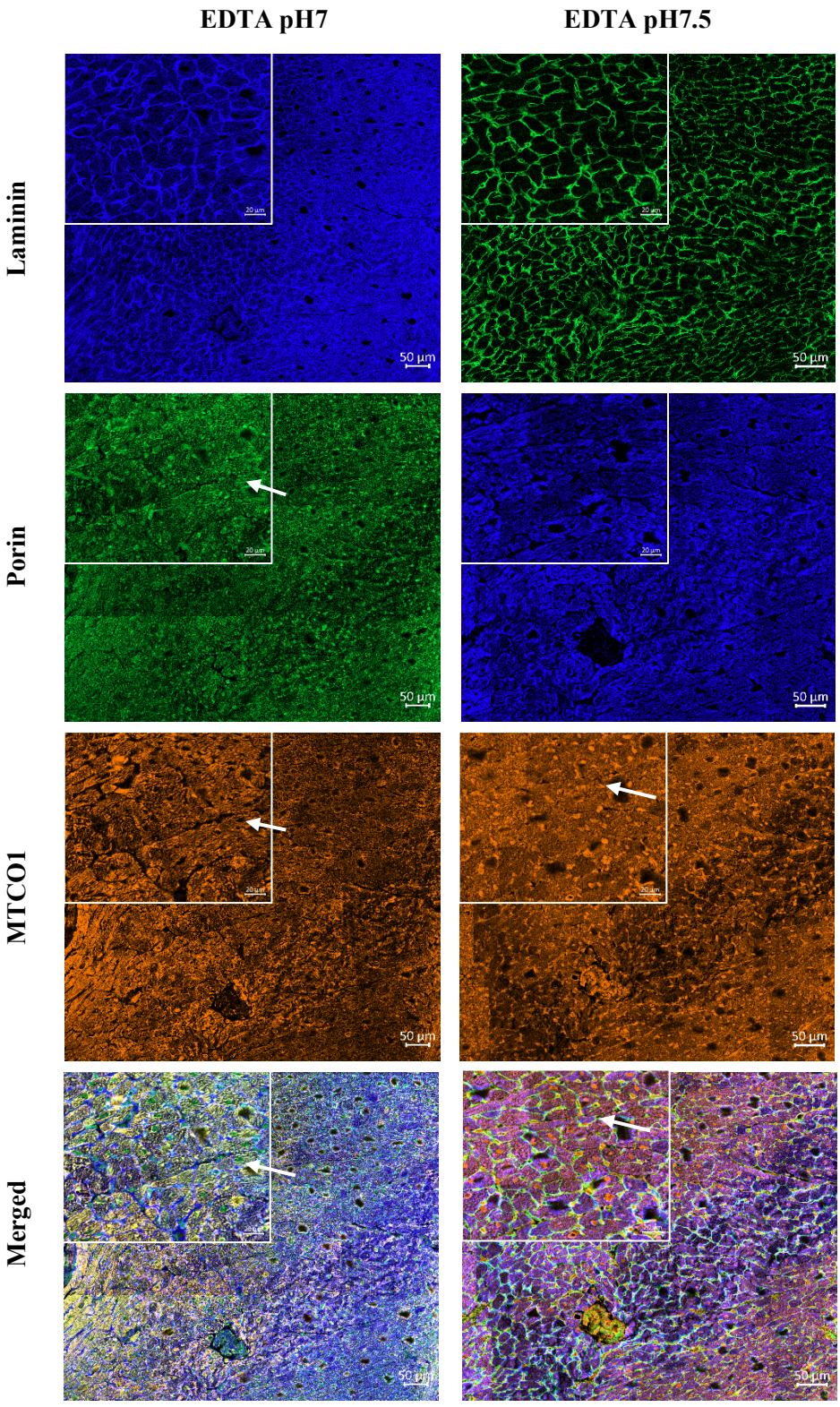


Figure 3.8 Representative images of heart tissue labelling of mitochondrial complex IV (MTCO1) using 1mM EDTA pH7 (left) and pH7.5 (right). On the left, AlexaFluor 405 labels anti-laminin primary antibody (cellular membrane marker); AlexaFluor 488 labels anti-porin primary antibody (mitochondrial mass); AlexaFluor 546 labels anti-MTCO1 primary antibody (mitochondrial complex IV) and finally, merged image representing fluorescence from all channels combined. On the right, AlexaFluor 488 labels anti-laminin primary antibody (cellular membrane marker); AlexaFluor 405 labels anti-porin primary antibody (mitochondrial mass); AlexaFluor 546 labels anti-MTCO1 primary antibody (mitochondrial complex IV). Magnification x20. Scale bars= 50um; 20um magnified inserts. Arrows denote lifted regions.

Due to failed previous optimisation attempts, Tris EDTA (i.e., 10mM Tris Base, 1mM EDTA) was trialled as an alternative antigen retrieval buffer. This buffer was used at pH7.5, pH8 and pH9. Antibodies used were the same as those described in table 3.1, omitting anti-NDUFB8 due to the additional experimental time this labelling requires. It can be observed that laminin labelling failed at pH7.5 and improved at both pH8 and pH9. Lifting was not as prominent using pH7.5 or pH8 Tris EDTA. Using pH9 tissue lifting was significant and therefore sections were unanalysable. Finally, an additional artefact appeared with the use of Tris EDTA pH7.5 in the AlexaFluor 488 labelling of anti- porin primary antibody (figure 3.9), These punctate regions of high levels of fluorescence were not observed with the use of Tris EDTA pH8. Given that the lifting artefact was significantly reduced with the use of Tris EDTA pH8 while laminin labelling was adequate, this buffer was selected for the final assay.

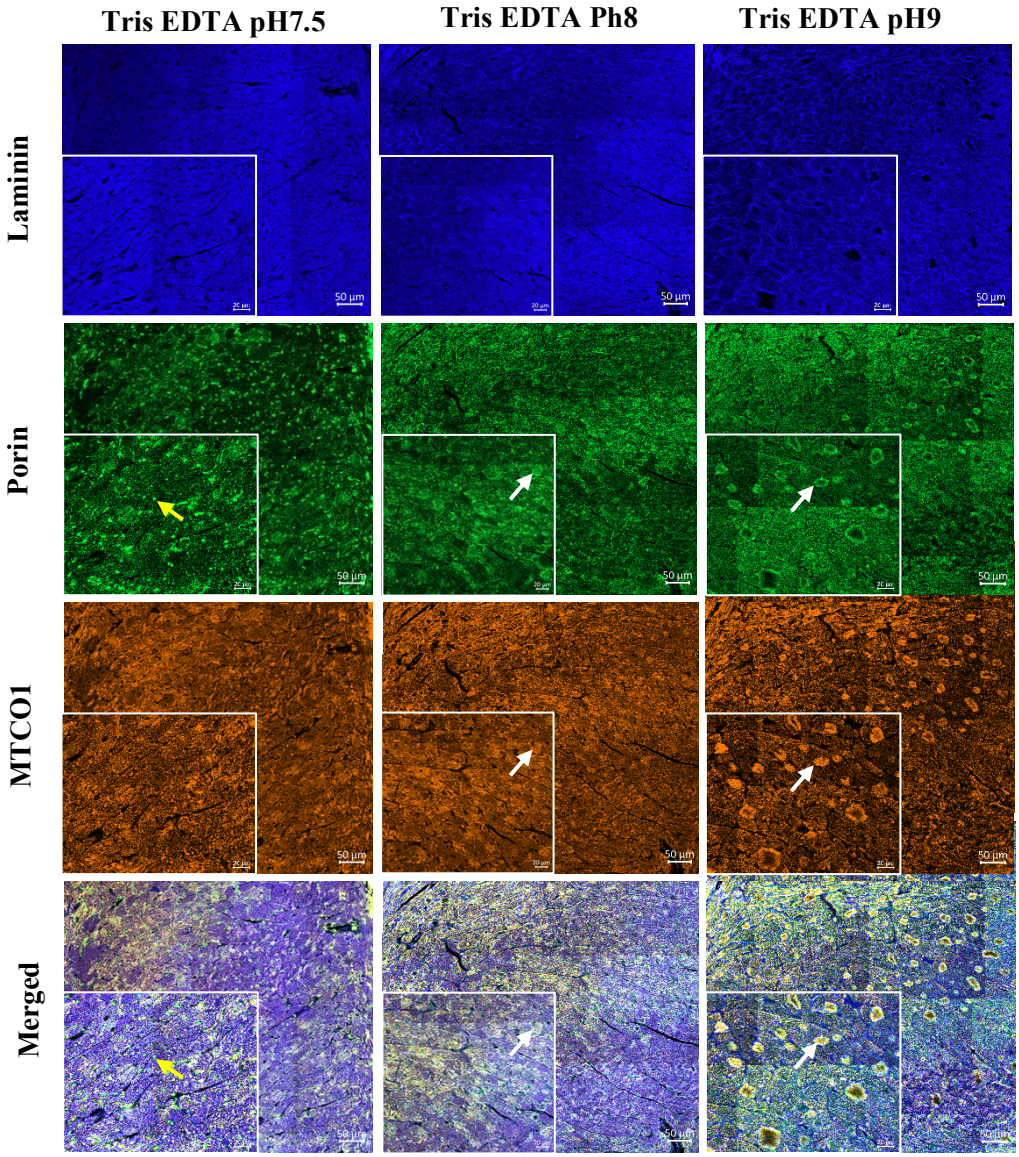


Figure 3.9. Representative images of heart tissue labelling of mitochondrial complex IV (MTCO1) using, using Tris EDTA (i.e., i.e., 10mM Tris Base), pH7.5 (left), and pH8(middle) and pH9 (right). For all three columns from top to bottom immunofluorescent staining: AlexaFluor 405 labels anti-laminin primary antibody (cellular membrane marker); AlexaFluor 488 labels anti-porin primary antibody (mitochondrial mass); AlexaFluor 546 labels anti-MTCO1 primary antibody (mitochondrial complex IV) and finally, merged image representing fluorescence from all channels combined. Scale bars= 50um; 20um magnified inserts. White arrows denote lifted regions, yellow arrows additional artefact.

Given the improvement of laminin labelling with AlexaFluor 488 in comparison to AlexaFluor 405, AlexaFluor 488 labelling of laminin was selected for the final assay. When AlexaFluor 405 was used to label porin however, the additional artefact observed in Figure 3.9 (yellow arrow) was observed once more (Figure 3.10). Considering this result and the generally weak signal with the use of this fluorophore it was therefore proposed that AlexaFluor 405 be omitted

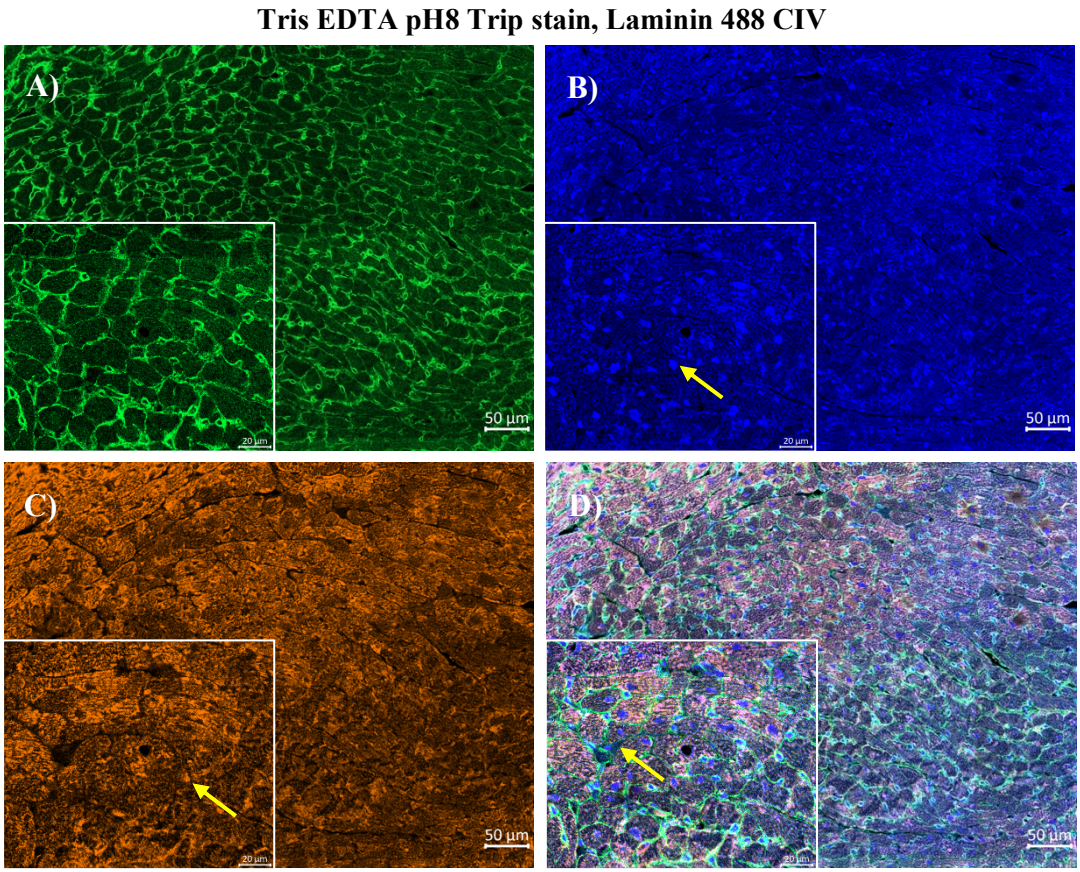


Figure 3.10. Representative images of heart tissue labelling of complex IV (MTCO1), using Tris EDTA (i.e., 10mM Tris Base) pH8. A) AlexaFluor 488 labels anti-laminin primary antibody (cellular membrane marker); B) AlexaFluor 405 labels anti-porin primary antibody (mitochondrial mass); C) AlexaFluor 546 labels anti-MTCO1 primary antibody (mitochondrial complex IV) and finally, D) merged image representing fluorescence from all channels combined. Magnification x20. Scale bars= 50um; 20um magnified inserts. Yellow arrows denote additional artefact.

from the experiment. To accommodate all target proteins using only three fluorophores, it then became necessary to label two serial sections from each mouse in the final assay. Laminin and porin were labelled with AlexaFluor 488 and AlexaFluor 546 respectively on both sections. NDUFB8 was labelled with AlexaFluor 647 on one section and MTCO1 was labelled with AlexaFluor 647 on the second section (Figure 3.11). This labelling was deemed the optimal method to assess NDUFB8 and MTCO1 protein levels in *nfkb1*−/− mice. Triple staining and the use of laminin 488 at pH8 Tris EDTA proved to be the optimal staining method to assess NDUFB8 and MTCO1 protein levels in 6 *nfkb1*−/− staining and the use of laminin 488 (figure 3.11).

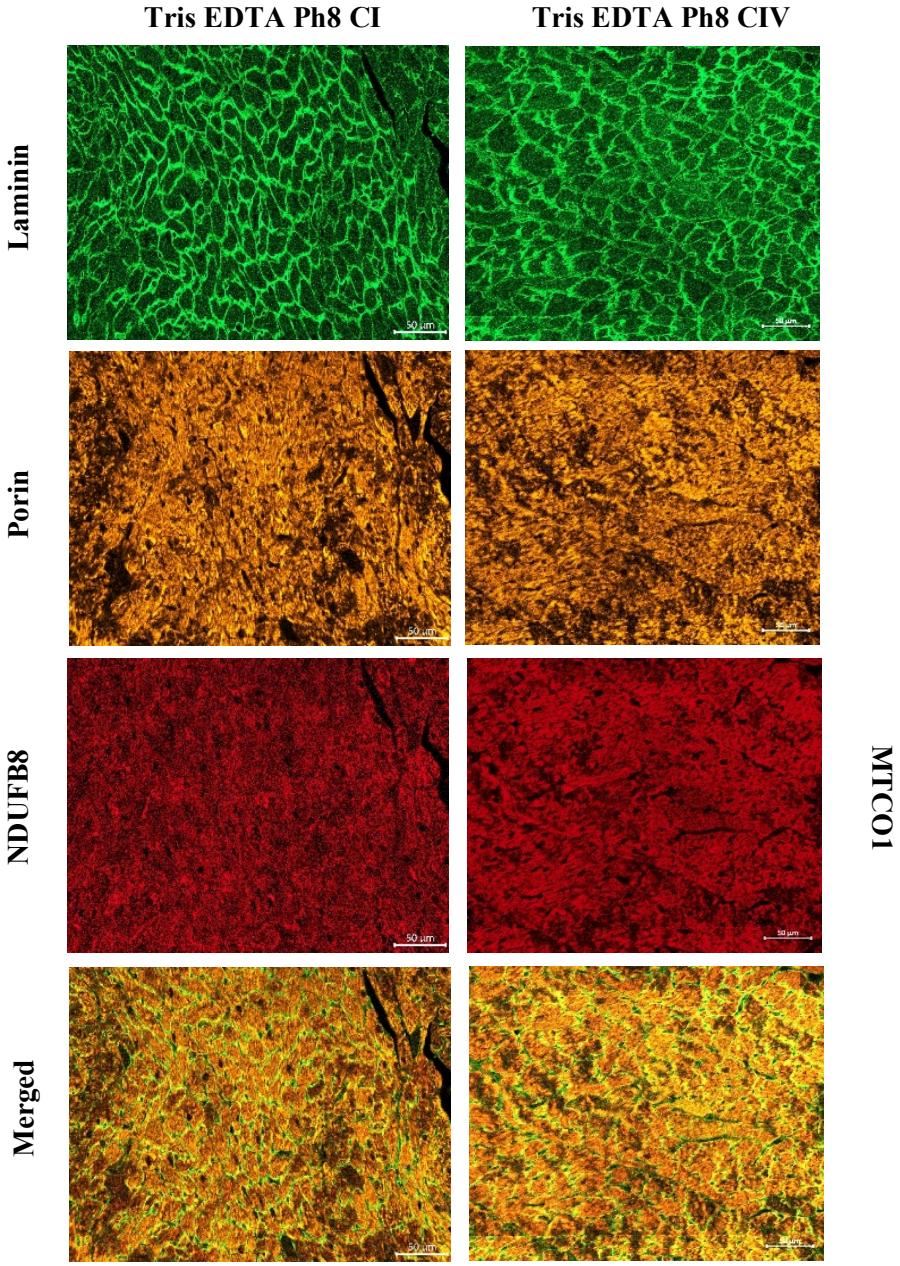


Figure 3.11. Representative images of heart tissue labelling of mitochondrial complex I (NDUFB8), left; and complex IV (MTCO1), right, using Tris EDTA (i.e., i.e., 10mM Tris Base) pH8. AlexaFluor 488 labels anti-laminin primary antibody (cellular membrane marker); AlexaFluor 405 labels anti-porin primary antibody (mitochondrial mass); AlexaFluor 546 labels anti-NDUFB8 primary antibody (mitochondrial complex I)/anti-MTCO1 primary antibody (mitochondrial complex IV) and finally, merged image representing fluorescence from all channels combined. Magnification x20. Scale bars= 50um.

3.13.5 Confocal microscopy

Confocal microscopy produces a point source of light whilst rejecting out of focus fluorescent light through the use of a confocal pinhole (Minsky, 1961). Subsequently allowing to image deep into tissue and reconstruct 3D images of samples by scanning many thin sections at a high resolution without blurring (Minsky, 1961). The first confocal microscope was designed by Marvin Minsky in the 1950s, with aims to map neural connections (Minsky, 1961). This design is still implemented today (Figure 3.12) (Bodyak *et al.*, 2002). 3D images are obtained by the laser focal point being scanned in z-axis (when collecting a stack/ series), x and y (Swaim, 2010; Elliott, 2020).

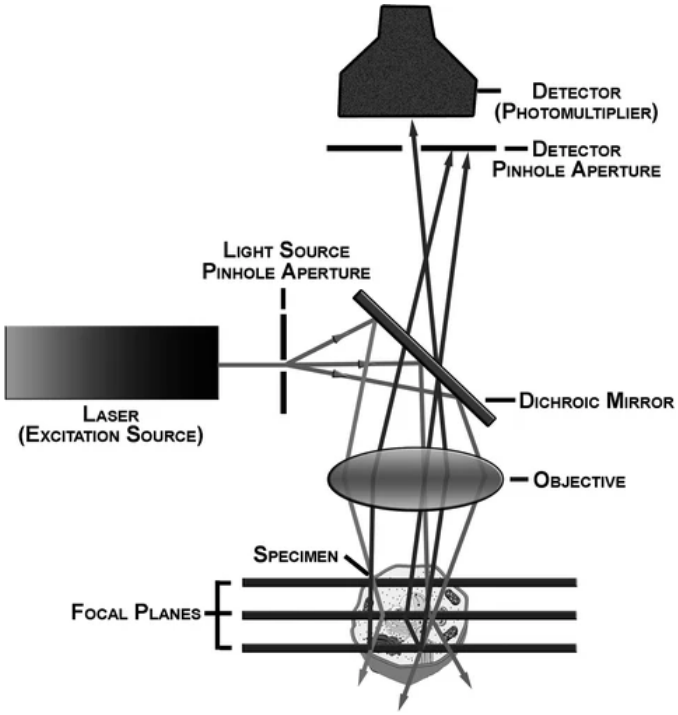


Figure 3.12 Diagram of a laser scanning confocal microscope, adopted from (Swaim, 2010)

Training was provided by the Bioimaging Unit at Newcastle University, and Dr Anna Smith prior to capturing images of the final assays myself. Dr Anna smith aided in the imaging of optimisation samples to ensure timely completion of the study.

Airyscan technology was used in the present thesis, with a 1.7x higher resolution in the x, y and z (Zeiss) (Elliott, 2020). Images were captured at x20 magnification using the confocal microscope (Zeiss LSM800 Airyscan), which was run through Zeiss' software package (Zen). Control slides were imaged alongside test samples and known as no primary controls (NPCs) (Swaim, 2010). This allowed setting of baseline parameters and avoidance of over or under-saturation of the signal.

Two tracks were used, with Alexa Fluor 546 (i.e., porin) measured on track 1 (i.e., channel 1) and Alexa Fluor 488 (Laminin) (i.e., channel 2)/ Alexa Fluor 647 (NDUFB8/MTCO1) (i.e., channel 3) measured on track 2. The pinhole remained consistent across all samples at 178 AU/ 50um for track 1 and 2.06 AU/ 50um for track 2. The microscopes' detectors conveyed signal digitally for detection of light at 555-645nm for Alexa Fluor 546 (excitation wavelength 557); 400-555nm for Alexa Fluor 488 (excitation wavelength 493); 645-700nm for Alexa Fluor 647 (excitation wavelength 653). Detector gain remained at 675V for channel 1, and 650V for channel 2 and 3 for all slides. Detector offset remained at 0. For all sections in the present study, laser scanning microscope scan speed was set to 9, a frame time of 1.27s with a bidirectional scan direction.

3.13.6 Microscope image analysis software- Imaris

Initially, the use of the in-house quadruple immunofluorescence analyser cell segmentation analysis coded for by MatLab 2015a software was trialled for the analysis of fluorescent murine heart tissue sections in the present study. However, MatLab was unable to accurately process and detect myocardium cells, this can be explained by the purpose of the software itself i.e., MatLab was designed for the analysis of skeletal muscle fibres, which are larger and have a more definitive outline than cardiac muscle (Walker and Schrodt, 1974; Sommer and Waugh, 1978).

The Imaris image analysis software (Imaris x64 9.7.2) is developed for images produced from confocal microscopy, and semi-automatically marks cells or muscle fibres along the plasma membrane. Errors including under and over segmentation, or incorrect cell identification are manually corrected by the user following semi-automated cellular marking. In the present thesis, I manually select areas most suitable for analysis through area selection, "create surface" and "mask all" functions. Prior to pressing "mask all", the channel for the membrane stain was selected. Once all cells were selected, data was extracted to an excel file for data analysis. Laminin was used to semi-automatically detect and outline transverse cardiomyocytes.

Figure 3.13 provides an example of the semi-automatic cardiomyocyte outlining prior to immunofluorescence mean fluorophore intensity (per cell) data extraction.

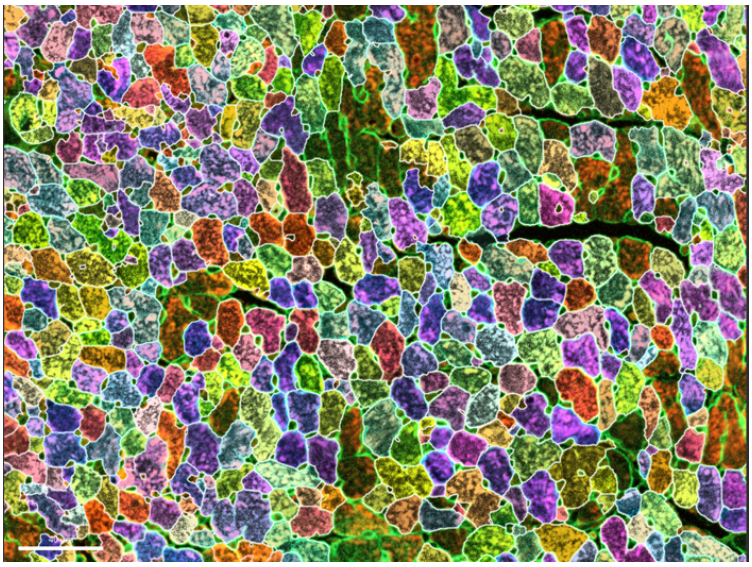


Figure 3.13 Representative image of cardiac muscle selection of cells using IMARIS prior to immune data extraction.

Chapter 4: Part A) The effect of age and sex on the physiological determinants of exercise tolerance in healthy individuals; Part B) The mechanisms of exercise intolerance in individuals with HCM

4.1 Abstract

Objective: The aim of the present study was two-fold: Part A) to evaluate the effect of age and sex on the physiological determinants of exercise tolerance in healthy individuals, with physical activity and body mass index (BMI) as the confounding factors, and Part B) to evaluate the determinants of exercise intolerance ($\dot{V}O_2$peak) in individuals with hypertrophic cardiomyopathy (HCM).

Methods: Part A) Firstly, seventy-one healthy individuals were divided into groups according to their age i.e., younger (<40 years of age, N=43, n=17 females); and older (≥55 years of age, N=28, n=13 females) and sex. Part B) Secondly, and in a cross-sectional, observational design, the present study recruited 29 individuals with HCM (age: 55±15 years old; n=7 female), and 23 sex-matched healthy controls (age: 52±18 years old; n=9 female). All participants underwent maximal graded cardiopulmonary exercise stress testing using a cycle ergometer coupled with simultaneous non-invasive gas-exchange and central haemodynamic measurements. Using the Fick equation, arteriovenous O_2 difference was calculated as the ratio between oxygen consumption and cardiac output.

Results: Part A) The mean age of younger participants was 26±6 years and for older participants was 65±7 years. $\dot{V}O_2$peak was significantly lower in older compared to the younger age group (21.2±11.6 vs 32.5±10.5 ml/kg/min, p<.01), but no interaction with sex was observed. With advancing age there was an acceleration in resting systolic blood pressure (SBP) and mean arterial blood pressure (MAP) in females when compared to males ((young 103±14.7 vs 112±13.0 mmHg; and old 118±13.9 vs 112±15.4 mmHg), p=.01), and ((young 77.6±10.3 vs 83.1±9.15 mmHg; and old 84.4±9.90 vs 78.6±10.7 mmHg), p=.01), respectively. Part B) $\dot{V}O_2$peak was significantly lower in individuals with HCM when compared to healthy individuals (20.0±11.4 vs 29.8±11.8 ml/kg/min, p=.01). Variability in $\dot{V}O_2$peak in individuals with HCM in comparison to healthy individuals was determined by reduced arteriovenous O_2 difference (β=0.93, p<.01), which was significantly reduced at peak in this patient population (9.19±4.46 vs 12.5±4.61 ml/kg/min, p<.01).

Conclusions: Part A) There was an accelerated age-related vascular change in females in comparison to males when physical activity was controlled for. The age-related reduction in exercise tolerance was aetiological of the reduced ability of skeletal muscles to extract delivered oxygen represented by reduced arteriovenous O_2 difference. However, there was no interaction between age and sex for $\dot{V}O_2$peak and determinants (i.e., cardiac output and arteriovenous O_2

difference). Part B), Exercise intolerance is aetiological of reduced arteriovenous O_2 difference at $\dot{V}O_2$peak in individuals with HCM compared to healthy individuals.

4.2 Introduction

$\dot{V}O_2$peak is recognised as a gold standard measure of cardiorespiratory fitness and functional capacity, and represents the point at which oxygen uptake no longer increases, despite further increase in the exercise intensity (Koutlianos *et al.*, 2013; Mandsager *et al.*, 2018; Riebe *et al.*, 2018).

As an individual ages, there is a significant correlation between chronological and biological age (Baker and Sprott, 1988). The Human Mortality Database (http://www.mortality.org/), which began in the year 2000, includes reliable data for over 38 countries. A trend of increasing lifespan is observed, with females living longer than males. Chronological age is the major risk factor for developing chronic conditions such as cardiovascular, metabolic and neuromuscular diseases (Franceschi *et al.*, 2018). A sedentary lifestyle compromises physiological function and accelerates cardiovascular and muscular function decline with ageing (Kwak, 2013). In addition, males have a higher rate of physiological and cardiovascular function decline with ageing compared to females (Hawkins and Wiswell, 2003; Paterson *et al.*, 2004; Betik and Hepple, 2008; Byberg *et al.*, 2009; Shephard, 2009; Shiroma and Lee, 2010; Wen *et al.*, 2011; Berry *et al.*, 2013; Mandsager *et al.*, 2018). For males, there is an estimated onset of cardiovascular disease 10 to 15 years earlier than in females (<45 years of age), hypothesised to be due to the cardioprotective effects of estrogen prior to menopause (Baker *et al.*, 2003).

4.2.1 Ageing and exercise tolerance

Older individuals (i.e., >65 years of age) with lower exercise tolerance have reduced functional independence, quality of life and increased incidence of cardiovascular and all-cause morbidity and mortality (Goldspink *et al.*, 2009). A loss of functional independence in older age most likely occurs when $\dot{V}O_2$peak drops below 18mL/kg/min in males and 15 mL/kg/min in females (Shephard, 2009). After the age of 40 years old, oxygen consumption along with cardiac output and heart rate at peak exercise declines more in males than females (Rivera *et al.*, 1989; Stathokostas *et al.*, 2004; Weiss *et al.*, 2006; Tanaka and Seals, 2008; Goldspink *et al.*, 2009). It has been suggested that such sex differences may be explained by hormonal factors including oestrogen (Hawkins and Wiswell, 2003). An important finding in females is that oestrogen's anti-apoptotic effects lead to reduced rate of cardiac ageing through less cardiomyocyte loss when compared to males (Olivetti *et al.*, 1995; Goldspink *et al.*, 2009). The described sex-specific protection reduces with age and following menopause (Merz and Cheng, 2016).

Older individuals demonstrate left ventricular (LV) and vascular remodelling manifested as lower peak heart rate, peak cardiac output, peak arteriovenous O_2 difference, early diastolic

filling rate, LV contractility, reduced blood volume, increased stiffness and thickness of the myocardium and arteries, as well as increased LV end-diastolic volume (Kim *et al.*, 2016; Jakovljevic, 2018). Aerobic exercise in particular leads to improvement of age-related cardiovascular adaptations that would otherwise hinder exercise tolerance (i.e., LV remodelling and vascular remodelling), resulting in improved cardiac efficiency and decreased myocardial oxygen demand (Manou-Stathopoulou *et al.*, 2015; Jakovljevic, 2018). However, whether peak cardiac output and stroke volume decline with age is controversial (Rodeheffer *et al.*, 1984a; McElvaney *et al.*, 1989; Ogawa *et al.*, 1992; McGuire *et al.*, 2001; Carrick-Ranson *et al.*, 2013; Houghton *et al.*, 2016; Pandey *et al.*, 2019).

A stronger understanding of the physiological mechanisms leading to reduced exercise tolerance/ intolerance in elderly individuals is important as it may lead to development of novel interventions that delay or prevent the onset of age-related physiological decline (Rodeheffer *et al.*, 1984b; Hawkins and Wiswell, 2003; Christou and Seals, 2008; Kim *et al.*, 2016).

4.2.2 Physical activity and ageing

Regular exercise leads to a higher level of cardiorespiratory fitness and has been proven to be an efficient strategy to slow down cardiovascular ageing as well as overall ageing (Blair *et al.*, 1995; Tanaka;DeSouza and Seals, 1998; Tanaka *et al.*, 2000; Seals, 2003; Gielen;Schuler and Adams, 2010). The age-related increase in oxygen cost of exercise and decreased exercise efficiency may be reversed in older individuals through increased physical activity (Woo *et al.*, 2006). In coherence, higher daily physical activity has been observed to preserve exercise capacity and cardiac metabolism in older people (Jakovljevic *et al.*, 2015).

4.2.3 Hypertrophic cardiomyopathy and exercise intolerance

The most inherited heart disease, hypertrophic cardiomyopathy (HCM), is characterised by left ventricular (LV) hypertrophy (Marian and Braunwald, 2017). The degree of LV hypertrophy is largely dependent on and abnormal myocardial calcium kinetics and leads to a variability in symptom presentation (i.e., dyspnea, chest pain, fatigue and syncope) in individuals with HCM (Marian and Braunwald, 2017; Coppini *et al.*, 2018).

In individuals with HCM, exercise intolerance is a clinical hallmark, resultant of a decrease in cardiovascular function (Del Buono *et al.*, 2019; Hwang *et al.*, 2022). Mechanisms of exercise intolerance in individuals with HCM include under-perfusion of peripheral muscular tissue, diastolic dysfunction, and left ventricular outflow tract (LVOT) obstruction (Finocchiaro *et al.*, 2015; Magri and Santolamazza, 2017). On a molecular level, the observed impaired diastolic

ventricular filling in individuals with HCM is hypothesised to be caused by molecular remodelling of cardiomyocytes (i.e., hypertrophy, disarray, and fibrosis) (Chacko *et al.*, 2018). In the most recent assessment of exercise capacity in individuals with HCM, factors associated with impairment were age, female sex, N-terminal-pro hormone BNP (NT-proBNP) levels, and ratio of mitral peak velocity of early filling (E) to early diastolic mitral annular velocity (e') (E/e') on echocardiography (Hwang *et al.*, 2022).

4.3 Rationale, Aim and Hypotheses of the Study

Before assessing the mechanisms of exercise intolerance in HCM, the mechanisms of exercise tolerance in healthy individuals must first be understood.

More studies are warranted to improve our understanding of the effect of ageing on physiological remodelling of the cardiovascular system, and their implications on exercise tolerance in older individuals. Based on the suggested sex differences in physiological function and age-related physiological changes, it is prudent to investigate the mechanisms of exercise tolerance in relation to sex. Most importantly and additionally, previous studies have not assessed the effect of physical activity as a confounding factor on the mechanisms of exercise tolerance associated with age and sex. Assessing results with physical activity as a confounder is key due to its ability to preserve age-related cardiac metabolism and exercise capacity decline, and the increase in sedentary behaviour associated with age (de Rezende *et al.*, 2014; Jakovljevic *et al.*, 2015).

The ability to measure $\dot{V}O_2$peak in individuals with HCM has important prognostic utility. At present, no previous study has assessed the mechanisms of exercise intolerance in HCM when compared to healthy individuals.

Therefore, the aim of the present chapter was two-fold: Part A) to evaluate the effect of age and sex on the physiological determinants of exercise tolerance in healthy individuals, with additional consideration of physical activity and body mass index as confounding factors, and Part B) to evaluate the determinants of exercise intolerance in individuals with HCM by comparing data to healthy individuals.

The hypotheses for the present study are detailed below:

i) Older individuals will demonstrate significantly lower exercise tolerance ($\dot{V}O_2$peak) and cardiac function (peak cardiac output) than younger individuals.

ii) There will be a significant interaction between age and sex, and males will demonstrate significantly higher absolute but not relative values of exercise tolerance and cardiac function.

iii) Cardiac output will be the major determinant of exercise tolerance in older age.

iv) Individuals with HCM will demonstrate significantly lower exercise tolerance and cardiac function compared to healthy individuals.

4.4 Participants and methods

4.4.1 Part A) The effect of age and sex on mechanisms of exercise tolerance in healthy individuals

For the original validation study (described in Chapter 3.3), 99 adults provided informed consent (Appendix 2) and participated in the study. From this dataset, data from seventy-one (72%) healthy adults, aged between 19-78 years, were analysed for the present observational study. Participant age groups were defined as <40 years of age and ≥55 years of age and were based on the age of recruited participants for the original validation study. Eligible participants attended one visit lasting between 90 and 120 minutes at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK. Therefore, the present study was a single centre, cross-sectional, observational study. Data collection began in January 2018 and was finalised in July 2019.

4.4.1.1 Inclusion and exclusion criteria

The inclusion criteria for the cross-sectional analysis was healthy individuals >18 years of age, and a body mass index >18 kg/m^{2}to ≤30kg/m^2. Participants also needed to be able to independently provide written informed consent. There was no upper age limit to the study.

The exclusion criteria for participant data assessed in the final analysis included self-reported coexisting medical conditions that would interfere with exercise testing, mild cognitive impairment or dementia, previous stroke, current major depressive or another psychiatric disorder, pre-existing cardiac disease (i.e., cardiomyopathy, myocardial infarction), intermittent claudication, musculoskeletal disease interfering with exercise, chronic respiratory conditions, active cancer and/or signs of cardiac ischemia or arrhythmia at rest or during exercise. Individuals were also excluded if they were using medication known to affect cardiovascular function, currently or previously smoked, or had an alcohol intake >21 units/week for men and >14 units/week for women.

4.4.2 Part B) The mechanisms of exercise intolerance in individuals with hypertrophic cardiomyopathy

For the present single centre, cross-sectional, observational study, the healthy data was collected prior to the completion of the present thesis, and gifted by Professor Djordje Jakovljevic (Houghton *et al.*, 2016). Healthy participants attended one visit lasting between 90 and 120 minutes at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK. Data collection began January 2018 and was finalised February 2022.

The HCM participant data included in the present analysis was the baseline data from the lifestyle intervention in individuals with HCM (Chapter 5). I led the data collection which was collected by myself and my research colleagues at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK. HCM individuals attended visits approximately 3 hours long. Data collection began January 2018 and was finalised February 2022.

4.4.2.1 Inclusion and exclusion criteria

The inclusion/ exclusion criteria for healthy individuals was as described in section 4.4.1.1.

Inclusion/ exclusion criteria for HCM individuals is described thoroughly in section 5.3.2.

4.4.3 Ethical approval

Ethical approvals were obtained prior to the start of the present PhD. The healthy data collection for the studies were approved under 'quality assurance and control' by the National Health Service, National Research Authority (Northeast- Tyne and Wear South Research Ethics Committee), number 15/NE/0190) (Jakovljevic *et al.*, 2015; Houghton *et al.*, 2016). Data collection from individuals with HCM was approved by the North East-Tyne and Wear Research Ethics Committee (18/NE/0318) and was part of the European In Silico trials for drug tracing the effects of sarcomeric protein mutations leading to familial cardiomyopathy (SILICOFCM) NCT03832660 (Tafelmeier *et al.*, 2020) (Chapter 5). All procedures were performed according to the declaration of Helsinki, and all participants provided written informed consent (Appendix 2).

4.4.4 Study protocol and measurements

All study participants were asked to abstain from alcohol or caffeine containing foods and beverages on test days and were asked not to perform vigorous exercise 24 hours prior to the test. However, participants did not attend the clinic fasted. Upon arrival at the laboratory, all participants completed a health related Physical Activity Readiness questionnaire (Appendix 6) (Riebe *et al.*, 2018).

Resting blood pressure from the brachial artery in triplicate and heart rate (Welch Allyn, USA) were measured in a seated position followed by height and weight. Measurements of body weight and height were taken using a weighing scale and stadiometer (Seca 769 electric column scale with telescopic measuring rod Seca 220, Seca, Hamburg, Germany). A resting 12-lead electrocardiogram in supine position was next recorded.

Non-invasive bioimpedance technology was used for haemodynamic measurements in part A, and non-invasive bioreactance technology for part B (i.e. cardiac output, stroke volume, total peripheral resistance and HR) at rest in supine position for 30 minutes and six minutes, respectively, including during exercise (Fortin *et al.*, 2006; Jakovljevic;Trenell and MacGowan, 2014). Table 4.1 depicts the protocol followed on visit days for part A, and healthy data part B. The full study day protocol for individuals with HCM can be found in section 5.3.3.1.

Table 4.1. Study day protocol for healthy participants (A and B) with approximate times

Time	Procedure	Location
30 mins	Informed consent Medical history and physical examination (electrocardiogram)	Assessment room
30 mins	i) Non-invasive bioimpedance, gas exchange and Smartcardia resting measurements. ii) Non-invasive bioreactance	Exercise lab
30 mins	Cardiopulmonary exercise stress testing	Exercise lab
Total duration of visit= 90 mins		

Table 4.2 details metabolic and non-invasive haemodynamic measurements acquired for assessment in the present study and physiological meaning.

Table 4.2. Non-invasive haemodynamic, and metabolic measurements assessed in the present study and their physiological meaning (Herdy *et al.*, 2016; Yildiz *et al.*, 2017; Homan;Bordes and Cichowski, 2023).

Measurement type	Measurement	Physiological meaning
Metabolic	Oxygen consumption	Amount of oxygen used by the body per minute (Absolute, L/min; relative, mL/kg/min)
	Peak oxygen consumption	Highest value of oxygen uptake an individual has reached during exercise testing following which the test is terminated (Absolute, L/min; relative, mL/kg/min)
	Respiratory exchange ratio	Indirect measurement of the muscle's oxidative capacity and exercise intensity. It represents the ratio of the volume of CO_2 produced to amount of O_2 consumed.
	Arteriovenous oxygen difference	The amount of oxygen extracted from the blood by tissues (mLo_2/ 100mL of blood).
	Work rate	Unit of power output (Watts).
Haemodynamic	Heart rate	The number of times that the heart beats per minute (beats/min).
	Stroke volume	The volume of blood pumped out of the LV of the heart during each systolic cardiac contraction (Absolute, mL/beat; relative, mL/beat/m^2).
	Cardiac output	The amount of blood ejected from the LV per minute (Absolute, L/min; relative, L/min/m^2).
	Cardiac power output	The measure of cardiac pumping capability (Absolute, W; relative, W/m^2)
	Systolic blood pressure	Maximum pressure within the aorta during heart contraction (mmHg).
	Diastolic blood pressure	Minimum pressure within the aorta during heart relaxation (mmHg).
	Mean arterial pressure	Average blood pressure in a single cardiac cycle (mmHg).

LV, left ventricle.

4.4.4.1 Categorical measurements

All categorical variables were collected using a case report form (Appendix 7) at the beginning of the study visit.

For Part A, physical activity level was split into three categories (i.e., low, medium, and high) to be used as a confounder in statistical analysis. Categories were defined using the World health organisation (WHO) guidelines which recommend that all adults complete 150-300 minutes of moderate-intensity exercise, 75-150min vigorous-intensity activity, or an equivalent

combination of aerobic physical activity weekly (Bull *et al.*, 2020). Based on such recommendations, low activity level was defined as being below such recommendations, moderate within/ equal to the upper and lower limit, and high an excess of.

Additional categorical values not included as confounding factors in analysis due to sample size but included in initial assessment between young/ old, male/ female groups were education status (University/ No University education- reported in table 4.2 as the percentage of those who attended University); social status (i.e., single/ married- reported in table 4.2 as the percentage of single individuals); and alcohol consumption based on NIH National Institute on Alcohol Abuse and Alcoholism (No consumption; binge consumption, i.e., five or more alcoholic drinks for males and four or more for females on the same occasion; moderate, i.e., less than five drinks on a single day, and <15 drinks per week for men, and less than four drinks on any single day and less than seven per week for women) (Gunzerath *et al.*, 2004). No participants took part in heavy drinking, i.e., binge drinking on five or more days in the past month as this was a healthy cohort.

4.4.4.2 Progressive exercise test and peak oxygen consumption measurements

All participants completed a progressive exercise using a semi-recumbent cycle ergometer (Corival, Lode, Groningen, Netherlands) with simultaneous gas exchange measurements (Cortex metalyzer 3B, Cortex, Leipzig, Germany). In addition, simultaneous 12-lead electrocardiography (Custo, CustoMed, HmbH, Ottobrunn, Germany) and an automated blood pressure (SunTech Tango, SunTech Medical, Inc., Morrisville, USA) were recorded.

The exercise test was terminated i) when respiratory exchange ratio (RER) exceeded 1.15 (i.e. universally acknowledged RER threshold (Issekutz Jr;Birkhead and Rodahl, 1962) ii) upon volitional exhaustion i.e., inability to maintain a cadence of 60 rpm, iii) when $\dot{V}O_2$peak was achieved, defined as the inability to increase oxygen consumption despite an increase in exercise intensity (watts), iv) if the participant requested termination of the test.

4.4.5 Research study team

Dr Nduka Okwose, Dr Jadine Scragg and I attained measurements for healthy individuals for part A. Professor Djordje Jakovljevic and team collected data for healthy individuals that informed the findings for part B. For data collection for individuals with HCM, Professor Djordje Jakovljevic, Dr Nduka Okwose and myself attained the data along with Miss Alaa Alyahya who joined the research team just prior to and following the coronavirus pandemic. Dr

Sarah Charman was present at the Clinical Research Facility after the coronavirus pandemic for study visits for additional research team support when required. Professor Guy MacGowan and Dr Kristian Bailey (consultant cardiologists) were present for safety reasons during the exercise testing. Professor Christopher Eggett and Mr Peter Luke collected the echocardiography data. For specific procedures followed for each measurement acquired, please refer to General Methodology, Chapter 3.

4.4.6 Sample size calculation

An analysis of sample sizes was completed using SAS version 9.4 (SAS Institute Inc).

To analyse the effect of age and sex on mechanisms of exercise tolerance, the sample size was calculated based on previous literature suggesting that there would be a significant interaction between age and sex on $\dot{V}O_2$peak in healthy individuals (Molgat-Seon *et al.*, 2018). The same study reported $\dot{V}O_2$peak values of 34.7±6.32 and 30.6±9.00 mL/kg/min in older males and females, and 53.4±10.3 and 51.7±9.29 mL/kg/min in younger males and females using a cycle ergometer exercise stress test. Assuming similar findings to account for age and sex differences, the present study would need to analyse at least 24 participants in total – 12 per age group (i.e., younger vs. older) to ensure that each age group consisted of at least 6 males and 6 females to allow sex differences to be evaluated. This ensures a 90% power for the study to detect a significant difference at a two-tailed significance level of .05 in $\dot{V}O_2$peak between younger and older age groups as well as between males and females. Post-hoc power analysis confirmed that the present study assessing the effect of age and sex on mechanisms of exercise tolerance where the alpha was .05 had a high power to determine whether there was a significant difference between groups (beta=0.98).

Previously, a cross-sectional study comparing the $\dot{V}O_2$peak in healthy individuals to that of individuals with HCM has not yet been completed. Statistical power was calculated using post-hoc analysis for a two-sided test where the alpha was 0.05. It was calculated that 29 individuals with HCM and 23 healthy individuals would provide a high power for the study (beta= 0.86).

4.4.7 Data analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, N.Y., USA). Dr Kim Pearce (senior statistician, Faculty of Medical Sciences) and Miss Alaa Alyahya provided statistical guidance, and I then completed all data analysis. Data is presented with no significant outliers when screened using standard Z-distribution cut-offs, boxplots and Mahalanobis distance test. A Kolmogorov-Smirnov test was

used to evaluate normality of distribution. All data was normally distributed. For all analysis, data is presented as mean ± SD unless stated otherwise. Statistical significance was indicated if $p<.05$.

4.4.7.1 Part A) The effect of age and sex on mechanisms of exercise tolerance in healthy individuals

A chi-square test of independence assessed categorical variables (i.e., sex, education, physical activity, and alcohol consumption) between the age and sex groups. For the assessment of physical characteristics, metabolic, and haemodynamic measurements at rest and peak exercise were compared using a 2 x 2 analysis of covariance (ANCOVA) for age and sex between the four groups with body mass index (BMI) (calculation described in Chapter 3.4) and physical activity category (i.e., low, medium and high) as confounding factors. Pairwise comparisons with Bonferroni correction was performed and reported for significant findings. A multiple linear regression analysis was calculated to determine predictors of $\dot{V}O_2$peak from age, sex, BMI, peak arteriovenous O_2 difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic blood pressure, and peak mean arterial blood pressure within age groups. Another multiple linear regression analysis was calculated to determine predictors of $\dot{V}O_2$peak from age, BMI, peak arteriovenous O_2 difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic blood pressure, and peak mean arterial blood pressure within sex groups.

4.4.7.2 Part B) The mechanisms of exercise intolerance in individuals with hypertrophic cardiomyopathy

A Chi-square test of independence assessed categorical variables (i.e., sex distribution, medication and medical intervention and comorbidity) between healthy individuals and individuals with HCM. Physical characteristics, metabolic, and haemodynamic measurements at rest and peak exercise were compared between healthy individuals and those with HCM using a one-way ANCOVA, with BMI (calculation described in Chapter 3.4) and drug total (i.e., the number of drugs taken by each individual) as confounding factors. A Bonferroni correction was performed and reported for significant findings. Multiple linear regression analysis was calculated to determine predictors of $\dot{V}O_2$peak from age, sex, body mass index, drug total, peak arteriovenous O_2 difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic

blood pressure, and peak mean arterial blood pressure within healthy individuals and those with HCM.

4.5 Results

4.5.1 Part A) The effect of age and sex on exercise tolerance and its determinants

A total of 71 participants / 30 females were recruited into the study and divided into two age groups, younger (<40 years of age) and older (≥55 years of age) as depicted in Figure 4.1.

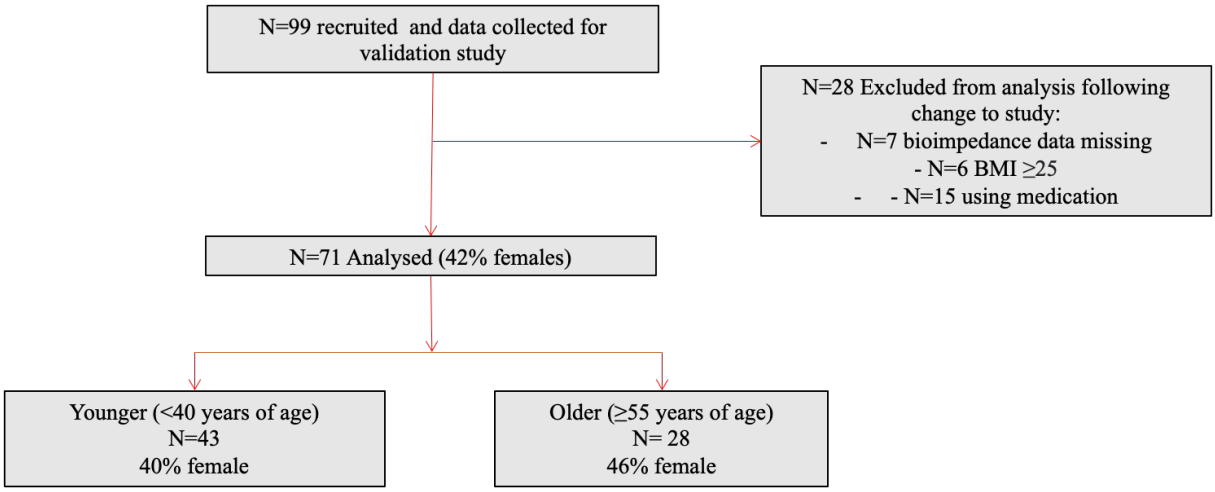


Figure 4.1. Flow chart of participant recruitment: the effect of age and sex on exercise tolerance and its determinants.

4.5.1.1 Demographic and physical characteristics

A total of 71 individuals, including 41 males and 30 females completed the study. Participants ranged from 19 to 78, and older individuals were 150% older than younger individuals (p<.01). Between age groups, there were no significant difference in sex distribution (X^2 (1, N=71)=0.33, p=.57). Participant's demographic and physical characteristics are presented in Table 4.3.

There was no significant interaction between age and sex for physical characteristic variables and weekly physical activity volume (p>.05). However, the main effect analysis showed that younger individuals were significantly taller than older individuals by 4% (p<.01). In addition, older individuals participated in significantly less physical activity than younger by 41% (p<.01), with a significant difference in distribution of low, medium, and high volume of physical activity, (X^2 (2, N=71)=31, p<.01) between younger and older age groups.

In contrast, older individuals had an 11% higher body weight (p<.01), and a 20% higher BMI (p<.01) compared to younger participants. Females had a significantly lower height, body

weight and BMI when compared to males by 5% (p<.01), 14% (p<.01), and 4% (p=.04), respectively.

The analysis of categorical variables showed that more younger participants attended University (X^2 (1, N=71)=48, p<.01), and were single (X^2 (2, N=71)=47, p<.01) in comparison to older participants.

Table 4.3. Demographic and physical characteristics of study participants.

Variables	<40 years (N=43)	≥ 55 years (N=28)	Females (N=30)	Males (N=41)
Age (years)	26±6	65±7**	42±21	41±20
Sex (Female/ (%))	17 (40)	13 (46)		
Height (m)	1.74±0.11	1.67±0.10*	1.66±0.08	1.75±0.11**
Body weight (kg)	69.5±12.0	77.9±10.8**	66.6±11.6	77.4±10.1**
Body mass index (kg/m^2)	22.8±1.97	27.9±2.89**	24.2±4.00	25.2±2.93*
Education status				
University (%)	42 (98)	23 (82)**	19 (63)	28 (68)
Relationship status				
Single (%)	37 (86)	1 (4)**	17 (57)	21 (51)
Physical activity				
Per week (mins)	389±231	231±148**	238±188	344±250
Physical activity (category)				
Low (%)	4 (9)	16 (2) **	10 (33)	10 (24)
Mod (%)	11 (26)	11 (39) **	10 (33)	12 (29)
High (%)	28 (65)	1 (4) **	10 (33)	19 (46)
Alcohol consumption				
No (%)	9 (21)	6 (21)	7 (23)	8 (20)
Binge (%)	15 (35)	5 (18)	8 (27)	12 (29)
Mod (%)	18 (42)	17 (61)	14 (47)	21 (51)

P-value defines between-group differences (young/ old; female/male); ****= p<.01** and ***=p<.05**

4.5.1.2 Effect of age and sex on resting metabolic and haemodynamic measurements

Regarding resting metabolic measurements (table 4.4), there was no statistically significant interaction between age and sex, whilst controlling for BMI and physical activity for relative oxygen consumption (F(1, 56)= 0.80 , p=.80, partial η^2 <0.01), absolute oxygen consumption (F(1, 65)=0.12 , p=.73, partial η^2 <0.01), and arteriovenous oxygen difference (F(1, 62)=0.83 , p=.37, partial η^2 =0.01.).

There was a statistically significant interaction between age and sex, whilst controlling for BMI and physical activity category for systolic blood pressure ($F(1, 61)=6.97$, $p=.01$, partial $\eta^2=0.10$), and mean arterial blood pressure ($F(1, 60)=7.30$, $p<.01$, partial $\eta^2=0.11$). For systolic blood pressure, pairwise comparison confirmed a significantly higher resting systolic blood pressure in younger males compared to younger females by 9% (112±13.0 vs 103±14.7 mmHg, $p=.02$), and non-significantly higher resting systolic blood pressure in older females compared to older males by 5% (118±13.9 vs 112±15.4 mmHg, $p=.20$). For mean arterial blood pressure, pairwise comparison confirmed a non-significantly higher resting mean arterial blood pressure in younger males compared to younger females by 6% (83.1±9.15 vs 77.6±10.3 mmHg, $p=.05$), and non-significantly higher resting mean arterial blood pressure in older females compared to older males by 6% (84.4±9.90 vs 78.6±10.7 mmHg, $p=.08$).

In contrast, there was no statistically significant interaction between age and sex, whilst controlling for BMI and physical activity level for resting heart rate ($F(1, 65)=.19$, $p=0.67$, partial $\eta^2<0.01$), cardiac output ($F(1, 63)=0.72$, $p=.40$, partial $\eta^2=0.01$), cardiac index ($F(1, 65)=0.31$, $p=.58$, partial $\eta^2=0.01$), cardiac power output ($F(1, 58)=3.91$, $p=.05$, partial $\eta^2=0.06$), cardiac power output index ($F(1, 59)=2.65$, $p=.11$, partial $\eta^2=0.04$), stroke volume ($F(1, 63)=3.70$, $p=.06$, partial $\eta^2=0.06$), stroke volume index ($F(1, 63)=1.73$, $p=.19$, partial $\eta^2=0.03$), or diastolic blood pressure ($F(1, 61)=3.33$, $p=.07$, partial $\eta^2=0.05$).

4.5.1.3 Simple main effects analysis of resting metabolic and haemodynamic data

The main effects analysis showed that males had a significantly higher relative oxygen consumption than females by 23% (0.25±0.06 vs 0.21±0.06 ml/kg/min, $p=.01$). Regarding haemodynamic measurements, the simple main effects analysis showed that resting stroke volume was higher in younger than older individuals by 18% (96.2±24 vs 78.6±26.4 mL/beat, $p=.02$), stroke volume index by 20% (52.5±11.8 vs 44.3±13.2 mL/beat/m^2, $p=.04$), cardiac output by 31% (6.17±1.81 vs 4.25±1.97 L/min, $p<.01$), cardiac index by 28% (3.39±1.02 vs 2.45±1.10 L/min/m^2, $p<.01$), cardiac power output by 35% (1.14±0.36 vs 0.74±0.40 W, $p<.01$), cardiac power output index by 32% (0.62±0.19 vs 0.42±0.21 W/m^2, $p<.01$) , respectively. In addition, resting heart rate, cardiac index, and cardiac power output index were significantly higher in females than males by 11% (63.2±10.0 vs 57.7±10.7 beats/min, $p=.03$), 12% (3.11±0.75 vs 2.73±0.79 L/min/m^2, $p=.04$) and 15% (0.56±0.14 vs 0.48±0.15 W/m^2, $p=.03$).

Table 4.4. Metabolic and haemodynamic variables at rest

	<40 years (N=43)	**≥ 55 years (N=28)**	**Female (N=30)**	**Male (N=41)**
Metabolics				
Oxygen consumption (L/min)	3.71±1.28	2.89±0.57	3.24±0.20	3.47±0.18
Oxygen consumption (mL/kg/min)	0.24±0.65	0.23±0.05	0.22±0.05	0.25±0.06*
Arteriovenous oxygen difference (mLo_2/ 100mL of blood)	6.38±2.70	7.02±2.02	6.35±0.48	7.19±0.42
Haemodynamics				
Heart rate (beats/min)	64.7±1.97	56.4±2.51	63.2±1.86	58.0±1.65*
Stroke volume (mL/beat)	102±19.4	71.7±15.0*	86.3±3.45	88.1±3.03
Stroke volume index (mL/beat/m^2)	55.8±9.23	38.8±9.30*	49.1±1.74	47.1±1.52
Cardiac output (L/min)	6.12±1.37	4.33±1.1**	5.40±0.26	5.04±0.23
Cardiac index (L/min/m^2)	3.41±0.78	2.36±0.65**	3.09±0.14	2.73±0.14*
Cardiac power output (W)	1.09±0.27	0.82±0.27**	0.99±0.56	0.91±0.48
Cardiac power output index (W/m^2)	0.60±1.34	0.45±0.14**	0.56±0.03	0.49±0.02*
Systolic blood pressure (mmHg)	107±11.2	117±13.1	111±2.35	113±2.00♦
Diastolic blood pressure (mmHg)	65.5±8.41	71.8±8.72	68.1±1.67	67.9±1.49
Mean arterial pressure (mmHg)	79.8±8.54	83.3±8.41	81.2±1.67	80.8±1.46 ♦◊

P-value defines between-group differences (young/ old; female/male): ****= *p*<.01** and ***=*p*<.05**
Age and sex interaction *p*-values: **♦◊=*p*<.01** and **♦=*p*<.05**

4.5.1.4 Effect of age and sex on peak metabolic and haemodynamic measurements

There was no statistically significant interaction between age and sex, whilst controlling for BMI and physical activity category, for absolute $\dot{V}O_2$peak (F(1, 60)=1.87, p=.18, partial η^2 =0.03), relative $\dot{V}O_2$peak (F(1, 59)=0.68, p=.41, partial η^2 =0.01), respiratory exchange ratio (F(1, 62)=0.45, p=.51, partial η^2 =0.01), peak arteriovenous oxygen difference (F(1, 53)=0.18 , p=.67, partial η^2 <0.01), and peak work rate (F(1, 60)=2.26 , p=0.14, partial η^2 =0.04).

In addition, there was no statistically significant interaction between age and sex, whilst controlling for BMI and physical activity category for peak heart rate (F(1, 62)=0.15, p=0.70, partial η^2 <.01), peak stroke volume (F(1, 57)=0.05 , p=0.82, partial η^2 <.01), peak stroke volume index (F(1, 57)=0.11, p=.74, partial η^2 <0.01), peak cardiac output (F(1, 57)=0.41, p=.52, partial η^2 =0.01), peak cardiac index (F(1, 59)=0.06 , p=.81, partial η^2 =<0.01), peak cardiac power output (F(1, 57)=2.29, p=.14, partial η^2 =0.04), peak cardiac power output index (F(1, 57)=1.76 , p=.19, partial η^2 =0.03), peak systolic blood pressure (F(1, 62)=1.07 , p=0.30,

partial η^2 =0.02), peak diastolic blood pressure (F(1, 62)=0.08 , p=0.78, partial η^2 <0.01) or peak mean arterial blood pressure (F(1, 62)=0.44 , p=0.51, partial η^2 =0.01).

The simple main effects analysis showed that younger individuals had a significantly higher relative $\dot{V}O_2$peak by 35% (32.5±10.5 vs 21.2±11.6 ml/kg/min*, p*<.01*),* and peak work rate by 27% (182±73.7 vs 132±77.7 watts*), p*=.04) compared to older individuals. Simple main effects analysis showed that males had significantly higher absolute $\dot{V}O_2$peak by 38% (2.27±0.67 vs 1.64±0.63 L/min, *p*<.01), relative $\dot{V}O_2$peak by 30% (30.4±7.86 vs 23.3±7.57 ml/kg/min*, p*<.01), arteriovenous oxygen difference by 31% (14.1±3.85 vs 10.8±3.83 mLo_2/ 100mL of blood*, p*<.01) and work rate by 33% (180±55.9 vs 135±52.7 watts*), p<.01)* than females, respectively. When simple main effects analysis was completed for haemodynamic data, peak heart rate was significantly higher in younger when compared to older individuals by 18% (160±27.4 vs 132±29.6 beats/min*, p*<.01). When sex was assessed, only systolic blood pressure was significantly different with males observed having a higher systolic blood pressure than females by 16% (184±50.0 vs 158±43.7 mmHg*, p*=.02). Results are depicted in table 4.5.

Table 4.5. Metabolic and haemodynamic variables at peak exercise

	<40 years (N=43)	**≥ 55 years (N=28)**	**Female (N=30)**	**Male (N=41)**
Metabolics				
Oxygen consumption (L/min)	2.38±0.76	1.58±0.57	1.65±0.52	2.37±0.83**
Oxygen consumption (mL/kg/min)	35.05±9.11	18.8±5.24**	24.6±9.20	31.8±11.5**
Respiratory exchange ratio	1.12±0.09	1.10±0.06	1.09±0.10	1.10±0.08
Arteriovenous oxygen difference (mLo_2/ 100mL of blood)	15.8±4.34	9.01±3.01	11.7±5.04	14.2±4.96**
Work rate (watts)	200±64.0	116±44.6*	136±50.0	190±75.0**
Haemodynamics				
Heart rate (beats/min)	162±19.9	129±18.3**	153±21.6	145±26.9
Stroke volume (mL/beat)	142±34.9	173±41.5	145±35.6	160±42.5
Stroke volume index (mL/beat/m^2)	78.3±19.5	92.1±18.1	83.8±17.1	83.4±21.9
Cardiac output (L/min)	22.7±4.96	22.1±3.87	22.1±4.81	22.7±4.46
Cardiac index (L/min/m2)	12.4±2.91	11.8±1.88	12.8±2.63	11.8±2.43
Cardiac power output (W)	5.31±0.82	6.06±1.52	5.15±2.04	5.71±1.33
Cardiac power output index (W/m2)	2.93±0.21	3.22±0.75	2.96±1.08	2.99±0.72
Systolic blood pressure (mmHg)	165±44.8	181±41.3	157±41.3	181±43.6*
Diastolic blood pressure (mmHg)	77.1±13.2	95.0±15.0	86.4±17.4	82.2±15.5
Mean arterial pressure (mmHg)	106±1.45	124±20.2	110±23.2	115±18.4

P-value defines between-group differences (young/ old; female/male): ****= *p*<.01** and ***=*p*<.05**

4.5.1.5 Predictors of exercise tolerance

Age, sex, body mass index, peak arteriovenous oxygen difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic blood pressure, and peak mean arterial blood pressure significantly predicted relative $\dot{V}O_2$peak for younger individuals ($F(9, 33)=14.5$, $p<.01$, adjusted $R^2=.74$), and older individuals ($F(8, 18)=37.9$, $p<.01$, adjusted $R^2=.93$).

In individuals <40 years of age, sex ($\beta=0.23$, $p=.02$), peak arteriovenous oxygen difference ($\beta=0.25$, $p<.01$) and systolic blood pressure ($\beta=0.25$, $p=.03$) added statistically significance to the prediction model.

In individuals ≥ 55 years of age, age ($\beta=-0.21$, $p=.01$), sex ($\beta=0.26$, $p=.01$), and peak arteriovenous oxygen difference ($\beta=0.93$, $p<.01$) added statistically significance to the prediction model.

Age, body mass index, peak arteriovenous oxygen difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic blood pressure, and peak mean arterial blood pressure significantly predicted relative VO_2peak for females ($F(8, 121)=33.3$, $p<.01$, adjusted $R^2=.90$), and males ($F(8, 32)=27.4$, $p<.01$, adjusted $R^2=.84$).

In females, peak heart rate ($\beta=0.69$, $p<.01$), stroke volume index ($\beta=0.78$, $p<.01$), and peak arteriovenous oxygen difference ($\beta=0.67$, $p<.01$) added statistically significance to the prediction model. In contrast, only peak arteriovenous oxygen difference ($\beta=0.67$, $p<.01$) added statistically significantly to the prediction in males.

4.5.2 Part B) The mechanisms of exercise intolerance in individuals with hypertrophic cardiomyopathy

4.5.2.1 Demographics and physical characteristics

The mean age of individuals with HCM was 55±15 years old and healthy individuals was 52±18 years old. Individuals with HCM had a 10% greater body weight ($p<.05$) compared to healthy individuals. Individuals with HCM were prescribed significantly more medication than healthy individuals (X^2 (2, $N=52$)=30.2, $p<.01$). The mean time since diagnosis of HCM for the study cohort was 7.52±4.34 years. Recruited participants' demographic and physical characteristics are presented in Table 4.6.

Table 4.6. Demographic and physical characteristics of individuals with HCM and healthy individuals

Variables	HCM (N=29)	Healthy Controls (N=23)	P-value
Age (years)	55±15	52±18	.53
Sex (Male/Female)	22/7	14/9	.25
Height (m)	1.73±0.08	1.64±0.33	.17
Body weight (kg)	85.6±18.0	76.7±11.1	**.04**
Body mass index (kg/m^2)	28.8±5.01	27.5±3.45	.31
Tobacco			
No (%)	26 (90)	23 (100)	.11
Medications N (%)			
Angiotensin-converting enzyme inhibitor	4 (14)	0	.06
Angiotensin receptor blocker	4 (14)	0	.06
Antidepressant	3 (10)	0	.11
Anti-arrhythmic	4 (14)	0	.06
Beta-blocker	17 (59)	0	**<.01**
Calcium channel blocker	7 (24)	0	**.01**
Diuretic	5 (17)	0	**.04**
Non-steroidal anti-inflammatory	4 (14)	0	.06
Anticoagulant	10 (34)	0	**<.01**
Statins	6 (21)	0	**<.01**
Insulin	3 (10)	0	.11
Medical intervention N (%)			
ICD/ Pacemakers	10 (34)	0	**<.01**
Comorbidities N (%)			
Hepatic dysfunction	1 (3)	0	.37
Type II diabetes	7 (24)	0	**.01**

4.5.2.2 Metabolic and haemodynamic measurements at rest in individuals with hypertrophic cardiomyopathy and healthy individuals

There was a statistically significant difference between individuals with HCM and healthy individuals whilst controlling for drug total in resting absolute oxygen consumption ($F(1, 49)=5.64$, $p=.02$) and arteriovenous oxygen difference ($F(1, 49)=5.28$, $p=.03$). Resting absolute oxygen consumption and arteriovenous oxygen difference were significantly higher in individuals with HCM by 22% (0.37±10 vs 0.29±0.11 L/min), and 55% (9.37±2.24 vs 4.18±2.32 mLo_2/ 100mL of blood). In contrast, there was not a statistically significant

difference between individuals with HCM and healthy individuals for resting relative oxygen consumption ($F(1, 49)=0.44$, $p=.51$).

There was a statistically significant difference between individuals with HCM and healthy individuals whilst controlling for drug total in resting stroke volume ($F(1, 49)=21.5$, $p<.01$), stroke volume index ($F(1, 49)=12.7$, $p<.01$), cardiac output ($F(1, 49)=19.1$, $p<.01$), cardiac index ($F(1, 49)=18.0$, $p<.01$), cardiac power output ($F(1, 49)=20.8$, $p<.01$), and cardiac power output index ($F(1, 49)=17.4$, $p<.01$).

Resting stroke volume and stroke volume index were significantly lower in individuals with HCM compared to healthy individuals by 58% (63.2±24.6 vs 100±25.4 mL/beat, $p<.01$)), 76% (32.8±21.6 vs 57.7±22.4 mL/beat/m^2, $p<.01$)). In addition, resting cardiac output and cardiac output index, cardiac power output and cardiac power output index were significantly lower in individuals with HCM by 53% (4.28±1.60 vs 6.53±1.65 L/min, $p<.01$)), 45% (2.07±1.24 vs 3.76±1.28 L/min/m^2, $p<.01$)), 39% (1.06±0.28 vs 1.47±0.29 W, $p<.01$)) and 46% (0.48±0.30 vs 0.88±0.31 W/m^2, $p<.01$)) compared to healthy individuals.

There was no significant differences between individuals with HCM and healthy individuals whilst controlling for drug total in resting heart rate ($F(1, 49)=0.10$, $p=.76$), systolic blood pressure ($F(1, 49)=0.47$, $p=.50$), diastolic blood pressure ($F(1, 49)=<0.01$, $p=.97$) or mean arterial blood pressure ($F(1, 49)=0.25$, $p=.62$). Results are depicted in table 4.7.

Table 4.7. Metabolic and haemodynamic variables at rest in individuals with HCM and healthy control participants.

Variables	HCM (N=29)	Healthy Controls (N=23)	*P*-value
Metabolic variables			
Oxygen consumption (L/min)	0.36±0.10	0.30±0.08	**.02**
Oxygen consumption (mL/kg/min)	4.34±0.87	3.92±0.90	.51
Arteriovenous oxygen difference (mLo_2/ 100mL of blood)	9.07±2.45	4.57±0.97	**<.01**
Haemodynamic variables			
Heart rate (beats/min)	68±10	67±8	.76
Stroke volume (mL/beat)	62.8±19.3	101±22.3	**<.01**
Stroke volume index (mL/beat/m^2)	33.2±13.7	57.3±22.9	**<.01**
Cardiac output (L/min)	4.21±1.42	6.62±1.27	**<.01**
Cardiac index (L/min/m^2)	2.07±0.61	3.76±1.43	**<.01**
Cardiac power output (W)	0.99±0.29	1.54±0.25	**<.01**
Cardiac power output index (W/m^2)	0.49±0.15	0.86±0.34	**<.01**
Systolic blood pressure (mmHg)	143±17.1	138±15.5	.50
Diastolic blood pressure (mmHg)	87.1±7.90	85.3±8.28	.97
Mean arterial pressure (mmHg)	106±9.46	103±7.96	.62

4.5.2.3 Effect of hypertrophic cardiomyopathy on peak metabolic and haemodynamic measurements

There was a statistically significant difference between individuals with HCM and healthy individuals whilst controlling for drug total in relative $\dot{V}O_2$peak (F(1, 49)=7.16, p=.01) and peak arteriovenous oxygen difference (F(1, 49)=5.28, p=.03), with healthy individuals having a significantly higher relative $\dot{V}O_2$peak and peak arteriovenous oxygen difference by 49% (20.0±11.4 vs 29.8±11.8 ml/kg/min), and 26% (12.5±4.61 vs 9.19±4.46 mLo_2/ 100mL of blood). In contrast, there was not a statistically significant difference between individuals with HCM and healthy individuals for absolute $\dot{V}O_2$peak (F(1, 49)=2.67, p=.11).

There was a statistically significant difference between individuals with HCM and healthy individuals whilst controlling for drug total in peak heart rate (F(1, 49)=15.8, p<.01) and systolic blood pressure (F(1, 49)=7.68, p=.01), with healthy individuals reaching a higher heart rate and systolic blood pressure than individuals with HCM by 22% (152±22.8 vs 118±26.7 beats/min), and 10% (201±22.7 vs 181±21.9 mmHg).

There was also a statistically significant difference between individuals with HCM and healthy individuals whilst controlling for medication in peak stroke volume (F(1, 49)=7.77, p=.01),

with individuals with HCM reaching a higher peak stroke volume by 32% (176±62.0 vs 120±64.1 mL/beat) versus healthy individuals.

There was no statistically significant difference between individuals with HCM and healthy individuals whilst controlling for drug total in peak stroke volume index ($F(1, 49)=2.60$, $p=.11$), cardiac output ($F(1, 49)=1.99$, $p=.17$), cardiac index ($F(1, 49)=0.01$, $p=.95$), cardiac power output ($F(1, 49)=0.93$, $p=.34$), cardiac power output index ($F(1, 49)=0.23$ $p=.64$), diastolic blood pressure ($F(1, 49)=0.02$, $p=.89$), peak mean arterial blood pressure ($F(1, 49)=3.31$, $p=.08$). Results are depicted in table 4.8.

Table 4.8. Metabolic and haemodynamic variables at peak exercise capacity in individuals with HCM and healthy control participants.

Variables	HCM (N=29)	Healthy Controls (N=23)	*P*-value
Metabolic variables			
Oxygen consumption (L/min)	1.54±0.53	2.47±1.10	.11
Oxygen consumption (mL/kg/min)	18.3±5.90	31.9±13.4	**.01**
Arteriovenous oxygen difference (mLo_2/ 100mL of blood)	10.2±5.48	13.1±4.03	**.03**
Haemodynamic variables			
Heart rate (beats/min)	118±28.9	160±24.5	**<.01**
Stroke volume (mL/beat)	137±40.7	125±34.5	**.01**
Stroke volume index (mL/beat/m^2)	66.9±18.7	69.9±25.5	.11
Cardiac output (L/min)	16.6±5.65	18.6±4.81	.17
Cardiac index (L/min/m^2)	8.25±2.83	10.4±3.79	.95
Cardiac power output (W)	4.67±1.48	5.35±1.57	.34
Cardiac power output index (W/m^2)	2.34±0.74	2.99±1.10	.64
Systolic blood pressure (mmHg)	186±21.2	198±17.0	**.01**
Diastolic blood pressure (mmHg)	95.6±12.1	94.7±15.2	.89
Mean arterial pressure (mmHg)	126±10.7	129±10.4	.08

4.5.2.4 Predictors of exercise intolerance in individuals with hypertrophic cardiomyopathy

Multivariate linear regression was run to predict relative VO_2peak from age, sex, BMI, drug total, peak arteriovenous oxygen difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic blood pressure, and peak mean arterial blood pressure within the healthy and HCM groups.

Age, sex, BMI, drug total, peak arteriovenous oxygen difference, peak heart rate, peak cardiac index, peak cardiac power output index, peak stroke volume index peak systolic blood pressure, peak diastolic blood pressure, and peak mean arterial blood pressure significantly predicted relative VO_2peak in the multivariate linear regression analysis for HCM individuals (F(12,

28)=24.3, p<.01, adjusted R^2 =.91), and healthy individuals (F(11, 22)=17.3, p<.01, adjusted R^2 =.86).

In individuals with HCM, peak arteriovenous oxygen difference (β=0.93, p<.01), systolic blood pressure (β=-5.62, p=.03), diastolic blood pressure (β=-6.52, p=.04), and mean arterial blood pressure (β=8.52, p=.04) added statistically significantly to the prediction model.

In healthy individuals, only peak arteriovenous oxygen difference (β=0.71, p<.01) added statistically significantly to the prediction model.

4.6 Discussion

The first aim of the present study was to evaluate the age-related haemodynamic and metabolic changes in healthy individuals. The study further assessed how sex influences exercise capacity, with BMI and physical activity included as confounding factors. The findings show that when physical activity is adjusted for, (i) Older people exhibited reduced cardiac function, shown by a significantly reduced resting cardiac output, cardiac power output and relative O_2 consumption, however there was no interaction with sex. In contrast, resting systolic blood pressure and mean arterial blood pressure was higher in younger males than females but higher in older females than males; (ii) A diminished exercise capacity in older people was through significant reduction in peak arteriovenous O_2 difference but not cardiac output, despite significant heart rate attenuation. Older individual's peak cardiac output did not significantly differ from younger individuals through an ability to increase resting stroke volume to peak stroke volume to a greater degree; (iii) There was no interaction between age and sex for peak metabolic and haemodynamic variables when physical activity level was taken into consideration.

The second aim of the present study was to evaluate the mechanisms of exercise intolerance in individuals with HCM. Results show that when medication is adjusted for, (i) Individuals with HCM exhibited a reduced cardiac function in comparison to healthy control participants as demonstrated by a significantly lower resting cardiac index, cardiac power output index, and stroke volume index and (ii) Individuals with HCM have a diminished $\dot{V}O_2$peak that is aetiological of significantly reduced peak arteriovenous O_2 difference.

Therefore, the major findings of the present study is that when physical activity level is adjusted for, there is an accelerated increase in resting systolic and mean arterial blood pressure with age in females when compared to males. However, there is no interaction between age and sex in

metabolic or haemodynamic parameters at peak exercise. Finally, exercise intolerance in individuals with HCM is attributed to peripheral (i.e., arteriovenous O_2 difference) factors.

4.6.1 The effect of age and sex on exercise tolerance and its determinants

Age-related differences in cardiovascular function are most apparent when the cardiac muscle is placed under physiological stress, such as during maximal exercise stress testing (Stratton *et al.*, 1994).

Males and females may have differing mechanisms that result in age-related $\dot{V}O_2$peak decline. Evidence from Goldspink and colleagues in a study of presbycardia concluded that males have a significantly greater degree of cardiac ageing than females (Goldspink *et al.*, 2009). The mechanisms leading to the accelerated decline of cardiorespiratory fitness in males is not clear, but it is hypothesised to be because of a significantly greater absolute cardiovascular function in youthful males (Merz and Cheng, 2016).

A decrease in cardiac power output index is hypothesised to be resultant of progressive cardiac myocyte apoptosis throughout the lifespan of males (Goldspink;Burniston and Tan, 2003). The surviving myocytes compensate through hypertrophy, but the loss of contractile cells leads to diminished cardiac function (Goldspink;Burniston and Tan, 2003). A significant age-related decreases in peak stroke volume, cardiac power output and cardiac reserve have been correlated with loss of left ventricular mass in males in contrast to females who do not lose any ventricular mass (Goldspink *et al.*, 2009). Progressive myocyte apoptosis with age does not occur in females due to higher IGF-1 levels (both local and systemic) and oestrogen (Olivetti *et al.*, 1995; Leri *et al.*, 2000).

Oestrogen is hypothesised to stimulate anti-apoptotic signals and delaying myocyte senescence, which protects the heart and vasculature of females to a greater degree (Guerra *et al.*, 1999). Older females also have an increased pulsatile afterload and work load on the left ventricle (Franklin *et al.*, 1997b). The described factors lead to preservation of left ventricular mass in females. The greater decline in cardiac function in males described could also be due to an initially greater function and factors such as a greater age-associated inotropic sensitivity to β-adrenergic agonist because of its greater role in stimulating left ventricular systolic function in younger males than females (Turner *et al.*, 1999).

As hypothesised and in agreement with other studies, $\dot{V}O_2$peak and peak heart rate were significantly lower in older age groups (Houghton *et al.*, 2016). Most previous studies show that $\dot{V}O_2$peak declines with age through reductions in both peak arteriovenous O_2 difference

(Rivera *et al.*, 1989; Ogawa *et al.*, 1992; McGuire *et al.*, 2001; Bell *et al.*, 2003; Weiss *et al.*, 2006) and peak cardiac output (McElvaney *et al.*, 1989; Ogawa *et al.*, 1992; Carrick-Ranson *et al.*, 2013; Pandey *et al.*, 2019)).

However, when results in the present study were adjusted for by confounding factors (i.e., BMI and physical activity level), peak cardiac output nor arteriovenous O_2 difference were significantly different between age groups, and there was no interaction with sex.

A number of studies have observed a significant decline in peak cardiac output for a given work level with age, aetiological of a decrease in active metabolic tissue (McElvaney *et al.*, 1989; Ogawa *et al.*, 1992; Carrick-Ranson *et al.*, 2013; Pandey *et al.*, 2019). In addition, age-associated change in hemodynamic mechanisms i.e., reduced cardiovascular response to catecholamine stimulation, has been observed to lead to the augmentation of cardiac output (Rodeheffer *et al.*, 1984a). Regarding resting cardiac output and stroke volume, the present study observed lower values associated with ageing, which are consistent with the findings of several other studies (Ogawa *et al.*, 1992; Houghton *et al.*, 2016; Pandey *et al.*, 2019). However, there was not a significant and negative relationship between peak cardiac output and age and sex interaction.

The least controversial of the mechanisms associated with age-related decline in $\dot{V}O_2$peak is heart rate. The decline in peak heart rate with age is unaffected by lifestyle or sex, and occurs at a rate of approximately 5% per decade (Hawkins and Wiswell, 2003). Because peak heart rate consistently shows significant relative contribution to decreased $\dot{V}O_2$peak in older individuals, it leaves little doubt that the mechanism has a major role (Ogawa *et al.*, 1992; Hawkins and Wiswell, 2003). Our results are consistent with this evidence, with a significantly higher peak heart rate in younger individuals by 26%, and no interaction between age and sex.

An age-associated change in stroke volume to compensate for the lower heart rate at $\dot{V}O_2$peak is a result of changes in the Frank Starling mechanism with age i.e., a significant increase in end-diastolic volume (Gagnon, 2009; Houghton *et al.*, 2016). It has been shown that in comparison to the increase in stroke volume through increase of end-diastolic volume in older males, younger healthy males rely on increased ejection fraction (Stratton *et al.*, 1994).

In agreement with previous findings, individuals in the older age group of the present study increased their stroke volume from rest to peak by 141%, whereas the younger individuals only increased their stroke volume by 39%. Leading to no difference in peak stroke volume or cardiac output between age groups. Such finding is further confirmed by studies with participants 20-80 years of age, which concluded that peak cardiac output is unaffected by age

through a significant rise in stroke volume under physiological stress, compensating for heart rate attenuation in older individuals (Rodeheffer *et al.*, 1984a; McGuire *et al.*, 2001; Houghton *et al.*, 2016).

However, a significantly lower resting stroke volume in females has previously been confirmed by Proctor and colleagues (Proctor *et al.*, 1998). Whereas, a study by Freedson and colleagues concluded that stroke volume is significantly lower in males when lean body weight was taken in to account (Freedson *et al.*, 1979). In the present study, no significant interaction between age and sex in peak stroke volume was observed when physical activity is confounded for. The greater percentage of body fat in females than in males in sedentary individuals has been shown to account for the sex differences in peak cardiac output and stroke volume (Ogawa *et al.*, 1992). The same study presenting these findings concluded that it is sex differences in stroke volume that lead to differing peak cardiac output, and so $\dot{V}O_2$peak (Ogawa *et al.*, 1992).

It is a common finding that lean body mass in males and oestrogen replacement after menopause/maintenance of training volume in females leads to a maintenance of $\dot{V}O_2$peak (Hawkins *et al.*, 2001; Katzel;Sorkin and Fleg, 2001; Eskurza *et al.*, 2002). However, females who do not replace oestrogen after menopause display greatest reduction in cardiorespiratory fitness over a given time (Hawkins *et al.*, 2001; Katzel;Sorkin and Fleg, 2001; Eskurza *et al.*, 2002).

The ability to increase arteriovenous O_2 difference during physical stress is hindered significantly by age (Ogawa *et al.*, 1992). In agreement, the present study showed that arteriovenous O_2 difference increased significantly less between resting and $\dot{V}O_2$peak in the older age group (i.e., 148% vs 28%). Arteriovenous oxygen difference was confirmed a significant predictor of $\dot{V}O_2$peak in older and younger individuals as well as males and females.

There is a trend of decreased training volume and intensity paired with an increase in sedentary behaviour in older athletes when compared to younger, as demonstrated in the present study (Rivera *et al.*, 1989). With ageing, an increasingly sedentary lifestyle is linked to a decrease in arteriovenous O_2 difference (MacIntosh;Gardiner and McComas, 2006). In agreement, the present study observed that arteriovenous O_2 difference was a stronger predictor of $\dot{V}O_2$peak in older than younger individuals. The learned behaviour of a sedentary lifestyle leads to a reduction in functional fitness that is equal with ageing for both males and females, exaggerating age-related changes in the cardiovascular system and leading to a significant reduction in peripheral oxygen extraction (Rivera *et al.*, 1989; Milanovic *et al.*, 2013).

Research shows that when training volume and intensity is increased in elderly individuals to match that of the younger athletes, the age-related decrease in arteriovenous O_2 difference observed is attenuated/ disappears through improved peripheral mechanisms i.e. microvascular and/or skeletal muscle (Rivera *et al.*, 1989; Haykowsky *et al.*, 2012). The present study confirms previous findings. When physical activity is included as a confounding factor, the age-related decrease in arteriovenous O_2 difference is insignificant.

As part of the cardiovascular response to exercise, blood pressure is altered to maintain perfusion pressure to vital organs (Joyner and Casey, 2015). Arteries stiffen with age, causing a significant increase in systolic blood pressure and mean arterial pressure through an increase in power to generate more pressure in response to vascular changes and increased afterload (Takazawa *et al.*, 1996; Houghton *et al.*, 2016). In the present study, there was a significant interaction between age and sex for systolic blood pressure and mean arterial blood pressure at $\dot{V}O_2$peak. However, no significant differences were found between older and younger age groups when physical activity level is adjusted for.

Using 24-hour ambulatory blood pressure monitoring, a study has previously confirmed that age-matched males have a higher blood pressure than females, as well as a greater incidence of uncontrolled hypertension (Wiinberg *et al.*, 1995). In the present study, a significantly higher systolic blood pressure and mean arterial blood pressure in males was only found when younger males were compared to younger females. In older individuals, resting systolic blood pressure and mean arterial blood pressure was significantly higher in females. Research confirms no difference between both sexes in blood pressure at ~60 years, with females showing increased acceleration beyond this age compared to males (Franklin *et al.*, 1997b).

Accelerated blood pressure in females after ~60 years suggests that greater arterial compliance and vasodilatory state in younger females is lost with menopause onset, however this also leads to the maintenance of left ventricular mass, preserving cardiac function (Franklin *et al.*, 1997b). The greater vasodilatory state in young females means they are less able to compensate for reductions in stroke volume by increasing their heart rate when compared to males, leading to greater reliance on mechanical efficiency, greater cardiac work and peripheral oxygen extraction (Wheatley *et al.*, 2014). Although, in the present study, arteriovenous O_2 difference determined $\dot{V}O_2$peak equally in older males and females.

The present study allows for insight into mechanisms leading to a decrease in $\dot{V}O_2$peak in association with age and sex.

4.6.2 Determinants of exercise tolerance in individuals with hypertrophic cardiomyopathy in comparison to healthy individuals

Relative $\dot{V}O_2$peak was significantly reduced in individuals with HCM when compared to healthy individuals. In a previous assessment of mechanisms leading to reduced exercise capacity in HCM, peak cardiac index was the major determinant of exercise capacity (Finocchiaro *et al.*, 2015). However, the present study demonstrated that variability in $\dot{V}O_2$peak was determined by peak arteriovenous O_2 difference in individuals with HCM, which was also significantly reduced when compared with healthy individuals.

Although cardiac index was not significantly reduced at peak exercise in the present study when medication was controlled for, the present study agrees that cardiac function is significantly reduced in individuals with HCM through the finding of significantly reduced resting cardiac index and cardiac power output index in comparison to healthy individuals (Finocchiaro *et al.*, 2015). In addition and in coherence with previous findings, peak heart rate was significantly reduced in individuals with HCM in the present study (Finocchiaro *et al.*, 2015). A finding that is still present heart-rate lowering medication is ceased (Wu *et al.*, 2019).

Resting stroke volume and stroke volume index were significantly lower at rest in individuals with HCM, but not peak exercise stroke volume. Stroke volume increased by 118% from rest to peak in HCM compared with 24% in control. The present cohort is a representation of early-stage disease, and such increase can be suggested to be a compensatory mechanism, of which may have future pathological consequence (Kishi, 2012).

In healthy individuals, blood pressure rises because of increased sympathetic activity and heart rate to increase cardiac output to meet oxygen demand of muscles (Schultz and Sharman, 2014). In the present study, the HCM cohort was slightly hypertensive (i.e., resting systolic blood pressure ≥140 mmHg) but had a significantly lower peak systolic blood pressure than healthy individuals when the effect of medication was controlled for. As medication was controlled for, the present finding suggests decreased ability of the cardiovascular system to respond to exercise stress when compared to healthy individuals. This finding should be assessed in future studies.

4.6.3 Study limitations

In the present study, the following limitations should be considered; haemodynamic measurements were non-invasive and thus not the ‘gold-standard’ technique for assessing

haemodynamic function, but validity and reproducibility of non-invasive measurement (bioimpedance and bioreactance) has been confirmed by past studies (Jakovljevic;Trenell and MacGowan, 2014; Brittain;Busk and Moller, 2018)

Additionally, part A of the present study was originally completed as a validation study and results were utilised as an observational study due to technical difficulty to extract data from the non-invasive haemodynamic Bluetooth device once returned to the third-party. Of the eligible data for analysis cohort, there was a lack of participants aged 35-55 years, and fewer participants in the older than younger age group, which meant that analysis between age and mechanisms of $\dot{V}O_2$peak may not be as accurate as we would like. The lack of participants between the defined ages provides rationale for why data was assessed within the defined age sub-groups.

Furthermore, time of data collection was not the same for everyone, as data was collected at a time suitable for each participant (between 9.30am and 4.30pm). It has been observed that total work rate is ~10% greater, and aerobic power ~5% when CPET is complete in the afternoon (Hill *et al.*, 1992). Therefore, results may have been slightly affected.

Regarding the assessment of the mechanisms of exercise intolerance in individuals with HCM, the following limitations should be considered: there was potential for sampling bias, because 88 out of the potential 118 study participants declined/ were excluded from participation (i.e., all participants' data was baseline data from the lifestyle intervention in HCM- Chapter 5). However, the clinical characteristics are comparable to published studies (Coats *et al.*, 2015; Saberi *et al.*, 2017).

Overall, the population studied was sufficient to further improve and add knowledge about the mechanisms leading to decreased exercise tolerance associated with chronological age in the healthy population. In addition, it was sufficient to further improve and add knowledge about the mechanisms leading to decreased exercise tolerance associated with individuals with HCM. In the future, a study should be conducted using the same haemodynamic measures to assess the interaction between age and sex as well as the effects of HCM on exercise intolerance.

4.6.4 Conclusions

In conclusion for part A, the findings of the present study indicate that $\dot{V}O_2$peak is reduced in older individuals when physical activity level is considered, and arteriovenous O_2 difference, not cardiac output, determines variability in $\dot{V}O_2$peak with advanced age. The data suggests that with advancing age that in both males and females, the skeletal muscle contributes greater

to the decline in exercise capacity than that of the central cardiovascular system. In addition, with advancing age there was an acceleration in systolic blood pressure and mean arterial blood pressure in females in comparison to males. However, there was no interaction between age and sex for $\dot{V}O_2$peak and other determinants.

Therefore, although physical activity may slow the age-related decrease in exercise tolerance, cardiovascular decline was still present. Deficiencies in cardiovascular function of older individuals require future studies to provide physiological knowledge so that clinical interventions can be improved and prevent a state of dependence in our ageing population.

In conclusion for part B, the present study indicated that the variability in exercise tolerance was determined by peak arteriovenous O_2 difference in individuals with HCM. In contrast, peak cardiac output did not differ between individuals with HCM and healthy individuals because of the compensatory increase in peak stroke volume in individuals with HCM. The data suggests that in individuals with HCM at an early-stage disease, reduced skeletal muscle capacity for oxygen extraction contributes greater than that of the central cardiovascular system to the decline in exercise capacity.

Research is in the early stages of data collection regarding exercise intolerance in individuals with HCM. Therefore, defining the underlying mechanisms of exercise intolerance in individuals with HCM using larger observational studies will help to define therapeutic targets to lessen the burden of HCM to those individuals affected.

Chapter 5. The effect of a novel lifestyle intervention on functional capacity and quality of life in individuals with hypertrophic cardiomyopathy

5.0 Abstract

Objective: The aim of the present study was to determine the effect of a novel lifestyle intervention on cardiovascular function and quality of life (QoL) in individuals with HCM.

Methods: In a single-centre, observational, pilot study design, individuals with HCM were randomised into intervention (N=21; 24% female) or control (N=8; 60% female) group. Individuals with HCM completed a cardiopulmonary exercise stress test (CPET), non-invasive cardiac measurements (bioreactance and echocardiography), quality of life, and blood chemistry assessments. at baseline and follow up (16-weeks). Individuals in the intervention group consumed 6mmol of dietary nitrate (NO_3^-) daily and maintained their daily physical activity level by at least ≥2000 steps/day from baseline for 16 weeks. Medication was controlled for throughout data analysis.

Results: In response to the 16-week lifestyle intervention, the intervention group significantly increased and maintained their physical activity by 2489 steps/day (6197±2363 vs 8686±2784 steps/day, *p*=.01), and the control group maintained usual step count (7559±3257 vs 7659±2965, *p*=.91). $\dot{V}O_2$peak did not significantly change when response to lifestyle intervention was compared between the intervention and control groups (*p*=.69); (i.e., 18.2±5.40 vs 19.9±4.72 ml/kg/min, *p*=.05 vs. 18.7±5.46 vs 20.2±4.75 ml/kg/min, *p*=.43), respectively. However, peak diastolic blood pressure significantly improved when intervention was compared to control (*p*=.03); (i.e., 97.1±12.2 vs 84.5±8.75mmHg, *p*<.01, vs. 90.5±12.3 vs 92.6±8.83mmHg, *p*=.70) respectively. There was an improvement in disease-specific health related QoL in the intervention group when compared to control (*p*=.04); (i.e., 22.4±19.4 vs. 13.9±13.7 MLHF score, *p*=0.04; vs 24.5±19.5 vs. 31.7±14.3, *p*=.26). Echocardiography measures of diastolic function remained unchanged in both groups, and serum biomarkers of kidney and liver function remained within the normal range.

Conclusions: In individuals with HCM, a 16-week lifestyle intervention improved QoL and blood pressure, a major risk factor for cardiovascular mortality and adverse cardiac remodelling. However, the intervention did not lead to significant improvements in exercise tolerance or diastolic dysfunction.

5.1 Introduction

Reduced cardiovascular fitness and quality of life (QoL) are common presentations in individuals with hypertrophic cardiomyopathy (HCM), and predict long-term outcomes (Cox *et al.*, 1997; Huff;Turer and Wang, 2013).

Data available assessing the effects of exercise moderate-intensity exercise training in HCM is limited. In an assessment of the response to a 16-week intervention incorporating moderate-intensity exercise training in individuals with HCM, Saberi et al. observed a significant increase in exercise tolerance ($\dot{V}O_2$peak), and no adverse events (Saberi *et al.*, 2017). An improved $\dot{V}O_2$peak in HCM is highly clinically important, because reductions are related to increased clinical decompensation (Coats *et al.*, 2015). In individuals with heart failure (HF), a 6% increase in $\dot{V}O_2$peak through exercise training is associated with 8% lower cardiovascular mortality risk and hospitilisation (O'Connor *et al.*, 2009).

Endothelial dysfunction is associated with reduced vasodilation of coronary and peripheral vasculature, and therefore increased pressure in the cardiovascular and peripheral vascular system, compromising the body's ability to redistribute blood during exercise (Alem, 2019; Giannitsi *et al.*, 2019). The described pathology is attributable to reduced nitric oxide (NO) bioavailability. Increased NO bioavailability in response to exercise observed in healthy individuals is attenuated in individuals with cardiovascular disease (Macdonald;Schyvens and Winlaw, 1996). In coherence, individuals with HCM are observed to have endothelial dysfunction (Dimitrow *et al.*, 2007; Alem, 2019). An increased NO bioavailability may be achieved through the consumption of NO_3^- through the NO_3^--nitrite (NO_2^-)-NO pathway, as defined in Chapter 1.11.3 and observed in Figure 5.1 (Lundberg;Weitzberg and Gladwin, 2008).

Dietary nitrate (NO_3^-), which is abundant in beetroot, is observed to result in improved factors linked to left ventricular (LV) diastolic dysfunction in heart failure with preserved ejection fraction (HFpEF), a key pathological characteristic of HCM that denotes poor prognosis and promotes adverse remodelling (Lundberg;Weitzberg and Gladwin, 2008; Zamani *et al.*, 2015; Eggebeen *et al.*, 2016; Chirinos *et al.*, 2017). Observations include increased exercise capacity, vasodilation, and reduction in arterial wave reflections (figure 5.1). In addition, NO_3^- supplementation has repeatedly been observed to have vasodilatory properties, resulting in a

reduced blood pressure in individuals with HF, and therefore may provide a similar therapeutic effect in individuals with HCM (Eggebeen *et al.*, 2016; Chirinos *et al.*, 2017).

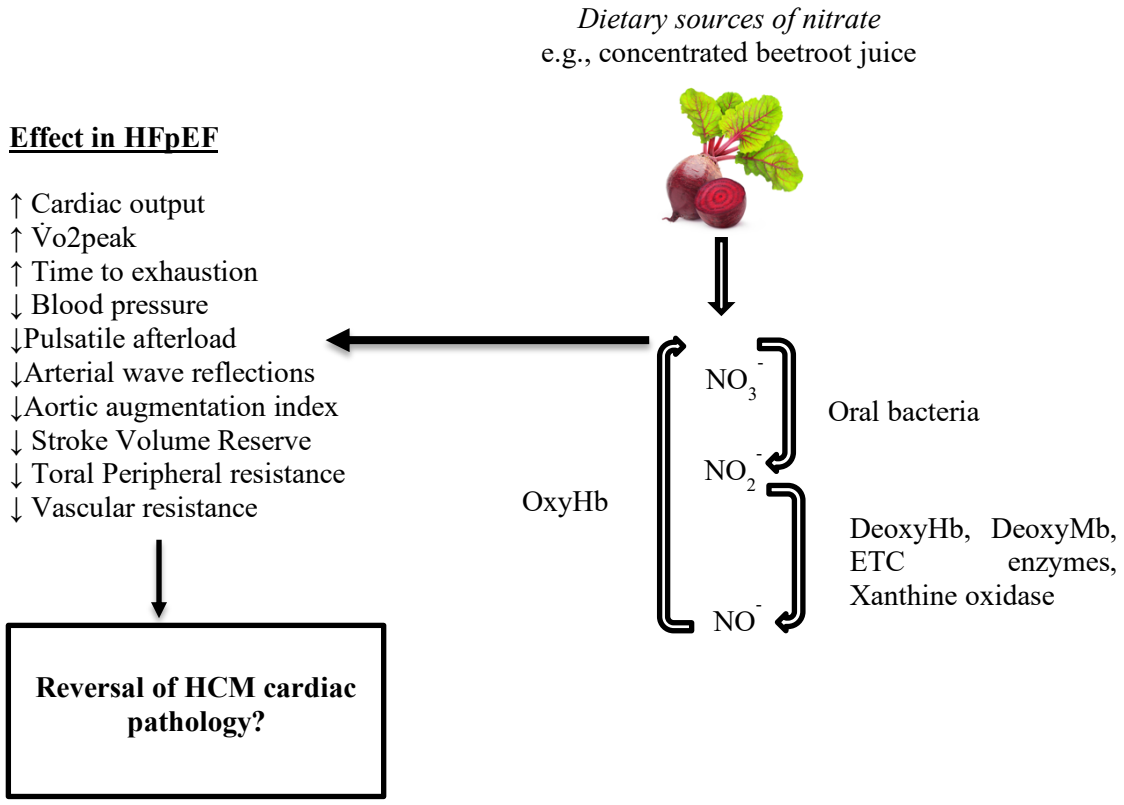


Figure 5.1 Nitrate-nitrite-NO pathway and benefits to individuals with heart failure with preserved ejection fraction (Zamani *et al.*, 2015). Adapted from Vanderpool and Gladwin, 2015 (Vanderpool and Gladwin, 2015). DeoxyHb, deoxygenated haemoglobin; DeoxyMb, deoxygenated myoglobin; ETC, electron transport chain; NO^-, nitric oxide; NO_2^- , nitrite; NO_3^- , nitrate; V̇o2peak, peak oxygen consumption.

Regarding safety and dose of NO_3^-, no adverse effects have been observed in interventions assessing a 12.9mmol single dose of NO_3^-, or one week of 6.1mmol NO_3^- in the form of a beetroot juice shot in HFpEF (Zamani *et al.*, 2015; Eggebeen *et al.*, 2016).

5.2 Rationale, Aim and Hypotheses of the Study

At present, there is no treatment for HCM progression. In addition, regular exercise and/or nutritional therapies are not usually prescribed or followed after HCM diagnosis.

NO_3^- supplementation has been observed to target cardiac remodelling and improve vascular function at rest and peak exercise in individuals with HF, and exercise training has been observed to improve exercise tolerance in HCM (Zamani *et al.*, 2015; Eggebeen *et al.*, 2016;

Saberi *et al.*, 2017). However, exercise training has been observed to have a limited effect on cardiac remodelling in HCM (Saberi *et al.*, 2017). The presence of endothelial dysfunction and reduced NO bioavailability in HCM may lead to a reduced effect of exercise (Dimitrow *et al.*, 2007).

In individuals with HFpEF, 6mmol of NO_3^- three times per week in addition to exercise training, in comparison to exercise training without NO_3^- supplementation lead to no additional effect on $\dot{V}O_2$peak (Shaltout *et al.*, 2017). However, an additive effect may have been observed it NO_3^- consumption occurred daily.

It is therefore hypothesised that daily NO_3^- supplementation will further improve NO bioavailability and reduce blood pressure. Leading to an additive benefit than moderate-intensity exercise training alone in individuals with HCM.

The **aim** of this chapter was to assess the effect of a physical activity and NO_3^- supplementation lifestyle intervention on cardiorespiratory fitness and QoL in individuals with HCM. The **hypothesis** was that a 4-month lifestyle intervention will result in significant improvements following the intervention in exercise tolerance, clinical phenotype, and QoL in HCM individuals when compared to control.

5.3 Methods

5.3.1 Design and outcomes

The present study was conducted at the Clinical Research Facility of the Royal Victoria Infirmary in Newcastle-Upon-Tyne. This randomised, single-centre, observational, pilot study that assessed the effect of a novel lifestyle intervention incorporating physical activity and NO_3^- supplementation in individuals with HCM. The lifestyle intervention in individuals with HCM included one primary outcome, and four secondary outcomes, as detailed below.

Primary outcome:

i) Peak oxygen consumption

Secondary outcomes:

i) Change in echocardiography markers of diastolic function (i.e., left atrial diameter, left atrial volume, left atrial volume index, E/e' ratio, LV end diastolic volume (EDVLV), LV internal diameter end-diastole (LVIDd), and posterior wall thickness at end-diastole (PLWd))

ii) Change in quality of life

iii) Change in anxiety and depression

iv) Change in biochemical markers of cardiac, liver, and kidney function

5.3.2 Participants

Potential and eligible participants were identified by a consultant cardiologist via the Cardiology clinics and North of England Cardiac Family History Service of Newcastle Upon Tyne Hospitals (NUTH). The recruitment process and acquiring of informed consent (Appendix 2) is described in Chapter 3.3. Participants were randomised at a ratio of 2:1 to either the intervention or control group. Randomisation numbers were computer generated and assigned to a strict sequence. At the point of randomisation, participants were assigned to the next number in the sequence. To maintain concealment and minimise selection bias, randomisation was performed after the baseline visit.

Consented participants attended two visits lasting approximately three hours, one at baseline and one at 16-weeks. After randomisation, exercise assignment and consumption of dietary nitrate was not blinded to participants or study staff. Study duration was from May 2019 to February 2022, with a pause in recruitment and study completion from March 2020 to April 2021 due to the coronavirus 2019 pandemic.

Inclusion criteria included clinically stable adults ≥18 years of age with obstructive/non-obstructive HCM (Ommen *et al.*, 2020) on optimal medical treatment; sinus rhythm on electrocardiogram at the time of screening; ability to provide written informed consent and association with NUTH; and willingness to participate in the study and attend screening and 16-week follow-up.

Exclusion criteria included being less than 3 months after or having scheduled septal reduction therapy, pacemaker or implantable cardioverter-defibrillator (ICD) placement; New York Heart Association class IV; previously displayed hypotensive response to exercise testing ((≥20 mmHg decrease of systolic blood pressure from baseline blood pressure or an initial increase in systolic blood pressure followed by a decrease of systolic blood pressure ≥20 mmHg); resting blood pressure >160/100mmHg; resting LVOT gradient >50 mmHg; LVEF <50% quantified by echocardiography; renal insufficiency i.e., glomerular filtration rate of less than 30 mL/min per 1.73m^2; present or planned pregnancy; life expectancy <12months ; severe obesity i.e., body mass index (BMI) >40 kg/m^2; a history of exercise induced syncope or

uncreated symptomatic ventricular arrhythmias; use of other investigational drugs at the time of enrolment inability to exercise due to orthopaedic or other non-cardiovascular limitations and participation in competitive/ organised sport activities (e.g., football, basketball, rugby, hockey, etc.), burst activity (such as sprinting, racket sports, etc.) or heavy isometric exercise (such as body building or bench-pressing) or opposition of refraining from the same for the duration of the study; and history of drug or alcohol abuse within the past 12 months.

The study was approved by the Northeast-Tyne and Wear Research Ethics Committee (18/NE/0318) and a part of the European In Silico trials for drug tracing the effects of sarcomeric protein mutations leading to familial cardiomyopathy (SILICOFCM) NCT03832660 (Tafelmeier *et al.*, 2020). All procedures were performed according to the declaration of Helsinki.

5.3.3 Study protocol and measurements

5.3.3.1 Interventional procedure

Participants randomised to the intervention were asked to consume 6mmol NO_3^- in the form of a 70ml concentrated beetroot juice shot (NO_3^-, BEET IT Sport, James White Drinks Ltd., Ipswich, UK) daily with breakfast for 16 weeks. I provided participants with instructions for self-administration of the beetroot juice shot.

Participants were educated on the effect of chewing gum, and use of mouthwash on NO_3^- supplementation. However, all current optimal medication was advised to be continued, therefore diuretics and use of antiacids were not asked to be ceased. Limitations to the study regarding the intake of drugs such as antiacids and diuretics are discussed in closing comments of the present chapter. No participants confirmed the consumption of organic nitrates.

In combination with daily NO_3^-, participants within the intervention group were asked to increase daily physical activity level from baseline (i.e., average steps/day for 7 days after initial appointment) by at least 2000 steps/day for the duration of the intervention. Control participants were asked to continue with their usual lifestyle and monitor daily step counts.

The number of steps was evaluated objectively using pedometers (Omron Health care, Model no: HJ-321-E, Japan) over a 7-day period. Following the baseline study visit, I provided participants with a self-reported physical activity diary for step-count documenting (Appendix 11).

I monitored participants weekly to measure adherence to the intervention/ usual activity via planned telephone calls. I input step counts into a excel spreadsheet and completed a weekly telephone follow-up sheet (Appendix 10) for each participant. In the intervention group, weekly phone calls also provided a means of motivation. As well as physical activity discussion, I asked participants about compliance to beetroot juice shot consumption. Participants were asked to return any beetroot juice shots not taken during the duration of the study at the follow-up visit (no beetroot juice shots were returned).

5.3.3.2 Study measurements

For intricate details of the following study measurements, please refer to the General methodology Chapter (Chapter 3).

Upon arrival at the Clinical Research Facility on the baseline study visit day, medical history, physical examination and physical activity readiness were assessed (Appendix 5 and 6) (Riebe *et al.*, 2018).

At baseline and 16 weeks, I collected blood pressure from the brachial-artery, body temperature and heart rate (Welch Allyn, NY, USA) in a seated position. This was followed by height and weight measurements (seca 769 electric column scale with telescopic measuring rod seca 220, seca gmbh & co, Hamburg, Germany).

Next, I recorded resting 12-lead electrocardiogram and resting haemodynamic measurements via bioreactance (NICOM, Cheetah Medical Inc., Indianapolis USA) in supine.

A professional echocardiographer then completed resting transthoracic echocardiography.

Prior to a light lunch, blood was collected by registered nurse in the Clinical Research Facility.

During lunch, I provided each participant with self-reported questionnaires (Appendix 9) related to QoL (Minnesota living with heart failure questionnaire (MLHFQ), the Short Form-36 Health Survey (SF-36), Hospital Anxiety and Depression Scale (HADS)) were completed.

Finally, I set up and supervised a CPET on a semi-recumbent cycle ergometer (Corival, Lode, Groningen, Netherlands) was completed with simultaneous gas exchange measurements (Metalyzer 3B, Cortex, Leipzig, Germany) (Zigmond and Snaith, 1983; Rector, 1987; Ware, 2000).

Before participants departed the Clinical Research Facility, for all participants I calculated step length to be input into the Omron Health pedometer and provided individuals in the intervention group with the total beetroot juice for study duration.

Table 5.1 outlines the lifestyle intervention study day protocol, with visits lasting approximately 3 hours.

Table 5.1. Lifestyle intervention in hypertrophic cardiomyopathy study protocol

Time (minutes)	**Procedure**	**Played major role in data collection (Y/N)**
45	Consent Medical History and physical examination	Y
20	ECG/ autonomic function (spacelab/ NICOM)	Y
35	ECHO	N- Registered Echocardiographer
15	Blood sample collection	Y (preparation for) N (collecting)- Dr Nduka Okwose/ Clinical Research Facility Nurses
40	Questionnaires with food: HADS/ MLHFQ/ SF-36	Y
30	CPET	Y (lead CPET but consultant cardiologist also attended)
10	Pedometer set up and beetroot juice collection	Y
Total visit time= ~3 hrs 15min		

CPET, Cardiopulmonary exercise testing; HADS, Hospital Anxiety and Depression Scale; ECG, electrocardiogram; ECHO, echocardiography; MLHFQ, Minnesota Living with Heart Failure Questionnaire; N, no; NICOM, Non-invasive cardiac output monitoring; SF-36, 36-item Short Form survey; Y, yes.

5.3.3.3 Equipment and Assessments

Non-invasive bioreactance technology was used for haemodynamic measurements (i.e. cardiac output, stroke volume, total peripheral resistance and HR) at rest in supine position for 5 minutes, and during exercise (Fortin *et al.*, 2006; Jakovljevic;Trenell and MacGowan, 2014). A full description of and theory behind bioreactance technology is described in Chapter 3.7.2.

Participants completed a progressive exercise protocol CPET using a semi recumbent cycle ergometer with simultaneous gas exchange measurements, as described in Chapter 3.8. Simultaneous 12-lead electrocardiography and blood pressure were recorded. Exercise test was terminated i) when respiratory exchange ratio exceeded 1.15 ii) upon volitional exhaustion i.e. inability to maintain a cadence of 60 rpm, iii) when maximum oxygen consumption was achieved, defined as the inability to increase oxygen consumption despite an increase in exercise intensity (watts), iv) if the participant requested termination of the test.

QoL measures included the self-reported MLHFQ, and SF-36 questionnaires (Rector, 1987; Ware, 2000). Anxiety and depression were assessed using HADS (Zigmond and Snaith, 1983). A full description of the questionnaires (Appendix 9), including score descriptions, can be found in Chapter 3.11.

A British Society of Echocardiography accredited echocardiographer performed transthoracic echocardiography including colour and tissue Doppler at rest, and in response to the Valsalva manoeuvre to allow analysis of measures related to diastolic dysfunction (i.e., left atrial diameter, left atrial volume, left atrial volume index, E/e' ratio, EDVLV, LVIDd and PLWd). The Echocardiography procedure is further described in Chapter 3.10.

A fasted blood sample (25ml) was taken from the antecubital vein before and after the 16-week intervention by a research nurse from the Clinical Research Facility of the RVI to assess the response of cardio-metabolic function in response to the lifestyle intervention. Methodology and further rationale is described in Chapter 3.9.

Blood biomarkers are useful for assessment of early progression of disease in HCM (such as LV dysfunction and myocardial fibrosis) (Matthia *et al.*, 2022). For example, c-reactive protein (CRP) is a marker of inflammation and increases are related to histopathological fibrosis in HCM, in whom tumor necrosis factor alpha (TNFα), is also increased (Matthia *et al.*, 2022). In addition, hypercholesterolemia is associated with cardiac hypertrophy and fibrosis, and therefore a potential contributor to worsening HCM pathology (Kang *et al.*, 2009). Cardiac function changes following the lifestyle intervention were also assessed (i.e., troponin-t and NT-proBNP), measures related to LV wall thickness and LA diameter (Matthia *et al.*, 2022).

Additional biomarkers assessed also included biomarkers of renal and liver function in response to the intervention (i.e., urea, creatinine, estimated glomerular filtration rate (eGFR)), although no adverse effects have previously been observed in healthy individuals of exercise and NO_3^- supplementation (Carpentier *et al.*, 2015). Lipid profile (total cholesterol (TC), high density

lipoprotein cholesterol (HDL-C), non-HDL-C, and T/HDL-C ratio, triglycerides), haemoglobin A1c (HbA1C)), and inflammation (i.e., albumin, bilirubin, alkaline phosphatase (ALP), and albumin, also a biomarker for nutritional status and inflammation were compared between and within groups (Meikle *et al.*, 2013; Lopez-Giacoman and Madero, 2015; Dorcely *et al.*, 2017; Liu *et al.*, 2017a; Hirata *et al.*, 2020). Finally, total protein (TP), a measure of the two major proteins in the blood (i.e., albumin and globulin) was also analysed.

5.3.4 Sample size calculation

Analysis of sample sizes was completed using SAS version 9.4 (SAS Institute Inc). Dr Kim Pearce (senior statistician, Faculty of Medical Sciences) provided guidance on the use of the software and explained sample size calculation procedure prior to me completing the analysis and reporting such findings. To analyse the effect of a lifestyle intervention on exercise intolerance in HCM, the sample size was calculated based on previous literature suggesting that there is a significant effect of 16-week moderate intensity exercise training on $\dot{V}O_2$peak in individuals with HCM (Saberi *et al.*, 2017). In the described study, there was a significant increase in $\dot{V}O_2$peak after the exercise condition when to control (i.e., 1.27±2.71ml/kg/min). Assuming a similar effect, n=219 individuals were required in total, with 146 individuals required in the intervention group, and 75 in the control at the recruitment ratio 2:1 to detect a significant difference at a power of 0.9. In the present study, n=29 individuals with HCM were recruited. Therefore, the power of the present study to detect a significant difference is 0.2 and should be used to confirm trends.

5.3.5 Data analysis

All statistical analysis was performed using IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., Armonk, N.Y., USA). Dr Kim Pearce (senior statistician, Faculty of Medical Sciences), and Miss Alaa Alyahya gave statistical guidance, and I then completed all data analysis. Prior to statistical analysis, Data presented with no significant outliers when screened using standard Z-distribution cut-offs, boxplots and Mahalanobis distance test. A Kolmogorov-Smirnov test was used to evaluate normality of distribution, all data was normally distributed other than SF-36 questionnaire results.

Chi-square test of independence assessed categorical variables (i.e., sex, tobacco or alcohol use, medications, medical intervention, comorbidity presence) between the intervention and control group. For the assessment of physical characteristics, metabolic, and haemodynamic measurements at rest and peak exercise, echocardiography measurements, MLHFQ, HADS,

and blood between intervention and control group, as well as pre vs post intervention/ control, repeated measures analysis of covariance (ANCOVA) was completed. Medication (drug total) was controlled for and included as the confounding factor during analysis. Due to non-normal data, differences in SF-36 change score following 16 weeks between the intervention and control conditions was assessed using the Mann-Whitney U test. Pre versus post intervention SF-36 scores within the intervention and control groups were assessed using the Wilcoxon signed-rank test. Missing post-intervention data occurred completely at random, because of this, the mean for the variable was inserted in such cases (Xu, 2017). Bonferroni correction was performed and reported for significant findings. Pearson's coefficient of correlation was used to assess the relationship between step-count and $\dot{V}O_2$peak. The strength of the relationship between the two variables was quantified using the absolute value of 'r' i.e. r<0.3 - indicating weak relationship, 0.4-0.6 – moderate, 0.7-1.0 strong (Akoglu, 2018). Statistical significance was indicated if $p<0.05$ for all calculations. All data is presented as mean ± SD other than SF-36 questionnaire data, which is presented as median (Q1-Q3).

5.4 Results

The consultant cardiologists at Newcastle upon Tyne Hospitals NHS Foundation Trust provided details for a total of 118 individuals diagnosed with HCM and eligible for participation in the study. Twenty-eight individuals did not answer when a telephone call was attempted three times and did not respond to voice message recording. Thirty-three individuals confirmed they were not interested in taking part in the research study following receipt of the patient information sheet (Appendix 4). Thirteen individuals were excluded due to awaiting ICD implantation (n=1); undergoing chemotherapy (n=1); physical limitation due to hip replacement (n=1) or being very elderly (n=1); medical diagnosis i.e., muscular dystrophy (n=1); not being present on the NUTH system (n=2); chest pain (n=2); atrial fibrillation (n=1); waiting for transplant (n=1); waiting for transoesophageal echocardiogram for mitral regurgitation (n=1); and recent cerebrovascular accident (n=1). Other reasons given for not participating in the study (n=15) included the inability to take time off work (n=13), not being able to attend clinic following the coronavirus pandemic due to occupation (n=1), and not wanting to increase current activity (n=1).

From the 118 individuals screened, a total of 29 individuals (i.e., 25%) with HCM (n=7 females), aged 55±15 years old, were successfully recruited and participated in the lifestyle intervention. The mean time since diagnosis of HCM at visit 1 for the study cohort was 7.52±4.34 years.

Two individuals had presentation of obstructive HCM (i.e., left ventricular outflow tract gradient ≥30 mmHg).

Five (17%) individuals with HCM had a BMI healthy BMI (i.e., 18.5 to <25), 13 HCM individuals (45%) were overweight (i.e., 25 to <30), and 11 HCM individuals (38%) were within the obese range (i.e., ≥30).

A flow-chart of the recruitment is depicted in Figure 5.2.

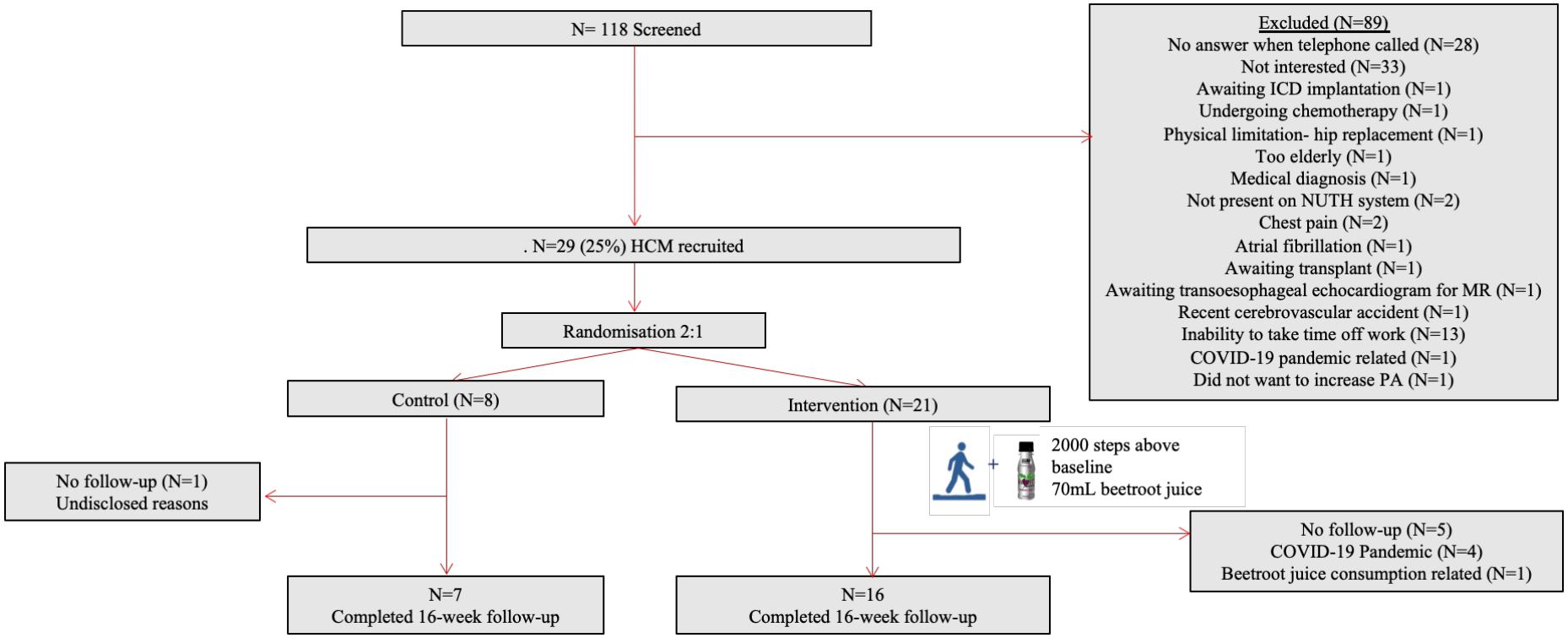


Figure 5.2. Flow chart of the recruitment for the lifestyle intervention in HCM

5.4.1 Baseline demographic and clinical characteristics of a novel lifestyle intervention in hypertrophic cardiomyopathy

The mean age of the 21 individuals randomised to the intervention group was 56±14 years old and in the control group (N=8) was 52±17 years. Of the participants, 16 (76%) successfully completed the intervention arm, and seven (88%) completed the control arm. Four individuals within the intervention group were unable to undergo a follow-up clinical examination due to the coronavirus pandemic, and two individuals dropped out (i.e., intervention group: n=1, related to beetroot juice shot consumption; control group: n=, 1 undisclosed reasons). Three individuals completed the follow-up clinical examination but were unable to complete the CPET measurements (intervention group: n=2, new onset atrial fibrillation investigation; control group: n=1, alert for ICD investigation).

When intervention and control groups were compared for differences in demographic and clinical characteristics at baseline, there were no significant differences found for any variable other than non-steroidal anti-inflammatory medication. There was significantly less (67%) individuals in the intervention group taking non-steroidal anti-inflammatory drugs than control (X^2 (1, N=29)=5.22, p=.02)). The demographic and physical characteristics are presented in Table 5.2.

Table 5.2. Demographic and clinical characteristics of study participants at baseline.

Variables	Intervention (N=21)	Control (N=8)	P-value
Age (years)	56.0±14.1	51.5±17.1	.49
Sex (Male/Female)	17/4	5/3	.30
Height (m)	1.73±0.86	1.72±0.71	.72
Weight (kg)	84.8±15.8	87.9±22.0	.68
Body mass index (kg/m^2)	28.3±3.38	29.8±7.61	.48
Tobacco	2	1	.81
Alcohol	14	6	.67
Medications N (%)			
Angiotensin-converting enzyme inhibitor	2	2	.28
Angiotensin receptor blocker	4	0	.18
Antidepressant	2	1	.81
Anti-arrhythmic	3	1	.90
Beta-blocker	14	3	.15
Calcium channel blocker	3	4	.05
Diuretic	4	1	.68
Non-steroidal anti-inflammatory	1	3	**.02**
Anticoagulant	8	2	.51
Statins	4	2	.72
Insulin	3	0	.26
Medical intervention N (%)			
ICD/ Pacemakers	7	3	.83
Comorbidities N (%)			
Hepatic dysfunction	1	0	.53
Type II diabetes	7	0	.06

5.4.2 The effect of a novel lifestyle intervention in individuals with hypertrophic cardiomyopathy

5.4.2.1 Changes in step count following the 16-week lifestyle intervention

Step count significantly altered when the intervention condition was compared to control ($F(1, 26)= 6.51$, $p=.02$, $\eta^2=.19$). The average number of steps/day significantly increased on average for the 16-weeks in the intervention group when compared to control. In the intervention group, participants significantly increased the number of steps by 2489 steps/day from baseline to follow-up (i.e., from 6197±2363 to 8686±2784 steps/day, $p<0.01$). The control group adhered

to the protocol on average and followed what was expected by maintaining usual activity for the duration of the study (i.e., non-significant increase of 100 steps/day, from 7559±3257 to 7659±2965, p=.90).

In the intervention group, step count continuously increased throughout the intervention, and was significantly higher than baseline at week 1, weeks 8 and 16 by 20% (1266±1775 steps/day, p=.03); 45% (2814±2600 steps/ day, p<.01); and 51% (3160±3466 steps/day, p<.01), respectively. This highlights a progressive improvement and maintenance of steps as participants completed each week of the intervention. In comparison, the control group maintained their daily step count throughout the 16-week period. There was however a trend of decrease in average step count in week 1 in the control group. This could be a result of being over-cautious of doing too much following instructions to maintain usual activity. Habitual step count returned by week 8. Results can be visualised in figure 5.3.

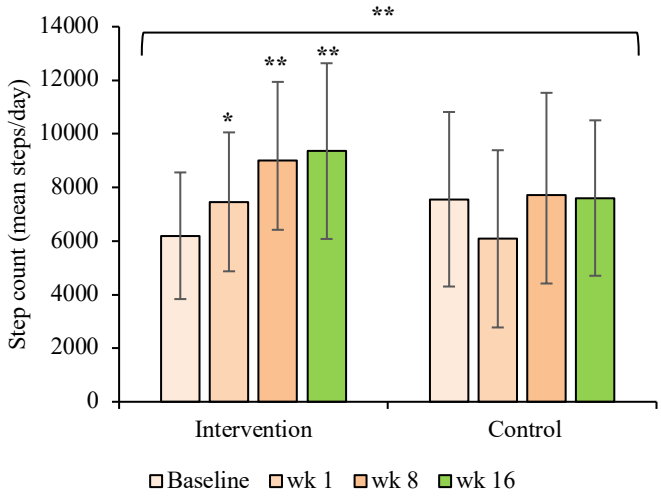


Figure 5.3. The difference between baseline step count and week (wk) 1, 8 and 16 for both the intervention and control groups (B). **= p<.01 and *=p<.05.

5.4.2.2 Changes in physical characteristics, and metabolic and haemodynamic function measurements at rest and peak exercise following the 16-week lifestyle intervention

When drug total was controlled for, resting systolic blood pressure was significantly affected by the intervention condition when change was compared to control ($F(1, 26)= 6.13$, p=.02, $\eta^2= .19$). Resting systolic blood pressure significantly decreased, indicated by a Bonferroni's corrected score decrease within the intervention group of 5% (143±19.5 vs 136±18.0mmHg, p=.04), but did not significantly change within the control (143±16.4 vs 151±18.1mmHg,

p=.13). The same finding was observed for diastolic blood pressure (F(1, 26)= 6.00, *p*=.02, η^2= .19). Resting diastolic blood pressure significantly decreased within the intervention group by 6% (88.2±5.36 vs 82.7±6.37mmHg, *p*<.01), but did not significantly change within the control (84.4±7.92 vs 85.6±6.40mmHg, *p*=.61). A significant effect of the intervention was also observed when compared to control for mean arterial blood pressure (F(1, 26)= 8.80, *p*<.01, η^2= .25). Resting mean arterial blood pressure significantly decreased within the intervention group by 6% (107±9.16 vs 101±8.66mmHg, *p*<.01) but did not significantly change within the control (104±9.20 vs 107±8.72mmHg, *p*=.20).

At rest, oxygen consumption was not affected by the intervention condition when change was compared to control (F(1, 26)= 0.181, *p*=.67, η^2= .01). Arteriovenous O_2 difference was also unaffected by the intervention (F(1, 26)= 0.30, *p*=.59, η^2= .01).

Resting heart rate was not affected by the intervention condition when change was compared to control (F(1, 26)= 0.755, *p*=.39, η^2= .03). Such finding was also observed for stroke volume (F(1, 26)= 1.84, *p*=.19, η^2= .07), stroke volume index (F(1, 26)= 0.923, *p*=.35, η^2= .03), cardiac output (F(1, 26)= 2.01, *p*=.17, η^2= .07), cardiac index (F(1, 26)= 0.911, *p*=.35, η^2= .03), cardiac power output (F(1, 26)= 0.888, *p*=.36, η^2= .03), and cardiac power output index (F(1, 26)= 0.118, *p*=.73, η^2= .01).

Finally, weight and BMI were not affected by the intervention condition when change was compared to control, (F(1, 26)= 0.96, *p*=.34, η^2=.04); and (F(1, 26)= 0.84, *p*=.37, η^2=.03). Physical characteristics and resting metabolic and haemodynamic measurements are shown in table 5.3.

Table 5.3. Physical characteristics and Metabolic and haemodynamic variables at rest before and after 16-weeks of lifestyle intervention and control condition.

Variables	Intervention		Control		*P*- value
	Pre (N=21)	Post (N=13)	Pre (N=8)	Post (N=6)	
Physical characteristics					
Weight (kg)	84.8±16.2	83.4±14.2	87.9±23.5	90.5±22.8	.34
Body mass index (kg/m^2)	28.3±3.46	28.0±2.65	29.8±8.13	30.5±7.87	.37
Metabolic function					
Oxygen consumption (mL/kg/min)	4.40±0.86	4.05±0.59	4.19±0.92	3.68±0.44	.67
Arteriovenous oxygen difference (mLo_2/ 100mL of blood)	9.30±2.72	8.53±2.65	8.44±1.49	8.83±2.63	.43
Haemodynamic function					
Heart rate (beats/min)	67.5±9.56	66.8±7.98	69.1±11.0	65.8±5.91	.39
Stroke volume (mL/beat)	60.9±15.3	68.9±21.4	67.6±29.0	60.4±20.2	.19
Stroke volume index (mL/beat/m^2)	30.6±8.24	33.8±9.77	32.9±12.3	30.2±9.02	.35
Cardiac output (L/min)	4.08±0.97	4.57±1.19	4.33±1.93	3.92±1.55	.17
Cardiac index (L/min/m^2)	2.05±0.54	2.24±0.53	2.10±0.81	1.95±0.70	.35
Cardiac power output (W)	0.99±0.26	1.05±1.94	0.98±0.39	0.91±0.33	.36
Cardiac power output index (W/m^2)	0.50±0.14	0.50±0.08	0.48±0.17	0.46±0.15	.73
Systolic blood pressure (mmHg)	144±12.4	137±12.4*	141±26.8	150±29.4	**.02**
Diastolic blood pressure (mmHg)	88.3±7.98	82.7±6.60**	84.1±7.32	85.8±5.33	**.02**
Mean arterial pressure (mmHg)	107±8.12	101±7.23**	103±12.6	107±11.5	**<.01**

P-value (right column) defines between-group change score differences; ****= p<.01** and ***=p<.05**
Within-group differences: pre vs. post 16-weeks in intervention or control group.

When drug total was controlled for, AT was significantly affected by the intervention condition when compared to control ($F(1, 26)= 5.78$, $p=.02$, $\eta^2=.18$). A significant Bonferroni's corrected score increase in peak AT was observed within the control group by 22% (11.6±3.14 vs 14.2±2.66 mL/kg/min, $p=0.02$), compared to no change following intervention (11.8±3.11 vs 11.4±2.66 mL/kg/min, $p=.52$). In addition, peak diastolic blood pressure was significantly affected by the intervention condition when compared to control ($F(1, 26)= 5.66$, $p=.03$, $\eta^2=.18$). Peak diastolic blood pressure significantly decreased within the intervention group by 13% (97.1±12.2 vs 84.5±8.75mmHg, $p<.01$) compared to no change after 16-weeks control condition (90.5±12.3 vs 92.6±8.83mmHg, $p=.70$).

$\dot{V}O_2$peak was not affected by the intervention condition when change was compared to control ($F(1, 26)= 0.16$, $p=.69$, $\eta^2=.01$). In addition, respiratory exchange ratio ($F(1, 26)= 0.14$, $p=.71$, $\eta^2=.01$); arteriovenous O_2 difference ($F(1, 26)= 0.15$, $p=.70$, $\eta^2=.10$); and peak work rate ($F(1, 26)= 2.94$, $p=.10$, $\eta^2= .10$) were unaffected.

Peak heart rate was not affected by the intervention condition when change was compared to control ($F(1, 26)= 3.22$, $p=.09$, $\eta^2=.11$). Such finding was also observed for peak stroke volume ($F(1, 26)= 0.47$, $p=.47$, $\eta^2=.02$); stroke volume index ($F(1, 26)= 0.14$, $p=.72$, $\eta^2=.01$); cardiac output ($F(1, 26)= 0.23$, $p=.64$, $\eta^2=.01$); cardiac index ($F(1, 26)= 1.67$, $p=.21$, $\eta^2= .06$); cardiac power output ($F(1, 26)= 3.17$, $p=.09$, $\eta^2=.11$); cardiac power output index ($F(1, 26)= 1.56$, $p=.22$, $\eta^2=.06$); systolic blood pressure ($F(1, 26)= 0.01$, $p=.93$, $\eta^2= .01$); and mean arterial blood pressure ($F(1, 26)= 3.68$, $p=.07$, $\eta^2= .12$).

Within the intervention group, peak heart rate, stroke volume index, cardiac power output and cardiac power output index significantly decreased, indicated by a Bonferroni's corrected score decrease post-intervention by 11%, (i.e., 118±23.4 vs 131±20.4 beats/min, $p=.04$); 19%, (71.3±17.9 vs 59.9±13.1 mL/beat/m^2, $p=.02$); 17% (4.92±1.37 vs 4.10±0.92 W, $p=.01$); and 23% (2.45±0.73 vs 1.88±0.27 W/m^2, $p<.01$), respectively. Peak mean arterial blood pressure also significantly decreased following the 16-week intervention by 19%, (126±10.6 vs 116±8.43 mmHg, $p<.01$). A trend towards improvement in $\dot{V}O_2$peak was observed within the intervention group (i.e., increase of 9% (18.2±5.41 vs 19.9±4.72 mL/kg/min, $p=.05$)). Furthermore, there was a trend towards improvement in arteriovenous O_2 difference and work rate within the intervention group (i.e., increase of 25% (9.27±5.45 vs 11.59±2.62 mLo_2/ 100mL of blood, $p=.05$); and decrease of 13% (112±71.0 vs 126±34.1 watts, $p=.08$), respectively.

Within the control group, Bonferroni's corrected peak cardiac index, cardiac power output, and cardiac power output index significantly decreased pre vs post intervention by 34% (i.e., 8.12±2.43 vs 5.36±1.50 L/min/m^2, $p=.01$); 48%, (i.e., 3.88±1.26 vs 2.02±0.93 W, $p<.01$); and 47%, (i.e., 2.02±0.71 vs 1.08±0.31 W/m^2, $p<.01$), respectively. Results are depicted in table 5.4.

Table 5.4. Metabolic and haemodynamic variables at peak exercise capacity before and after 16-weeks of lifestyle intervention and control condition.

Variables	Intervention		Control		*P*- value
	Pre (N=21)	Post (N=13)	Pre (N=8)	Post (N=6)	
Metabolic function					
Oxygen consumption (mL/kg/min)	18.0±4.25	19.8±2.57	19.3±9.28	20.2±8.84	.69
Anaerobic threshold (mL/kg/min)	11.8±3.33	11.4±2.29	11.7±2.25	14.2±3.27*	**.02**
Respiratory exchange ratio (VCO_2/VO_2)	1.07±0.11	1.09±0.04	1.05±0.11	1.06±0.07	.71
Arteriovenous oxygen difference (mLo_2/ 100mL of blood)	9.22±3.04	11.5±2.82	13.3±9.25	14.9±2.72	.70
Work rate (W)	110±41.4	124±36.9	131±62.3	120±66.9	.10
Haemodynamic function					
Heart rate (beats/min)	130±27.1	117±19.7*	123±34.8	113±30.6	.09
Stroke volume (mL/beat)	142±45.3	126±39.9	122±20.9	88.2±13.1	.50
Stroke volume index (mL/beat/m^2)	70.7±21.1	60.2±15.4*	60.4±10.6	43.4±3.85	.72
Cardiac output (L/min)	17.4±5.20	15.5±4.00	14.7±6.70	11.3±4.08	.64
Cardiac index (L/min/m^2)	8.69±2.76	7.51±1.38	8.33±2.76	5.38±1.72*	.21
Cardiac power output (W)	4.88±1.50	4.10±1.01*	3.98±1.14	2.01±0.45**	.09
Cardiac power output index (W/m^2)	2.44±0.77	1.89±0.33**	2.06±0.55	1.06±0.21**	.22
Systolic blood pressure (mmHg)	186±21.3	179±22.2	186±22.4	181±20.9	.93
Diastolic blood pressure (mmHg)	97.1±11.3	84.5±10.0**	96.3±13.7	92.8±1.12	**.03**
Mean arterial pressure (mmHg)	127±11.2	116±8.50**	122±9.30	122±7.61	.07

P-value (right column) defines between-group change score differences; **= **p<0.01** and *=**p<0.05** Within-group differences: pre vs. post 16-weeks in intervention or control group.

When the relationship between peak oxygen consumption and average step count was assessed at baseline and after the 16-week study duration, no relationship was observed (Figure 5.4).

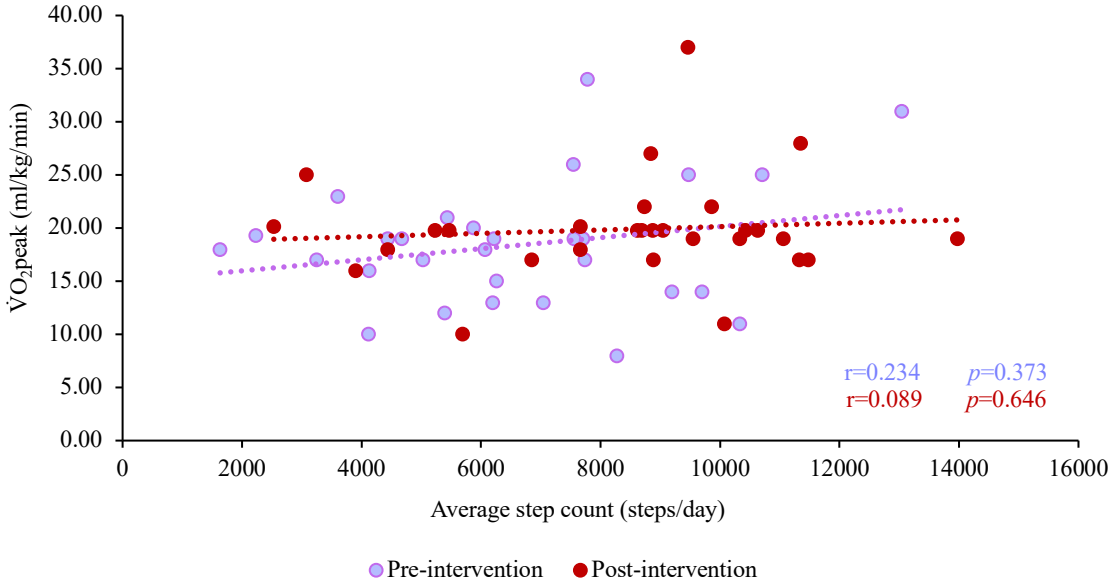


Figure 5.4. Relationship between number of steps and peak oxygen consumption ($\dot{V}O_2$peak) pre (i.e., baseline) and average step count of the study period (i.e., post-intervention) for all participants.

5.4.2.3 Changes in echocardiographic measurements of diastolic function following the 16-week lifestyle intervention

Table 5.5 demonstrates the change in echocardiographic measurements of diastolic function pre and post intervention for both groups.

Left atrial diameter, left atrial volume and left atrial volume index were not affected by the intervention condition when change was compared to control ($F(1, 26)= 0.05$, $p=.82$, $\eta^2<.01$); ($F(1, 26)= 0.04$, $p=.85$, $\eta^2<.01$); ($F(1, 26)= 0.07$, $p=.90$, $\eta^2<.01$), respectively. The same finding was observed for E/e' ratio ($F(1, 26)= <.01$, $p=.97$, $\eta^2<.01$); EDVLV ($F(1, 26)= 3.27$, $p=.08$, $\eta^2=.11$); LVIDd ($F(1, 26)=0.09$, $p=.77$, $\eta^2<.01$); and PLWd ($F(1, 26)= 1.04$, $p=.32$, $\eta^2=.04$).

Table 5.5. Echocardiographic measurements of diastolic function before and after 16-weeks of lifestyle intervention and control condition

Variables	Intervention		Control		*P*- value
	Pre (N=21)	Post (N=16)	Pre (N=8)	Post (N=7)	
Left atrial diameter (cm)	4.15±0.70	4.07±0.32	3.81±0.34	3.77±0.37	.82
Left atrial volume (mL)	68.1±26.1	69.7±18.6	67.3±18.8	65.5±14.9	.85
Left atrial volume index (mL/m^2)	34.9±15.0	35.3±11.1	33.3±9.47	32.2±8.51	.90
E/e' ratio	10.9±6.18	10.8±5.13	7.99±3.38	7.70±1.94	.97
EDVLV (mL)	74.6±14.4	79.5±10.5	87.7±31.9	80.7±21.3	.08
LVIDd (cm)	2.91±0.74	3.20±1.63	4.34±0.64	4.44±0.59	.77
PLWd (cm)	1.00±0.19	0.99±0.13	0.96±0.09	1.02±0.10	.32

E/e', ratio between early mitral inflow velocity and mitral annular early diastolic velocity; EDVLV, left ventricular end diastolic volume; LVIDd, left ventricular internal diameter end-diastole; PLWd, posterior wall thickness at end-diastole. *P*-value (right column) defines between-group change score differences.

5.4.2.4 Changes in disease-related quality of life (Minnesota living with heart failure questionnaire), general quality of life (36-item short form survey), and anxiety and depression (Hospital anxiety and depression questionnaire) following the 16-week lifestyle intervention

Disease-related QoL (MLHF score) was significantly affected by the intervention condition when compared to control ($F(1, 26)= 4.57$, $p=.04$, $\eta^2=.15$). In the intervention group, MLHF score significantly improved, indicated by a Bonferroni's corrected score decrease of 38% (22.4±19.4 vs. 13.9±13.7, $p=0.04$), whereas in control group, MLHF score was not significantly affected (24.5±19.5 vs. 31.7±14.3, $p=.26$.). Figure 5.5 demonstrates the changes in MLHF score between intervention and control groups pre and post intervention.

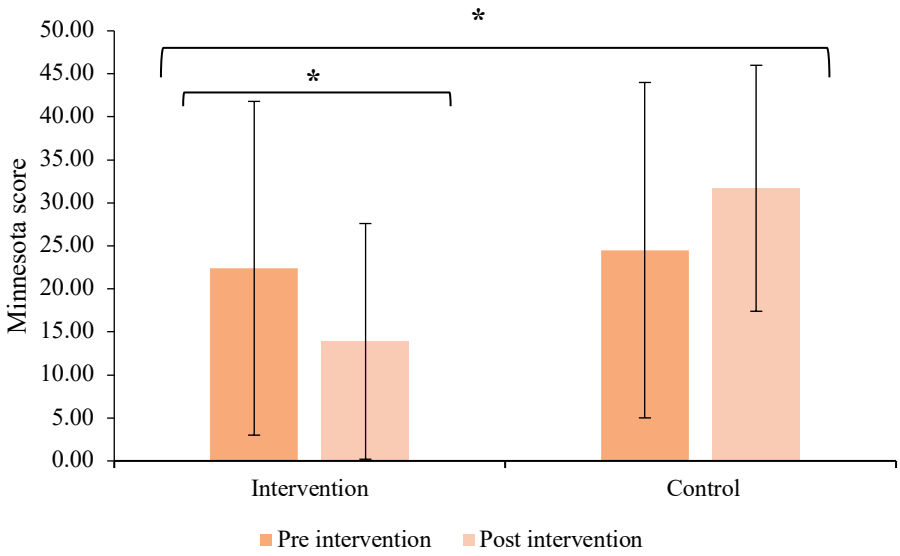


Figure 5.5. Depiction of Minnesota living with heart failure questionnaire results pre and post intervention in the intervention and control groups.

Changes in measurements of general QoL (i.e., SF-36 score) pre and post intervention for the lifestyle intervention and control groups is presented in Table 5.6. A significant difference was found between the intervention group and control group change scores for role limitations due to physical health pre vs post study period (U=66.5, p=0.04) (i.e., significantly higher median post-test ranks in intervention group (Z=20.0, p=0.04) vs lower median post-test ranks than pre-test ranks in the control (Z=3.00, p=0.22)). Scores that also significantly differed include energy/fatigue (U=40.5, p=0.03), (i.e., significantly higher post-test ranks in intervention group (Z=162, p<0.01) vs lower median post-test ranks than pre-test ranks in the control (Z=6.50, p=0.40)); social functioning (U=34.5, p=0.01), (i.e., higher post-test ranks in intervention group (Z=61.5, p=0.08) vs significantly lower median post-test ranks than pre-test ranks in the control (Z=1.00, p=0.04)); and general health (U=38.0, p=0.02) (i.e., significantly higher post-test ranks in intervention group (Z=164, p=0.03) vs lower median post-test ranks than pre-test ranks in the control (Z=6.00, p=0.18)).

In addition to the dimensions previously discussed, a significantly higher post-test ranks than pre-test ranks (Z=152, p=0.02) was observed for physical functioning in the intervention group. Within the Control group, no significant changes occurred pre vs post intervention using any scale of the SF-36.

Table 5.6. 36-Item short form survey scores at baseline and 16-weeks within and between intervention and control groups. Data presented as median (Q1-Q3).

Scale	Intervention		Control		*P*- value
	Pre (N=21)	Post (N=16)	Pre (N=8)	Post (N=7)	
Physical functioning	75 (50-95)	85 (80-90)*	75 (58-93)	73 (65-83)	0.40
Role limitations due to physical health	100 (25-100)	100 (82-100)	75 (56-100)	50 (35-56)	**0.04**
Role limitations due to emotional health	100 (67-100)	100 (80-100)	83 (58-100)	86 (58-100)	0.79
Energy/ fatigue	42 (15-60)	59 (50-75)**	53 (40-69)	52 (41-58)	**0.03**
Emotional well being	76 (68-84)	77 (64-92)	78 (71-85)	69 (63-85)	0.46
Social functioning	88 (50-100)	100 (85-100)	100 (88-100)	76 (69-91)*	**0.01**
Pain	90 (48-100)	90 (87-100)	90 (74-100)	71 (58-90)	0.13
General Health	50 (35-60)	60 (55-68)*	70 (64-71)	55 (45-60)	**0.02**

P-value (right column) defines between-group change score differences;.**= **p<.01** and *=**p<.05** pre-test ranks vs post-test ranks within intervention or control group.

Anxiety and depression were not affected by the intervention condition when change was compared to control ($F(1, 26)<0.01$, $p=.99$, $\eta^2<.01$); and ($F(1, 26)=0.034$, $p=.86$, $\eta^2<.01$), respectively (Table 5.7).

Table 5.7. Hospital Anxiety and Depression scale scores at baseline and 16-weeks within and between intervention and control groups.

Scale	Intervention		Control		*P*- value
	Pre (N=21)	Post (N=16)	Pre (N=8)	Post (N=7)	
Anxiety	6.19±4.88	5.25±4.42	7.13±4.97	6.29±4.03	.99
Depression	4.00±3.19	3.06±2.33	4.38±3.07	3.71±2.37	.86

5.4.2.5 Changes in blood chemistry following the 16-week lifestyle intervention

As shown in Table 5.8, the analysis of pre-post blood chemistry revealed a significant difference in several parameters when the intervention group was compared to the control group. Whilst controlling for drug total, bilirubin was significantly affected by the intervention condition when compared to control ($F(1, 26)= 4.51$, $p=.04$, $\eta^2=.15$). In the intervention group, bilirubin significantly decreased by 29% (12.1±6.78 vs. 8.60±4.12μmol/L, $p=.03$), whereas in the control group, bilirubin was not significantly affected (6.10±6.82 vs. 8.80±6.69μmol/L, $p=.29$). Total protein was also significantly affected ($F(1, 26)= 5.46$, $p=.03$, $\eta^2=.17$). In the intervention group,

total protein significantly decreased by 7% (70.9±4.03 vs. 65.9±4.40g/L, p<.01), whereas in the control group, total protein was not significantly affected (70.4±4.04 vs. 71.0±4.41g/L, p=.77). Urea level was significantly affected by the intervention condition when compared to control (F(1, 26)= 6.05, p=.02, η^2=.18). In the intervention group, urea level non-significantly increased by 8% (5.10±1.10 vs. 5.49±1.19mmol/L, p=.10), and in the control group, urea level non-significantly decreased by 12% (5.66±1.10 vs. 5.00±1.19mmol/L, p=.08).

Regarding lipid profile, T/HDL-C was significantly affected by the intervention condition when compared to control (F(1, 26)= 4.87, p=.04, η^2=.16). In the intervention group, T/HDL-C significantly decreased by 12% (3.67±1.01 vs. 3.23±0.73mmol/L, p=.01)), whereas in the control group, T/HDL-C was not significantly affected (3.51±0.99vs. 3.76±0.67mmol/L, p=.36).

In contrast, NT-proBNP was not affected by the intervention condition when change was compared to control (F(1, 26)= 1.68, p=.21, η^2=.06). The same finding was observed for troponin-T (F(1, 26)= 0.53, p=.48, η^2=.02), albumin (F(1, 26)= 1.84, p=.19, η^2=.07), alkaline phosphatase (F(1, 26)= 0.19, p=.66, η^2=.01), HDL-C (F(1, 26)= 0.36, p=.55, η^2=.01),), non-HDL-C (F(1, 26)= 3.47, p=.07, η^2=.12), TC (F(1, 26)= 2.32, p=.14, η^2=.08), T/HDL-C ratio (F(1, 26)= 0.77, p=.39, η^2=.03), triglycerides (F(1, 26)= 0.77, p=.39, η^2=.03), creatinine (F(1, 26)= 0.78, p=.39, η^2=.03), and eGFR (F(1, 26)= 0.11, p=.74, η^2<.01).

Within the intervention group, HDL levels significantly increased when pre and post intervention levels were compared by 14% (1.31±0.32 vs. 1.49±0.23g/L, p<.01).

Table 5.8. Blood chemistry at baseline and 16-weeks within and between intervention and control groups.

Blood chemistry	Intervention		Control		*P*- value
	Pre (N=21)	Post (N=16)	Pre (N=8)	Post (N=7)	
Cardiac function					
NT-proBNP (ng/L)	753±996	1021±1693	333±252	288±235	.52
Troponin-T (ng/L)	19.2±27.3	12.6±6.76	11.7±6.43	11.0±5.26	.48
Glucose					
HbA1c (mmol/mol)	41.3±8.59	41.8±9.31	36.8±3.41	37.6±2.61	.81
Inflammation					
Albumin (g/L)	46.8±26.2	45.7±11.8	46.4±2.44	47.3±2.37	.19
Alkaline Phosphatase (IU/L)	81.6±23.9	83.1±19.1	66.1±16.0	70.2±10.2	.66
Bilirubin (μmol/L)	12.2±7.65	8.58±3.34*	6.00±1.77	8.83±5.54	**.04**
Total protein (g/L)	70.9±3.92	65.7±4.77**	70.6±4.34	71.5±5.20	**.03**
Lipid profile					
HDL (mmol/l)	1.31±0.34	1.49±0.26**	1.41±0.12	1.50±0.14	.36
Non-HDL Cholesterol (mmol/L)	3.28±1.07	3.15±0.94	3.61±1.10	4.20±1.28	.07
Total Cholesterol (mmol/L)	4.59±0.34	4.64±0.99	5.03±1.16	5.70±1.28	.14
Total/ HDL Cholesterol Ratio (mmol/L)	3.66±1.06	3.20±0.78*	3.54±0.71	3.84±0.86	**.04**
Triglycerides (mmol/L)	1.39±0.53	1.27±0.48	1.24±0.45	1.31±0.34	.39
Liver and Renal Function					
Creatinine (μmol/L)	88.0±20.8	82.9±19.7	73.5±8.77	73.4±9.44	.39
eGFR (ml/min/1.73 m^2)	78.8±14.2	79.9±12.5	88.9±4.16	88.1±3.56	.74
Urea (mmol/L)	5.14±1.27	5.51±1.26	5.55±0.86	4.93±0.97	**.02**

ALT, Alanine transaminase; eGFR, estimated glomerular filtration rate; HbA1C, hemoglobin A1c; HCM, high density lipoprotein; NT-proBNP, n-terminal-pro hormone BNP. *P*-value (right column) defines between-group change score differences;.****= p<.01** and ***=p<.05** pre vs post 16-weeks in intervention or control group.

5.5 Discussion

The major finding of the present study was that when differences between intervention and control groups following the 16-week lifestyle intervention were assessed, peak diastolic blood pressure significantly improved following the intervention. In contrast, anaerobic threshold increased significantly in the control group. When within-group pre vs post intervention changes were assessed, peak diastolic blood pressure and mean arterial blood pressure significantly improved in the intervention group.

Regarding secondary endpoints, resting systolic, diastolic, and mean arterial blood pressure, as well as QoL significantly improved following 16-weeks of intervention condition compared to control. However, no significant differences in measurements of diastolic function, or anxiety and depression were observed when intervention was compared to control following the study period. However, several significant differences were observed in blood chemistry when intervention was compared to control (i.e., improved T/HDL-C ratio, decreased TP and bilirubin, but increased urea).

5.5.1 The effect of a novel lifestyle intervention on exercise tolerance in hypertrophic cardiomyopathy

The majority of individuals with HCM follow a sedentary lifestyle due to anxiety concerning risk of exercise-induced arrhythmia (Snir *et al.*, 2021). Yet, in a prospective study of SCD in children and young adults, it was a structurally normal heart that represented the largest cohort (Bagnall *et al.*, 2016). HCM accounted for only four percent of SCD cases (Bagnall *et al.*, 2016).

No adverse events occurred throughout the present study. In coherence, mild-to-moderate exercise training is deemed safe by recent guidelines for individuals with HCM (Ommen *et al.*, 2020). In individuals with chronic diseases such as HCM, HF and chronic obstructive pulmonary disease, an increase in exercise capacity, lowered all-cause mortality, and lowered hospitalisation have been observed when regular physical activity is adhered to (O'Connor *et al.*, 2009; Saberi *et al.*, 2017; Qiu *et al.*, 2018). In the present study, individuals participating in the intervention arm significantly increased step count, and HCM individuals in the control condition adhered to what was asked of them and maintained usual activity throughout.

In the general population, an increase in step count, but not intensity has been observed to lower all-cause mortality and cardiovascular event risk (Kraus *et al.*, 2019; Saint-Maurice *et al.*,

2020). In the present study, relative $\dot{V}O_2$peak showed a trend of increase by 1.7 mL/kg/min in the intervention group when pre vs post intervention values were compared. However, when $\dot{V}O_2$peak change score was compared between intervention and control groups, no statistical difference or trend was observed. Limited research is available for the assessment of exercise training, such as increased step count in HCM. In a preliminary evaluation of the effect of 16-weeks moderate-intensity exercise training in individuals with HCM, a significantly modest increase in exercise capacity was observed when compared to usual activity (Saberi *et al.*, 2017). In such study, VO_2peak increased by 6%, (1.29 mL/kg/min, p=0.02) when intervention and control groups were compared. Similar findings were observed in the largest trial assessing exercise in individuals with HF (O'Connor *et al.*, 2009). The lack of significance between groups in the present study could be related to low participant number. Saberi and colleagues (Saberi *et al.*, 2017) reported a study completion number of 113, in comparison to a completion number of 29 in the present pilot study. However, the described study did adjust results to account medication as the present thesis did.

Regarding determinants of $\dot{V}O_2$peak (i.e., peak cardiac output and arteriovenous O_2 difference), no significant change was observed for either determinant when the effect of the intervention vs control condition was assessed. However, a trend towards improvement was observed in peak arteriovenous O_2 difference when within the intervention group pre vs post study data was assessed. Previously, peak arteriovenous O_2 difference was significantly compromised when compared to the general population. Factors such as pulmonary diffusing capacity, skeletal muscle capillarisation and mitochondrial density determine peak arteriovenous O_2 difference (Bassett and Howley, 2000). Our results suggest physiological adaptation to reduce oxygen cost of exercise and improve skeletal muscle perfusion.

A trend towards increased work rate was demonstrated in the intervention group when pre- vs post-intervention differences were calculated. No assessment of the effect of NO_3^- supplementation (beetroot juice) on exercise tolerance in HCM has been completed, however, increased time to exhaustion during high-intensity training in healthy adults, and improved endurance exercise capacity under clinical hypoxic conditions i.e., HF, has been observed (Eggebeen *et al.*, 2016; McMahon;Leveritt and Pavey, 2017; Van De Walle and Vukovich, 2018).

In the intervention group when compared to control group, systolic, diastolic, and mean arterial blood pressure at rest, as well as diastolic blood pressure at peak exercise improved. Regular

NO_3^- supplementation has also been shown to improve vasodilatation thereby compensating for under-perfusion (Bentley *et al.*, 2017). Assessment of haemodynamic profile in individuals with HF and/or controlled hypertension who followed regular endurance training and/or daily NO_3^- demonstrate reduced resting systolic blood pressure (Shaltout *et al.*, 2017). This is an important finding due to the relationship between hypertension, symptom prevalence, adverse cardiac remodelling, and clinical outcome in individuals with HCM (Dimitrow *et al.*, 1998; Guttmann *et al.*, 2017).

5.5.2 The effect of a novel lifestyle intervention on secondary outcomes in hypertrophic cardiomyopathy: diastolic function, quality of life, and blood chemistry

No measures of diastolic function were significantly altered in the present study, however a trend toward improved EDVLV was observed. In agreement, recent research concluded that habitual levels of physical activity are not been associated with lifetime phenotypic transition in individuals with HCM (Aengevaeren *et al.*, 2019). In addition, LV wall thickness, cardiac morphology and function is unaffected following 16-weeks of moderate-intensity exercise training, and phenotype non-significantly differs between athletes and non-athletes with HCM (Saberi *et al.*, 2017; Dejgaard *et al.*, 2018). In contrast to exercise training that has demonstrated to have limited effect on cardiac morphology, and to the present study, NO_3^- supplementation in individuals with hypertension and HF with preserved ejection fraction has demonstrated to decrease LV volume, improved central haemodynamics, arterial wave reflections and LV filling pressures, all of which are linked to LV diastolic dysfunction and cardiac remodelling (Zamani *et al.*, 2015; Eggebeen *et al.*, 2016).

When general QoL was assessed using the SF-36 questionnaire in the present study, physical functioning significantly improved, along with energy/fatigue, social functioning, and general health. Disease-related QoL (MLHF) also significantly improved in the intervention group. Exercise intolerance is a primary pathological symptom in HCM and is related to reduce QoL (Gupte and Hamilton, 2016; Zaiser *et al.*, 2020). Physical training alone has been observed to improve the physical functioning SF-36 dimension in individuals with HCM, but not MLHF (Saberi *et al.*, 2017). Therefore, it can be hypothesised that improvements disease-related and general QoL may have been aided by the combined effects of NO_3^- supplementation and increased physical activity. This hypothesis is further strengthened by within-group pre- vs post-intervention changes being statistically significant for intervention group only. Limited studies are available for the effect of NO_3^- supplementation on QoL in individuals with HCM.

Therefore, further studies are needed to determine whether improvement in exercise tolerance and/or NO_3^- supplementation interventions translate to QoL in such clinical populations.

In the present study, there was a maintenance of a normal level of anxiety and depression in the intervention and control group (Zigmond and Snaith, 1983). In contrast to the normal levels of anxiety and depression in the present study, individuals with HCM commonly present with increased levels of anxiety and depression when compared to the general population, which increases regardless of disease status following a recent familial SCD (Cox *et al.*, 1997; Hamang *et al.*, 2011). No research was available assessing the effect of exercise training or NO_3^- supplementation on anxiety in HCM. Those in HF assessing anxiety levels and exercise capacity are contradictory (Chien *et al.*, 2011; Patron *et al.*, 2017; Uszko-Lencer *et al.*, 2017). Regarding symptoms of depression, exercise training in individuals with HF has been observed to lead to a significant decrease, and to be independently associated with 6-minute walk test distance (Tu *et al.*, 2014; Chiala *et al.*, 2018). Recently, it has also been confirmed that regular physical activity may alleviate anxiety and depression symptoms, as demonstrated in individuals with cardiovascular disease (Wang *et al.*, 2021). More research is needed before a conclusion can be made regarding exercise, NO_3^- supplementation and improved anxiety and depression due to methodological weakness and a lack of clinical studies.

Bilirubin was significantly reduced in the intervention but not control group, which is likely due to adherence to NO_3^- supplementation. NO_3^- supplementation has anti-inflammatory properties, and has also been observed to lower serum bilirubin as reported by the United States National Health and Nutritional Examination Survey 1999-2008 (Ong *et al.*, 2011; Peleli *et al.*, 2020). Bilirubin is also a biomarker of liver function and was significantly reduced in the intervention group when compared to control, but also remained within normal range (i.e<17.5umol/L) (Kringen *et al.*, 2014). The present study also demonstrated a significant decrease in TP and increase in urea serum levels post intervention in the intervention group. Even though changes were significant, they remained within the normal range i.e., TP, 65.7±4.77g/L; urea, 5.51±1.26mmol/L.

No significant relationship has been identified between NO_3^- supplementation and chronic kidney disease development, of which is a major risk factor for adverse cardiovascular events (Bahadoran *et al.*, 2016). Elevated urea and TP levels are diagnostic of both kidney and liver health. A normal urea level is defined as 1.8-7.1mmol/L and TP level 60-80g/L (Busher, 1990; Hosten, 1990). Due to serum biomarkers of liver and kidney function remaining within normal

range, it can be confirmed that the intervention had no adverse effect on liver and kidney function in individuals with HCM. In agreement, NO_3^- supplementation has been observed to be safe in general and clinical populations (Bahadoran *et al.*, 2016; Stanaway *et al.*, 2017).

Moderate-intensity exercise previously demonstrated no significant differences in serum biomarkers in individuals with HCM (Saberi *et al.*, 2017). There is also a lack of studies assessing the effect of NO_3^- supplementation on serum biomarkers in humans that are related to cardiac stress. However, in the first study assessing the effects of NO_3^- supplementation on ischemia-reperfusion injury there was no effect on plasma biomarkers of organ injury following cardiac surgery (Eriksson *et al.*, 2021).

TC/HDL-C significantly decreased when difference in change between the intervention and control group were assessed. Cholesterol-related risk is quantified by assessing TC, HDL-C and non-HDL-C (Grundy *et al.*, 2019). TC/HDL-C ratio has been defined as one of the strongest cardiovascular risk markers in numerous population-based studies (Ingelsson *et al.*, 2007; Lewington *et al.*, 2007; McQueen *et al.*, 2008; Elshazly *et al.*, 2016). Lower HDL-C levels have been associated with increased cardiovascular risk due to HDL-C's cardioprotective effects such as inflammation moderation via reverse cholesterol transport (Ference *et al.*, 2012; Ouimet;Barrett and Fisher, 2019). More research is needed regarding cardioprotective effects of serum HDL-C, as there are reports which challenge the hypothesis that increased levels (>60mg/dL) always results in lowered myocardial infarction risk (Voight *et al.*, 2012)..

Exercise training has been demonstrated to lower serum LDL-C, and TC/HDL-C ratio, whilst increasing HDL-C levels in the general population and individuals with cardiovascular disease (Muscella;Stefano and Marsigliante, 2020). The improved serum cholesterol profile in response to exercise may be the result of the enhanced skeletal muscle utilisation of lipids rather than glycogen (Earnest *et al.*, 2013). The reduction in LDL-C following regular exercise training is hypothesised to occur due to an increase in the enzyme that is responsible for converting ester to HDL-C (lecithin-cholesterol acyltrans) (Riedl *et al.*, 2010). An increase in HDL-C levels is resultant of exercise volume, rather than intensity with a minimum effective exercise dose of 120 minutes of exercise per week (Kodama *et al.*, 2007). An increase in physical activity at a low intensity (i.e., increase of 2000 steps/day) in the present study resulted in a significant increase in HDL-C level. Due to controversial data and lack of research, further studies are needed regarding the effect of exercise and NO_3^- supplementation on blood chemistry and associated cardiovascular benefits.

5.5.3 Study limitations

In the present study, the following limitations should be considered; there is potential for sampling bias, because 88 out of the potential 118 study participants declined/ were excluded from participation. However, the clinical characteristics are comparable to published studies (Coats *et al.*, 2015; Saberi *et al.*, 2017). Secondly, the effect of exercise training and NO_3^- supplementation on primary and secondary outcomes can only be used to describe trends because of low recruitment and study completion number. A lower recruitment/ study completion number was because of the coronavirus 2019 pandemic pausing study activities from March 2020 to March 2021. Thirdly, compliance assessment in the present study was poor, step-counts from participants were self-reported using pedometers and weekly follow-up calls. Beetroot juice shot consumption was also self-reported, with participants asked to return any beetroot juice shots not consumed but the consumption of all shots could not be fully confirmed. Therefore, step count information may have been reported differently and beetroot juice shots at times not consumed.

Fourth, although the study did not record any adverse events, the study was not powered for safety assessment. A substantial sample size is required to assess safety due to the rare and infrequent occurrence of adverse events in individuals with HCM (Maron *et al.*, 1999). In addition, longer follow-up studies would be needed to fully assess the effect of exercise training and NO_3^- supplementation on disease progression. Fifth, the inability to blind the experiment may have led to bias in self-reporting of QoL and anxiety and depression. This may have been because of participant expectation. Sixth, the time between when the final beetroot juice shot was consumed and when testing was administered may have attenuated any nitrate-mediated performance benefits. To try to avoid this, each participant attended the clinic within seven days of completing the supplementation to complete CPET.

Finally, the physical activity and NO_3^- supplementation intervention was combined but not also assessed separately. Including a combination and dissecting of interventions such as in that by Shaltout and colleagues in individuals with heart failure would allow for the assessment of any additional benefits from NO_3^- supplementation, as well as which improvements were owed to which intervention component (Shaltout *et al.*, 2017).

Overall, the population studied was sufficient to provide a platform for larger trials assessing the effect of the combination of exercise training and NO_3^- supplementation on exercise tolerance and QoL in HCM.

5.5.4 Conclusions

In conclusion, the 16-week lifestyle intervention incorporating an increase of physical activity and NO_3^- supplementation significantly improved disease-related QoL (MLHF) and parameters of general QoL (i.e., physical functioning, energy/fatigue, social functioning, and general health) when compared to the control group. The lifestyle intervention was associated with improved vasodilation, represented by significantly reduced peak diastolic blood pressure and resting systolic, diastolic and mean arterial blood pressure when compared control condition. A trend towards improved exercise capacity when the effect of the lifestyle intervention was compared to control was observed.

The primary marker of diastolic function (i.e., E/e' ratio) was unchanged following the lifestyle intervention. Regarding blood biochemistry, serum lipid levels also improved. Finally, a normal score for anxiety and depression was maintained in the intervention and control groups.

Overall, the present and novel pilot study significantly improved QoL and had no adverse events. In addition, vasodilation was improved in response to the present lifestyle intervention, indicating less load placed on the heart at rest and during physical exertion, which may lead to slowed progression of pathological cardiac remodelling. In the future, a fully powered study should be performed to explore the observed trend in results and explore the clinical importance and long-term safety of increased physical activity and NO_3^- supplementation in individuals with HCM. In addition, a comparison of dissection of the lifestyle intervention compared to combined should be performed.

Chapter 6. The role of the mitochondria in the ageing heart and hypertrophic cardiomyopathy

6.0 Abstract

Objective: Ageing and hypertrophic cardiomyopathy (HCM) are characterised by chronic inflammation. Chronic inflammation is a driver of left ventricular (LV) hypertrophy, diastolic dysfunction, and therefore adverse cardiovascular prognosis. The mitochondria play a central role in the initiation and development of inflammation. Therefore, the aim of the present study was to assess the association between mitochondrial function and pathological phenotype in ageing and HCM.

Methods: The present pilot study compared the abundance of key mitochondrial proteins within complex I (CI); NDUFB8 and complex IV (CIV); MTCO1, in a mouse model of premature ageing, cardiac hypertrophy and inflammageing lacking the P50 subunit of NF-kB (*nfkb-/-* mouse) to age-matched and healthy controls; WT (C57BL/6J) young (3mo) and WT old (12mo) mice. Using antibodies against NDUFB8 and MTCO1, complex I and IV were labelled in two separate batches by immunofluorescence prior to being imaged via confocal microscopy and single cell data extraction using IMARIS. Mitochondrial mass was assessed with the use of an antibody against porin (voltage dependant anion channel 1 (VDAC1)) of the outer mitochondrial membrane, and myocyte cellular membranes were labelled using an antibody targeting laminin. Linear mixed models after log transformation of data with analysis of deviance (type II sum of squares) using R calculated the likelihood ratio chi squared and statistical significance. Statistical analysis analysed whether age or genotype was a significant predictor of logNDUFB8, logPORIN or logNDUFB8 normalised to logPORIN, with mouse ID as random effect.

Results: When genotypes were compared for NDUFB8 (complex I) levels, a significant reduction was observed in *nfkb1−/−* mice compared to WT ($\chi 2$ (1) = 5.74, p=0.02). Furthermore, a significant decrease with age was observed when NDUFB8 was normalised to mitochondrial mass (porin) ($\chi 2$ (1) = 4.28, p=0.04). MTCO1 (complex IV) levels were not significantly altered by age or mouse genotype i.e., ($\chi 2$ (1) = 0.05, p=0.09) and ($\chi 2$ (1) = 1.05, p=0.31), respectively. In addition, porin (mitochondrial mass) levels were unchanged with age ($\chi 2$ (1) = 3.25, p=0.07), and when *nfkb1−/−* mice were compared to the WT mice ($\chi 2$ (1) = 3.10, p=0.08).

Conclusions: The present pilot study suggests that CI dysfunction, as indicated by reduced NDUFB8 levels, may be associated with the pathological alterations in the aged and HCM cardiac phenotype.

6.1 Introduction

By the year 2050, 22% of the global population are expected to be >60 years of age (Newgard and Sharpless, 2013). Age is the primary risk factor for chronic disease and results in a progressive decline of molecular and physiological processes (Franceschi *et al.*, 2000). A biological decline is observed in normal and pathological ageing (Khan;Singer and Vaughan, 2017). In the general population, a trend of increased cardiovascular (CV) disease prevalence is observed in the ageing population (increased lifespan) (Roth *et al.*, 2020). Recently, the American Heart Association confirmed that ~90% of men and ~92% women over 80 years of age are affected by CV disease (Benjamin *et al.*, 2019). The present thesis has explored physiological mechanisms implicated in the age-related decline in CV function. However, an exploration of the molecular mechanisms associated with age-related CV alterations leading to decline in exercise tolerance is imperative to fully understand the aetiology of pathology progression and define potential therapeutic targets.

Interconnecting processes such as inflammageing, mitochondrial dysfunction, senescence, and autophagy are observed to cause the reduction in the regenerative capacity of organ tissue during ageing (Murtha *et al.*, 2019). Parainflammation (i.e., chronic inflammation) is triggered in ageing tissue as an attempt to restore homeostasis and a response to tissue malfunction (Medzhitov, 2008). Cardiac structure and function subsequently decline, regardless of contributing risk factors (Murtha *et al.*, 2019). The aged heart is characterised by fibrosis, stiffening and hypertrophy of the LV wall, because of an increase in cell size, and reduced tolerance for stress (Lesnefsky;Chen and Hoppel, 2016; Murtha *et al.*, 2019; Li *et al.*, 2020). Therefore, age-related reduction in exercise capacity occurs because of the described parainflammation.

The mitochondria have their own genome (mtDNA) and are key regulators of inflammatory response (Anderson *et al.*, 1981; Javadov;Kozlov and Camara, 2020). The organelle also supplies >90% of the energy (i.e., ATP) required for cellular metabolism in eukaryotes (Javadov;Kozlov and Camara, 2020). Mitochondrial dysfunction is a feature of cardiac ageing and pathological remodelling (Muller-Hocker, 1989; Wang *et al.*, 2019). Additional major functions regulated by mitochondria include reactive oxygen species (ROS) generation, calcium regulation, senescence, cell growth and cell death (Javadov;Kozlov and Camara, 2020). Consequently, as mitochondrial function is perturbed during ageing, mitochondria are implicated in the progression and pathogenesis of CV disease (Javadov;Kozlov and Camara,

2020). Therefore, it may be hypothesised that the mitochondria may be play a key role in the aged cardiac phenotype (i.e., LV hypertrophy).

Adult cardiomyocytes are abundant in mitochondria (i.e., 20-40% cardiomyocyte volume); the primary energy source of the heart and a regulator of cardiomyocyte survival (Chaudhary;El-Sikhry and Seubert, 2011). This makes them particularly susceptible to the effects of mitochondrial dysfunction. The heart contains two populations of mitochondria; the first under the sarcolemmal membrane (SMM), and the other between myofibrils, also known as interfibrillar mitochondria (IFM) (Lesnefsky;Chen and Hoppel, 2016). Age-related mitochondrial defects in the heart have been shown to be localised to the IFM and cause increased ROS generation (Lesnefsky;Chen and Hoppel, 2016).

Under normal conditions, cardiomyocytes predominantly use fatty acid oxidation to obtain ATP (i.e., >60%), a process known as oxidative phosphorylation (OXPHOS) (Belitzer and Tsybakova, 1939; Bing *et al.*, 1954; Bing, 1955; Hatefi, 1985). The remainder of ATP is produced using glucose and lactate oxidation, as well as ketone bodies (Bing *et al.*, 1954). Utilisation of glucose and lactate to produce cardiac ATP is observed to increase during exercise, and glycolysis is increased at the expense of fatty acid oxidation in the failing and aged heart (Lakatta and Yin, 1982; Gertz *et al.*, 1988; Kato *et al.*, 2010). Regarding the use of fatty acids to produce ATP, acyl-coenzyme A (CoA) synthases firstly synthesise fatty CoAs (Belitzer and Tsybakova, 1939). Next, fatty CoAs enter the inner membrane of the mitochondria after conversion to acylcarnitines by carnitine palmitoyl transferase 1 (Eaton;Bartlett and Pourfarzam, 1996). B-oxidation is then initiated and produces acetyl CoA after the acylcarnitines are liberated to fatty CoAs (Eaton;Bartlett and Pourfarzam, 1996). Acetyl CoA enters the Krebs cycle to produce nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) (Krebs and Johnson, 1937). These products then transfer electrons through the electron transport chain (ETC), which is located on the inner membrane of the mitochondria and comprised of four complexes (Hatefi, 1985). Electron flow along the ETC and efflux of protons activates ATP-synthase to produce ATP (Mitchell, 1961).

6.1.1 Mitochondrial dysfunction, inflammation, and telomere attrition

Age-related parainflammation has been observed to promote cellular senescence through elevating telomere dysfunction (Jurk *et al.*, 2014). OXPHOS is significantly compromised in the aged heart (Tatarkova *et al.*, 2011; Zhang *et al.*, 2014). Dysfunctional mitochondria lead to

attrition of telomeres, and telomere damage leads to mitochondrial biosynthesis alteration and mitochondrial dysfunction (Zheng;Huang and Wang, 2019). Telomeres protect the ends of eukaryotic chromosomes and are comprised of repetitive DNA sequences and binding proteins (i.e., shelterin) (Turner;Vasu and Griffin, 2019). The telomere length predicts lifespan and health status in early and later life (Vera *et al.*, 2012; Aviv and Shay, 2018). In most eukaryotic cells the absence of telomerase (i.e., the enzyme which replicate the telomeric ends of linear DNA) causes DNA to progressively shorten with each cellular division with an endpoint of replicative senescence (Hayflick and Moorhead, 1961; Harley;Futcher and Greider, 1990). This phenomenon is known as the telomere hypothesis of ageing (Harley *et al.*, 1992).

In a self-amplifying loop, telomere dysfunction is a driver of pathological inflammation and related to increased DNA damage, impairment of mitochondrial biogenesis and function as well as increased ROS (Passos;Saretzki and von Zglinicki, 2007). The subsequent increased oxidative stress and compromised OXPHOS/ cellular respiration occur through repression of the peroxisome proliferator-activated receptor gamma coactivator (PGC) network, (i.e., transcription factors PGC 1 alpha, PGC 1 beta and downstream genes) which has a vital role in energy homeostasis, metabolic function (beta-oxidation, fatty acid metabolism and gluconeogenesis), and mitochondrial biology regulation (Wu *et al.*, 1999; Sahin *et al.*, 2011). Degenerative phenotypes in the liver and hearts of aged mice are suggested to be caused by telomere dysfunction (Sahin *et al.*, 2011). The degenerative phenotypes are a consequence of a compromised PGC network, resulting in a reduction in mitochondrial DNA content, NADH:ubiquinone oxidoreductase (CI) and Cytochrome *c* oxidase (i.e., CIV) activity, and ATP levels (Sahin *et al.*, 2011).

CI is the major producer of ROS and therefore driver of parainflammation in the ETC (Turrens and Boveris, 1980; Kushnareva;Murphy and Andreyev, 2002). The complex is encoded by seven of the 13 genes of mtDNA that encode the four OXPHOS enzyme complexes (Linnane *et al.*, 1989). Due to CI genes comprising a larger proportion of the genome, and according to the mitochondrial theory of ageing, ROS is damage is most likely to affect this mitochondrial complex than others (Harman, 1956; Harman, 1972; Lenaz *et al.*, 1997). CIV is the second most likely enzyme to be affected by age-related increase in ROS. This is because it is encoded by three of the 13 genes of mtDNA that encode the four complexes (Linnane *et al.*, 1989). This is in comparison to CIII which is encoded by one gene and ATP synthase that is encoded by two genes (Linnane *et al.*, 1989).

Figure 6.1 demonstrates the bioenergetics of the electron transport chain and pathological implications of reduction in CI and CIV function caused by parainflammation.

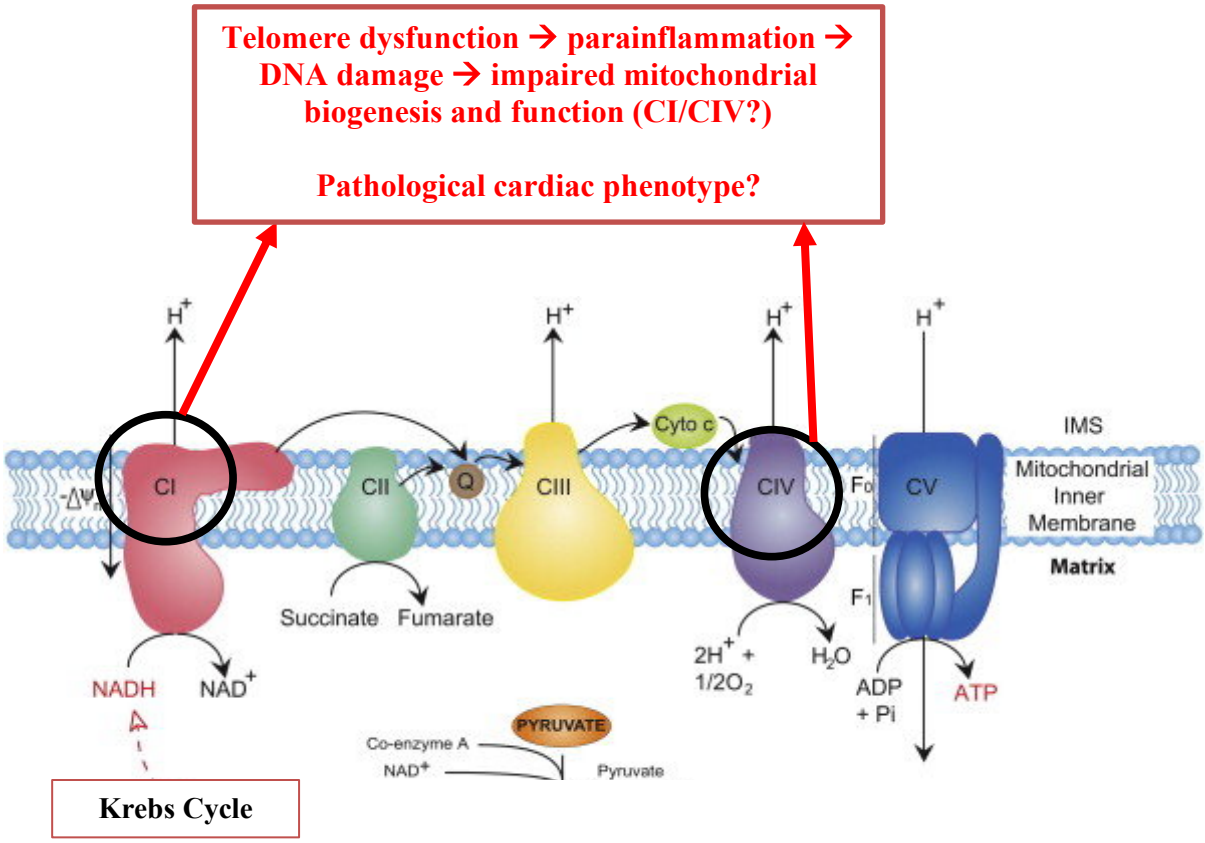


Figure 6.1. The electron transport chain and parainflammation. Adapted from Osellame and colleages to highlight hypothesised mechanisms aetiological of pathological cardiac phenotype (Osellame;Blacker and Duchen, 2012). Pyruvate is converted to high-energy molecules including nicotinamide adenine dinucleotide plus hydrogen (NADH), guanosine triphosphate-binding proteins and flavin adenine dinucleotide through catalysation by Krebs cycle enzymes. NADH generated is shuttled to complex I (CI) and is converted to nicotinamide adenine dinucleotide (NAD+) driving oxidative phosphorylation. Electron transfer through the chain maintains membrane potential via proton pumping into the intermembrane space (IMS). In this final step ADP (adenosine diphosphate) is phosphorylated to form ATP (adenosine triphosphate) via complex V (ATP synthase). CII, complex 2; CIII, complex 3; CIV, complex 4; IMS, intermembrane space, IMS; Q, coenzyme Q.

6.1.2 Mitochondrial function and hypertrophic cardiomyopathy

The inflammatory response in HCM is significantly associated with myocardial fibrosis, a contributor to sudden cardiac death, LV dysfunction and progression to heart failure (HF) in HCM (Raman *et al.*, 2019). Therefore, much like the aged heart, the disease pathophysiology

is believed to be actively modified by inflammation (Kuusisto *et al.*, 2012). In individuals with HCM, higher levels of low-grade inflammation markers (i.e., high-sensitivity C-reactive protein) and interleukins (IL-1β, IL-1RA, IL-6, IL-10) are observed when compared to healthy individuals (Kuusisto *et al.*, 2012).

Much like in ageing, mitochondrial oxidative stress may be a trigger for inflammation in HCM due to the mitochondria's role in inflammation regulation (Becker;Owens and Sadayappan, 2020). In individuals with HCM, there is an increase in energy demand because of increased force production (Ranjbarvaziri *et al.*, 2021). Histopathologically, the presence of hypertrophied cardiomyocytes in HCM coincides with an increase in the number of mitochondria, but a decrease in mitochondrial size (Pisano *et al.*, 2016; Tejado and Jou, 2018). Mitochondrial biogenesis is demonstrated to be a maladaptive mechanism in both human and murine models to ensure mitochondrial density matches energy demand but leads to progression of cardiac dysfunction (Sebastiani *et al.*, 2007; Rosca;Tandler and Hoppel, 2013). The pathological relationship between an increase in mitochondria and impaired contractile function is only observed in cardiomyocytes (Taylor *et al.*, 2003). Additionally, mitochondria in individuals with HCM appear enlarged, subsarcolemmal and swollen with irregular cristae (Meyers;Basha and Koenig, 2013). Golgi apparatus prominence is also observed, along with identifiable progressive glycogen accumulation (Meyers;Basha and Koenig, 2013; Tejado and Jou, 2018). When disease progresses to HF (i.e., dilated cardiomyopathy), blunted mitochondrial biogenesis signalling is observed (Dai *et al.*, 2011).

Available human, murine and feline model research surrounding cardiac energetics and HCM is limited. However, there is increasing evidence to support the hypothesis that mitochondrial dysfunction is a common pathogenic mechanism in individuals with HCM (Abdulhag *et al.*, 2015; Ranjbarvaziri *et al.*, 2021). Donor hearts assessed using integrated omics analysis are observed to have global energetic decompensation (i.e., lowered ATP, ADP and phosphocreatine), decreased expression of genes required for the synthesis of creatine kinase and ATP, an increase in damaged mitochondria with reduced density of cristae, and reduced mitochondrial oxidative respiration (Ranjbarvaziri *et al.*, 2021). ROS elevation and antioxidant defence reduction are related to the described pathological mitochondrial characteristics and are biomarkers of inflammation and indicators of oxidative stress (Kowalczyk *et al.*, 2021). It is known that HCM is primarily caused by impaired sarcomeres, however secondary hypertrophic remodelling of the heart and HCM progression are suggested to occur because of oxidative stress (Nakamura *et al.*, 2005). Development and progression of HCM in feline

models has been observed to occur because of mitochondrial membrane damage and oxidative stress caused by ROS (Christiansen *et al.*, 2015). In a human individual with HCM, it has also been observed that mutations in genes encoding mitochondrial complex subunits such as COX6B1 cause mitochondrial complex IV deficiency and are associated with LV hypertrophy and pathological cardiomyopathy (Abdulhag *et al.*, 2015).

6.1.3 Mouse model of ageing and hypertrophic cardiomyopathy

Nuclear factor-kB (NF-kB) is the transcriptional regulator of genes related to cell survival and fate, and others associated with immune and inflammatory responses (Hayden and Ghosh, 2004; Liu *et al.*, 2017c). The transcription regulator is suggested to be a key functional link between inflammation and CV disease (Fiordelisi *et al.*, 2019). Inflammatory genes affected include those related to pro-inflammatory cytokines, macrophages, chemokines, interleukins and adhesion molecules (Liu *et al.*, 2017c). The Nf-kB/Rel family is comprised of RelA (i.e., p65), NF-kB1 (i.e., p105/P50), NF-kB2 (p100/p52), c-Rel, and RelB (Gaspar-Pereira *et al.*, 2012). Activation of NF-kB occurs via the canonical (i.e., using RelA, P50 and c-Rel subunits) and noncanonical pathway (using RelB and p100/p52). The former pathway has been suggested to be associated with cardiovascular disease progression i.e., cardiac remodelling, hypertrophy, and HF (Frantz *et al.*, 2003; Gaspar-Pereira *et al.*, 2012).

In the present study, *nfkb1−/−* mice lacking the P50 subunit of NF-kB were used as a model of ageing and HCM to assess whether mitochondrial function (i.e., via complex I and complex IV immunofluorescence) may be affected in these clinical populations (Jurk *et al.*, 2014). The described mice present with premature ageing resultant of progressive low-grade inflammation, mortality at ~20 months, telomere dysfunction, and spontaneous cardiac hypertrophy (Gaspar-Pereira *et al.*, 2012; Jurk *et al.*, 2014). The previously described 5 subunit components of NF-kB function as homo- or heterodimers; the canonical RelA:P50 heterodimer has pro-inflammatory function and RelA activates target gene transcription (Gaspar-Pereira *et al.*, 2012). In contrast, the P50 homodimer is a pro-inflammatory gene transcription repressor and acts via recruitment of histone deactylase 1 (HADC1) (Wilson *et al.*, 2015). The P50:P50:HDAC1 complex competes with RelA-containing dimers for kB DNA binding motifs and deacetylase histones (Elsharkawy *et al.*, 2010). Consequentially, *nfkb1−/−* mice are unable to actively repress the pro-inflammatory gene transcription using P50 homodimers but continue produce Rel-A containing dimers. The described factors account for a parainflammatory phenotype.

Due to the presentation of an aged and HCM-like pathophysiology in *nfkb1−/−* mice, the mice were an ideal model to assess mitochondrial function in ageing and HCM. Hypotheses for the present study are:

i) There will be a significantly compromised mitochondrial structure and function, defined by key mitochondrial protein levels (i.e., Porin (mitochondrial mass); NDUFB8 (CI); and MTCO1 (CIV)) in older WT and P50 mice.
ii) There will be a significantly compromised mitochondrial structure and function, defined by key mitochondrial protein levels (i.e., Porin (mitochondrial mass); NDUFB8 (CI); and MTCO1 (CIV)) in P50 mice when compared to WT.

6.2 Materials and Methods

All materials used are listed in Appendix 1.

6.2.1 Mice

Animals used were C57BL/6 (WT) or genetically modified P50ko i.e., C57BL/6 *nfkb1−/−* mice gifted from Professor Fiona Oakley (Jurk *et al.*, 2014; Bianchi *et al.*, 2021), as described in Chapter 3.14.1. Table 6.1 depicts the number of formalin-fixed, paraffin-embedded 4µm mouse cardiac sections utilised (cut by Dr Anna Smith) in the present study within the WT 3mo, WT 12mo, P50 3mo and P50 12mo groups.

Table 6.1. Number of mice within the WT 3mo, WT 12mo, P50 3mo and P50 12mo groups

Mouse group	N (Complex I)	N (Complex IV)
WT 3mo	4	4
WT 12mo	5	5
P50 3mo	4	4
P50 12mo	5	5

6.2.2 Protocol overview

Using the described sections, an extensive optimisation process took place to define the optimal immunofluorescence protocol by myself and Dr Anna Smith. The optimal staining technique completed by me, with guidance from Dr Anna Smith when required. The optimisation process and intricate details of the present study are described in detail in 3.14.1 to 3.14.3.

After confocal microscopy imaging of stained sections, immunofluorescence data extraction using IMARIS, and first run through of R analysis performed by Dr Julia Whitehall, I omitted one mouse in the WT 12mo, two mice in the P50 3mo, and one mouse in the P50 12mo groups (i.e., four mice) from the final CI assay analysis due to weak immunofluorescence staining of NDUFB8. The study protocol is depicted in figure 6.2.

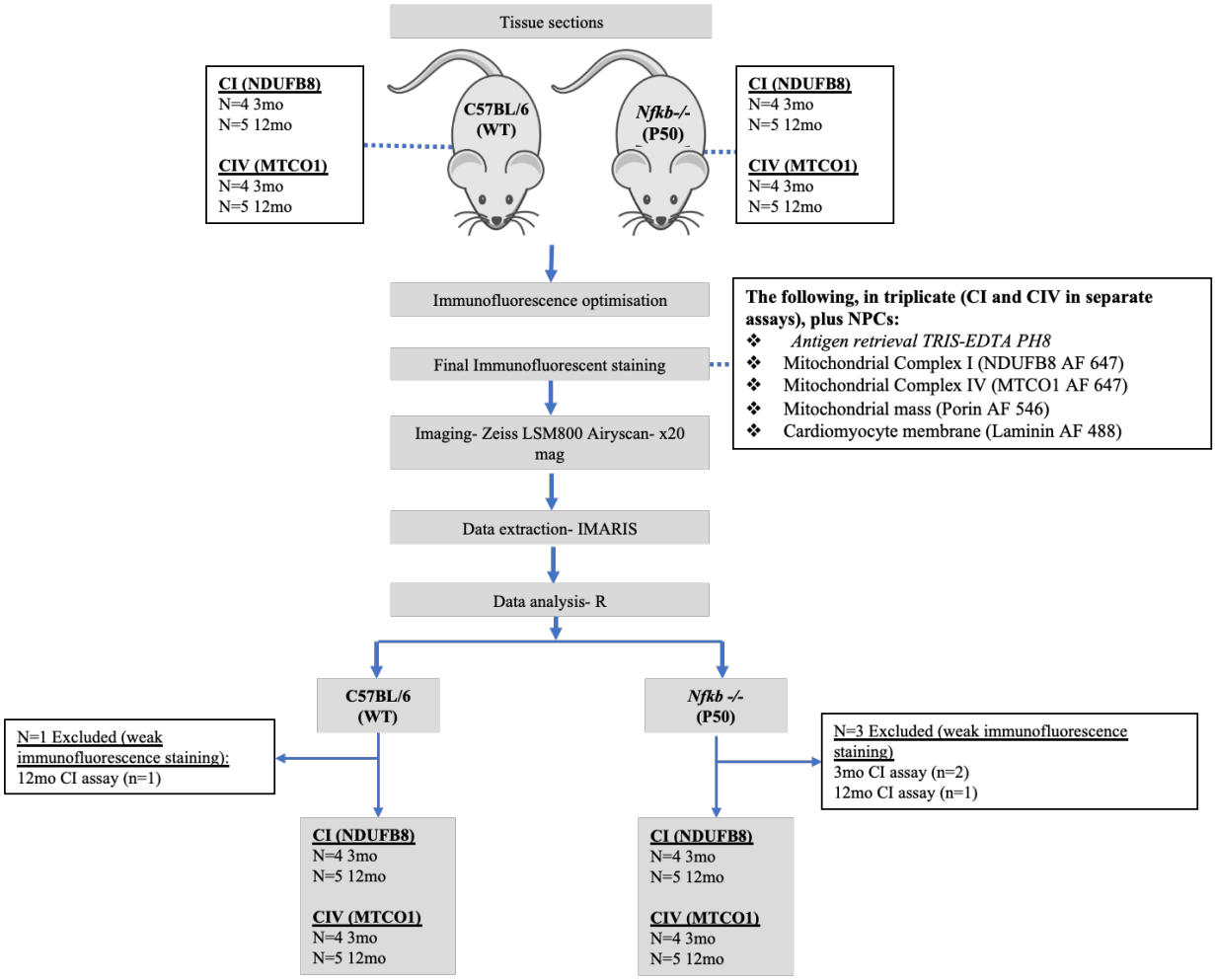


Figure 6.2. Study protocol for the assessment of mitochondrial function in a mouse model of ageing and hypertrophic cardiomyopathy. CI, Complex I; CIV, Complex IV; EDTA, Ethylenediamine tetra-acetic acid; mo, months; MTCO1, Cytochrome c oxidase subunit I; NDUFB8, NADH: ubiquinone oxidoreductase subunit B; Nfkb-/-, Nuclear factor-kB knockout; NPC, no primary control; WT, wild-type.

6.2.3 Final oxidative phosphorylation immunofluorescence assay

For the final immunofluorescence assay, I performed quantitative immunofluorescent labelling on formalin-fixed, paraffin-embedded 4µm mouse cardiac sections to assess levels of mitochondrial CI (NDUFB8) and CIV (MTCO1) proteins and total mitochondrial mass (porin).

Laminin was also used to mark membrane boundaries. Final antibody cocktails and concentrations are detailed in Table 6.2.

Table 6.2 Primary and secondary antibodies for immunofluorescent detection of OXPHOS complexes I and IV (NDUFB8 and MTCO1), mitochondrial mass (porin) and cardiomyocyte membrane/ boundary marker (Laminin)

Antibody	Host	Dilution	Manufacturer
Primary antibodies			
Laminin	Rabbit	1/50	L9393 Sigma Aldrich
Porin	Mouse	1/100	ab14734 Abcam
NDUFB8	Mouse	1/40	ab110242 Abcam
MTCO1	Mouse	1/50	ab14705 Abcam
Secondary antibody			
Biotinylated IgG1	Goat	1/200	A10519 Invitrogen
Tertiary antibodies			
Anti-rabbit IgG Alexa Fluor 488	Goat	1/200	A11008 Invitrogen
Anti-mouse IgG2b Alexa fluor 546	Goat	1/200	A21143 Invitrogen
Anti-mouse Streptavidin conj Alexa Fluor 647	Goat	1/200	S32357 Invitrogen
Anti-mouse IgG2a Alexa Fluor 647	Goat	1/200	A21241 Invitrogen

As described in 3.14.4, I captured images using the confocal microscope (Zeiss LSM800 Airyscan). Alexa Fluor 546 (i.e., porin) was measured on track 1 (i.e., channel 1), and Alexa Fluor 488 (Laminin) (i.e., channel 2)/ Alexa Fluor 647 (NDUFB8/MTCO1) (i.e., channel 3) measured on track 2.

6.2.4 Image analysis

I completed analysis using the immunoanalyser software, IMARIS (additional information available in Chapter 3.14.5). Five random snapshots of x20 magnification were selected from each mouse for analysis, with laminin used to semi-automatically detect and outline transverse cardiomyocytes.

6.2.5 Data analysis

After I extracted data from IMARIS into excel spreadsheets, mean fluorophore intensity data were assessed using the statistical software R with the guidance of and by statistician Dr Julia Whitehall and second opinion from statistician Dr Alasdair Blain.

First, data plots using raw data were produced to ensure that the raw intensities of NDUFB8/MTCO1, porin and laminin differed from the NPCs (i.e., to confirm that specific immunofluorescence was present and not merely background signal). Data plots confirmed that the immunofluorescent labelling was successful for both assays (i.e., NDUFB8 and MTCO1). Box cox analysis suggested log transformation was required to meet the normality assumption. Log transformation emphasised the difference between small values, optimal for looking at deficient cells. Laminin data was fitted using a linear mixed effect model (lme); (https://www.rdocumentation.org/packages/nlme/versions/3.1-152/topics/lme package in R), allowing for an lme model to be fitted for all variables (i.e., logNDUFB8, logPORIN and log (NDUFB8/log (PORIN)) with genotype (WT/P50) and age (young or old) as fixed.

An lme model was also performed using residual maximum likelihood (REML) after being confirmed as the better model by Akaike information criterion in R statistics (AIC). REML allowed consideration of mouse number as a random effect (type II sum of squares) for NDUFB8 and MTCO1 ($p<0.05$ was the threshold set for assigning statistical significance). ANOVA (type III sum of squares) computed p-values ($p<0.05$ was the threshold set for assigning statistical significance) when assessing whether age has a different impact on NDUFB8 or MTCO1 levels depending on mouse genotype (i.e., WT/P50) for CIV. Type III sum of squares was not complete for CI because of omission of the P50 mouse group due to low group numbers. The mice omitted in CI were due to weak immunofluorescence staining of NDUFB8. All data was background corrected to porin. Data was positively transformed prior to analysis.

6.3 Results

6.3.1 Mitochondrial complex I (NDUFB8) protein levels in P50 knockout old (12 month) and young (3 month) mouse hearts

Figure 6.3 shows representative images obtained using the Zeiss LSM800 Airyscan microscope of WT young (3mo), and P50 young (3mo), WT old (12mo) and P50 old (12mo) mouse heart tissue labelling of mitochondrial CI (NDUFB8).

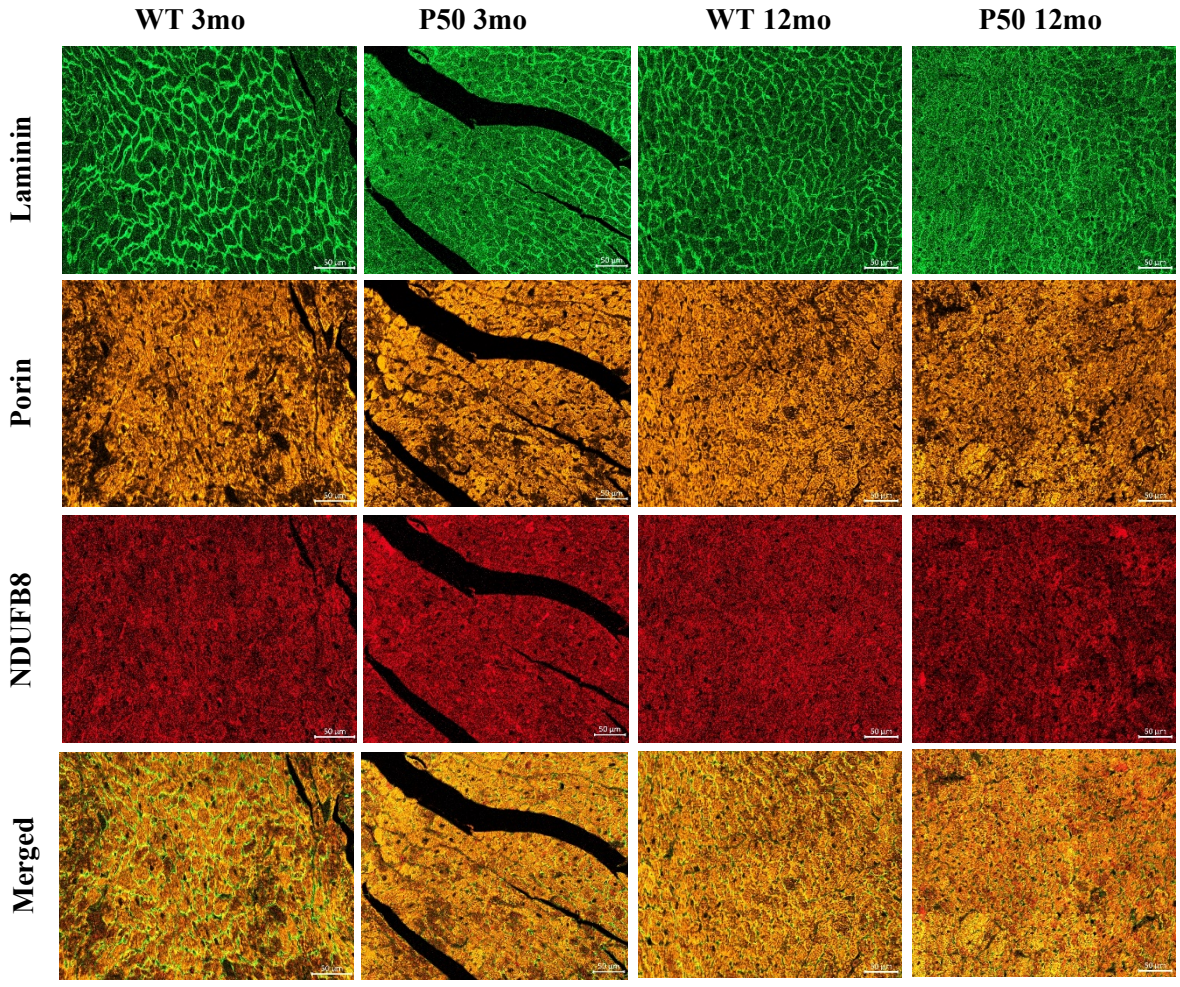


Figure 6.3 Mitochondrial NDUFB8 (CI) subunit expression levels. Representative images of cardiac tissue sections from mice for immunofluorescent detection of NDUFB8 (complex I), Laminin (cardiomyocyte membrane marker) and porin (mitochondrial mass marker) in WT young (3mo), P50 young (3mo), WT old (12mo) and P50 old (12mo) mice. Magnification x20. Scale bars=50um

Firstly, log transformation and lme was first fitted for all variables (i.e., logNDUFB8, logPORIN and log(NDUFB8/log(PORIN)) with genotype (WT/P50) and age (3mo or 12mo), and visualised on strip plots (figure 6.4A-F)

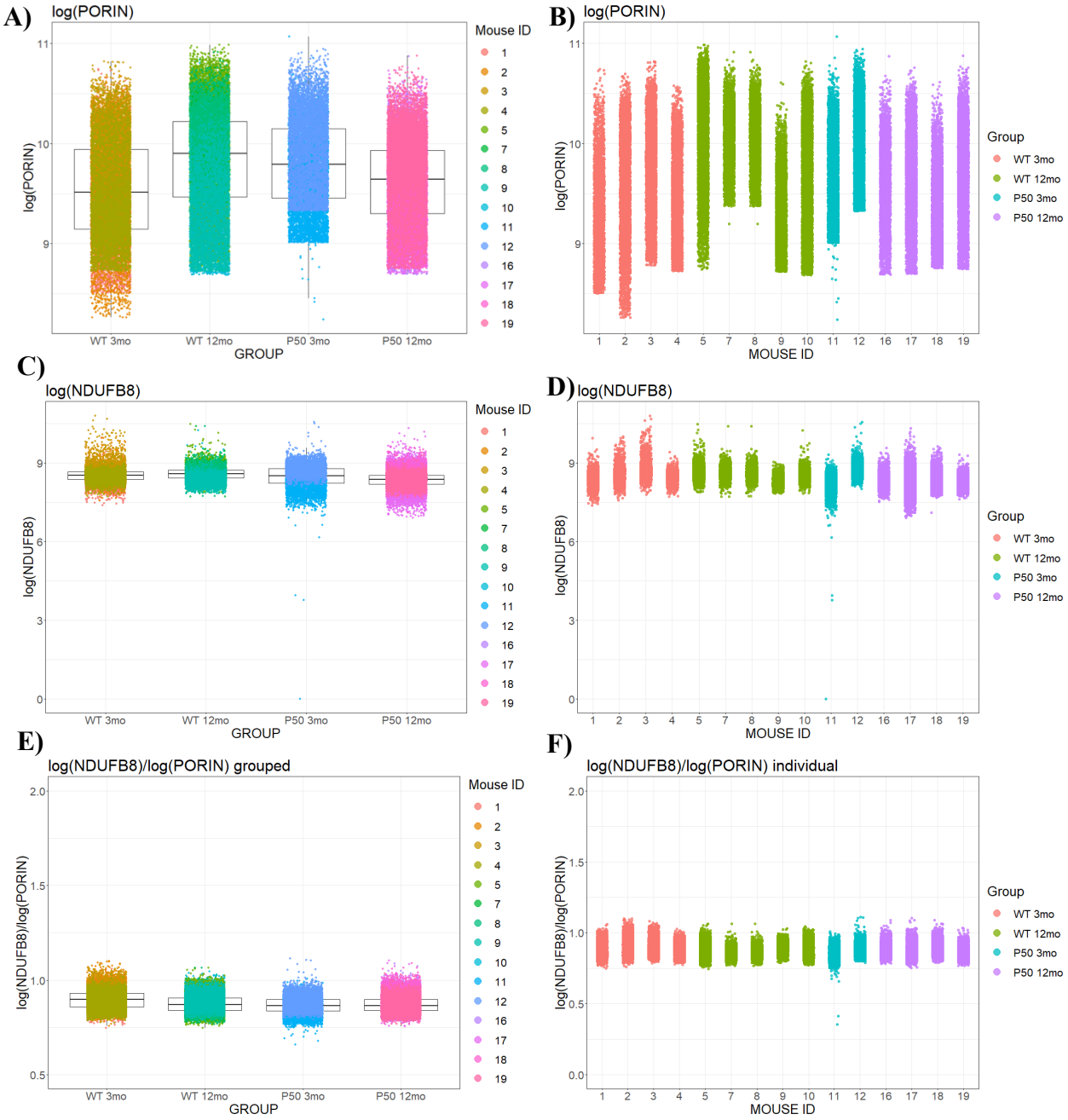


Figure 6.4: Strip charts for A, B) logPORIN; C), D) logNDUFB8; and E), F) log(NDUFB8/log(PORIN)) with genotype (wt/P50) and age (young i.e., 3mo or old i.e., 12mo).

When REML was performed, a significant reduction in logNDUFB8 was observed in P50 mice when compared to WT i.e., ($\chi 2$ (1) = 5.74, p=0.02). In addition, when logNDUFB8 was normalised to logPORIN, a significant decrease was observed with age ($\chi 2$ (1) = 4.28, p=0.04), but no significant difference was found for mouse genotype ($\chi 2$ (1) = >0.01, p=0.99). Similarly, logPORIN values did not differ between 12mo and 3mo mice ($\chi 2$ (1) = 3.25, p=0.07). In addition, no statistical difference was observed based on genotype ($\chi 2$ (1) = 1.79, p=0.18). Finally, no significant difference was observed when the lme assessed whether age influenced logNDUFB8 with mouse ID as the random effect i.e., ($\chi 2$ (1) = 0.40, p=0.53). Figure 6.5A-F depict the described findings.

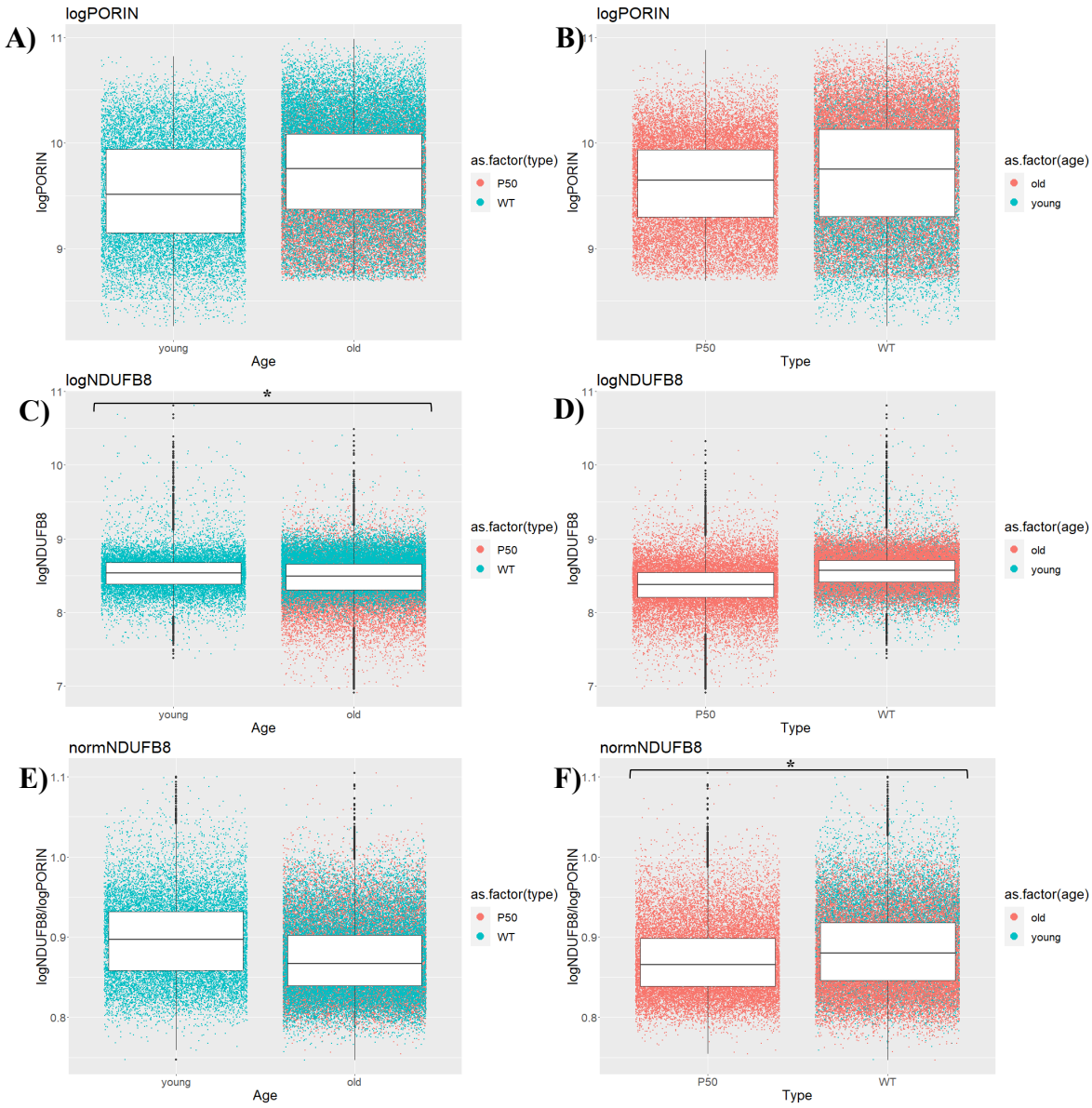


Figure 6.5: Box plots of linear mixed model, assessing whether biological age (old i.e., 12mo/ young i.e., 3mo) and type (WT/P50) has an impact on A/B) logPORIN C/D) logNDUFB8 levels or E/F) normalised logNDUFB8, with mouse number as a random effect. ***= p<0.05**

6.3.2 Mitochondrial complex IV (MTCO1) protein levels in P50 knockout old (12mo) and young (3mo) mouse hearts

Figure 6.6 shows representative images obtained using the Zeiss LSM800 Airyscan microscope from WT young (3mo), and P50 young (3mo), WT old (12mo) and P50 old (12mo) mouse heart tissue labelling of mitochondrial CIV (MTCO1).

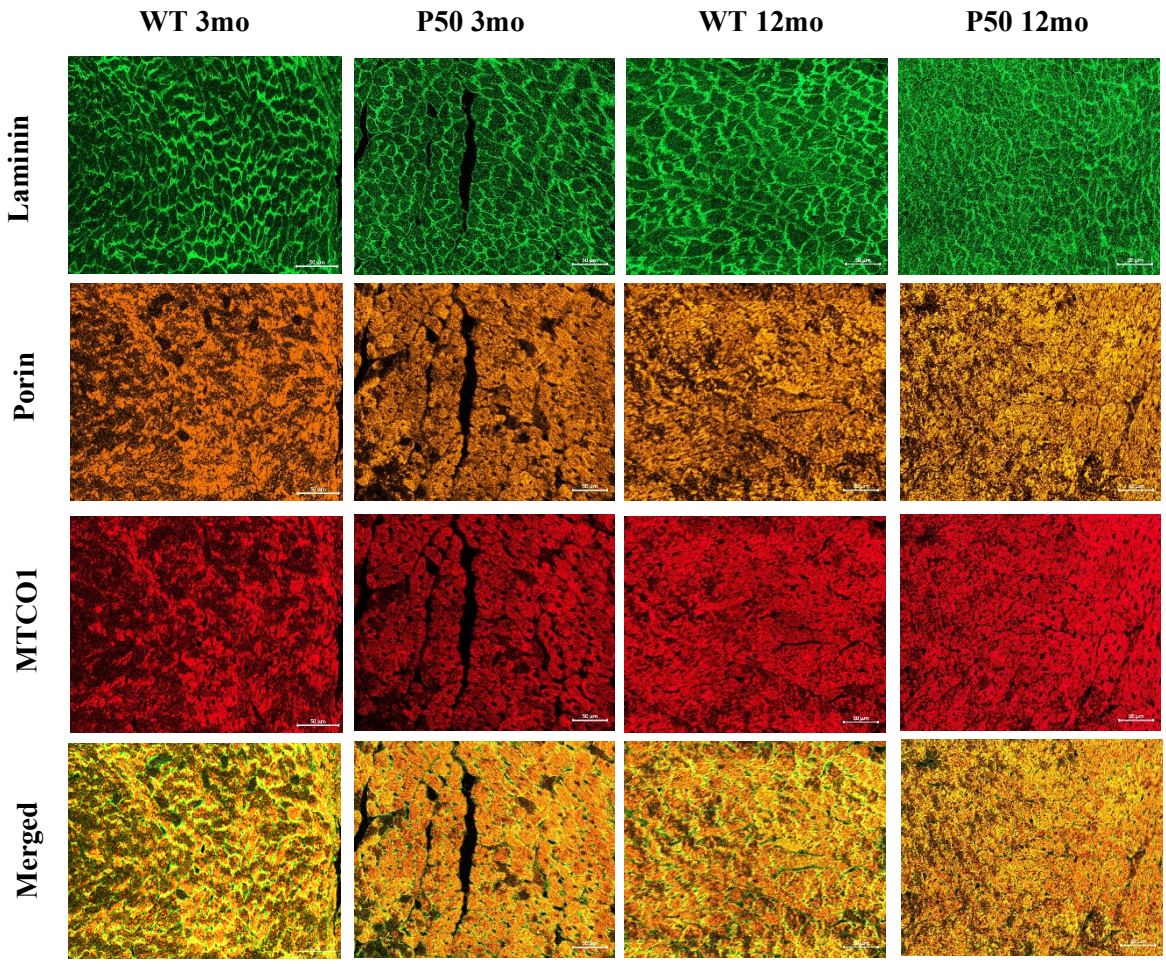


Figure 6.6. Mitochondrial MTCO1 (CIV) subunit expression levels. Representative images of cardiac tissue sections from mice for immunofluorescent detection of MTCO1 (complex IV), Laminin (cardiomyocyte membrane marker) and Porin (mitochondrial mass marker) in WT young (3mo), P50 young (3mo), WT old (12mo) and P50 old (12mo) mice. Magnification x20. Scale bars=50um

Firstly, log transformation and lme was first fitted for all variables (i.e., logMTCO1, logPORIN and log(MTCO1/log(PORIN)) with genotype (WT/P50) and age (3mo or 12mo), and visualised on strip plots (figure 6.7A-F).

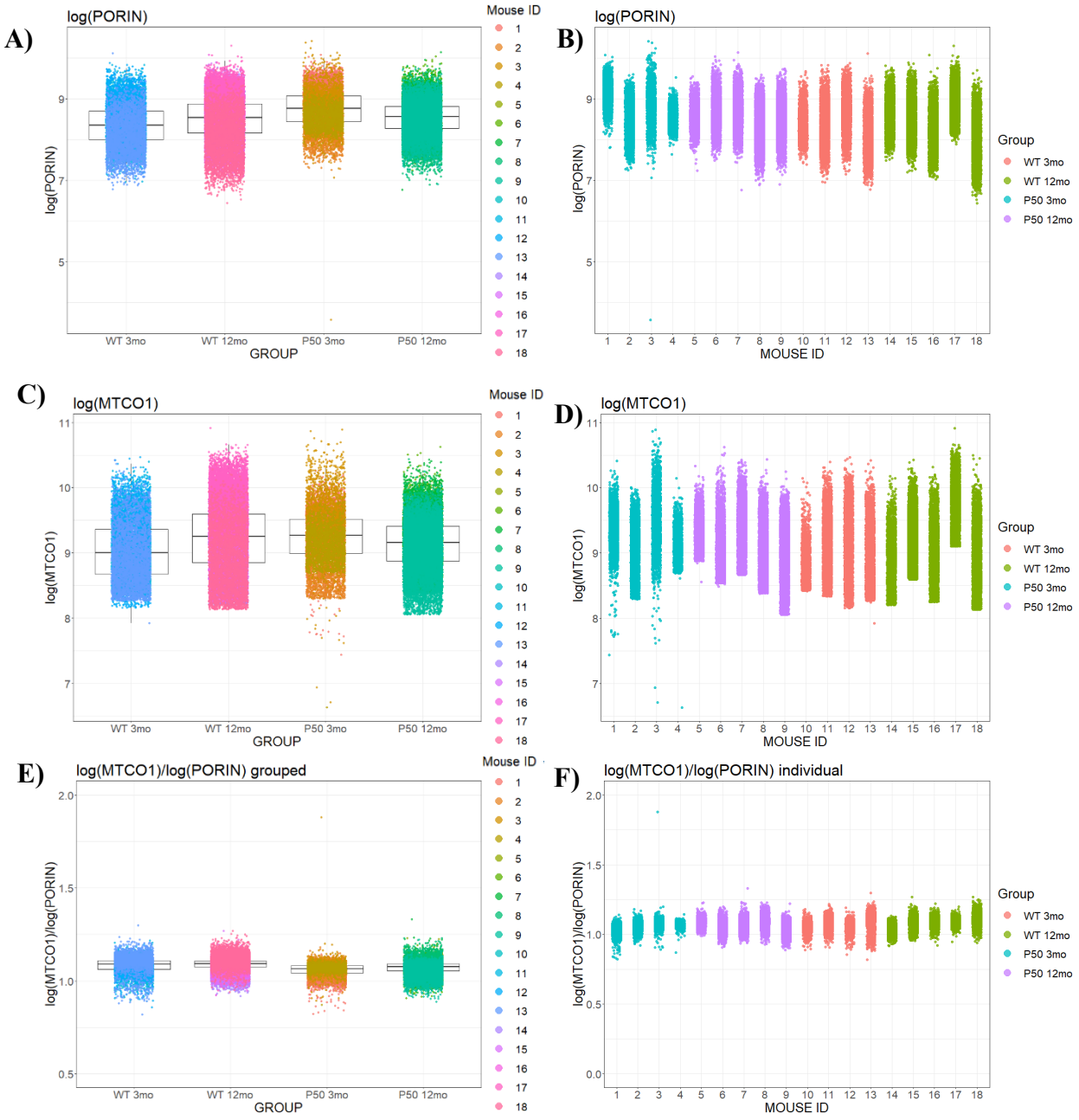


Figure 6.7. Strip charts for A, B) logPORIN; C), D) logMTCO1; and E), F) log(MTCO1/log(PORIN)) with type (wt/P50) and age (young i.e., 3mo or old i.e., 12mo).

No statistically significant differences were found for log(MTCO1) and log(PORIN) or logMTCO1/logPORIN when REML was performed with mouse number or age taken into account. The same result was observed for logPORIN when P50 mice were compared with WT i.e., ($\chi 2$ (1) = 3.10, p=0.08). Interaction between age and type was not significant for logPORIN ($\chi 2$ (1) = 1.11, p=0.29), and neither was the difference when age was considered ($\chi 2$ (1) = 0.22,

p=0.64). No significant differences were observed when the lme assessed whether age and type influenced logMTCO1 with mouse ID as the random effect i.e., age: ($\chi 2$ (1) = 0.05, *p*=0.09); type: ($\chi 2$ (1) = 1.05, *p*=0.31); interaction between age and type: ($\chi 2$ (1) = 1.23, *p*=0.27). When logMTCO1 was normalised to logPORIN, no significant differences were observed for age, type or interaction between age and type i.e., age: ($\chi 2$ (1) = 0.67, *p*=0.42); type: ($\chi 2$ (1) = 1.81, *p*=0.18); interaction between age and type: ($\chi 2$ (1) = 0.02, *p*=0.89). Figure 6.8 A-F depict the described findings.

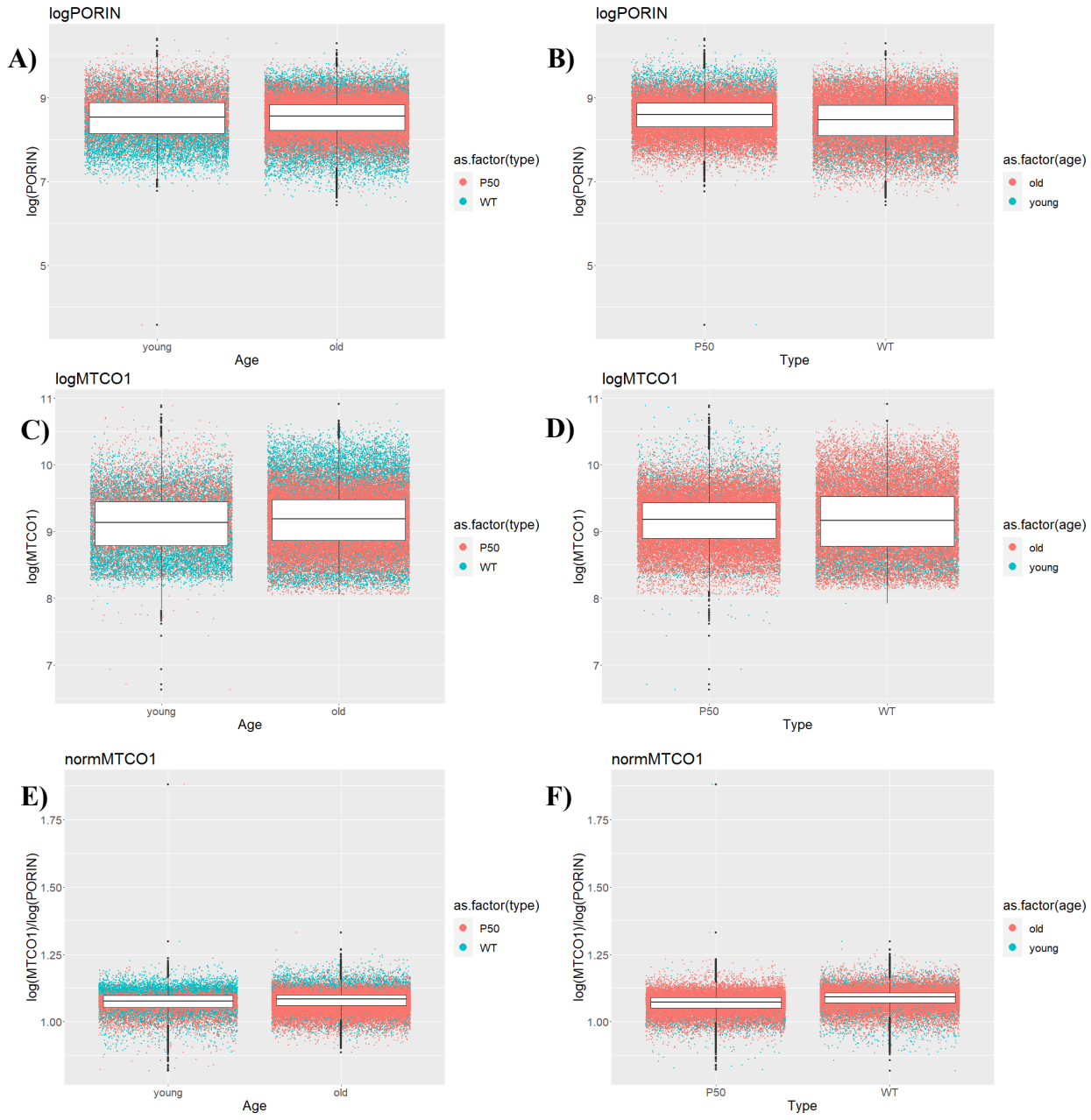


Figure 6.8. Box plots of linear mixed model, assessing whether biological age (Old i.e., 12mo/ young i.e., 3mo) and type (WT/P50) has an impact on A/B) logPORIN C/D) logMTCO1 levels or E/F) normalised logMTCO1, with mouse number as a random effect.

6.4 Discussion

The present study observed significantly decreased mitochondrial OXPHOS CI protein levels in the aged and HCM phenotype mouse model (i.e., C57BL/6J *nfkb1−/−* mice) when compared to WT (i.e., C57BL/6J mice). In addition, when CI protein levels were normalised to mitochondrial mass, a significant decrease was observed in older (i.e., 12mo) mice. However,

regarding normalised CI protein levels, no significance was found between genotypes. No change was observed when CI was normalised to mitochondrial mass between mouse types. CIV function was not significantly altered by age or type.

Cardiac ageing in mice has been demonstrated to resemble human cardiac ageing, with mitochondrial ROS playing a central role (Dai and Rabinovitch, 2009). The parainflammatory phenotype in *nfkb1*−/− mice has been previously described and leads to premature ageing and hypertrophy of the heart i.e., HCM phenotype (Gaspar-Pereira *et al.*, 2012; Jurk *et al.*, 2014). An investigation in to whether mitochondrial OXPHOS protein levels are affected in an inflammatory mouse model of ageing and HCM has not yet been explored.

6.4.1 Mitochondrial mass in an ageing and hypertrophic cardiomyopathy mouse model

As an individual ages, the process of mitochondrial fusion, fission, biogenesis and mitophagy (i.e., mitochondrial dynamics) mediated by dynamin-related protein 1 is altered and cell cycle regulation, quality control and transmission of energy status are disrupted (Liu *et al.*, 2020). In the aged heart, cardiomyocyte number decreases by up to 30%, and the remaining cardiomyocytes hypertrophy as a compensatory mechanism, with an additional 39% loss occurring on the left side of the heart (18% in the LV) (Olivetti *et al.*, 1991). There is no damage observed in the right ventricle, however the compensatory LV hypertrophy leads to age-related diastolic dysfunction (Olivetti *et al.*, 1991; Akasheva *et al.*, 2015). Similarly, HCM is characterised by LV hypertrophy and diastolic dysfunction (Geske;Ommen and Gersh, 2018).

A number of research studies demonstrate compensatory upregulated mitochondrial number via increased biogenesis signalling in ageing and HCM (Pisano *et al.*, 2016; Tejado and Jou, 2018; Ranjbarvaziri *et al.*, 2021). The described finding is accompanied by a trend of decreased mitochondrial size in response to ROS-induced mitochondrial damage (Pisano *et al.*, 2016; Tejado and Jou, 2018; Ranjbarvaziri *et al.*, 2021). However, the present study observed no change in mitochondrial mass between ages or genotypes.

The effect of the parainflammatory phenotype on mitochondrial mass in the heart is controversial. In contrast to the described findings and the present study, a decrease in mitochondrial mass and number and localisation of decreased fatty acid oxidation and cytochrome oxidase activity in the IFM with age has been described (Fannin *et al.*, 1999; Barazzoni;Short and Nair, 2000). This is where the most age-related adaptations have been observed (Fannin *et al.*, 1999). Studies assessing mitochondrial function in only the SSM found

no age-related differences in protein import or ROS function (Chen;Warshaw and Sanadi, 1972; Takasawa *et al.*, 1993). It can therefore be suggested from research assessing mitochondrial function in the SSM and IFM, that unaffected SSM during ageing may mask the defected IFM if not assessed separately, and lead to observations of unaltered mitochondrial mass in research (Manzelmann and Harmon, 1987).

6.4.2 Mitochondrial complex I defects in an ageing and hypertrophic cardiomyopathy mouse model

Alterations in CI are significant for mitochondrial respiration and ROS accumulation as aerobic NADH oxidation is almost completely controlled by CI and complex III (CIII) (Lenaz *et al.*, 2006). Previous research in rodent hearts has demonstrated an age-related reduction in CI activity (Petrosillo *et al.*, 2009). The slowing of electron transfer rate with age is caused by a decrease in CI function and enhances superoxide (i.e., primary oxygen free radical produced in mitochondria with high instability) production (Boffoli *et al.*, 1994; Petrosillo *et al.*, 2009). This feeds the positive-feedback loop of CI dysfunction, ROS production, mtDNA mutation, and protein damage (Hsieh *et al.*, 1994; Petrosillo *et al.*, 2009).

An age-related decline in CI activity is suggested to be a result of ROS-induced cardiolipin peroxidation (Petrosillo *et al.*, 2009). Binding of mitochondrial cardiolipin to CI is required for functional activity of CI, and this phospholipid that helps form the inner mitochondrial membrane is significantly decreased with age (Petrosillo *et al.*, 2009; Jussupow;Luca and Kaila, 2019). A reduction in cardiolipin leads to impaired mitochondrial respiratory activity, electron leakage and subsequently increased ROS levels (Petrosillo *et al.*, 2009). In rats, the exogenous addition of cardiolipin almost completely restores CI activity in older hearts to that of younger (Petrosillo *et al.*, 2009). In agreement, significant reduction in CI protein was observed in the present study in older mouse hearts when compared to younger when normalised to mitochondrial mass. In the present study, there is no evidence for the effect of ageing on cardiolipin. Therefore, this could be explored next.

Much like in ageing, a ROS-induced reduction in cardiolipin is observed in HCM (Ranjbarvaziri *et al.*, 2021). HCM is characterised by hypercontractability, which is associated with increased ROS production via OXPHOS (Cohn *et al.*, 2018). The increase in oxidative stress leads to mitochondrial damage, which is significantly higher in donor hearts of individuals with HCM when compared to controls (Ranjbarvaziri *et al.*, 2021). There is also an

increase in biomarkers for oxidative stress such as cysteine and reduced levels of major antioxidant transcripts such as glutathione reductase, superoxide dismutase and glutathione peroxidase 1 in individuals with HCM (Ranjbarvaziri *et al.*, 2021). As hypothesised and in agreement with understanding from models of ageing related to oxidative damage and CI function, the present study concluded significantly reduced CI NDUFB8 levels in mice with HCM pathological phenotype (i.e., *nfkb1−/−*) mice in comparison to WT.

Taking the present study findings and previous literature into account, CI dysfunction may be a contributing factor to the inflammatory pathway and therefore cardiac pathological phenotype in ageing and HCM.

6.4.3 Mitochondrial complex IV defects in an ageing and hypertrophic cardiomyopathy mouse model

In a study assessing CIV function in the human heart, CIV activity was unaltered between the ages of 10 days and 67 years (Marin-Garcia;Ananthakrishnan and Goldenthal, 1998). In coherence, the same has been observed in a mouse model of ageing. In agreement, the present study demonstrated no change in CIV activity in *nfkb1−/−* mice, and no alterations in WT old compared with WT young. However, this finding contrasts with research in 140 hearts from men and studies assessing CIV proteins in rat hearts, where CIV was observed to be significantly affected by age (Muller-Hocker, 1989; Tatarkova *et al.*, 2011; Lesnefsky;Chen and Hoppel, 2016). In addition, reductions in cardiolipin have been demonstrated to impair CIV activity in the rat heart (Paradies *et al.*, 1997). Cardiolipin, as previously described, is reduced with age and therefore CIV function would have been expected to decrease in the aged and *nfkb1−/−* mouse heart (Paradies *et al.*, 1997). Due to controversial research findings, more data is needed to confirm the presence and importance of CIV dysfunction in ageing and HCM.

6.4.4 Limitations

The present study presents with several limitations. Firstly, it is limited by the number of mice included in the cohort. Due to low numbers, the study is a pilot study and should be used to confirm trends and provide a study design for completion of a larger investigation with optimal labelling methods. The present study has also provided rationale to further explore the contribution of mitochondrial dysfunction (i.e., CI and CIV) to the pathological phenotype of the heart in ageing and HCM.

Secondly, tissue degradation of the provided mouse hearts was observed around the outer edges of the cardiac tissue, thought to potentially because of tissue drying prior to fixation. In these areas, immunolabelling was unanalysable. In future investigations, fixing after harvest should be completed more promptly.

Thirdly, CI analysis was limited by the omission of 4 mice i.e., n=1 WT 12mo; n=2 P50 3mo; n=1 P50 12mo because of failed immunolabelling of porin and NDUFB8. The omission of the mice meant that analysis of interaction between age and type could not be completed because the P50 3mo group had to be removed from statistical analysis. Due to the time that it took to complete optimisation and the need to label CI and CIV in separate batches, there was not sufficient time available to repeat the experiment and analysis of CI.

Finally, regarding the age of older mice, 18mo instead of 12mo may have provided a significant difference in results, this would allow significantly more time for age-related pathophysiological changes to manifest. In a previous study, mature adult mice have been defined as 3-6months, middle-aged mice 10-14months and old as 18-20months (Shoji *et al.*, 2016). Therefore, in the future an experiment analysing differences in mitochondrial OXPHOS protein levels could be completed in young (3mo) and old (18mo) WT and *nfkb1−/−* mice.

6.4.5 Conclusion

In conclusion, the present pilot study suggests that mitochondrial OXPHOS dysfunction may be implicated in parainflammation within the aged heart and individuals with HCM. Levels of the CI protein NDUFB8, are significantly reduced in pathologically aged cardiac tissue of mice, i.e., *nfkb1−/−* mice. The study presents as a starting point for further investigation using a larger sample size to confirm the contribution of mitochondrial dysfunction to pathological phenotype of the heart in ageing and HCM.

Chapter 7. General discussion

The present thesis explored the effects of age and sex on the mechanisms of exercise tolerance whilst controlling for physical activity level, the mechanisms of exercise intolerance in individuals with hypertrophic cardiomyopathy (HCM), the effects of a novel lifestyle intervention in individuals with HCM, and finally, the role of the mitochondria in a mouse model (*nfkb-/-* mouse) of ageing and HCM cardiac pathological phenotype.

Physical activity increases lifespan and health-span through the decreased risk of secondary ageing (i.e., aetiological of disease and environmental factors), altering life expectancy (Vina *et al.*, 2016). Aerobic exercise attenuates age-related declines in cardiorespiratory fitness through improvement of cardiovascular disease risk factors such as endothelial dysfunction (Malbut;Dinan and Young, 2002; Hawkins and Wiswell, 2003; Huang *et al.*, 2005). Non-linear decline in exercise tolerance ($\dot{V}O_2$peak) in sedentary individuals begins earlier (i.e., mid-twenties), in comparison to trained individuals that only present with a non-linear decline once they cease training (Hawkins and Wiswell, 2003). To assess the mostly primary (biological) age-related determinants of exercise tolerance, Chapter 4 assessed determinants of exercise tolerance in healthy individuals whilst controlling for physical activity level. In Chapter 4 Part A there was an age-related reduction in peak oxygen consumption ($\dot{V}O_2$peak), but no interaction between age and sex. The determinant of age-related reduction in $\dot{V}O_2$peak was arteriovenous O_2 difference, and not cardiac output. The described finding was consistent for older males and females. However, females also presented with a significant age-related worsening in blood pressure compared to males.

As described, Chapter 4 Part A highlighted the age-related determinants of exercise tolerance without physical activity level interaction. Based on the present findings, future research should look to assess the molecular mechanisms leading to age-related reduction in the ability of skeletal muscle to extract oxygen. Such mechanisms could then be targeted in addition to prescribing exercise training in practice to reduce secondary/ primary ageing pathological progression. In addition, the observed accelerated worsening of blood pressure in females in the present study should be further examined. In the future, a longitudinal study assessing a large cohort of females could determine risk factors such as hypertensive disorders of pregnancy on age-related blood pressure control. This is important to evaluate due to the association between hypertension and adverse cardiac remodelling (Tomek and Bub, 2017). Such women that present with lifetime risk factors leading to hypertension in old age could then be followed-up in clinic on a more regular basis and have preventative measures provided.

Reduction in $\dot{V}O_2$peak is associated with worsening pathophysiology in individuals with HCM (Coats *et al.*, 2015). Determination of the mechanisms of exercise intolerance in individuals with HCM allows insight into how disease pathology affects the body's ability to transport and utilise oxygen (i.e., cardiac output and arteriovenous O_2 difference), thus identifying potential therapeutic targets (Fick, 1870; Bassett and Howley, 2000). No research has previously assessed the mechanisms of exercise intolerance in adults with HCM when compared to healthy adults. The present thesis observed that impaired transport and extraction of oxygen from the blood by skeletal muscles (peak arteriovenous O_2 difference) is the major determinant of exercise capacity in individuals with early-stage HCM (Chapter 4, Part B). The observed could be hypothesised to be aetiological of factors such as reduced mitochondrial density of skeletal muscle and deconditioning because of reduced physical activity in the patient population (Critoph *et al.*, 2014).

In individuals with cardiovascular disease, improved peripheral pathophysiology (i.e., skeletal muscles) is the major mechanism leading to improved $\dot{V}O_2$peak (Pandey *et al.*, 2021). In coherence, exercise training in HCM has not been observed to improve cardiac function (Saberi *et al.*, 2017). The present thesis suggests that in individuals with early-stage HCM, targeting of mechanisms associated with reduced arteriovenous O_2 difference can be hypothesised to improve $\dot{V}O_2$peak, and attenuate progression to heart failure (Desai *et al.*, 2014). Because of a low cohort number analysed in Chapter 4 Part B, a study of a greater sample size comparing the mechanisms of exercise intolerance between individuals with early-stage HCM, and age-and-sex matched healthy individuals should be completed to confirm the targeting of reduced arteriovenous O_2 difference to slow progression of disease at an early stage in this clinical population.

There have been recent advancements in the treatment of HCM. Most of these advancements have been pharmacological, e.g., Mavacamten (Olivotto *et al.*, 2020). However, we are now seeing more research emerging investigating the therapeutic effects of lifestyle interventions incorporating physical activity (Saberi *et al.*, 2017). Contrary to previous findings by Saberi and colleagues regarding 16-weeks exercise training in individuals with HCM, the present thesis (Chapter 5) observed no significant changes in $\dot{V}O_2$peak or determinants (i.e., cardiac output and arteriovenous O_2 difference (Saberi *et al.*, 2017). This may be owed to the present thesis controlling for medication.

The effects of dietary nitrate (NO_3^-) supplementation in isolation and combined with exercise have been explored in diseases with hypoxic conditions (Hendgen-Cotta *et al.*, 2012; Eggebeen *et al.*, 2016; McMahon;Leveritt and Pavey, 2017; Van De Walle and Vukovich, 2018). Lifestyle interventions in HFpEF have demonstrated that acute dietary nitrate NO_3^- supplementation, but not exercise, has resulted in improvements in pathophysiological characteristics associated with left ventricular diastolic dysfunction and cardiac remodelling, but no studies are available exploring the response in individuals with HCM (Zamani *et al.*, 2015; Eggebeen *et al.*, 2016). However, when hypothesised additive effects of NO_3^- supplementation to exercise tolerance was assessed in comparison to exercise training alone in HFpEF, no such additional benefit was observed (Shaltout *et al.*, 2017). This may have been due to NO_3^- supplementation only occurring three times per week in the research completed by Shaltout and colleagues (Shaltout *et al.*, 2017).

The present and novel lifestyle intervention incorporating daily 6.1mmol NO_3^- supplementation and increased physical activity led to a significantly improved peak diastolic blood pressure and resting systolic, diastolic, and mean arterial blood pressure. Representative of improved vasodilation aetiological of improved endothelial function (Kenchaiah and Pfeffer, 2004; Takimoto *et al.*, 2005; Tzemos;Lim and MacDonald, 2009; Gaudreault *et al.*, 2013). In addition, significant improvement in serum lipid levels (i.e., total/ high density lipoprotein cholesterol) have been observed to occur as a result of increased skeletal muscle utilisation of lipids in place of glycogen in the general population, and those with cardiovascular disease, and has now been further corroborated in individuals with HCM in the present study (Chapter 5) (Riedl *et al.*, 2010; Earnest *et al.*, 2013; Muscella;Stefano and Marsigliante, 2020).

Sign and symptom presentation is related to quality of life (QoL) in HCM (Zaiser *et al.*, 2020). Previously, a 16-week moderate intensity training intervention in individuals with HCM failed to improve all parameters of QoL when assessed using the Minnesota Living with Heart Failure questionnaire (MLHFQ), and the 36-item Short Form survey (SF-36), other than physical functioning (SF-36) (Saberi *et al.*, 2017). However, in response to the present and combined lifestyle intervention, disease-specific health related QoL (MLHFQ), and parameters of general health QoL (SF-36) i.e., physical functioning, energy/fatigue, social functioning, and general health), significantly improved when change scores between intervention and control groups were compared. It can therefore be suggested that improvements in QoL were resultant of the effect of the present lifestyle intervention and provides rationale for the completion of a higher-powered study.

Chapter 5 observed no significant alterations in markers of diastolic dysfunction when change scores between intervention and control were assessed. However, NO_3^- supplementation has demonstrated ability to promote revascularisation of the heart in mice with chronic ischemia after 14 days (one human year is equivalent to 9 mouse days (Dutta and Sengupta, 2016)), and deemed safe up to 17 months of supplementation (Hendgen-Cotta *et al.*, 2012; Hezel *et al.*, 2015). Although this is an animal model, it could be hypothesised from research in mice that longer-term NO_3^- supplementation could reduce the common ischemia-induced changes in LV compliance associated with HCM, and therefore improve relaxation of the heart through increased blood-flow (Cannon *et al.*, 1985; Hendgen-Cotta *et al.*, 2012). In addition, previously described available research regarding acute NO_3^- supplementation and pathophysiological characteristics associated with diastolic dysfunction in HFpEF, allow to hypothesise that a significant improvement in diastolic function may have occurred with an acute, larger dose (ie., 12.9 mmol) (Zamani *et al.*, 2015). Based on such research, a trial of a longer duration and/or higher dose of NO_3^- could be trialled in individuals with HCM in the future.

In order to assess whether NO_3^- supplementation adds additional benefit to that observed with exercise training in individuals with HCM, future studies should first assess a larger cohort of participants, and include three intervention arms (i.e., increased physical activity; combined physical activity and daily NO_3^- supplementation, and a usual activity (control) group for 16-weeks). At present, lifestyle interventions are not prescribed to individuals with HCM to manage symptoms and disease progression. However, if significant benefits were observed in factors associated with symptom prevalence and disease progression in the clinical population, lifestyle interventions may be prescribed in clinical practice to manage individuals with HCM.

Chapter 4 and 5 assessed cardiovascular pathophysiology associated with ageing and HCM. However, a deeper understanding of molecular pathways and their contribution to pathological cardiac phenotype in the ageing and HCM heart is imperative for further therapeutic advancements. Previous research demonstrates reduction in Complex I (CI) and complex IV (CIV) activity because of oxidative stress in ageing in rodent models (Petrosillo *et al.*, 2009; Tatarkova *et al.*, 2011). Similarly, HCM is characterised by oxidative stress and therefore a similar finding was hypothesised (Ranjbarvaziri *et al.*, 2021). Studies assessing CIV function in the aged human heart are conflicting (Muller-Hocker, 1989; Miró *et al.*, 2000). Chapter 6 compared the abundance of key mitochondrial proteins within complex I (CI); NDUFB8 and complex IV (CIV); MTCO1, in a mouse model of premature ageing, cardiac hypertrophy and inflammageing lacking the P50 subunit of NF-kB (*nfkb-/-* mouse) to age-matched and healthy

controls; WT (C57BL/6J) young (3mo) and WT old (12mo) mice. In the present pilot study, NDUFB8 protein levels decreased, but MTCO1 was not. Therefore, the research in this thesis suggests that reduced mitochondrial CI activity (i.e., defined by reduced NDUFB8 protein levels), may be a causal mechanism of cardiac pathological phenotype in ageing and HCM. The present pilot study provides rationale for a larger study to be completed to confirm the role of the mitochondria in the ageing heart and HCM, and therefore provide a therapeutic target of adverse cardiac hypertrophy.

7.1 Conclusion

The present thesis is timely and important because it i) provides insight into the physiological mechanisms of primarily primary ageing leading to reduced exercise tolerance (of which is associated with QoL, morbidity and mortality, and disease progression/development) in an ageing population for therapeutic target. In addition, it highlights the need for investigation into the accelerated age-related decline in vascular function in females; ii) demonstrates the important role that a novel lifestyle intervention incorporating increased physical activity and NO_3^-supplementation may have in managing factors associated with disease progression (i.e., blood pressure) and QoL in HCM, and; iii) provides insight in to the molecular (i.e., mitochondrial) mechanisms aetiological of aged and HCM pathological phenotype, and subsequently beginning to define new therapeutic targets.

Currently, no intervention is available that alters the natural course of HCM, a disease characterised by reduced functional capacity, diastolic dysfunction, lowered QoL, and progression to heart failure. Therefore, development of effective strategies targeting HCM-induced adverse cardiac remodelling is imperative to improving disease outcomes in this clinical population. Lifestyle interventions have the potential to become part of therapeutic recommendation in clinical care. For that reason, the present thesis recommends further evaluation of a combined lifestyle intervention incorporating increased physical activity and NO_3^- supplementation to improve exercise tolerance, QoL, measures of diastolic dysfunction and serum lipid levels in HCM.

How the coronavirus pandemic affected my PhD

The coronavirus 2019 pandemic significantly impacted the completion of studies 2: "The effect of physical activity and dietary nitrate (NO_3^-) supplementation on functional capacity in individuals with hypertrophic cardiomyopathy (HCM)" (Chapter 5), and 3 "The role of the mitochondria in the ageing heart and HCM" (Chapter 6) of the present thesis.

Firstly, recruitment for assessment of the effect of physical activity and NO_3^- supplementation on functional capacity in individuals with HCM was placed on hold from March 2020 to March 2021. The pause in study completion also resulted in 5 individuals with HCM being unable to attend follow-up. Due to the pause on study completion, the PhD data collection deadline was extended from September 2021 to February 2022. As a result, study participant number was reduced.

Secondly, the laboratory induction to complete the final study (Chapter 6) of the present thesis on the role of the mitochondria in the ageing heart and HCM in the mitochondrial research centre was not possible until 11th March 2021. Following induction, extensive optimisation of murine tissue sections had to be completed to determine the optimal method for immunolabelling of the mouse cardiac tissue sections. The combination of significantly delayed study completion to the third year of the present PhD, and extensive optimisation process meant that there was only time to complete data collection and analysis of complex I (NDUFB8 protein levels) and complex IV (MTCO1 protein levels) correctly once. Four mice i.e., n=1 WT 12mo; n=2 P50 3mo; n=1 P50 12mo were omitted from complex I analysis because of unanalysable immunolabelling of Porin and NDUFB8. This meant that interaction between age and type could not be completed because the P50 3mo group had to be removed from statistical analysis. Additional analysis on the effect of exercise training on mitochondrial function in aged mice had been planned. There was not sufficient time to complete such data collection and analysis.

Overall, the coronavirus pandemic placed a limit on the timeframe for data collection in the present thesis, and therefore the amount of data available for analysis. Due to this, studies 2 and 3 are deemed as pilot studies and a means to describe trends in results to provide rationale for future, larger studies with the same aims.

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Appendices

Appendix 1: Chapter 6 equipment, consumables, and antibodies

Equipment

2100 Antigen Retriever (Aptum Biologics Ltd)

Electrothermal MH8517 paraffin section flotation bath (Cole-Parme)

Immunoanalyser software (IMARIS)

MatLab (MathWorks)

Microm HM 325 rotary microtome (Thermo Fisher Scientific)

Microwave (Igenix)

Zeiss LSM 800 confocal laser scanning microscope (Zeiss)

Zen 2.5 Blue microscope software (Zeiss)

Zeiss Zen Lite microscope software (Zeiss)

Consumables

Avidin/Biotin blocking kit (SP-2001, Vector laboratories)

Coverslips (22x22mm, 22x40mm) (MSD)

Ethylenediamine tetra-acetic acid, (EDTA) Tetrasodium Salt, Dihydrate SDS (J15700-A1, Affymetrix)

Falcon tubes (15ml, 50ml) (BD Biosciences)

Pipettes (P2, P10, P20, P100, P200, P1000) (Star Lab)

Gloves (Star Lab)

Histoclear clearing agent (HS-200, National Diagnostics)

Hydrochloric acid (BDH7237-1, VWR International Ltd)

Hydrophobic pap pen (CellPath)

Microfuge tubes (0.6ml, 1.5ml, 2.0ml) (Star Lab)

Normal Goat Serum (G9023, Sigma Aldrich)

Pipette tips (10µl, 20µl, 200µl, 1000µl) (Star Lab)

Prolong Gold antifade mountant (P36930, Invitrogen)

Reagent reservoirs (Fisher Scientific)

Slide mailers (Cell path)

Superfrost glass slides (MSD)

Tris Buffered Saline (TRIS-RO, Sigma-Aldrich)

Tri-sodium citrate dihydrate (27833.29, VWR International Ltd)

Tween (100316964, Sigma-Aldrich)

Weight boats (VWR)

Antibodies

Goat anti-mouse IgG2a-Alexa Fluor ®647 (A21241, Invitrogen)

Goat anti-mouse IgG2a-Alexa Fluor ®546 (A21143, Invitrogen)

Goat anti-mouse IgG2b-Alexa Fluor ®546 (A21143, Invitrogen)

Goat anti-mouse IgG2b-Alexa Fluor ®488 (A11008, Invitrogen)

Goat anti-rabbit IgG-Alexa Fluor ®405 (A31556, Invitrogen)

Goat-anti-rabbit IgG-Alexa Fluor ®488 (A11008, Invitrogen)

Goat anti-mouse streptavidin conjugate- Alexa Fluor ®488 (S32357, Invitrogen)

Goat anti-mouse streptavidin conjugate- Alexa Fluor ®647 (S32357, Invitrogen)

Goat biotinylated IgG1 (A10519, Invitrogen)

Mouse anti-MTCO1 (ab14705, Abcam)

Mouse anti-NDUFB8 (ab110242, Abcam)

Mouse anti-VDAC1 (ab14734, Abcam)

Rabbit anti-Laminin (L9393, Sigma-Aldrich)

Appendix 2. Consent forms

Written Consent Form / Potential Participant
EPFL Innovation Park
Bâtiment C, 1015 Lausanne, Switzerland

SmartCardia

WRITTEN CONSENT STATEMENT FOR PARTICIPATION IN A RESEARCH PROJECT

Participant's Code: ☐☐☐☐☐

Please read this form and feel free to ask questions when you do not understand something or you want to have more details.

Study's short name:	Newcastle Smart Cardia Validation
Title of the study:	Validity of the wireless Smart Cardia monitor to measure electrocardiography, oxygen saturation, blood pressure, activity and metabolic rate.
Project Identifier:	SmartCardia SA EPFL Innovation Park, Bâtiment C 1015, Lausanne Switzerland
Place for the conduction of the study:	Newcastle University Medical School Clinical Research Facility of the Royal Victoria Infirmary
Project Leader:	Dr Djordje Jakovljevic
Participant No: (Name and Surname): Date of birth:	☐ female ☐ male

- I declare to have been informed by the research team of the undersigned study, orally and in writing, for the objectives and project progress as well as the alleged effects, benefits, possible disadvantages and potential risks.
- I accept to take part in this study voluntarily and I agree with the content of the information sheet given to me on the above study. I had enough time to make my decision.
- I received satisfactory answers to the questions I raised in relation to my participation in the project. I retain the information sheet and get a copy of my written consent.
- I agree that the relevant experts of the institution, the project identifier, the competent Ethics Commission for this study can check my raw data to carry out controls, provided that the confidentiality of such data is strictly ensured.
- I know that my personal data can be transmitted to research purposes **as part of the project only and under anonymized manner**.
- I can, at any time and without having to justify myself, revoke my consent to participate in the study, without this having an adverse effect on the result of taking my usual medical care.
- I am informed that the liability of the project management covers the improbable project caused damage that I may suffer.
- I am aware that the obligations mentioned in the information sheet for participants must be respected throughout the duration of the study. The direction of the study can exclude me at any time in my health interest.
- For the personal data protection, in an absolute strict manner and according to the **GDPR** regulation.

Each Subject / Participant should always have the full knowledge and awareness that his / her data can be used in ***ONLY IN ONE*** out of the following three manners, **STRICTLY:**

- **I do not accept that my Data / Sensitive Information, even in a fully anonymised format to be used anywhere either for Scientific, Research, Epidemiological - Societal or Commercial Purposes.** ☐
- **I do accept the use of my Fully ANONYMISED Personal & Sensitive Data ONLY FOR SCIENTIFIC, RESEARCH, EPIDEMIOLOGICAL AND SOCIETAL PURPOSES / NEVER FOR COMMERCIAL ONES, upon the written approval of the Scientific / Medical Supervisor.** ☐
- **I do accept the use of my Fully ANONYMISED Personal & Sensitive Data ONLY FOR SCIENTIFIC, RESEARCH, EPIDEMIOLOGICAL AND SOCIETAL PURPOSES / NEVER FOR COMMERCIAL ONES, upon the written approval of the Scientific / Medical Supervisor and STRICTLY UPON MY WRITTEN INDIVIDUAL APPROVAL Per CASE / REQUEST .** ☐

Place, date: Newcastle, _ _ / _ _ / _ _	Signature of the participant:

Attestation of Investigating Physician: I hereby certify that I have explained to the participant the nature, extent and scope of the project. I declare that I fulfil all obligations in relation to this project in accordance with the legislation. In case that I become aware of any elements that may affect the Participant's consent to participate in the Project at any time during the Project, I undertake to inform the authorities immediately.

Place, date: Newcastle, _ _ / _ _ / _ _	Name and signature of the investigator responsible for the project providing information to the participants in block letters Name: Signature:

Newcastle University

The Newcastle upon Tyne Hospitals NHS
NHS Foundation Trust

Clinical Cardiovascular Research Group
Faculty of Medical Sciences
Newcastle University
Newcastle upon Tyne, NE2 4HH

T: +44 (0)191 208 6935

Patient Identification number for this trial:_____

CONSENT FORM

Title of Project: Clinical and genetic determinants of disease progression and response to lifestyle intervention in patients with hypertrophic cardiomyopathy

Name of researchers: Drs Djordje Jakovljevic, Guy MacGowan, Paul Brennan, Kristian Bailey, Nduka Okwose, Mario Siervo, Kieren Hollingsworth, Christopher Eggett

Please initial box

1. I confirm that I have read and understand the information sheet dated ..... (version .....) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participant 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 agree to my GP being informed of my participation in the study. ☐

4. I understand that my results will be kept confidential and anonymous. ☐

5. I understand that relevant sections of my medical notes and data collected during the study,may be looked at by members of the research team or individuals from the NHS Trust (including regulatory bodies for auditing purposes). I give permission for these individuals to have access to my records. ☐

6. I understand that after this study, any samples that I donate will be destroyed. All samples will be anonymised, meaning that no one will be able to identify me from the sample or from the information that accompanies it. ☐

7. I understand that in this study, anonymised data may be shared with collaborating research partners and used in another research project. ☐

9. I agree to take part in the above study ☐

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Researcher	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 3: Consultant letter

Dr Djordje Jakovljevic PhD MSc BSc (Senior Lecturer, Newcastle University & Newcastle upon Tyne Hospitals)
Dr Guy MacGowan MBBCH, MD, FRCPI, FACC (Consultant Cardiologist and Honorary Reader in Heart Failure, Freeman Hospital)
4th Floor William Leech Building, ICM
Newcastle University, Medical School, Framlington Place
Newcastle upon Tyne, NE2 4HH

Tel: 0191 208 8257

Date:

GP name:
GP address:

Patient Details:

Dear Dr _____,

RE: Study participation

I am writing to let you know the above named patient has agreed to participate in a research study "Clinical and genetic determinants of disease progression and response to lifestyle intervention in patients with hypertrophic cardiomyopathy" at Newcastle Upon Tyne Hospitals NHS Foundation Trust in collaboration with Newcastle University.
At baseline and at 4-month follow-up the above patient will undergo the following assessments: medical history, physical examination, blood sampling, body composition, cardiac autonomic function, arterial stiffness assessment, electrocardiography, cardiopulmonary exercise testing, echocardiography, cardiac magnetic resonance imaging.
The study is a randomized control trial to assess the effect of a lifestyle intervention incorporating physical activity and dietary supplementation with inorganic nitrate (beetroot juice) for 4 months. Therefore, the above patient may be allocated to the intervention or a control (usual care) group.
The patient has been provided with appropriate contact details for any questions or complications arising from the study. The study does not affect the current management and on-going care. Any incidental findings from the study will be reported to you.
If you have any questions or require any further information please do not hesitate to contact me on 0191 208 8257 or email djordje.jakovljevic@newcastle.ac.uk

Yours sincerely,

Dr Djordje Jakovljevic

Appendix 4: Lifestyle intervention in hypertrophic cardiomyopathy Patient Information Sheet

The Newcastle upon Tyne Hospitals NHS Foundation Trust

Newcastle University Cardiovascular Research Centre

Cardiovascular Exercise Research Group
NIHR Clinical Research Facility
Level 6, Leazes Wing
Royal Victoria Infirmary
Queen Victoria Road
Newcastle upon Tyne, NE1 4LP
T: 0191 208 6935

Patient Information Sheet

Study Title: Clinical and genetic determinants of disease progression and response to lifestyle intervention in patients with hypertrophic cardiomyopathy

Research study run by the Clinical Exercise Research Group of Newcastle University and Newcastle upon Tyne Hospitals NHS Foundation Trust evaluates the feasibility of a novel lifestyle intervention which includes increase in daily physical activity and dietary supplementation with beetroot juice on exercise tolerance, function of the heart and quality of life in people living with hypertrophic cardiomyopathy.

You are invited to participate in this research project. Please take time to read the following information carefully. It explains why the research is being done and what it involves. If you have any questions about the information, you are very welcome to ask for further explanation. Thank you for reading this.

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

Discuss with others if you wish and take time to decide regarding your participation.

Part 1

What is the purpose of the research project?

Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiac disease, affecting one in 500 individuals. The diagnosis of HCM is based on enlargement of the left wall of the heart. The course of the disease is highly unpredictable, ranging from no symptoms and a

normal life expectancy to a progressive disease characterised by chest pain, heart failure, abnormal heart rhythm, or sudden death. Disease progression can relate to increasing the mass of the heart leading to worsening of function of the heart. No medical treatment has been shown to halt or reverse disease progression. Lifestyle interventions including physical activity and dietary supplementation with beetroot juice are safe and can potentially improve symptoms and signs in patients with heart problems. However, their combined effect in those living with hypertrophic cardiomyopathy has not been previously investigated. The aim of the present study is to evaluate the effect of a 4 months lifestyle intervention incorporating increase in daily physical activity and consumption of beetroot juice.

Why have I been chosen?

You have been chosen because you have been diagnosed with HCM. The project will involve up to 60 people with HCM.

Do I have to take part?

Your participation is purely voluntary and all results will be strictly anonymous. If you decide to take part, you are still free to withdraw at any time without giving reasons and without your medical care being affected. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form.

What will the research project involve?

You will be asked to attend the Clinical Research Facility at the Royal Victoria Infirmary and Newcastle Magnetic Resonance Centre. There will be in total 5 visits spread across 7 months, detailed below.

Visit 1:

- You will be asked to avoid eating food or drinking anything other than water, for at least 8 hours prior to the morning visit. After reading the information sheet (previously sent out in the post/email) and after having had time to make a decision and ask any questions to the researcher, you will be asked to sign the consent form saying that you would like to take part in this research study.

- You will be asked to complete a screening, medical history and physical activity questionnaire, and undertake a short physical examination with resting ECG and anthropometric measurements which includes height and weight, and waist circumference.

• We will then measure the amount of fat and fat free mass (muscle, bone) in your body using the BODPOD. This includes sitting still for 5 minutes in your swimwear, and is a painless procedure.

A cannula will then be placed in your arm and a small amount (25 ml) of fasted blood sample will be collected by a trained phlebotomist. Only a small amount of blood is taken during the test so you shouldn't feel any significant after-effects. However, some people feel dizzy and faint during and after the test. If this has happened to you in the past, tell the person carrying out the test so they're aware and can help you feel more comfortable. After the test, you may have a small bruise where the needle went in. Bruises can be painful, but are usually harmless and fade over the next few days.

• Following blood sampling, the breakfast will be provided. Following breakfast, you will be asked to complete questionnaires about you quality of life, food intake and wellbeing.

• Your heart and blood vessels function and body temperature will be assessed using simple, painless non-invasive methods. For these measures we will ask you to lie still for around 30minutes.

• Echocardiography (ultrasound of the heart) will be used to assess heart function and structure while you are resting, blowing against resistance and also following progressive exercise test detailed below.

• We will then ask you to perform a progressive exercise test, during which your breathing and heart will be assessed. You will be wearing a facemask and we will ask you to cycle between 8-12 minutes, or until you cannot cycle any more. A blood sample will also be collected from a finger prick at peak exercise and body temperature from the ear will be recorded.

• You will be provided with heart monitor, physical activity monitor (wrist watch), small step-counting device and explanation how to use the same will be given.

• Lunch will also be offered to you at the end of research Visit 1.

Total Visit 1 duration: 3.5-4 hours

Visit 2:

Will be performed at Newcastle Magnetic Resonance Centre on different day but during the same week as Research Visit 1 depending on availability of the scanner, and will last up to 2

hours. It is expected that some people will not be eligible for the scan, particularly if they have implanted a metal device, or if they feel claustrophobic (fear of being enclosed in a small space). In those selected, cardiac magnetic resonance imaging assessment will be include the following:

- Completion of the magnetic resonance imaging screening questionnaire to ensure you have no contraindication to the scan. You will have opportunity to ask questions about the magnetic resonance imaging procedures.

- You will than undergo magnetic resonance imaging examination. First measurements will be taken with the participants laying supine within the scanner for assessment of heart structure and function. After a short break, participants will lie in the scanner prone to evaluate metabolism of the heart. In some study participants (based on previous clinical notes) further assessment may be performed with injection of small amount (0.1. mmol/kg) of gadolinium - contrast agent to help evaluate the level of fibrosis (scar) within the heart muscle.

Total Visit 2 duration: up to 2 hours

Lifestyle Intervention and Control Group

Following Visit 1, study participants will be grouped into a 4-month intervention or control group using computer generated numbers. Those in the intervention group will perform physical activity and consume a beetroot juice daily (as detailed below) in addition to a standard treatment recommended by their doctor. Participants in the control group will continue their standard treatment recommended by their doctor.

All participants will be provided with physical activity diaries and step counting devices and will be asked to record their activity level on a daily basis. Participants in both the intervention and control group will receive a weekly telephone call from the member of the research team to discuss the ongoing participation in the study and inform their recordings over the previous week.

Physical activity and beetroot juice intervention: Participants in the intervention group will be asked to increase their physical activity level by 2000 steps per day (e.g. walking for approximately 30 minutes), at least 5-7 days per week. We have previously shown that this increase in physical activity is safe and can improve functional capacity in those living with heart failure. The increase in physical activity of 2000 steps per day can be divided into several bouts of shorter activity duration throughout the day (e.g. 3 x 10 min or similar). To control for exercise intensity you will be instructed to use standardised Scale (0-20) to rate perceived

exertion aiming for achieving the levels between 11 – 13 (easy-light-to somewhat hard). Participants in the intervention group will also be asked to consume small amount of nitrate enriched beetroot juice (bottle of 70 ml per day) with breakfast every morning for 4 months. If you are in the intervention group, you will be supplied with 120 small bottles each containing 70 ml of beetroot juice to be taken daily with breakfast. At the end of visit 1, you will be given 60 bottles, totalling 4.2 litres. If this appears to be challenging to carry, we will be able to ship this to you. The remaining 60 bottles will be shipped to your home address at the beginning of week 8 of your intervention, so you can continue using beetroot juice on daily basis without interruption.

Participants in the control group will be asked to continue with their ordinary physical activity level for period of 4 months.

Visit 3: will be performed within 3 days after the intervention i.e. 4 months after Visit 1 and will include assessments undertaken during Visit 1.

Visits 4: will be performed within 7 days after the intervention i.e. 4 months after Visit 2 and will include assessments of the cardiac magnetic resonance imaging undertaken during Visit 2.

Visit 5 (follow-up): will be performed 7 months after study initiation when quality of life questionnaires and cardiopulmonary exercise testing will be completed, and physical activity reviewed. You will receive a telephone call one month before to the follow-up visit to identify suitable date and time for follow-up visit. **Total Visit 5 duration is up to 1.5 hours.**

What are the side effects of treatment received when taking part?

As a consequence of increasing physical activity it is expected your heart will beat faster and breathing will increase. It may cause you experience feelings of tiredness, fatigue, and / or shortness of breath, depending on your initial health status and fitness. This is a normal response to exercise and it should quickly resolve once session is completed. You will be able to phone and communicate with the members of research team as needed. No side effects have been reported to be linked to consumption of beetroot juice.

What you should do if you feel unwell?

All assessments are supervised by fully qualified research team members. Consultant cardiologists, who are part of the research team, will be consulted on the day of your assessment as needed and will advise on the best care plan. In case you are feeling unwell while at home after examinations, you should contact the research team on 0191 208 8264.

You should also contact your GP and seek further advice as needed. In case of emergency you should follow the normal process for accessing medical care urgently.

Are there any other possible disadvantages of taking part?

There are no anticipated disadvantages to taking part in this study but you will need to attend all of the study visits which takes time.

Expenses and payments

Any travel / parking costs for helping with this research will be refunded. Taxi will be arranged for you as needed.

What are the possible benefits of taking part?

Lifestyle intervention that is subject of this study may lead to improvements in your exercise tolerance and heart function. It may also lead to improved quality of life and overall wellbeing. As part of the study you will undergo comprehensive clinical assessments. You will be supported throughout the study by the research team member who will educate you about the effects of physical activity and dietary supplementation using beetroot juice and encourage you to become more physically active.

What happens at the end of the research project?

At the end of the project, we will be able to explain to you how the lifestyle intervention affected your physical and heart function.

What if there is a problem?

If you have any concern or complaint about any aspect of the study this will be dealt with immediately by the study research team members. Contact details for the primary researcher are given at the end of Part 1.

How incidental findings would be managed?

As part of the clinical assessments it may be possible that some of the incidental findings occur. We will inform your GP about your participation in the research study. If any incidental findings occur we will report these to your GP and suggest appropriate specialist referral as needed.

What will happen to the results of the research study?

The results of the project will be presented in national and international meetings and will be published in scientific journals. You will not be identified in any report or publication. You will be welcome to have a copy of the results once they are published.

Who are the contacts for further information?

Further information can be obtained from:

Dr Nduka Okwose, Research Associate (nduka.okwose@ncl.ac.uk)
Dr Djordje Jakovljevic, Senior Lecturer (djordje.jakovljevic@ncl.ac.uk)
Dr Guy MacGowan, Consultant Cardiologist (guy.macgowan@nuth.nhs.uk)
Cardiovascular Exercise Research Group
Faculty of Medical Sciences
4th Floor William Leech Building
Newcastle University
Newcastle upon Tyne
NE2 4HH
Tel: 0191 208 6935

Thank you.

****

Part 2

What if relevant new information becomes available?

If new information is published during the course of a study this can sometimes change how the research should go forward. If this were to happen we would inform you of this revised information and ask you to confirm your consent to participate in the study. For this study it is highly unlikely that this would occur.

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. Any data already obtained from you would still be used if you were to agree to this.

What if there is a problem?

a) Complaints

If you have any concern or complaint about any aspect of this study you should contact the study team directly by phone on 0191 208 8264, or write to them at the address at the end of Part 1 of this document.

If you prefer to raise your concerns with someone not involved in your care, you can contact the Patient Advise and Liaison Service (PALS). This service is confidential and can be contacted on Freephone: 0800 032 0202

Alternatively, if you wish to make a formal complaint you can contact the Patient Relations Department through any of the details below:

Telephone: 0191 223 1382 or 0191 223 1454

Email: patient.relations@nuth.nhs.uk

Address: Patient Relations Department

The Newcastle upon Tyne Hospitals NHS Foundation Trust

The Freeman Hospital, Newcastle upon Tyne, NE7 7DN

Will my taking part in the project be kept confidential?

All information obtained during the course of the research project will be kept strictly confidential. We are bound by very strict rules about the use of confidential information - any

information will be treated confidentially and will only be accessed by the research team. Your name will be replaced by an identification number on any documentation.

None of your personal information will be identified in any reports about the research. At the end of the study your personal information will be deleted.

In accordance with Newcastle University's policy on data protection and storage, all information you provide groups will have all names and other identifiable information removed. Personal information will be kept in a locked filing cabinet within the Clinical Research Facility and Newcastle University, and accessed only by members of the research team. Any electronic information will be securely stored on password protected computers in the Institute of Cellular Medicine at Newcastle University. Again only members of the research team will have access to this information. Unless you request otherwise, data and samples you provide will be retained for up to 10 years.

Your own GP will be informed of your participation in the project, and this is normal practice. If any unexpected results are found throughout the study, we will inform yourself and your GP. The detailed results of the research tests will not be sent to anybody outside the University and Hospital. Your anonymised data may be shared with collaborating research partners and used in another research project.

The General Data Protection Regulation (GDPR)

Newcastle upon Tyne Hospitals NHS Foundation Trust is the sponsor for this study based in Newcastle upon Tyne, England. We will be using information from you and/or your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. Newcastle upon Tyne Hospitals NHS Foundation Trust will keep identifiable information about you for 10 years after the study has finished.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

What will happen to results of the research?

The results will be presented at scientific meetings for discussion by other experts in this field. They will be written up in the form of a scientific paper and this will be intended to be published in a suitable scientific journal. As soon as the results are fully analysed after the end of the

entire study you will receive a letter describing what we have found, and what implications it has for people with hypertrophic cardiomyopathy. We will also hold a feedback evening for participants at which we will present the results of the study.

In case you decide to withdraw from the study, your already collected data will be kept strictly confidential and anonymized, together with other study participants data, and will be used for analyses as appropriate. Your blood samples will be destroyed.

What will happen to blood samples?

The samples will be tested for liver function, insulin, sugar, fat, inflammation and other food derived substances. DNA may be extracted from whole blood for genetic analysis. All samples will be anonymised, meaning that no one will be able to identify you from the sample or from the information that accompanies it. Members of the study team will only have access to the blood samples during the research. At the end of research study, blood samples will be destroyed.

Who is organising and funding the research?

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no. 777204.

The design and organisation of the study is the responsibility of Drs Djordje Jakovljevic and Guy MacGowan, internationally recognised experts in this field.

There is no payment to any of the researchers involved in this study. They are employed by Newcastle University and Newcastle upon Tyne Hospitals NHS Foundation trust to work in the NHS, to teach and to research and have no financial link with the study.

The research project is insured by the University for its entire duration. The Newcastle University will provide legal liability cover for the design of the study. A letter from the University's insurers confirming the details of the University's Public Liability insurance policy can be obtained as needed.

Who has reviewed the study?

Ethical review of the study has been conducted by the North East – Tyne and Wear South Research Ethics Committee.

Appendix 5: Physical examination and Medical History document

THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

Surname	Patient i.d.No.
Forename	D.O.B.
Address	NHS No.
	Sex. Male / Female
Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

CLINICAL NOTES

Appointment 1 Physical examination

ID: **DOB:**

Body weight (kg): _____ **Waist Circumference** (cm): _____

%Fat Free Mass: _____ **% Fat Mass**: _____

Apical pulse rate(min): _____ **Rhythm**: OK / Not OK

Resting blood pressure, seated. ___/ ____

Auscultation of the lungs *with specific attention to uniformity of breath* **Palpation of cardiac apical impulse**	**OK / Not OK** *Comment:*_____ *sounds in all areas (abs* **OK / Not OK**
point of maximal impulse	*Comment:*_____
Auscultation of the heart *with specific attention to murmurs,* **Evaluation of the abdomen**	**OK / Not OK** *Comment:*_____ *gallops, clicks and rubs* **OK / Not OK**
Bowel sounds, masses, visceromegaly,	*Comment:*_____ *and tenderness.*
Evaluation of lower extremities *Oedema and presence of arterial pulse.*	**OK / Not OK** *Comment:*_____
Inspection of the skin focus on lower extremities in people with diabetes.	**OK / Not OK** *Comment:* _____
Neurologic function *Reflexes*	**OK / Not OK** *Comment:*_____
Any orthopedic or medical condition that would limit exercise.	**YES / NO** *Comment:*_____
Ventricular tachycardia	**OK / Not OK** *Comment:*_____

SOP MRC PETL 02 Pre-exercise training stress testing v1-1 03 02 2009

Date _____ Name _____ Designation _____

Signature _____

Potts Print (UK). Mar 2015 NUTH406

THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

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Forename	D.O.B.
Address	NHS No.
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Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

ST elevation (+1.0 mm) in leads without diagnostic Q-waves (other than V_1 or aVR) **OK / Not OK**
*Comment:*_____

ST or QRS changes *such as excessive ST suppression >2mm horizontal or down sloping depression* **OK / Not OK**
*Comment:*_____

Arrhythmias other than: *sustained ventricular tachycardia, including multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block or bradyarrhythmias.* **OK / Not OK**
*Comment:*_____.

Cleared to start exercise test **YES / NO**

Competed by: _____ **Date** _____

_____

SOP MRC PETL 02 Pre-exercise training stress testing v1-1 03 02 2009

Date _____ Name _____ Designation _____

Signature _____

Potts Print (UK). Mar 2015 NUTH406

THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

Surname	Patient i.d.No.
Forename	D.O.B.
Address	NHS No.
	Sex. Male / Female
Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

CLINICAL NOTES

Medical History Questionnaire

ID: Date of Birth:

Do you have or suffer from a:			**Details:**
History of heart disease (eg. heart attack, surgery, angina etc)	YES	NO	
Problems with the circulation	YES	NO	
High blood pressure	YES	NO	
Diabetes	YES	NO	
Lung disease/breathing problems (eg. asthma, COPD etc)	YES	NO	

Have you ever suffered from:			**Details:**
Discomfort in the chest, jaw, neck, back or arms (e.g. pressure, tingling, pain, heaviness, burning, tightness, squeezing or numbness)	YES	NO	
Light headedness, dizziness or fainting?	YES	NO	

Have you had any recent Illnesses? YES NO **Details:**
(including hospitalisation, new medical diagnosis, surgery)

Do you have any joint problems or anything which would make exercising difficult?
YES NO **Details:**

What medication are you taking?
(Please list)

Other habits

Caffeine	YES	NO	if yes, units per week ___
Alcohol	YES	NO	if yes, units per week ___
Tobacco	YES	NO	if yes, units per week ___

Date _____ Name _____ Designation _____

Signature _____

Potts Print (UK). Mar 2015 NUTH406

THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

Surname	Patient i.d.No.
Forename	D.O.B.
Address	NHS No.
	Sex. Male / Female
Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

CLINICAL NOTES

Family History **Details:**

Heart disease	YES	NO
Lung disease	YES	NO
Diabetes	YES	NO
Stroke	YES	NO
Sudden death	YES	NO

Do you have any known allergies? YES NO **Details**

Any additional information:

Completed by _____ Date _____

Date _____ Name _____ Designation _____

Signature _____

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Appendix 6: Physical Activity Readiness Questionnaire

THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

Surname	Patient i.d.No.
Forename	D.O.B.
Address	NHS No.
	Sex. Male / Female
Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

CLINICAL NOTES

Physical Activity Readiness Questionnaire

ID: **DOB:**

		Please choose	
1	Has your doctor ever said that you have a *heart condition* and that you should only do physical activity recommended by a doctor?	YES	NO
2	Do you ever feel *pain* in your chest when you do physical activity?	YES	NO
3	Have you ever had chest pain when you are not doing physical activity?	YES	NO
4	Do you ever feel *faint or have spells of dizziness?*	YES	NO
5	Do you have a *joint problem* (also back problem) that could be made worse by exercise?	YES	NO
6	Have you ever been told that you have high blood pressure?	YES	NO
7	Do you have any breathing problems?	YES	NO
8	Do you have any problems with your liver, thyroid, kidneys or have diabetes?	YES	NO
7	Are you currently taking any *medication*? If so, what?_____Reason _____	YES	NO
8	Are you pregnant, have you had a baby in the last 6 months, or do you plan to have a baby this year?	YES	NO
9	Has your mother or father had any heart problems?		
10	How many times a week do you do exercise: _____		
11	Is there any other reason why you should not participate in physical activity? If so, what? _____	YES	NO

Date _____ Name _____ Designation _____

Signature _____

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THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

Surname	Patient i.d.No.
Forename	D.O.B.
Address	NHS No.
	Sex. Male / Female
Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

CLINICAL NOTES

Appendix 2 **Risk Stratification Sheet**

ID: _____

DOB: _____ **Age:** _____

Study ID: _____

General Practitioner: _____

GP Address: _____

_____

Height _____

Weight _____

Waist _____

Hip circ. _____

Blood Pressure _____

Smoker **YES** **NO** **or** **given up <6month**

Blood Taken: **YES** **NO**

Comment: _____

_____

_____

_____

_____ **To be completed**

by investigator:

Date _____ Name _____ Designation _____

Signature _____

Potts Print (UK). Mar 2015 NUTH406

THE NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST UNIT No.

.....HOSPITAL

RESEARCH HISTORY SHEET

Affix patient identification label in box below or complete details

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Forename	D.O.B.
Address	NHS No.
	Sex. Male / Female
Postcode	

ELECTRONIC NOTES ONLY - NOT TO BE HAND WRITTEN

CLINICAL NOTES

Risk Stratification (circle): **Low** **Moderate** **High**

Action Taken: Commence exercise(Low Risk) **YES / NO**

Referred for Exercise ECG (moderate and high risk) **YES / NO**

Competed by _____ **Date** _____

Date _____ Name _____ Designation _____

Signature _____

Potts Print (UK). Mar 2015

Appendix 7: Case report form

CASE REPORT FORM

Clinical Validation of the SmartCardia (Newcastle, United Kingdom)

Principal Clinical Investigator: Dr Djordje Jakovljevic

Co-Clinical Inverstigators: Dr Kate Hallsworth, Dr Sophie Cassidy, Dr Nduka Okwose, Miss Jadine Scragg

Name of site: Newcastle

CRF Version Number: 2.0 Newcastle site

Participant's Code: ☐☐☐☐☐☐ Subject No. ☐☐☐

VISIT 1 (SCREENING) **DEMOGRAPHIC DATA**

Date of Assessment: __ __ / __ __ / __ __ __ __

(DD / MMM / YYYY)

Informed Consent:			
Date participant/signed written consent form:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)	**Date of first trial-related procedure:**	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)

Demographic Data:					
Ethinicity:					
Sex:	☐ Male	☐ Female			
Social Status	☐ Single	☐ Married	☐ Divorced/Separated		
Educational Status	☐ High School	☐ College	☐ University	☐ NS	(Not Specified)

Has the patient had any relevant medical history?	☐ **No** ☐ **Yes,** Complete below		
Condition / illness /surgical procedure	**Start date** (DD/MMM/YYYY)	**Stop date** (DD/MMM/YYYY)	**Or tick if ongoing at Screening Visit?**
	___/____/____	___/____/____	☐
	___/____/____	___/____/____	☐
	___/____/____	___/____/____	☐
	___/____/____	___/____/____	☐

VISIT 1 (SCREENING) **PHYSICAL EXAM**

Was Physical Examination performed? ☐ **No** ☐ **Yes, Complete below**				
System	***Abnormal**	**Normal**	**Not done**	***If noted ABNORMAL, please provide brief description and comment if clinically significant or not (CS/NCS)**
General Appearance	☐	☐	☐	
Skin	☐	☐	☐	
Eyes, Ears, Nose & Throat	☐	☐	☐	
Head, Neck & Thyroid	☐	☐	☐	
Cardiovascular	☐	☐	☐	
Respiratory	☐	☐	☐	
Abdomen	☐	☐	☐	
Extremities	☐	☐	☐	
Muscular-Skeletal	☐	☐	☐	
Neurological	☐	☐	☐	
Others (please specify)	☐	☐	☐	

VISIT 1 (SCREENING) **VITAL SIGNS & ECG**

Were Vital Signs performed? (please follow the attached file SmartWearable Validation checklist)	☐ **No (comment below)** ☐ **Yes,** Complete below Comment*: _____	
Date of Vital Signs:	__ __ / __ __ __ / __ __ __ __ (DD / MMM / YYYY)	
Time of Vital Signs:	**Staring Time** _____:_____ **(HH:MM)**	**Closing Time** _____:_____ **(HH:MM)**
Blood Pressure has taken in which positions: supine/standing/seating SBP: _____ DBP_____		

Pulse: ____ ___ ___ beats/min **Tympanic Temperature:** ___ ___ . ___ **°C** **Oxygen Saturation (SpO2:** _____	Please insert all the vital signs parameters on the provided checklist protocol
Weight: ___ ___ ___ . ___ kg **Height:** ___ . ___ ___ m	

Was an ECG performed?	☐ **No (comment below)** ☐ **Yes,** Complete below Comment*: _____
Date & Time of ECG: (please follow the attached file SmartWearable Validation checklist)	__ __/ __ __ __/ __ __ __ __ (DD / MMM / YYYY) _____:_____ HH:MM
The ECG is:	☐ **Within normal limits** ☐ **Abnormal, NOT clinically significant** ☐ **Abnormal, clinically significant, please specify:** _____

VISIT 1 (SCREENING) **SCREENING CONCOMITANT MEDICATIONS**

Date of Assessment: __ __/ __ __ __/ __ __ __ __
(DD / MMM / YYYY)

Is the participant taken any concomitant medications at screening					☐ **No** ☐ **Yes,** Complete below		
Medication (Record Generic or trade name)	**Reason for use** (Medical History diagnosis or other reason, e.g. Prophylaxis)	Dose and units	**Frequ e-ncy**	**Route**	**Start Date** (DD/MMM/YYYY)	**Stop Date** (DD//MMM/YYY)	**Or tick if ongoing at Screening Visit**
1.					___/____/____	___/____/____	☐
2.					___/____/____	___/____/____	☐
3.					___/____/____	___/____/____	☐
4.					___/____/____	___/____/____	☐

5.					___/___/___	___/___/___	☐

VISIT 1 (SCREENING) SMOKING / ALCOHOL

Date of Assessment: __ __ / __ __ __ / __ __ __ __
(DD / MMM / YYYY)

Has the participant ever smoked? ☐ **No** ☐ **Yes,** Complete below	
☐ **Current Smoker**	**Participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___ **Smoked for ___ ___ months/years**
☐ **Former smoker**	**Smoked for ___ ___ months/years** **Date when smoking ceased: __ __ / __ __ __ / __ __ __ __** (DD / MMM / YYYY) **When smoking, participant's average daily use:** - Number of cigarettes : ___ ___ - Number of cigars : ____ ___ - Number of pipes : ___ ___

Participant's alcohol consumption (based on NIH, National Institute on Alcohol Abuse and Alcoholism)

Binge Consumption ☐	Moderate Consumption ☐	Heavy Consumption ☐	No Consumption ☐
5 or more alcoholic drinks for males or 4 or more alcoholic drinks for females on the same occasion (i.e., at the same time or within a couple of hours of each other) on at least 1 day in the past month.	no more than 3 drinks on any single day and no more than 7 drinks per week. For men, it is defined as no more than 4 drinks on any single day and no more than 14 drinks per week	binge drinking on 5 or more days in the past month	

Participant's personal exercise history

Do you do any vigorous-intensity sports, fitness or recreational (leisure) activities that cause large increases in breathing or heart rate like [running or football] for at least 10 minutes continuously?
☐ Yes ☐ No

In a typical week, on how many days do you do vigorous intensity sports, fitness or recreational (leisure) activities? Number of days _____

How much time do you spend doing vigorous-intensity sports, fitness, or recreational activities on a typical day? _____Hours or _____minutes

Do you do any moderate-intensity sports, fitness or recreational (leisure) activities that cause a small increase in breathing or heart rate such as brisk walking, [cycling, swimming, volleyball] for at least 10 minutes continuously?
☐ Yes ☐ No

In a typical week, on how many days do you do moderate intensity sports, fitness or recreational (leisure) activities? Number of days ___

How much time do you spend doing moderate-intensity sports, fitness or recreational (leisure) activities on a typical day? _____Hours : _____minutes

We provide an Annex to help you complete this section (Annex Exercise Intensity Levels)

TRIAL COMPLETION

Did participant complete the trial?	☐ **Yes,** Please provide **date of last visit**: __ __ / __ __ __ / 2 0 __ __ (DD / MMM / YYYY) ☐ **No,** Please provide **date of withdrawal** and complete below: __ __ / __ __ __ / 2 0 __ __ (DD / MMM / YYYY)

<u>Early Withdrawal:</u> please tick most appropriate reason for participant not completing the trial:

☐ **Adverse Events related:** please state related AE: _____
(add details to AE page)
☐ **Participant's decision, specify: _____**
☐ **Investigator's decision, specify: _____**
☐ **Sponsor's decision**
☐ **Lost to follow up**
☐ **Patient deceased**
☐ **Other, specify: _____**

NEWCASTLE PROTOCOL

Has the participant completed the protocol? ☐ **No** ☐ **Yes,** Complete below								
Date: DD/MMM/YYYY **Recording Start Time:** 00.00 hrs	**Body Temperatura**	**O_2 Saturation**	**Blood Pressure (SBP/DBP)**	**Heart Rate**	**Glucose Level mmol/L**	**Pain VAS 0-10**	**Borg Scale**	**Time**
Rest (20-30 min)								
0 min	__	__	__/__	___	___	_	__	
10 min	__	__	__/__	___		_	__	
20 min	__	__	__/__	___		_	__	
30 min	__	__	__/__	___		_	__	
Exercise (8-18 min)								
6 min	__	__	__/__	___		_	__	
Peak (min:sec)	__	__	__/__	___	___	_	__	
Recovery (10 min)	__	__	__/__	___		_		

Recording Stop time: 00.00 hrs ☐ **Please check box if this is the last page used**

PRINICIPAL INVESTIGATOR'S SIGN OFF	
Principal Investigator's Signature Statement:	
I have reviewed this CRF and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this participant. All entries were made either by me or by a person under my supervision who has signed the Delegation and Signature Log.	
Principal Clinical Investigator's Signature: _____ Principal Clinical Investigator's Name: _____	**Date of Signature:** __ __/ __ __ __/ __ __ __ __ (DD / MMM / YYYY)
ONCE SIGNED, NO FURTHER CHANGES CAN BE MADE TO THIS CRF WITHOUT A SIGNED DATA QUERY FORM.	

Appendix 8: Borg Rating of Perceived Exertion (RPE) Scale

Borg RPE	
Score	**Level of exertion**
6	**No exertion at all**
7	**Extremely light**
8	
9	**Very light**
10	
11	**Light**
12	
13	**Somewhat hard**
14	
15	**Hard (heavy)**
16	
17	**Very hard**
18	
19	**Extremely hard**
20	**Maximal exertion**

Appendix 9: Questionnaires

Minnesota Living With Heart Failure Questionnaire

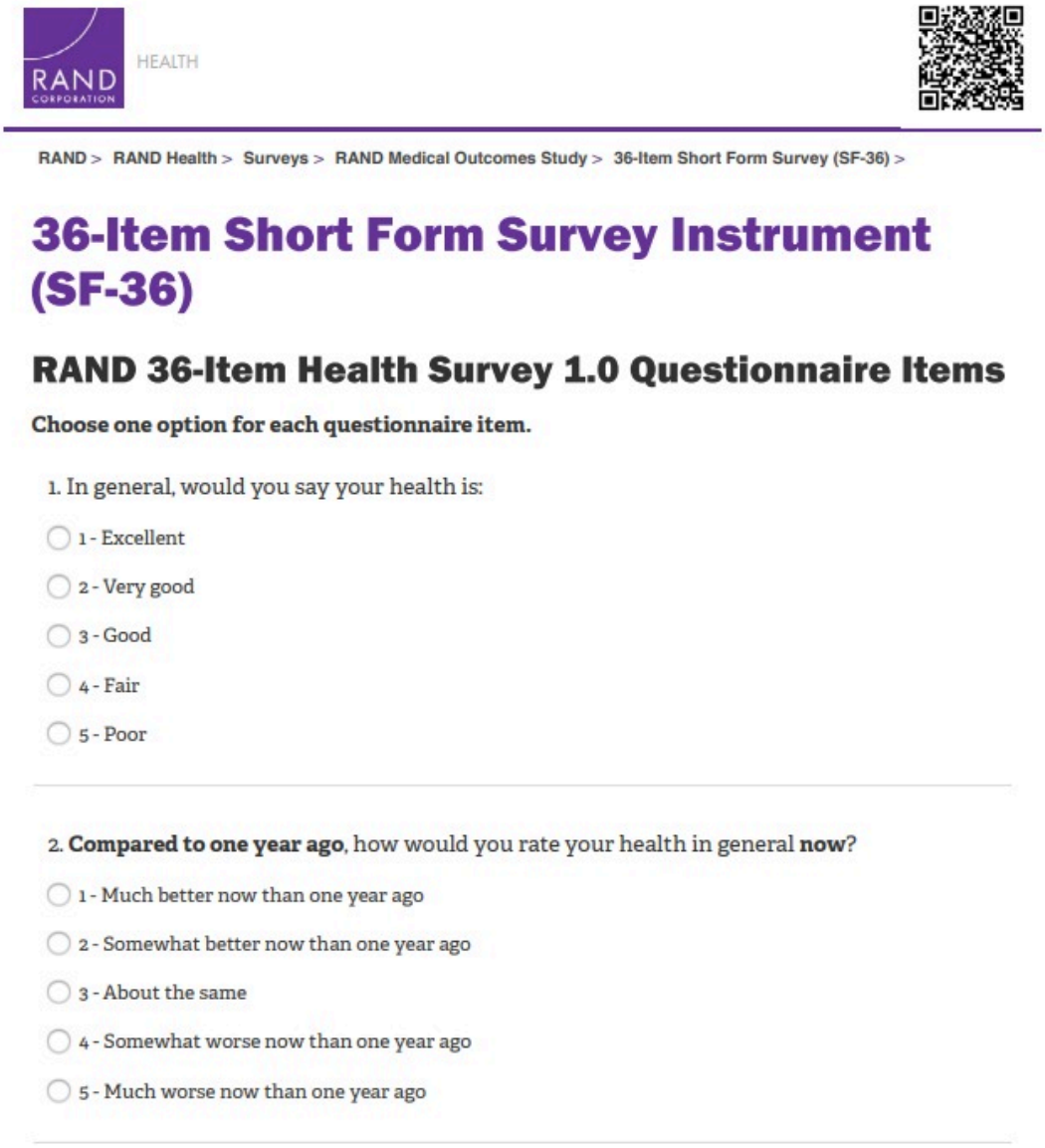
MINNESOTA LIVING WITH HEART FAILURE® QUESTIONNAIRE

The following questions ask how much your heart failure (heart condition) affected your life during the past month (4 weeks). After each question, circle the 0, 1, 2, 3, 4 or 5 to show how much your life was affected. If a question does not apply to you, circle the 0 after that question.

Did your heart failure prevent you from living as you wanted during the past month (4 weeks) by -	**No**			**Very Little**					**Very Much**
1. causing swelling in your ankles or legs?	0			1	2	3	4	5	
2. making you sit or lie down to rest during the day?	0			1	2	3	4	5	
3. making your walking about or climbing stairs difficult?	0			1	2	3	4	5	
4. making your working around the house or yard difficult?	0			1	2	3	4	5	
5. making your going places away from home difficult?	0			1	2	3	4	5	
6. making your sleeping well at night difficult?	0			1	2	3	4	5	
7. making your relating to or doing things with your friends or family difficult?	0			1	2	3	4	5	
8. making your working to earn a living difficult?	0			1	2	3	4	5	
9. making your recreational pastimes, sports or hobbies difficult?	0			1	2	3	4	5	
10. making your sexual activities difficult?	0			1	2	3	4	5	
11. making you eat less of the foods you like?	0			1	2	3	4	5	
12. making you short of breath?	0			1	2	3	4	5	
13. making you tired, fatigued, or low on energy?	0			1	2	3	4	5	
14. making you stay in a hospital?	0			1	2	3	4	5	
15. costing you money for medical care?	0			1	2	3	4	5	
16. giving you side effects from treatments?	0			1	2	3	4	5	
17. making you feel you are a burden to your family or friends?	0			1	2	3	4	5	
18. making you feel a loss of self-control in your life?	0			1	2	3	4	5	
19. making you worry?	0			1	2	3	4	5	
20. making it difficult for you to concentrate or remember things?	0			1	2	3	4	5	
21. making you feel depressed?	0			1	2	3	4	5	

11/10/04

36-Item Short Form Survey



RAND HEALTH

RAND > RAND Health > Surveys > RAND Medical Outcomes Study > 36-Item Short Form Survey (SF-36) >

36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- 1 - Excellent
- 2 - Very good
- 3 - Good
- 4 - Fair
- 5 - Poor

2. **Compared to one year ago**, how would you rate your health in general **now**?

- 1 - Much better now than one year ago
- 2 - Somewhat better now than one year ago
- 3 - About the same
- 4 - Somewhat worse now than one year ago
- 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
14. **Accomplished less** than you would like	○ 1	○ 2
15. Were limited in the **kind** of work or other activities	○ 1	○ 2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	○ 1	○ 2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
18. **Accomplished less** than you would like	○ 1	○ 2
19. Didn't do work or other activities as **carefully** as usual	○ 1	○ 2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- ○ 1 - Not at all
- ○ 2 - Slightly
- ○ 3 - Moderately
- ○ 4 - Quite a bit
- ○ 5 - Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

- ◯ 1 - None
- ◯ 2 - Very mild
- ◯ 3 - Mild
- ◯ 4 - Moderate
- ◯ 5 - Severe
- ◯ 6 - Very severe

---

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- ◯ 1 - Not at all
- ◯ 2 - A little bit
- ◯ 3 - Moderately
- ◯ 4 - Quite a bit
- ◯ 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
24. Have you been a very nervous person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
25. Have you felt so down in the dumps that nothing could cheer you up?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
26. Have you felt calm and peaceful?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
27. Did you have a lot of energy?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
28. Have you felt downhearted and blue?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
29. Did you feel worn out?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
30. Have you been a happy person?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6
31. Did you feel tired?	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

○ 1 - All of the time

○ 2 - Most of the time

○ 3 - Some of the time

○ 4 - A little of the time

○ 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	◯ 1	◯ 2	◯ 3	◯ 4	◯ 5
34. I am as healthy as anybody I know	◯ 1	◯ 2	◯ 3	◯ 4	◯ 5
35. I expect my health to get worse	◯ 1	◯ 2	◯ 3	◯ 4	◯ 5
36. My health is excellent	◯ 1	◯ 2	◯ 3	◯ 4	◯ 5

Hospital Anxiety and Depression Scale

Hospital Anxiety and Depression Scale (HADS)

Tick the box beside the reply that is closest to how you have been feeling in the past week.
Don't take too long over you replies: your immediate is best.

D	A		D	A	
		I feel tense or 'wound up':			**I feel as if I am slowed down:**
	3	Most of the time	3		Nearly all the time
	2	A lot of the time	2		Very often
	1	From time to time, occasionally	1		Sometimes
	0	Not at all	0		Not at all
		I still enjoy the things I used to enjoy:			**I get a sort of frightened feeling like 'butterflies' in the stomach:**
0		Definitely as much		0	Not at all
1		Not quite so much		1	Occasionally
2		Only a little		2	Quite Often
3		Hardly at all		3	Very Often
		I get a sort of frightened feeling as if something awful is about to happen:			**I have lost interest in my appearance:**
	3	Very definitely and quite badly	3		Definitely
	2	Yes, but not too badly	2		I don't take as much care as I should
	1	A little, but it doesn't worry me	1		I may not take quite as much care
	0	Not at all	0		I take just as much care as ever
		I can laugh and see the funny side of things:			**I feel restless as I have to be on the move:**
0		As much as I always could		3	Very much indeed
1		Not quite so much now		2	Quite a lot
2		Definitely not so much now		1	Not very much
3		Not at all		0	Not at all
		Worrying thoughts go through my mind:			**I look forward with enjoyment to things:**
	3	A great deal of the time	0		As much as I ever did
	2	A lot of the time	1		Rather less than I used to
	1	From time to time, but not too often	2		Definitely less than I used to
	0	Only occasionally	3		Hardly at all
		I feel cheerful:			**I get sudden feelings of panic:**
3		Not at all		3	Very often indeed
2		Not often		2	Quite often
1		Sometimes		1	Not very often
0		Most of the time		0	Not at all
		I can sit at ease and feel relaxed:			**I can enjoy a good book or radio or TV program:**
	0	Definitely	0		Often
	1	Usually	1		Sometimes
	2	Not Often	2		Not often
	3	Not at all	3		Very seldom

Please check you have answered all the questions

Scoring:
Total score: Depression (D) _____ Anxiety (A) _____
0-7 = Normal
8-10 = Borderline abnormal (borderline case)
11-21 = Abnormal (case)

Appendix 10: Telephone Record Sheet

Record Sheet

Patient ID _____ Researcher _____

Week of intervention _____ Today's date ____/____/____

Time call started _____ Time call finished_____ Duration _____

Self-monitoring

Steps each day (record day of week)

Day 1 _____ Day 2 _____ Day 3 _____ Day 4 _____

Day 5 _____ Day 6 _____ Day 7 _____

Goal setting

Agreed goal

Look at days of the week (weekday vs. weekend day). Active days?

Ideally, we would like you to achieve 2000 steps more that what you would do normally each day

How do you feel about this target? Achievable? Experience any problems? Positive reinforcement (i.e. any increase is positive, but how do you think you could increase further?).

Reflect upon baseline. May need to reassess goal, record new goal

Agreed goal: Try where possible to record the t**ype of activity** undertaken, **how much** (number of steps/minutes) where, **how often** and **with who.** Encouraging this level of detail will increase the likelihood that the goal is reached.

Reassessment of goal: Check if the person is happy with what they are **currently achieving** (if reaching their target for instance) and **ask would you like to make any changes to your goal?** They might say 'no, I'm happy with it as it is' close by saying something like, that's great, its working really well for you.

Barrier identification and problem solving

Barriers to increasing activity? Friends/Family?

Can you think of a solution? Get patient to think about this rather than providing the answer
You may want to go back over this in the follow up calls

Problem solving: If they don't identify any solutions to problems, and you struggle to help them, simply ask them to have a **think about how they could overcome those problems for next time.**

Prompt review of behavioural goals

Pedometer information
Calculate if patient is meeting targets

Are any changes needed?

Goals: To increase self-efficacy comment on **how close a person is to their goal even if they aren't reaching it** – i.e. they've made progress from last time – how could they increase further next time?

Does the person want to **amend their goal to attain a higher target** when things are going well, or a more realistic target when they are having trouble reaching their previous goal.

Prompt focus on past success

Patient to reflect

Increased levels of activity through cardiac rehab? Refer back to cardiac rehab techniques to increasing levels of activity.
When I was succeeding... I can do it again

Success: Use this to reassure participants they can succeed again.

Planning social support

Patient planning their future physical activity/exercise

Will this involve partner/family/friends/community support?
Emotional support of others?

Summary

Go through agreed action points
Make sure patient has understood – reflected upon issues
Patient has agreed to the summarised points

As well as making sure the patient has understood, **summarise to check with the patient that you have understood**. By briefly summarising what has been agreed, you show that you have **listened**. Use phases like, 'you said you weren't a fan of XXX activity, therefore you're going to give XXX a go instead. Have I got that right?

Agreed date and time of next phone call

Appendix 11: Activity Planner

Activity Planner

Planner start date:

DAY	GOAL Minutes / Steps / Other	ACTIVITY When? Where? Who with? How long for?	ACHIEVED Minutes / Steps / Other
MONDAY			
TUESDAY			
WEDNESDAY			
THURSDAY			
FRIDAY			
SATURDAY			
SUNDAY			