Heart Rate Variability in Ageing and Hypertrophic Cardiomyopathy

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Declaration

This thesis is submitted for the degree of Doctor of Philosophy at Newcastle University. I, Alaa Alyahya, declare that data and all other information presented in this thesis is the result of my own original research. I confirm that work done by others is clearly acknowledged and any prior published work is referenced. I certify that this thesis contains no material that has been submitted for any other academic degree and published material presented was the result of the research carried out during the present doctoral study.

Abstract

Heart rate variability (HRV) reflects the interaction between sympathetic and parasympathetic components of the autonomic nervous system. It is an indicator of cardiovascular function in normal and pathophysiological conditions. Hypertrophic cardiomyopathy (HCM) is the most common genetic heart disease caused by a single mutation in one of the sarcomeric protein genes, leading to cardiac hypertrophy and predominantly affecting the interventricular septum. Autonomic dysfunction is common in individuals with HCM and could lead to potentially life-threatening arrhythmias and sudden cardiac death. There is limited evidence about effects of lifestyle interventions on HRV in individuals with HCM.

The present thesis firstly, investigated the effect of age and sex on HRV measures and functional capacity in healthy individuals. Secondly, it assessed HRV measures and cardiometabolic function in individuals with HCM. Finally, it evaluated the effect of a novel home-based lifestyle intervention incorporating physical activity and dietary nitrate supplementation on HRV measures and functional capacity in individuals with HCM.

The major findings of this thesis can be summarized as the following three points. Firstly, among several time- and frequency-domain measures of HRV, it appears that the mean RR interval is the only measure influenced by sex. Data showed that there was not any effect of age on HRV measures. Secondly, vagal indices of HRV are increased in individuals with HCM compared to healthy individuals. Thirdly, the lifestyle intervention incorporating physical activity and dietary nitrate supplementation improved parasympathetic measures of HRV and mean arterial blood pressure in individuals with HCM. The research contained in this thesis is important as it improves understanding of the pathophysiology and its malleability with lifestyle intervention in individuals with HCM.

Dedication

This thesis is dedicated to my mother, Eman Altalib, my brother Abdulrahman Alyahya and my dearest friend Altaf Alramyan.

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First and foremost, I would like to thank Almighty Allah for giving me strength and encouragement throughout all the challenging moments of completing this thesis. I surely will be lost without Allah's guidance, mercy and grace.

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List of abbreviations

ACC/AHA	American College of Cardiology/American Heart Association
ACE	Angiotensin-converting enzyme
ACEI	Angiotensin-converting enzyme inhibitor
AF	Atrial fibrillation
ANS	Autonomic nervous system
ARB	Angiotensin receptor blocker
BMI	Body mass index
BP	Blood pressure
BPM	Beats per minute
BSA	Body surface area
CABG	Coronary artery bypass graft/grafting
CAD	Coronary artery disease
CCB	Calcium-channel blocker
CCV_{HF}	Coefficient of component variance of high frequency power
CI	Cardiac index
CMR	Cardiac magnetic resonance
CO	Cardiac output
CPO	Cardiac power output
DCM	Dilated cardiomyopathy
ECG	Electrocardiogram/ Electrocardiography
ESC	European society of cardiology
EF	Ejection fraction
HCM	Hypertrophic cardiomyopathy
HF	Heart failure
HF power	High frequency power
HFpEF	HF with preserved ejection fraction
HFrEF	HF with reduced ejection fraction
HR	Heart rate
HRV	Heart rate variability
ICD	Implantable cardioverter-defibrillator
IHD	Ischaemic heart disease
LF power	Low frequency power
LF:HF	low to high frequency power

LV	Left ventricular/left ventricle
LVEF	Left ventricular ejection fraction
LVOT	Left ventricular outflow tract
MABP	Mean arterial blood pressure
NYHA	New York Heart Association
Peak VO_2	Peak oxygen uptake
PNS	Parasympathetic nervous system
QRS	Q, R, and S waves (combination of three of the graphical deflections)
RAAS	Renin–angiotensin–aldosterone system
RCT	Randomized controlled trial
SBP	Systolic blood pressure
SCD	Sudden cardiac death
SDRR	Standard deviation of RR interval
SV	Stroke volume
SNS	Sympathetic Nervous System
TPR	Total peripheral index
VT	Ventricular tachycardia

List of publications and presentations from the thesis

Manuscripts – published

AI. Alyahya, SJ. Charman, NC. Okwose, AS. Fuller, C. Eggett, P. Luke, K. Bailey, GA. MacGowan, DG. Jakovljevic. Heart rate variability and haemodynamic function in individuals with hypertrophic cardiomyopathy. *Clinical Physiology and Functional Imaging*. Published 18 June 2023. **https://doi.org/10.1111/cpf.12840** .Accepted manuscript is in appendix K.

Manuscripts – under review

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Manuscripts – in preparation

AI. Alyahya, SJ. Charman, NC. Okwose, AS. Fuller, C. Eggett, P. Luke, K. Bailey, GA. MacGowan, DG. Jakovljevic. Effect of a novel lifestyle intervention on heart rate variability in individuals with hypertrophic cardiomyopathy. It will be submitted for *European Journal of Applied Physiology*.

Abstract- presented at international conferences and published in proceedings

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Alyahya A, Charman SJ, Okwose NC, Fuller A, Eggett C, Luke P, Bailey K, MacGowan GA, Jakovljevic DG. Differences in heart rate variability and haemodynamic function between patients with hypertrophic cardiomyopathy and healthy controls. In *European Journal of Heart Failure* 2022 Jul 1 (Vol. 24, pp. 29-29). 111 River ST, Hoboken 07030-5774, NJ USA: Wiley. Presented in Madrid, Spain on 21 May 2022 during the Heart Failure 2022 Congress.

Alyahya A, Charman SJ, Okwose NC, Fuller A, Eggett C, Luke P, Bailey K, MacGowan GA, Jakovljevic DG. The effect of a novel lifestyle intervention on autonomic and haemodynamic function in individuals with hypertrophic cardiomyopathy. *European Journal of Preventive*

Cardiology. Presented in Malaga, Spain on 14 April 2023 during the ESC Preventive Cardiology 2023.

Chapter 1. Impact of COVID-19 pandemic on the research studies

The present thesis has been negatively impacted by the COVID-19 pandemic causing a significant loss in time from the clinic and data collection. One of the aims of my PhD thesis was to assess the reproducibility of heart rate variability (HRV) measures at rest and during exercise in healthy individuals. From October until November 2019, preparation for the healthy prospective study was made including learning about HRV measures, calculating sample size, writing up protocol, applying for research passport and ethical approval. On 6th March 2020, the study commenced, and I completed data collection for the first healthy volunteer. Alongside preparation for the reproducibility of HRV measures in healthy study, I collected data from individuals with hypertrophic cardiomyopathy studies (study two and three of the present thesis). Due to COVID-19 pandemic and national lockdown in the United Kingdom (UK), Newcastle University was shut and all clinical studies were placed on hold from 16th March 2020. After receiving email from the administration of Newcastle University advising students to return home if possible and the Saudi Arabian government announcing that its residents living outside the country will be evacuated and flown back to Saudi Arabia, I decided to go back home to Saudi Arabia. However, on my journey home, on 23 March 2020, I underwent quarantine for one month in hotels in Bahrain and in Saudi until I met my family on 24 April 2020. During the quarantine time in hotels and before meeting my mom and brother, I was lonely and uncertain about my PhD aims.

I was not able to collect data and return to Newcastle because of two reasons: firstly, Saudi Arabia placed all international flights on hold. Secondly, there were two more additional lockdowns in the UK (Lockdown 2 (5th November – 2nd December) and lockdown 3 (4th January- 8th March 2021). During that time, I was in contact with my supervisors, Prof. Djordje Jakovljevic, Dr. Sarah Charman, and Dr. Nduka Okwose via email and Zoom meetings who were thankfully guiding and supporting me. I also wrote up my literature review, submitted my first year PhD progress report on 19 June 2020 and met with my PhD progression review assessors Prof. Chris Ward and Prof. Tom Hill who thankfully suggested to meet my supervisory team and find alternative objectives due to uncertainties about resuming data collection of the clinical studies in Newcastle. Therefore, after meeting with my supervision team we agreed on working on the influence of age and sex on HRV measures from data collected previously.

After nearly one year and three months of suspension and of being home, the clinical studies resumed on 11th May 2021, but the Saudi Arabian government allowed international flights on 17 May 2021. When I came back to Newcastle, I was subject to self-quarantine for 10 days, which meant I could not immediately resume the data collection. I restarted collecting data for the hypertrophic cardiomyopathy study from 1st June 2021 until 31st January 2022. During data

collection, the clinical research facility of the Royal Victoria Infirmary allowed us to recruit only one patient per week and to follow strict safety measures to avoid COVID-19 in this high-risk group of patients.

Chapter 2. Introduction and literature review

2.0 Introduction to thesis

This thesis is divided into eight chapters. Chapter one describes the impact of COVID-19 pandemic on my research programme and studies. Chapter two provides an overview of the thesis and a review of existing literature related to hypertrophic cardiomyopathy (HCM) and heart rate variability (HRV), including up-to-date evidence regarding lifestyle interventions as well as HRV in HCM. Chapters three and four describe the aims, hypotheses and main methodologies used in the thesis. Chapter five assesses the effect of age and sex on HRV measures and cardiometabolic variables and investigates the relationship between HRV measures and peak exercise cardiac function and functional capacity in healthy individuals. Chapter six evaluates the differences in HRV and haemodynamic variables between individuals with HCM and healthy controls and the relationship between these variables is examined in the individuals with HCM. Chapter seven evaluates the effect of a novel lifestyle intervention on HRV, haemodynamic and cardiopulmonary exercise testing performance in individuals with HCM. Chapter eight summarises the main thesis findings, highlights limitations and provides recommendations for future HRV research.

2.1 Hypertrophic cardiomyopathy

2.1.1 Definition and Epidemiology

Sixty years ago, hypertrophic cardiomyopathy (HCM) was called idiopathic hypertrophic subaortic stenosis by Braunwald group who made a comprehensive clinical description of the pathology based on 64 examined patients (Braunwald *et al.*, 1964). Since then, the condition has become well known as HCM resulting from an improved understanding of the disease complexity, heterogeneity and genetic involvement (Gersh *et al.*, 2011). According to Ommen *et al.* (2020), HCM refers to a predominant left ventricular hypertrophy (LVH) in the absence of another cardiac, systemic or metabolic disorder and for which a sarcomere-related variant is designated or genetic test is undetermined.

HCM is the most common heritable cardiovascular disease, affecting approximately 1:200-1:500 (20 million) people worldwide (Maron, 2018b) (Figure 2.1). In the United Kingdom, the recent reported prevalence of HCM is 1:517 affected individuals (0.19%) (de Marvao *et al.*, 2021). HCM is predominant in men than women with a ratio of 3:2 but women are usually diagnosed at an older age and are likely to be symptomatic later in life compared with men (Olivotto *et al.*, 2005; Canepa *et al.*, 2020). Despite the high estimated prevalence of HCM in the general population, only 10-20% of the cases are diagnosed (Maron *et al.*, 2016a; Maron *et al.*, 2022b). The reasons for under diagnosis of HCM are asymptomatic individuals, subtle clinical expression of the disease phenotype, lack of experience with HCM and insufficient diagnostic modalities (Maron *et al.*, 2018).

Figure 2.1 Epidemiology of hypertrophic cardiomyopathy. The disease affects approximately 20 million people worldwide (distribution of the disease is shown in red). Adapted from (Maron *et al.*, 2018).

2.1.2 Aetiology

Among individuals with HCM, approximately 30-60% are identified with a pathogenic variant, whilst the majority are without any evidence of genetic aetiology including a subgroup who have no familial HCM (Ingles *et al.*, 2017; Ommen *et al.*, 2020). The most common pathogenic variant occurs in beta myosin heavy chain 7 (MYH7) and myosin-binding protein C3 (MYBPC3), identified in 70% of variant-positive individuals, while other genes (TNNI3, TNNT2, TPM1, MYL2, MYL3, ACTC1) each account for 1-5% of individuals with HCM (Ommen *et al.*, 2020). Mutant sarcomere genes stimulate myocardial hypertrophy and fibrosis which result in systolic and diastolic heart failure (HF) (Ommen *et al.*, 2020). However, the diverse disease features including mitral valve abnormalities and myocardial bridging appear to be not only related to the sarcomere variants since the exact mechanisms by which the sarcomeric variants lead to the clinical expression have not been fully clarified yet (Ommen *et al.*, 2020).

2.1.3 Mortality rate

The mortality rate in HCM is influenced by age and sex, increasing with advancing age and peaking earlier in women than men (Östman-Smith *et al.*, 1999). Between 1999 and 2019, the reported HCM-related deaths were 39,200 in the United states (Minhas *et al.*, 2022). Around 30-40% of individuals with HCM experience complications such as sudden cardiac death (SCD), limiting symptoms of left ventricular outflow tract obstruction (LVOTO), diastolic dysfunction, HF with systolic impairment and atrial fibrillation (AF) with risk of thromboembolic events, all of which contribute to mortality in HCM (Ommen *et al.*, 2020). Nonetheless, the development of contemporary cardiovascular interventions such as the evolution of the SCD risk stratification scheme has lowered the mortality rate by more than 10-fold from 6% to 0.5% annually worldwide (Maron, 2018b; Maron *et al.*, 2019).

2.1.4 Symptoms and Signs

Approximately 80% of individuals with HCM are asymptomatic and have a normal lifespan due to the broad spectrum of phenotypic expression of the disease (Ommen *et al.*, 2020). Symptoms and signs of HCM includes chest pain (angina), dyspnoea, palpitation, arrhythmias, and syncope (Zamorano *et al.*, 2014).

In individuals with HCM, chest pain and dyspnoea may occur at rest or during exercise and they are often due to LVOTO, mitral regurgitation (MR), ischemia and increased left ventricular (LV) wall tension (Gersh *et al.*, 2011). Syncope is caused by hypovolaemia, complete heart block, sinus node dysfunction, ventricular tachycardia (VT), LVOTO and abnormal vascular reflexes (Counihan *et al.*, 1991; Prasad *et al.*, 2008). The symptoms and signs of HF are common in individuals with HCM, yet may vary in functional expression between individuals due to differences in LV ejection fraction (Melacini *et al.*, 2010).

The most common sign in individuals with HCM is cardiac remodelling which is characterised by myocardial fibrosis, impaired LV diastolic and systolic function, LV dilation and wall thinning (Melacini *et al.*, 2010). Since symptoms and signs of HCM vary among individuals, it is important to assess every individual comprehensively to inform the most appropriate therapy.

2.1.5 Pathophysiology

The pathophysiology of HCM is complex and composed of multiple linked factors which are all related to the hypertrophied myocardium as the cardinal manifestation of HCM. These factors include: LVOTO, diastolic dysfunction, MR, myocardial ischemia and autonomic dysfunction (Ommen *et al.*, 2020).

2.1.5.1 Left ventricular outflow tract obstruction

Left ventricular outflow tract obstruction (LVOTO) accounts for approximately 75% of individuals with HCM which presents at either rest or with provocation such as exercise, Valsalva strain manoeuvre, or amyl nitrate inhalation (Maron *et al.*, 2006; Ommen *et al.*, 2020). The diagnosis of LVOTO is made by echocardiography in the presence of peak LVOT gradient of ≥ 30 mmHg at rest or ≥ 50 mmHg at provocation (Ommen *et al.*, 2020). Generally, the presence of peak LVOT gradient of ≥ 50 mm Hg, either at rest or with provocation is the cut-off point for surgical invasive septal reduction therapy or percutaneous alcohol septal ablation in individuals who have drug- refractory symptoms (Ommen *et al.*, 2020). The LVOTO is attributed by two main mechanisms: 1) anatomical hypertrophied basal segment of the ventricular septum leads to abnormal blood flow direction that displaces the mitral leaflets anteriorly "known as systolic anterior motion abnormality (SAM)" (Figure 2.2); and 2) anatomical deformation in mitral valve apparatus including elongation of leaflets, leading to abnormal coaptation of the leaflets and abnormal anterior insertion of papillary muscle which results in MR (Ommen *et al.*, 2020). Consequently, the LV systolic pressure is increased, LVH is exacerbated, myocardial ischemia and prolonged ventricular relaxation time occurr (Sherrid *et al.*, 2000; Patel *et al.*, 2015). LVOTO is associated with low cardiac output, increased risk of HF and poor survival (Maron *et al.*, 2003; Sorajja *et al.*, 2009).

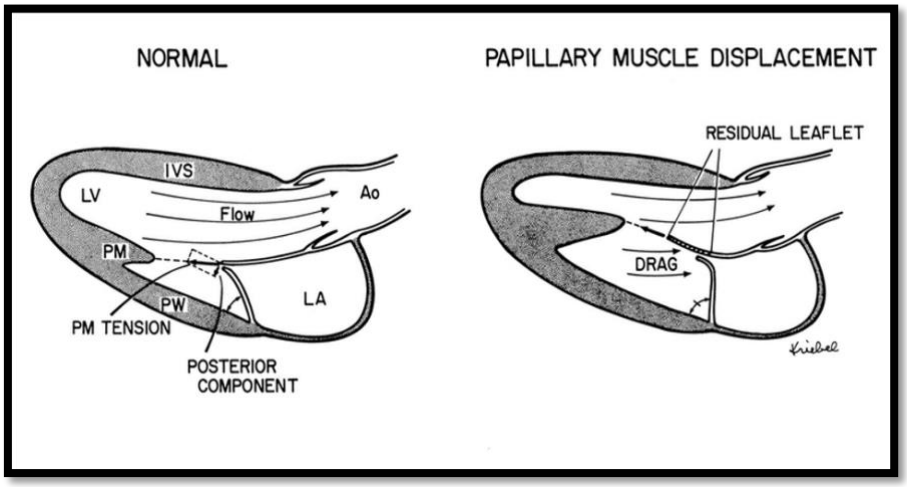

Figure 2.2 Systolic Anterior Motion of Mitral Valve (SAM). Ao: Aorta, IVS: interventricular septum, LA: left atrium, PM: papillary muscle, PW: posterior wall. Adapted from (Sen-Chowdhry *et al.*, 2016).

2.1.5.2 Diastolic dysfunction

Diastolic dysfunction in individuals with HCM could be related to nonuniform ventricular contraction and relaxation, abnormal intracellular calcium uptake, chamber stiffness which is caused by myocardial ischemia, severe myocardial hypertrophy and increased LV contraction caused by LVOTO (Paulus *et al.*, 1983; Soullier *et al.*, 2012; Villemain *et al.*, 2019). Alteration in systolic-diastolic coupling and impaired cardiac cellular oxygen uptake also contribute to reduced exercise capacity in individuals with HCM, leading to poor prognosis (Soullier *et al.*, 2012; Desai *et al.*, 2014; Dass *et al.*, 2015). A variety of echocardiographic measurement methods can be used to estimate LV filling pressure. However, the most common used method to evaluate diastolic function is the ratio between early mitral inflow velocity and mitral annular early diastolic velocity (E/e') (Park and Marwick, 2011).

2.1.5.3 Mitral regurgitation

Mitral regurgitation (MR) can occur in HCM either due to LVOTO or leaflets abnormalities and result in dyspnoea (Ommen *et al.*, 2020). In MR related to LVOTO, the SAM is the main mechanism of MR which leads to incomplete leaflets coaptation resulting in abnormal back flow of blood during mid-to-late systolic time (Hang *et al.*, 2019). Since the factors that influence LVOTO may also influence the degree of MR, the severity of MR will be evident by provocation for LVOTO and SAM of the mitral valve (Ommen *et al.*, 2020).

2.1.5.4 Myocardial ischemia

Myocardial ischemia is a common abnormality in HCM. Myocardial hypoperfusion in individuals with HCM occurs due to imbalance between myocardial oxygen supply and demand which is related to thick myocardial wall (Ommen *et al.*, 2020). Ischemia in HCM could also

be secondary to loading conditions such as MR, microvascular dysfunction, and myocardial bridging (Maron *et al.*, 2009b; Zamorano *et al.*, 2014; Hostiuc *et al.*, 2017).

2.1.5.5 Autonomic dysfunction

Cardiac autonomic function may be dysregulated in individuals with HCM, which could be evident by impaired heart rate recovery and inappropriate vasodilatation (Frenneaux *et al.*, 1990; Sadoul *et al.*, 1997; Olivotto *et al.*, 1999; Patel *et al.*, 2014b). Approximately 25% of individuals with HCM have an abnormal exercise vasodilatory response (Frenneaux *et al.*, 1990). The abnormal exercise response in individuals with HCM is defined as either failure to increase systolic blood pressure (SBP) by >20mmHg or a drop in SBP whilst exercising by >20mmHg (Ommen *et al.*, 2020). The presence of this abnormal mechanism is associated with poor prognosis and it could be secondary to autonomic dysregulation, LVOTO, abnormal diastolic filling or systemic vasodilation (Sadoul *et al.*, 1997; Olivotto *et al.*, 1999).

2.1.6 Diagnosis

The clinical diagnosis of HCM is mainly based on the increased LV end diastolic wall thickness by ≥15 mm in one or more myocardial segments in the absence of another cause of hypertrophy which can be established by imaging modalities such as echocardiography or cardiovascular magnetic resonance (CMR) imaging (Maron, 2018b; Ommen *et al.*, 2020). Minimal wall thickness of 13-14 mm can be also diagnostic of HCM in the presence of positive family history of HCM or in conjunction with a positive genetic testing (Ommen *et al.*, 2020). The disease is also composed of abnormalities which are not diagnostic of HCM but can be related to the phenotypic expression of the HCM such as myocardial fibrosis, mitral valve apparatus morphologic changes, right ventricular hypertrophy, electrocardiographic abnormalities, abnormal coronary microcirculation and myocyte disarray (Zamorano *et al.*, 2014). There are other conditions which can cause secondary hypertrophy, leading to confusion with HCM such as myocardial remodelling secondary to training (athletic heart), chronic systemic hypertension, valvular or sub valvular stenosis and stress induced cardiomyopathy (Keegan *et al.*, 2000; Sherrid *et al.*, 2020). Therefore, several testing approaches are used to differentiate between HCM and conditions that mimic HCM. Testing approaches include family history and genetic testing, electrocardiography (ECG), echocardiography and CMR imaging (Ommen *et al.*, 2020).

2.1.6.1 Family history and genetic testing

The individual's family history contributes essentially to diagnostic possibility of HCM especially in a family with a history of heart disease at young age (Wigle, 2001). An assessment of family history includes determination of a family tree to establish a genetic source of HCM and other family members who are predisposed to HCM (Zamorano *et al.*, 2014). The main

emphasis of pedigree analysis is the adverse events and the age of the presentation of SCD, HF, stroke, heart transplantation and the pattern of inheritance (Zamorano *et al.*, 2014).

The genetic testing is another useful diagnostic tool in prenatal cases, doubtful cases and in verifying high risk cases and it should be applied as per family rather than per one person because HCM may be seen in 50% of offspring (Zamorano *et al.*, 2014). Genetic testing should be used alongside clinical examinination and it is likely to be positive in young adults below 45 years of age with maximal LV thickness > 20mm, family history of HCM and SCD, absence of systemic hypertension (Bos *et al.*, 2014). Indeed, genetic testing is particularly useful in case of phenotypic overlaps between individuals with HCM and athletes, in individuals of Black African heritage and in hypertensive individuals (Jacoby *et al.*, 2013).

2.1.6.2 Electrocardiography

ECG is one of the most recommended initial tests for known or suspected individuals with HCM at rest and during exercise and to monitor progressive changes of symptoms in already diagnosed individuals with HCM (Gersh *et al.*, 2011). Since approximately 75% to 95% of individuals with HCM have abnormal ECG findings (Localised or diffused T- wave inversion and LVH criteria of Sokolow Lyon index of S or R wave >35mm on V1 +V5 or V6), recent guidelines have recommended 24 hour ambulatory ECG monitoring to detect supra ventricular arrhythmias, VT and to identify candidates for implantable cardioverter defibrillator (ICD) therapy (Antikainen *et al.*, 2006; Ommen *et al.*, 2020).

2.1.6.3 Echocardiography

Transthoracic echocardiography (TTE) is an imaging modality used mainly to assess LV wall thickness and function and it remains the gold standard imaging modality to diagnose individuals with HCM (Geske *et al.*, 2018). TTE includes an assessment of severity and distribution of LVH, LVOT pressure gradient, velocity and mechanism of obstruction, mitral valve morphology and haemodynamic (Ommen *et al.*, 2020). Echocardiography is recommended as an initial assessment in all suspicious and diagnosed individuals with HCM, during the family screening, and it as a repeated screening every 12-18 months for HCM offspring (Gersh *et al.*, 2011).

2.1.6.4 Cardiac Magnetic Resonance Imaging

Cardiac Magnetic Resonance (CMR) imaging provides detailed diagnostic information about cardiac structure and function and it can identify cardiac tissue in individuals with HCM (Rickers *et al.*, 2005). CMR imaging is indicated in suspected individuals with HCM when findings of echocardiography are inadequate for definite diagnosis, to obtain additional

information such as the degree and pattern of LVH and to rule out other differential diagnosis (Rickers *et al.*, 2005).

2.1.7 Management

There is no convincing evidence to date of any treatment that can alter the natural history of the HCM (Ommen *et al.*, 2020). The principal goal of the management of HCM is to alleviate symptoms and prevent disease progression pathways such HF, SCD and ventricular arrhythmias. The management of HCM follows either pharmacological or none-pharmacological approach.

2.1.7.1 Pharmacological therapy

Pharmacological therapy is the most commonly used approach for individuals with HCM, particularly individuals with LVOTO (Elliott *et al.*, 2001). Since the outflow obstruction is significantly varied throughout daily life, the success of medications is related to symptoms response but not to the measured gradient (Ommen *et al.*, 2020). The standard approach for pharmacological therapy is the combination of three types of medication: Beta adrenergic receptors blocker (BB), calcium channel blocker (CCB) and antiarrhythmic drugs (Zamorano *et al.*, 2014).

Beta blockers

Generally, non-vasodilating Beta adrenergic antagonist agents (BBs) are the first line treatment for individuals with HCM (Ommen *et al.*, 2020). BBs reduce LVOT gradient at rest and during exercise, they also improve symptoms of angina, breathlessness, palpitations and syncope (Fifer and Vlahakes, 2008). Propranolol was shown to significantly reduce LVOT gradient under rest and provocation and alleviate symptoms (Stenson *et al.*, 1973). Also, Sotalol is effective in inhibiting supraventricular arrhythmias and improving exercise capacity in individuals with HCM (Tendera *et al.*, 1993).

Calcium channel blockers

Calcium channel blockers (CCBs) are also promising in managing symptoms of obstructive HCM and could be used when BBs are contraindicated (Zamorano *et al.*, 2014). The most commonly prescribed CCB is Verapamil, which decreases LVOT pressure gradient and improves LV diastolic function (Zamorano *et al.*, 2014). CCBs have beneficial therapeutic effects against microvascular coronary artery disease (Frey *et al.*, 2011). However, care should be taken when prescribing vasodilatory CCBs because they might exacerbate LVOTO and result in hypotension, pulmonary edema and increase risk of mortality in severe LVOT gradient (Gersh *et al.*, 2011).

Antiarrhythmic drugs

Disopyramide which is a class IA anti-arrhythmic agent and is most frequently prescribed in combination with either BBs or CCBs to improve symptoms and prognosis in individuals with HCM (Zamorano *et al.*, 2014). Disopyramide has negative inotropic effect, leading to lower LVOT pressure gradient, alleviate HF symptoms and improve exercise tolerance and functional capacity (Sherrid *et al.*, 2005; Sherrid *et al.*, 2013). The dose of Disopyramide should be adjusted gradually and with intensive monitoring as it has anti-cholinergic side effects such as dry mouth and constipation (Sherrid *et al.*, 2005; Sherrid *et al.*, 2013). Disopyramide should be used with caution in individuals with HCM who have AF because of its potential augmentation of atrioventricular conduction, which can increase ventricular rate and cause VT or ventricular fibrillation (VF) (Adler *et al.*, 2017).

2.1.7.2 Non-pharmacological therapy

The use of non-pharmacological treatment is an uncommon approach in individuals with HCM, indicated in only approximately 5% of individuals with obstructive HCM. It mainly targets recurrent symptoms of LVOTO despite medications (Ommen *et al.*, 2020). Other non-pharmacological therapy includes: surgical myectomy, alcohol septal ablation and dual chamber pacing (Enriquez and Goldman, 2014).

Surgical myectomy

Surgical myectomy is an invasive procedure which repairs the valvular and sub valvular abnormalities and therefore relieves symptoms of LVOTO (Brown and Schaff, 2008). Surgical myectomy is safe with 1% perioperative mortality rate (Ommen *et al.*, 2005). The most common complication associated with surgical myectomy is ventricular septal defect (VSD), which occurs mostly in individuals with HCM who have mild septal hypertrophy (Xin *et al.*, 2001). Surgical myectomy has been associated with long-term favourable prognosis involving 90-95% absence of LVOT gradient, substantial reduction in symptoms of HF and improvement of MR (Sigwart, 1995).

Alcohol septal ablation

Septal ablation was introduced in 1994 and refers to an interventional, transcatheter procedure in which the ventricular septum is indirectly destroyed using ethanol (Sigwart, 1995). The procedure is performed as part of coronary angiography by injecting ethanol into proximal large septal perforators, causing an infarction of septum and therefore reduces LVOT pressure gradient, controls symptoms and improves survival rate (Park *et al.*, 2002; Van Dockum *et al.*, 2005). The procedure may be complicated by death (1-4%), ventricular arrhythmias (5%) and heart block (10-20%) (Enriquez and Goldman, 2014).

Dual chamber pacing

Dual chamber pacing was introduced as an alternative to septal myectomy for individuals with HCM who are unsuitable or unwilling to consider surgical myectomy (Zamorano *et al.*, 2014). Dual chamber pacing involves permanent sequential pacing with programmed short atrioventricular (AV) intervals (Nishimura *et al.*, 1997). Atrial synchronous pacing at an AV delay of 60 milli seconds, has been shown to reduce the LVOT gradient by 5mmHg (Nishimura *et al.*, 1997). Therefore, subaortic gradient and symptoms of HF might be improved by two mechanisms (Maron *et al.*, 2009a). The acute mechanism of the reduction in LVOT gradient is attributable to the altered ventricular activation time which decreases the projection of the basal segment of ventricular septum toward the LVOT whilst the long-term mechanism is related to improvement in the LV remodeling (Nishimura *et al.*, 1997). There are numerous observational studies and clinical trials which have reported a significant reduction in the LVOT gradient, improvement of symptoms and quality of life as main outcomes for dual chamber pacing in individuals with HCM (Slade *et al.*, 1996; Kappenberger *et al.*, 1997; Nishimura *et al.*, 1997; Maron *et al.*, 1999; Mickelsen *et al.*, 2004).

Implantable cardioverter de defibrillator

Implantable cardioverter defibrillator (ICD) is a device which is designed to detect heart rhythm and releases shock when it recognizes any lethal rhythms (Maron *et al.*, 2022a). SCD is the most common risk factor for considering ICD therapy in adults with HCM (Elliott *et al.*, 2014; Ommen *et al.*, 2020). ICD is indicated in 3-4% of HCM cases annually as a primary prevention strategy of sustained VT and VF and in 10% of HCM cases per year as secondary prevention (after resuscitated cardiac arrest) (Maron, 2018b; Maron *et al.*, 2021). The most recent HCM risk stratification criteria for considering ICD implantation includes family history of SCD, severe LVH $\geq$ 30 mm, unexplained recent syncope, non-sustained VT, evidence of LV fibrosis by CMR imaging, and end-stage HF with ejection fraction <50% and LV apical aneurysm (Maron *et al.*, 2019; Ommen *et al.*, 2020).

Heart transplantation

Heart transplantation is indicated for individuals with end-stage HF (with or without impaired systolic function) in the absence of other therapeutic options (Maron *et al.*, 2021; Maron *et al.*, 2022a). Although transplant has been shown to improve quality of life and longevity of individuals with HCM (Kato *et al.*, 2012; Maron *et al.*, 2016b; Rowin *et al.*, 2020), it is limited by donor availability, the need for immunosuppressants and the reliance on peak oxygen consumption values for transplant candidacy (Maron *et al.*, 2022a). The reported survival rate post-transplantation for individuals with HCM is 85%, 75% and 61% at 1, 5, and 10 years respectively (Rowin *et al.*, 2021).

2.1.8 Lifestyle modification

Lifestyle modification includes healthy diet and exercising. General lifestyle recommendations for individuals with HCM include maintenance of normal body mass index, avoidance of vigorous exercise, dehydration and excessive salt, alcohol and caffeine intake especially in patients with LVOTO (Zamorano *et al.*, 2014). There are several trials with evidence that proper nutrition has an important restorative influence on cardiovascular diseases (Burr *et al.*, 1989; Krone and Nitschmann, 2014; Filippou *et al.*, 2020).

Regarding physical activity (PA), there is a consensus on avoiding competitive and intense exercise for individuals with HCM because it may increase the risk for SCD particularly in individuals with major risk factors for SCD, LVOTO or history of unexplained syncope (Gersh *et al.*, 2011; Maron, 2018a; Ommen *et al.*, 2020). Previous American and European consensus reports have suggested that young athletes with HCM should be ineligible for any competitive sporting activity (Maron *et al.*, 2015; Pelliccia *et al.*, 2019). However, the 2020 guidelines of the American Heart Association and the American College of Cardiology (AHA/ACC) suggested that the decision of participating in competitive exercise should involve a shared discussion between individual athletes with HCM and their physicians in order to evaluate the possible potential risks and that every individual with HCM should take the responsibility of their final decision because the general and precise risks for participation in sports activities cannot be easily quantified across several sport types (Ommen *et al.*, 2020). In addition, participation in regular, aerobic, mild-to-moderate intensity recreational PA is acceptable but controversial as there is no evidence yet that aerobic PA elevates risk of arrhythmias or promotes disease progression in HCM (Saberi *et al.*, 2017; Maron and Thompson, 2018). However, mild to moderate intensity PA improves cardiorespiratory fitness, quality of life and overall health in individuals with HCM (Saberi *et al.*, 2017; Sweeting *et al.*, 2018). Accordingly, more clinical trials need to evaluate the effects of different types of sports activities on symptoms and disease progression in individuals with HCM (Ommen *et al.*, 2020).

2.2 Heart rate variability

2.2.1 Autonomic Nervous System

The autonomic nervous system (ANS) is an efferent system that carry nerve impulses from the central nervous system (CNS) to peripheral organs and muscles (Singh *et al.*, 2018). It controls and regulates heart rate, myocardial contractility, smooth muscle contraction and relaxation in various organs and constriction and dilatation of blood vessels through its two major nervous components: parasympathetic and sympathetic systems (Singh *et al.*, 2018). The anatomical

and functional differences between sympathetic (SNS) and parasympathetic (PNS) nervous systems on the cardiovascular system are shown in Table 2.1.

Table 2.1 Sympathetic and parasympathetic effects on the cardiovascular system through various receptor interactions. Adapted from (Singh *et al.*, 2018).

	Sympathetic		**Parasympathetic**	
Organ	Receptor Subtype	Effects	Receptor Subtype	Effects
Heart	Beta-1, beta-2	↑ Heart rate ↑ Force of contraction ↑ Conduction velocity ↑ Automaticity (beta-2) ↑ Excitability	Muscarinic - M_2	↓ Heart rate ↓ Force of contraction ↓ Conduction velocity
Arteries	Beta-2 Alpha-1	Vasodilatation Vasoconstriction	Muscarinic - M_1	Vasodilatation
Veins	Beta-2 Alpha-1	Vasodilatation Vasoconstriction	-	-

2.2.2 Heart rate variability

Heart rate variability (HRV) can be defined as the physiological variation in the time intervals between consecutive heart beats and it reflects the combined effects of sympathetic and parasympathetic tone on heart rate and serves as a measurable non-invasive tool of cardiovascular integrity and disease prognosis (Malik *et al.*, 1996; Singh *et al.*, 2018). The establishment and standards of HRV and its measures was developed almost three decades ago by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Malik *et al.*, 1996). Since then, thousands of HRV-related studies have been published (Sassi *et al.*, 2015). The growing popularity of HRV-related publications is possibly facilitated by the ease of measuring HRV measures, non-invasive, cost-effective and its recording feasibility across a wide range of settings (Grégoire *et al.*, 2023). The major HRV measures can be divided into time domain and frequency domain HRV measures which can be recorded over either short-term (typically 5-minutes) or long-term (24-hours) (Malik *et al.*, 1996; Sassi *et al.*, 2015).

2.2.3 Time domain heart rate variability measures

Time domain indices of HRV estimate the variation of the heart rate over different time ranges and is expressed in units of time (milliseconds) (Malik *et al.*, 1996) (Figure 2.3). There are numerous time domain HRV measures, yet the major variables used in medical research and clinical settings are listed in Table 2.2 (Bravi *et al.*, 2011; Smith *et al.*, 2013b; Smith *et al.*, 2013a).

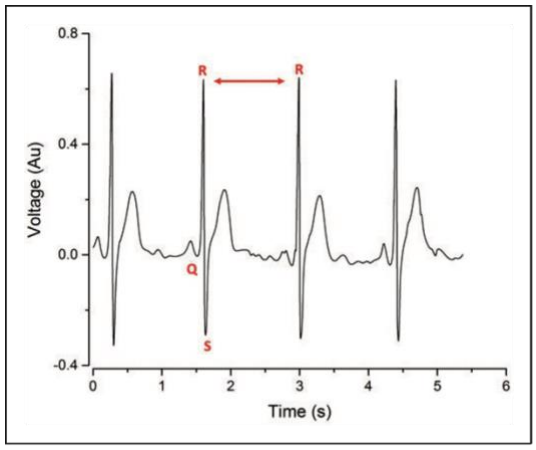

Figure 2.3 Electrocardiogram recording showing the interval between R waves. The consecutive time intervals between each R wave is known as time domain HRV. Adapted from (Johnston *et al.*, 2020).

Table 2.2 Main time domain measures of heart rate variability. Adapted from (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017)

Variables	Units	Description	Physiologic origin
RR interval	milliseconds (ms)	Time estimated between two successive QRS waves in the ECG	Overall autonomic cardiovascular regulation (Vagal and sympathetic contributions)
SDNN*	ms	Standard deviation of the sequence of sinus beats intervals measured by the NN distance during a certain period	Cyclic components responsible for heart rate variability (Vagal and sympathetic contributions)
SDRR*	ms	Standard deviation of the sequence of all intervals (sinus and non-sinus beats) measured by the RR distance during a certain period	Vagal and sympathetic contributions, but mainly vagal when recorded over five minutes
RMSSD	ms	The square root of the mean of the sum of the squares of differences between adjacent NN intervals	Vagal tone
PNN50	%	Proportion of successive NN interval differences larger than 50 ms	Vagal tone
NN50 count	-	Number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording	Vagal tone

NN; refers to RR for normal, sinus and free of artefacts R wave peaks. RR; refers to normal, sinus, free of artefacts beats and abnormal beats due to arrhythmias, ectopic beats, interference and artefacts.

It has been recommended by the Task force joint committee that the time domain measures of HRV should be recorded over 24 hours (Malik *et al.*, 1996), yet several studies have shown that the short-term recorded time domain HRV measures could also be used in clinical settings (Dekker *et al.*, 1997; Bruyne *et al.*, 1999; Nunan *et al.*, 2010b). Although time domain HRV measures are simple to derive and to calculate, they are sensitive to artefacts and non-sinus ectopic beats (Malik *et al.*, 1996; Seely and Macklem, 2004). Therefore, careful ECG signal filtering is indeed needed to ensure removal of ectopic beats and interference (Johnston *et al.*, 2020). Also, the recording of time domain HRV measures requires stationarity i.e. no significant changes in the mean heart rate, which is difficult to achieve in the biological system (Seely and Macklem, 2004). Consequently, most of time domain measures cannot discriminate between alterations in sympathetic nervous system (SNS) or parasympathetic nervous system (PNS) outputs but can be used to assess overall ANS activity and provide useful clinical outputs (Draghici and Taylor, 2016).

2.2.4 Frequency domain heart rate variability measures

Frequency domain HRV decomposes and separates RR variability using Fast Fourier Transform (FFT) or Autoregressive (AR) models into four main oscillatory components seen as four peaks in the power spectra and operated within different frequency ranges (Draghici and Taylor, 2016; Shaffer and Ginsberg, 2017) (Figure 2.4). The major frequency domain HRV measures are described in table 2.3.

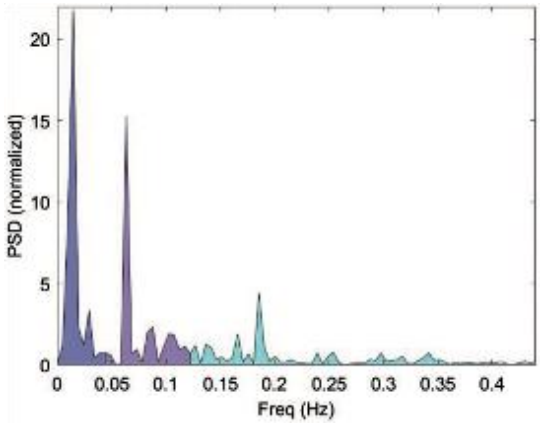

Figure 2.4 Power spectral density (PSD) analysis of frequency domain HRV measures. It was taken from five-minutes recording of healthy male. Three peaks are shown corresponding to low frequency power (0.03–0.15 Hz), high frequency power (HF) 0.15–0.40 Hz and very low frequency power 0.003–0.04 Hz. Adapted from (Johnston *et al.*, 2020).

Table 2.3 Main frequency domain measures of heart rate variability. Adapted from (Malik *et al.*, 1996; Shaffer *et al.*, 2014; Shaffer and Ginsberg, 2017)

Variables	Units	Description	Physiologic origin
HF power	ms^2	Absolute high-frequency component represents a peak ranging between 0.15 and 0.40 Hz in the power spectra	Vagal tone
HF power (n.u)	-	Relative power of the high frequency band. ‡HF (n.u) = HF (ms^2) / (LF (ms^2) +HF (ms^2)- VLF) *100	Should be used with caution when referring to vagal tone
LF power	ms^2	Absolute low-frequency power component represents a peak ranging 0.04 and 0.15 Hz in the power spectra	Vagal and sympathetic contributions, but mainly vagal and baroreceptor activity when recorded at rest and over five minutes
LF power (n.u)	-	Relative power of the low frequency band. ‡LF (n.u) = LF (ms^2) / (LF (ms^2) +HF (ms^2)- VLF) *100	Should be used with caution when referring to vagal tone and baroreceptor activity when recorded at rest and over five minutes
VLF power (less common)	ms^2	Absolute power of the very low frequency band ranging 0.0033–0.04 Hz in the power spectra	The exact mechanism not yet established, but could be related to RAAS, thermoregulation, and/or peripheral vasomotor tone
ULF power (less common)	ms^2	Absolute power of the ultra-low frequency band (≤0.003 Hz). It requires at least 24-hr recording time to be obtained	
LF/HF	-	Ratio of low-frequency power-to high-frequency	Controversially represent absolute and relative changes in sympathovagal balance

‡ Note that if VLF power is not recorded, the calculation of LF power (nu) and HF power (nu) is LF (ms^2) +HF (ms^2) without subtracting the VLF power; Hz: hertz; nu: normalised unit; RAAS: renin-angiotensin-aldosterone-system.

Frequency domain HRV measures can be recorded over short-term or long-term, yet they are usually performed over a short-term (5-minute) (Malik *et al.*, 1996; Grégoire *et al.*, 2023). Unlike time domain HRV measures, the frequency domain methods facilitate a more precise evaluation of directions and magnitude of ANS than time domain methods (Pumprla *et al.*, 2002). The physiologic origin of HRV measures was primarily investigated in frequency domain HRV measures than time domain measures (Akselrod *et al.*, 1981; Akselrod *et al.*, 1985). Since frequency domain HRV measures are analysed based on RR interval, they are highly sensitive to artefacts, interference and non-stationarity (Nunan *et al.*, 2010b). The distribution of frequency domain HRV values usually lacks normality, and it is preferable to transform data using natural logarithm (Ln) to achieve normality (Grégoire *et al.*, 2023). The most important frequency domain HRV measures to report are values of HF power and LF power because they represent the largest parts of the short-recording signals (Grégoire *et al.*, 2023).

2.2.5 The duration of heart rate variability measures recording

Analysis of HRV measures can be classified based on the length of data recording into short-term or long-term recording. The short-term recording ranges from 1-60 minute (Malik *et al.*, 1996; Heathers, 2014). However, it is recommended that short-term recording is kept over 5-minutes for comparison between clinical studies and convenience (Malik *et al.*, 1996; Laborde *et al.*, 2017; Hayano and Yuda, 2019). The main disadvantage of short-term HRV recording is instability as it cannot estimate the ultra-low (ULF) frequency power of HRV (Malik *et al.*, 1996; Kleiger *et al.*, 2005).

The long-term recording of HRV is typically over 24-hours, yet analysis of the 24-hour HRV data can be based either on a single segment of 24-hours or on a five-minute epoch over a 24-hours period (Malik *et al.*, 1996; Laborde *et al.*, 2017). Long-term HRV recording is stable, describes the changes in ANS over hours or even longer time periods, is a reliable method for predicting risk of mortality and is used to record both frequency and time domain measures (Li 2019). However, there are multiple limitations/disadvantages that researchers/clinicians need to be aware of when interpreting the long-term recorded HRV data. The long-term recording of HRV measures is usually performed for mobile individuals during daily activities which affect interpretation of the mechanisms by which data are related to clinical risks and mortality (Hayano and Yuda, 2019). Also, the 24-hours recording may not solely reflect ANS activity as it may be affected by circadian rhythm, metabolism, sleep cycle, body temperature, and renin-angiotensin system (Shaffer *et al.*, 2014). Thus, long-term recorded frequency domain HRV measures are at higher risk of inaccurate computation and instability which is time consuming and expensive (Nicolini *et al.*, 2012).

In summary, short-term HRV analyses are advantageous for four main reasons, 1) easy to perform, 2) convenient to control for confounding factors such as body posture, physical activity, medications, respiration and environmental factors such as room temperature, 3) requires least time for data editing and processing and 4) ability to describe the dynamic changes in HRV within a short period (Li, 2019).

2.2.6 Factors influences the interpretation of heart rate variability

There are several variables which researchers/clinicians need to be aware of when recording and interpreting HRV data in research and clinical settings. These factors can be classified as environmental factors and subjects-related factors (Laborde *et al.*, 2017; Shaffer and Ginsberg, 2017).

2.2.6.1 Environmental factors

The environmental factors include contextual variables such as: recording time and devices, sampling frequency, removal of artefacts, respiration and paced breathing (Shaffer and Ginsberg, 2017). HRV measures can be recorded via a wide range of ambulatory medical and fitness devices which are either based on photoplethysmography (PPG) or ECG, yet ECG remains the gold standard and most accurate method to record HRV data during rest and exercise (Singh *et al.*, 2018). Also, when HRV data is recorded a minimum of 500 Hz sampling frequency may be required to detect QRS complex on the ECG and artefacts should be eliminated by either selecting an artefacts free period for analysis or by editing the affected RR intervals manually (Laborde *et al.*, 2017). Respiration has a major influence on the HRV which is known as respiratory sinus arrythmias whilst during paced breathing, values of HRV data vary profoundly to the values obtained during normal breathing (Nunan *et al.*, 2010b; Shaffer and Ginsberg, 2017).

2.2.6.2 Subject-related factors

The subject factors that may influence the interpretation of HRV measures includes: age, sex, heart rate, health status, medications, body mass index and waist-to-hip ratio and lifestyle habits (Laborde *et al.*, 2017; Shaffer and Ginsberg, 2017). Age and sex are known to influence HRV measures as HRV might decline with age (Bonnemeier *et al.*, 2003; Nunan *et al.*, 2010b; Abhishekh *et al.*, 2013; Almeida-Santos *et al.*, 2016; Accardo *et al.*, 2020) and decrease in women than men (Koenig and Thayer, 2016a). Heart rate is inversely related to HRV, which means that faster heart rate reduces the time between successive RR interval (McCraty and Shaffer, 2015). Also, with poor health HRV might decline (Bigger *et al.*, 1995; Agelink *et al.*, 2002). Therefore, HRV has been shown to be a useful tool in predicting cardiovascular diseases, myocardial infarction, stroke and overall mortality (Tsuji *et al.*, 1996; Dekker *et al.*, 1997; Thayer *et al.*, 2010; Aeschbacher *et al.*, 2017).

All medications that influence ANS activity can also affect HRV measures such as beta blockers, calcium channel blockers, anti-arrhythmic agents, angiotensin-converting-enzyme inhibitors (ACEIs) and Angiotensin receptor blockers (ARBs) (Zhemanyuk and Syvolap, 2018). Also, It has been shown that all HRV measures (time and frequency domain) are significantly and inversely correlated with all adiposity measures including body mass index (BMI), percentage of body fat mass (PBF) and waist-to-hip ratio (WHR) regardless of age and sex (Yi *et al.*, 2013).

There are certain lifestyle habits which influence HRV measures and therefore may affect data interpretation and conclusion. These habits include cigarette smoking, alcohol consumption, caffeine intake, PA and diet (Laborde *et al.*, 2017).

Heavy smoking and alcohol dependence are associated with reduced vagal indices of HRV (Barutcu *et al.*, 2005; Quintana *et al.*, 2013) whilst acute caffeine intake increases vagal parameters of HRV (Zimmermann-Viehoff *et al.*, 2016). In relation to PA, most evidence showed that PA improved vagal indices of HRV measures in young, middle aged and old healthy adults (Grässler *et al.*, 2021a; Grässler *et al.*, 2021b). Also, diet rich with inorganic nitrate such as green leafy vegetables and beet root juice (BRJ) may influence HRV. It has been shown that acute consumption of dietary nitrate rich BRJ increases vagal indices of HRV in healthy individuals (Bond *et al.*, 2014) and intensifies the return of vagal control during recovery after exercise (Benjamim *et al.*, 2021).

2.2.7 Heart rate variability in hypertrophic cardiomyopathy

HRV in individuals with HCM has been widely applied in the following: 1) assessing baseline autonomic function, 2) evaluation of the response of autonomic function to pharmacological drugs and/or as prognostic tool to predict clinical events and HF.

Regarding baseline HRV in individuals with HCM, the majority of available evidence has shown that individuals with HCM have a reduction in PNS and increased SNS indices of time and frequency domain HRV measures (Ajiki *et al.*, 1993; Counihan *et al.*, 1993; Tanabe *et al.*, 1995; Bonaduce *et al.*, 1997; Limbruno *et al.*, 1998; Döven *et al.*, 2001). However, Fei *et al.* (1995) reported reduction in SNS indices of 24-hour recorded frequency domain HRV measures. When the effect of BB drugs was evaluated on HRV measures in individuals with HCM, the BBs-treated individuals with HCM had higher vagal indices of time (RMSSD and PNN50) and frequency domain (HF power) HRV measures compared with non-BBs-treated individuals with HCM (Mörner *et al.*, 2005). Furthermore, HRV has been shown to be a robust prognostic tool in HCM studies. For example, the reduction in PNS indices of HRV(RMSSD) in the work of Bonaduce *et al.* (1997) predicted enlarged left atrial size and abnormal LV end

systolic dimensions. In addition, reduced HF power was associated with cardiac events including progressive heart failure and hospitalisation in individuals with HCM (Kawasaki *et al.*, 2003).Recently, HRV measures have been reported as a reliable tool for SCD risk stratification in individuals with HCM (Yan *et al.*, 2023).

2.3 Lifestyle intervention

A healthy lifestyle is a major contributor in the prevention of cardiovascular diseases and modification of disease progression (Masana *et al.*, 2017). Healthy lifestyle recommendations include increased PA, eating a healthy diet, quitting smoking and avoiding or limiting alcohol and caffeine intake (Masana *et al.*, 2017).

PA and exercise training have been shown to improve cardiac autonomic function in various clinical populations such as individuals with myocardial infarction, HF, coronary artery bypass graft (CABG) surgery and percutaneous coronary intervention (PCI) (Malfatto *et al.*, 1996; Lucini *et al.*, 2002; Tsai *et al.*, 2006; Sandercock *et al.*, 2007; Pearson and Smart, 2018). The improvement in autonomic function in previous studies has been shown as a reduction in sympathetic indices of HRV, enhancement of parasympathetic indices of HRV and increase in overall HRV indexed by RR interval. There are several possible mechanisms by which exercise may modulate HRV (Routledge *et al.*, 2010).

One of the mechanisms is that exercise increases vagal tone which reduces resting heart rate and the myocardial contractility, leading to a reduction in the workload and oxygen consumed by the heart and therefore reducing the risk of lethal arrhythmias (Watanabe *et al.*, 1978; Casado *et al.*, 1994; Buch *et al.*, 2002). Another mechanism is that exercise could modulate the level of Angiotensin II (vasoconstrictor) by supressing its expression and synthesis and possibly mediates vagal enhancement (Townend *et al.*, 1995; Buch *et al.*, 2002). Also, exercise training has been found to improve endothelial function via release of nitric oxide (NO) which increases the NO bioavailability and potentially mediate exercise induced cardiac vagal tone stimulation (Chowdhary and Townend, 1999; Hambrecht *et al.*, 2000; Kingwell, 2000). Furthermore, exercise is known to reduce chronic inflammation thus inhibiting SNS activity, which is triggered by chronic inflammation (Kingwell, 2000; Jüttler *et al.*, 2002).

There are multiple dietary recommendations from the European Society of Cardiology (ESC) and ACC/AHA for the prevention and management of cardiovascular diseases (Lichtenstein *et al.*, 2021; Visseren *et al.*, 2022) with recent focus on inorganic dietary nitrate supplementation (Baião *et al.*, 2020). Recently, BRJ has been used in research as the most common method of providing dietary source of the inorganic nitrate anion (NO_3^-) (Gee and Ahluwalia, 2016; Ashor *et al.*, 2017; McMahon *et al.*, 2017). The physiologic mechanism by which BRJ releases NO is

based on the entero-salivary circulation of inorganic nitrate without involving nitric oxide synthase (NOS) activity so that once BRJ is consumed, the NO_3^- is reduced to NO_2^- by anaerobic oral bacteria by the action of nitrate reductase enzymes and then to nitric oxide (NO) in the stomach (Duncan *et al.*, 1995; Lundberg and Govoni, 2004). In the stomach nitrite is metabolised by the stomach acid into NO and other nitrogen oxides performing determinant physiological functions (Domínguez *et al.*, 2017). Nitric oxide (NO) induces several physiologic mechanisms which potentially improve cardiovascular health and muscle performance (Domínguez *et al.*, 2017). These benefits include reduction of blood pressure, inhibition of platelet aggregation, enhancement of endothelial function and reduction of inflammation, enhancement of vascular dilatation, improvement of blood flow in hypoxic and ischemic tissues, reduce pulmonary artery pressure, improve exercise performance and oxygen consumption and inhibition of SNS activity and/or increase PNS (Chowdhary *et al.*, 2000; Buch *et al.*, 2004; Domínguez *et al.*, 2017).

Available evidence on the effect of dietary nitrate rich BRJ on HRV in cardiovascular diseases is limited. However, it has been reported recently that acute intake of BRJ did not influence the short term recorded time or frequency domain HRV (Carrijo *et al.*, 2021). In individuals with coronary slow flow, a diet containing high amounts of NO_3- has been associated with an improvement in the 24hr recorded vagal indices (RMSSD, PNN50) of time domain HRV measures (Pekdemir *et al.*, 2004). A recent study reported no significant changes in the 5-minutes recorded frequency domain HRV measures after the acute dose of nitrate rich BRJ in individuals with peripheral artery disease (Pekas *et al.*, 2023).

Specific to HCM, there is a lack of evidence for the chronic effect of lifestyle intervention, combining both PA and inorganic dietary nitrate supplementation with BRJ on HRV, haemodynamic and cardiometabolic measures (Tafelmeier *et al.*, 2020).

Chapter 3. Aims, objectives, and hypotheses

3.1 Aims

The aims of this thesis are: 1) to understand the roles of age and sex on HRV, and the relationship between HRV and cardiometabolic function in healthy and individuals with HCM and 2) to understand the physiological implications of a personalised, home-based, novel lifestyle intervention incorporating physical activity and dietary nitrate supplementation on HRV and cardiometabolic function in individuals with HCM.

3.2 Objectives

To achieve these aims, five main objectives were considered:

1. To evaluate the effect of age, sex and their interaction on resting HRV measures and on peak exercise cardiometabolic variables in healthy individuals.
2. To assess the relationship between resting HRV measures and peak exercise functional capacity (VO_2) and cardiac function in healthy individuals.
3. To compare resting HRV and haemodynamic variables between individuals with HCM and healthy controls.
4. To investigate the relationship between resting HRV and haemodynamic variables including stroke volume (SV), total peripheral resistance (TPR) and mean arterial blood pressure (MABP) in individuals with HCM.
5. To evaluate the effect of the personalised, home-based, novel lifestyle intervention incorporating physical activity and dietary nitrate supplementation on HRV primarily and on resting and peak exercise cardiometabolic variables in individuals with HCM.

3.3 Hypotheses

The following research hypotheses were tested:

1. There will be a significant influence of age and sex on HRV and cardiometabolic variables in healthy individuals.
2. There will be a significant correlation between resting HRV and peak exercise VO_2 and cardiac power output in healthy individuals.
3. There will be a significant difference in resting HRV and haemodynamic measures including cardiac output (CO), SV, TPR, MABP) between individuals with HCM and healthy individuals.
4. There will be a significant relationship between resting HRV and haemodynamic measures in individuals with HCM.
5. The personalised, home-based, novel lifestyle intervention will lead to significant improvements in HRV and cardiometabolic variables at rest and peak exercise in individuals with HCM.

Chapter 4. Methods

4.0 Methods

This chapter details the main equipments and techniques used in the research to generate data for the present thesis. These include The Task Force Monitor which integrates bioimpedance and electrocardiography measurements to generate haemodynamic and heart rate variability data, bioreactance for rest and exercise cardiac output assessment, and cardiopulmonary exercise stress testing procedure. In addition, this chapter provides information about the lifestyle intervention in individuals with hypertrophic cardiomyopathy. Information regarding study design, screening of participants, recruitment, randomisation, inclusion and exclusion criteria, consent, the novel home-based lifestyle intervention, funding and ethical approval, sample size calculation and statistical analysis are detailed independently (Please refer to Sections 5.4, 6.4 and 7.4 in Chapters 5, 6 and 7, respectively).

4.1 Bioimpedance

4.1.1 Background

Bioimpedance, also called impedance cardiography (ICG), is a simple, transthoracic, non-invasive and continuous beat-to-beat method for estimating stroke volume (SV) and cardiac output (CO) (Woltjer *et al.*, 1997; Critchley and Critchley, 1999). Bioimpedance was established in 1966 (Kubicek *et al.*, 1966) and is based on the theory that the thoracic cavity is cylindrical and perfused with blood which has specific resistivity (haemodynamic resistance) (Khalil *et al.*, 2014). This resistance is to a high frequency low amplitude current called bioimpedance. Bioimpedance is measured using electrodes placed on the upper and lower thorax (Funk *et al.*, 2009). Impedance (Z_0) is then obtained from the voltage difference between cardiac events and aortic blood flow which is inversely related to the thoracic volume (V_{th}) (Funk *et al.*, 2009). Therefore, SV is calculated as the product of ventricular ejection time and change in the impedance ($dZ_0/dtmax$) (Fortin, 2004). The bioimpedance method was used in Chapters 5, 6 and 7 (Please refer to methods section of these chapters for further details).

4.1.2 Haemodynamic Measurements

The ICG method is imbedded in the Task Force Monitor (CNSystems, Graz, Austria), a device which integrates continuous blood pressure measurement and beat-to beat SV measurement using the vascular unloading method on the finger and impedance cardiography as shown in Figure 4.1(Kubicek *et al.*, 1966; Penaz, 1973; Sramek *et al.*, 1983; Fortin *et al.*, 1998b).

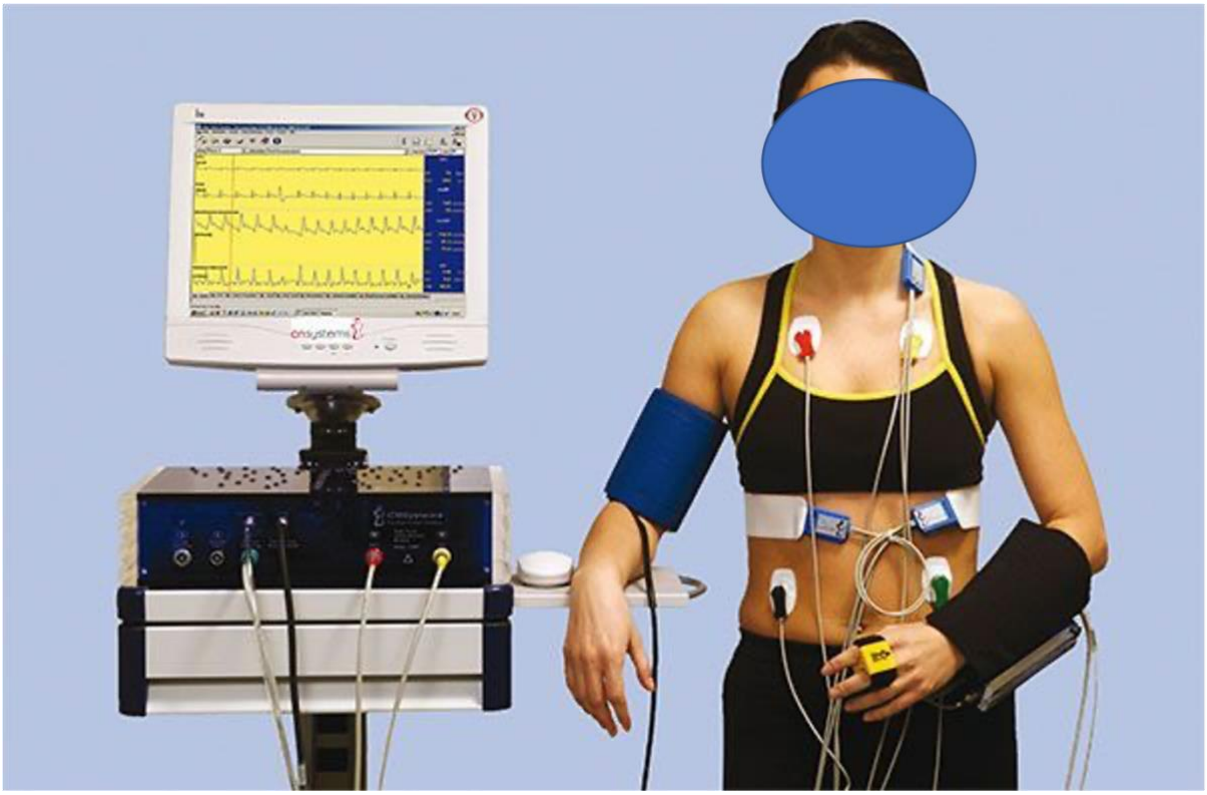

Figure 4.1 The Task Force (CNSystems, Graz, Austria) monitor and its connection to the body, adapted from (Fortin *et al.*, 2000).

The values of continuous blood pressure (cBP) measurements are automatically corrected to the oscillometeric blood pressure (oscBP) values, which are recorded from the contralateral arm (Fortin *et al.*, 2000). Therefore, the relative changes in blood pressure are derived from the finger plethysmographic device while the absolute values are derived from the oscillometric device in which the latter meet the standards for sphygmomanometers (Fortin *et al.*, 2000). The formula for the correction is shown below according to (Fortin *et al.*, 2000):

$$SBP(i) = SBP_{contBP}(i) - \overline{SBP}_{contBP} + \overline{SBP}$$

$SBP_{contBP}(i)$ = Systolic BP of beat I recorded from plethysmographic device

$\overline{SBP}_{contBP}$ = Mean BP recorded from finger plethysmographic device

$\overline{SBP}$ = Mean BP recorded from oscillometric device

Haemodynamic beat-to-beat measurements, listed in Table 4.1 below, are estimated along with continuous blood pressure measurements and displayed in real time (Kubicek *et al.*, 1966; Fortin *et al.*, 2000).

Table 4.1 Beat-to beat haemodynamic variables obtained from the Task Force monitor (CNSystems, Graz, Austria) (adapted from Fortin *et al.* (1998b); Jeong *et al.* (2010)).

Parameter	Title	Units	Description	Calculation formula
RRI	RR-Interval	(ms)	Time distance between 2 R-peaks in the ECG	RRI
HR	Heart rate	beats per minute (bpm)	Number of heart beats per minute	HR= 60*1000/ RRI
SBP	Systolic Blood Pressure (beat to beat)	(mmHg)	Systolic blood pressure (continuous and oscillometric measurement)	see Formula of BP correction above
DBP	Diastolic Blood Pressure (beat to beat)	(mmHg)	Diastolic blood pressure (continuous and oscillometric measurement)	see Formula of BP correction above
MBP	Mean Arterial Blood Pressure (beat to beat)	(mmHg)	Mean blood pressure (continuous and oscillometric measurement)	MAP = DBP + 1/3(SBP – DBP)
SV	Stroke Volume	(ml)	Stroke volume of the left ventricle per heartbeat	$SV= V_{th}*LVET* (dZ/dt_{max}/Z_0)$ $V_{th}= f (H, W, Z_0)$
SI	Stroke Volume Index	(ml/m²)	SV/BSA (body surface area)	SI= SV/BSA
CO	Cardiac Output	(l/min)	Circulating blood volume per minute	CO= SV*HR
CI	Cardiac Index	(l/(min*m²))	CO/BSA (body surface area)	CI= CO/BSA
TPR	Total Peripheral Resistance	(dyne*s/cm^5)	Note: CVP- Central Venous Pressure is fixed at 3 mmHg	TPR= (BP mean- CVP)/ (CO)*80
TPRI	Total peripheral resistance index	(dyne*s*m^2/cm^5)	Note: CVP- Central Venous Pressure is fixed at 3 mmHg	TPRI= (BP mean- CVP)/ (CI)*80

(dZ/ dt_{max})*:* the maximum rate of change of the transthoracic impedance wave form; ECG: electrocardiography; *H:* height; LVET: left ventricular ejection time; V_{th}: thoracic volume; *W:* weight; Z_0*:* transthoracic base impedance.

4.1.3 Heart rate variability measurements

In addition, the Task Force monitor (CNSystems, Graz, Austria) includes a 2-channel electrocardiography (ECG) for RR interval recording at the sample frequency of 1000 Hz and 16-bit resolution in which frequency domain (i.e., low frequency power (LF power), high frequency power (HF power) and LF/HF) heart rate variability (HRV) measurements are obtained (Fortin *et al.*, 2000). The digitally implemented filter within the Task Force monitor (CNSystems, Graz, Austria) eliminates any artefact and noise (Fortin *et al.*, 2000). The frequency domain approach of estimating HRV uses the spectral method to analyse a series of RR intervals as a total complex wave forms with different frequencies which are decomposed with auto-regressive (AR) modelling into three main frequency bands (Malik *et al.*, 1996; Fortin *et al.*, 1998b). The HRV frequency bands included are: (i) low frequency (LF) region of power analysis, (ii) high frequency (HF) power and (iii) very low frequency power (VLF) which were given in both normalised (nu) and absolute units (ms^2) (Malik *et al.*, 1996). The HF power ranges from 0.15 to 0.4 Hz, LF power ranges from 0.04 to 0.15 Hz and VLF power ranges from 0.003 to 0.04 Hz(Pham *et al.*, 2021; Grégoire *et al.*, 2023). Both the AR modelling measurement and Fast Fourier Transformation (FFT) are measurement techniques which convert a signal (i.e. RR interval) into spectral components and hence provides frequency information about the recorded signal (Clifford, 2002). The AR modelling analysis of the frequency domain measure has demonstrated more effective resolutions of sharp peaks, provides smoother and more interpretable curve than Fast Fourier Transformation (FFT) estimation of the frequency bands (Cowan *et al.*, 1992). It is widely recommended that HF and LF bands should be obtained by the AR modelling analysis since the FFT analysis overestimates the frequency bands components compared with AR (Fagard *et al.*, 1998; Pichon *et al.*, 2006). Normalised (nu) values of both LF power and HF power are expressed as a percentage and are calculated by dividing raw values of either short term frequency band (LF or HF) by the total spectral power (typically LF+HF) (Heathers, 2014).

In summary, the Task Force monitor (CNSystems, Graz, Austria) provides accurate and real time measurements of cardiovascular parameters including autonomic control and haemodynamic function by integrating ECG, ICG, cBP and oscBP into the same instrument.

4.2 Bioreactance

4.2.1 Background

Bioreactance is a non-invasive method of measuring CO and SV, which is based on the principle that an electric current travels through the thoracic cavity and generates beat-to-beat changes (phase shifts) (Keren *et al.*, 2007). The phase shifts mainly occur due to pulsatile blood flow, which is generated from the aorta (Marik, 2013). Therefore, bioreactance refers to electrical capacitance and inductance, which result from volume changes in the thoracic cavity and the bioreactance signal correlates strongly with aortic flow (Marik, 2013). The NICOM system (Starling^{TM} SV, Baxter International Inc, USA) applies the bioreactance technique to measure haemodynamic variables. The system consists of a radiofrequency generator, which produces a high frequency electrical current that transmits through the thorax (Keren *et al.*, 2007). Four dual surface electrodes are placed either on the chest or back with two placed above the heart and two below it to establish electrical body contact (Keren *et al.*, 2007). An amplifier to record transthoracic voltage which exist due to the injected current and electrical circuits determine the relative phase shift between the injected current and the recorded voltage (Keren *et al.*, 2007). One side of the dual surface stickers (electrodes) is used to introduce the high frequency (75 kHz) current to the thorax whilst the other end is used by a voltage input amplifier (Figure 4.2). The bioreactance method was used in Chapters 5 and 7, please refer to the methods section of these chapters for more details.

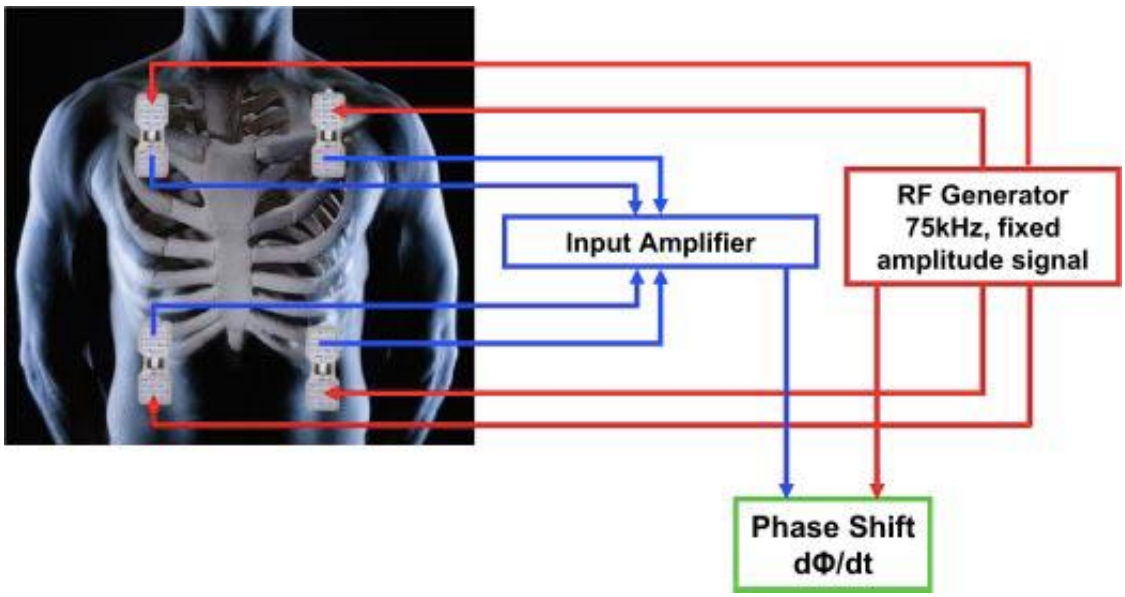

Figure 4.2 The NICOM system (Starling^{TM} SV, Baxter International Inc, USA) and its electrode attachment to the body; adapted from (Keren *et al.*, 2007).

4.2.2 Haemodynamic Measurements

Estimation of CO is based on the formula; CO = (C x VET x ΔΦ/ dtmax) x HR , where C is a constant of proportionality, VET is ventricular ejection time which is determined from the bioreactance and the ECG signals, ΔΦ/ dtmax is the relative phase shift of current, and HR is heart rate (Keren *et al.*, 2007; Marik, 2013). Several other haemodynamic parameters can be measured from the NICOM system (Starling^{TM} SV, Baxter International Inc, USA), which includes: CI, SV, SVI, HR, SBP, DBP (Bodys-Pełka *et al.*, 2021).

4.3 Comparison between bioimpedance and bioreactance

Bioimpedance and bioreactance are non-invasive and continuous CO and haemodynamic monitoring that have been used in clinical settings (Jakovljevic *et al.*, 2014). Both techniques are based on the analysis of impedance signals, yet bioreactance has been developed with an advanced modification of bioimpedance (Lee *et al.*, 2011; Marik, 2013). Bioimpedance analysis is based on the transthoracic voltage amplitude measurement in response to a high frequency current, which is significantly limited by electric noise, pericardial and/or pleural effusion (Critchley *et al.*, 2000; Raue *et al.*, 2009). Bioimpedance is also influenced by body weight and any physical factors that affect electrodes conductivity such as temperature or humidity (Wang and Gottlieb, 2006). Unlike bioimpedance, the signal analysis of bioreactance is based on the variation measurement of the frequency spectra of the delivered oscillating current and it averages the signals received over ten seconds so that allow accurate assessment of CO and has a higher signal-to-noise ratio (Keren *et al.*, 2007).

Studies comparing bioimpedance and bioreactance methods are limited (Jakovljevic *et al.*, 2014). For example, it has been reported in a large multicentre study that bioimpedance systematically underestimated long-term recorded CO and showed a higher degree of variability than bioreactance in patients who needed haemodynamic monitoring in the cardiac catheterization laboratory, cardiac care unit and in surgical and medical intensive care units (Raval *et al.*, 2008). Also, Jakovljevic *et al.* (2012) showed that there was no significant difference between the two methods at rest, yet peak exercise CO from bioimpedance was underestimated in healthy participants compared to bioreactance.

4.4 Electrocardiography

The Electrocardiography (ECG) is the process by which electrical activity of the heart is recorded using an electrocardiogram (Alghatrif and Lindsay, 2012). Electrical activity of the heart is generated through the movement of ions inside and across the cardiac myocytes membrane, which is recorded by applying electrodes on to the chest and upper and lower limbs,

which are then connected to the ECG device (Rautaharju, 1987). The standard ECG recorded electrical activity of the heart at a speed of 25 mm per second and 1 milli volt (mV) so that duration is measured horizontally and voltage is measured vertically (Becker, 2006).

Since 1887, the ECG has been used as a standard diagnostic tool for structural and/or valvular heart diseases (Waller, 1888; Rautaharju, 1987). The ECG is non-invasive, simple, safe and feasible and remains the most crucial indicator for myocardial infarction and has been widely used during rest and exercise testing (De Bacquer *et al.*, 1998; Bourque and Beller, 2015).

Although an ECG has been widely used as a screening tool for cardiovascular diseases, it should be incorporated with other diagnostic markers in order to make a comprehensive diagnosis (Ashley *et al.*, 2001). It has been shown that the presence of cardiovascular diseases may not always be ruled out by a normal resting ECG (Barraclough, 2008). For example, Froelicher *et al.* (1998); Sekhri *et al.* (2008) reported low sensitivity of an ECG (53%) for identifying individuals who would have future ischemic events and Bressman *et al.* (2020) showed that an ECG had a sensitivity of 30.7% for left ventricular hypertrophy. The ECG however still remains the gold standard method for evaluation of cardiac electrical activity (Stracina *et al.*, 2022). In the Chapters 5, 6 and 7 of the present thesis, the CardioExpress SL18A (Spacelabs Healthcare) ECG device was used (Figure 4.3) Please refer to methods section of the respective chapters for further details.

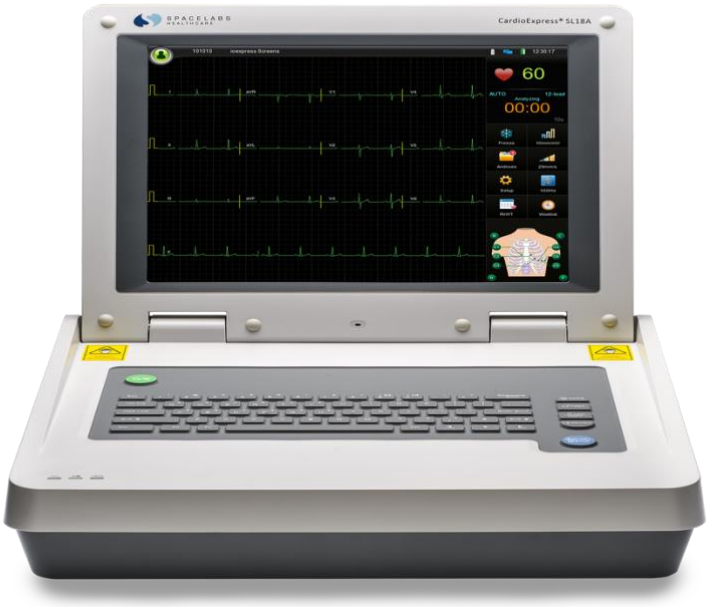

Figure 4.3 The CardioExpress SL18A electrocardiography device. Adapted from (Spacelabs Health Care).

4.5 Cardiopulmonary exercise test

A cardiopulmonary exercise test (CPET) refers to a non-invasive test that assesses the integrative response of exercise on the heart, lungs, blood vessels and skeletal muscle (Albouaini *et al.*, 2007). The CPET permits an evaluation of both submaximal and maximal exercise capacity, allowing physicians to differentiate between cardiac or pulmonary causes of exercise intolerance, or make prognosis, risk stratification, and determine response to treatment (Guazzi *et al.*, 2016; Glaab and Taube, 2022). There are variables that can be generated from a CPET such as oxygen consumption (VO_2), carbon dioxide output (VCO_2), tidal volume (VT), minute ventilation (VE), ventilatory efficiency slope that reflects the increase in ventilation in response to CO_2 production (VE/VCO_2) and oxygen consumption (VE/VO_2). However, the main parameters measured during a CPET and used in the present thesis are presented in Table 4.2.

Table 4.2 Cardiopulmonary exercise test variables (adapted from (Albouaini *et al.*, 2007)).

Parameter	Title	Units	Description
VO_2	Oxygen consumption or functional capacity	(L/min) (ml/kg/min)	- Ability to inhale, transport and use oxygen - Define individuals' aerobic capacity and cardiorespiratory fitness - Calculated from Fick equation; VO_2= (SV X HR) X (CaO_2 -CvO_2)
HR	Heart rate	beats per minute (bpm)	Number of heart beats per minute
RER	Respiratory exchange ratio	-	The ratio of carbon dioxide output/oxygen uptake (VCo_2/Vo_2)
WR	Work rate	(Watt)	The workload achieved during exercise
BP	Mean BP (beat to beat)	(mmHg)	Continuous blood pressure measurement
AT	Anaerobic threshold	(ml/kg/min)	- Estimate functional capacity at anaerobic metabolism. - During AT, lactic acid production increase in muscles and blood lactate concentration.

CaO_2: arterial oxygen content; CvO_2: venous oxygen content; SV: stroke volume

4.5.1 Cardiopulmonary exercise testing protocols

Participants in the present thesis completed a symptom limited graded CPET using a cycle ergometer. The cycle ergometer is used instead of the treadmill because of the following advantages: it is easier for blood gas collection, it provides lower noise and artefacts signals, it is safer than the treadmill and is favourable in patients than in healthy participants as it is associated with lower risk of falls compared to treadmill, obese and individuals with arthritis

(Pritchard *et al.*, 2021). Whilst exercising, simultaneous 12-lead ECG and automated blood pressure were recorded. All CPET variables represented in Table 4.2 were acquired from breath-by-breath expired air analysis and expressed in ten seconds intervals. Peak VO_2 and peak respiratory exchange ratio (RER) were expressed as the highest ten second averaged sample obtained during the last twenty seconds of testing. VE and VCO_2 values acquired from the beginning of exercise to peak were inputted into the spreadsheet software (Microsoft Excel, Microsoft Corp., Bellevue, WA) to calculate the VE/VCO_2 slope via a least squares linear regression (y = mx + b, m=slope). The test termination criteria consisted of symptoms (i.e., dyspnea and/or fatigue), ventricular tachycardia, > 2 mm of horizontal or down sloping ST segment depression, a fall of systolic blood pressure > 20 mm Hg during progressive exercise, or RER $\geqslant$1.15. A full description of exercise tests is presented in the respective data Chapters 5 and 7. Figure 4.4 shows an example of the CPET machine being used during our study (Chapters 5 and 7). Details on CPET procedure and protocol used for healthy participants and individuals with HCM is provided in methods section for Chapters 5 and 7.

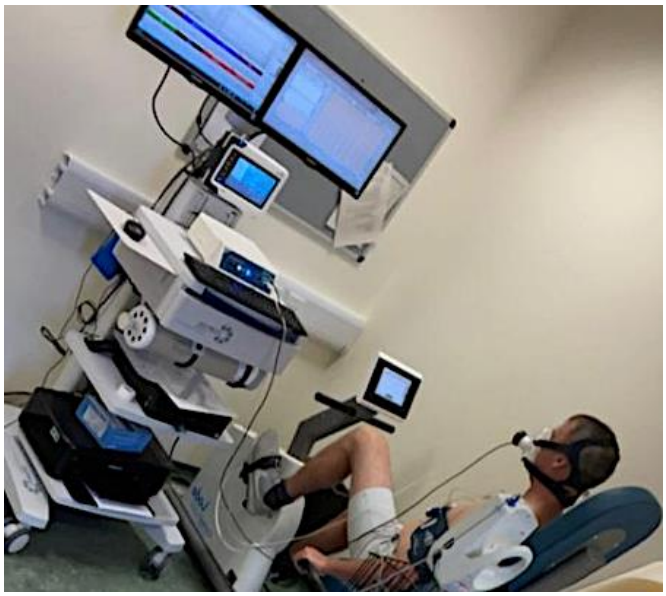

Figure 4.4 The cardiopulmonary exercise testing machine using a semi-recumbent cycle ergometer. Informed consent was obtained for publication of this figure.

4.6 Assessment of physical activity

Physical activity (PA) was self-reported in healthy participants and device-based (Pedometer Omron Health, Japan) in individuals with HCM. To enable comparison in PA between healthy participants and individuals with HCM, participants were categorised as active and inactive as described previously (WHO, 2022; Jordan *et al.*, 2023). Please refer to the methods in Chapters 5 and 6 for further details. PA was assessed in Chapters 5 and 6 as a covariate, since PA affects the primary outcome of the present thesis (HRV measures) and may increase vagal activity in

healthy participants (Grässler *et al.*, 2021a) and in clinical populations (Pearson and Smart, 2018). In Chapter 7, PA was used as part of the lifestyle intervention for individuals with HCM and it was assessed and recorded in step counts using a pedometer (Omron Health, Japan). Pedometers contain a lever arm that deflects with acceleration (the up-and-down motion) of the hips during motion (for example during walking) (Tudor-Locke *et al.*, 2002). With each deflection detected, an electrical circuit opens and closes and step count is accumulated and hence displayed digitally on a feedback screen (Tudor-Locke *et al.*, 2002). Although the use of pedometers for recording steps/day has been criticised for not considering the intensity of PA which can be measured by accelerometers (Garber *et al.*, 2011), pedometers can be motivating for increasing PA in clinical populations as it provides instant feedback of step count recording (Bravata *et al.*, 2007; Okwose *et al.*, 2019). It has been shown recently in the ChemoFit study that pedometers had high compliance rate (98%) when utilised as part of a home-based PA programme (Chmelo *et al.*, 2022). Please refer to the methods section of Chapter 7 for further details on the use of pedometers in the present thesis.

4.7 Lifestyle intervention

The lifestyle intervention used in the present thesis consisted of two components: PA and dietary supplementation with inorganic nitrate (NO3−), which was completed over 16 weeks for individuals with HCM (Tafelmeier *et al.*, 2020). PA was based on a home-based PA regimen aimed at increasing daily PA by at least 2000 steps/day from baseline for 7 days/week for 16 weeks using a pedometer (Omron Health, Japan). Individuals with HCM recorded daily step counts at the end of each day using a paper-based activity tracker and results were communicated weekly to a member of the study team.

A dietary supplementation with inorganic nitrate (NO3−) was given in a form of concentrated nitrate-rich beet root juice (BRJ). A single dose of the nitrate-rich BRJ (NO3−, BEET IT Sport, James White Drinks Ltd., Ipswich, UK) contained 6 mmol of NO3− in a 70 ml bottle. Figure 4.5 provides details of the two components of the lifestyle intervention used in the present thesis. Please refer to methods section in Chapter 7 for further details on the lifestyle intervention.

Figure 4.5 The pedometer (Omron Health, Japan) and the beetroot juice.

Chapter 5. The effect of age and sex on heart rate variability in healthy individuals

5.1 Abstract

Background and objectives: Heart rate variability (HRV) is a simple, non-invasive measure of cardiac autonomic function. The aim of the present study was twofold: 1) to evaluate the effect of age and sex on HRV measures, cardiac and metabolic functions, and (2) to determine the relationship between resting HRV and peak exercise functional capacity and overall cardiac function in healthy individuals.

Methods: Sixty-eight healthy participants were stratified according to their age into the younger age group (<40 years old, N=43, females, N=17, mean age: 26±6 years old) or the older age group (>55 years old, N=25, females, N=11, mean age: 64±6 years old). Frequency domain HRV measures (i.e. absolute and normalised low frequency power (LF), (HF) and LF/HF ratio) were derived from RR interval and recorded at rest (supine position) for 30 minutes. Non-invasive gas-exchange and central haemodynamic (bioimpedance) measurements were collected at rest and during exercise. Functional capacity was represented by peak oxygen consumption (VO_2), while overall cardiac function was represented by peak exercise cardiac power output (CPO) – the product of mean arterial blood pressure and cardiac output, expressed in watts.

Results: After controlling for body mass index and physical activity**,** mean RR interval was significantly affected by sex as males had significantly higher mean values of RR interval than females (males=1043±165; females=952±128 ms, p=0.02). There was no significant main effect of age, sex or their interaction on any of the other HRV measures. In younger and older females, resting RR interval had a significant relationship with peak exercise CPO (young females: r=0.54, p<0.05; old females: r=0.81, p<0.01). There was also a significant relationship between resting HF power and peak exercise CPO in younger females (r=0.70, p<0.01). There was no significant relationship between resting RR interval, HF power and peak VO_2 in any age or sex groups.

Conclusions: HRV was not influenced by age. RR interval was affected by sex and is positively associated with overall cardiac function in females regardless of age.

Key words: Ageing, Sex, Heart Rate Variability, Frequency Domain

5.2 Introduction

In Europe, more than one in five (20%) individuals were aged ≥ 65 years old at the beginning of 2018 and this figure will increase to 29% by 2050 (Ageing Europe, 2019). Age is a major risk factor for the development of chronic diseases (Niccoli and Partridge, 2012). The most common cause of death and disabilities among the elderly in Europe and North America are cardiovascular diseases, such as coronary artery disease and heart failure (Ungvari *et al.*, 2010; Balakumar *et al.*, 2016). It has become evident that ageing leads to well-defined phenotypic changes that cause coronary artery disease in the absence of traditional risk factors such as hypertension, metabolic diseases and smoking (Ungvari *et al.*, 2018). Vascular endothelial function is also compromised by ageing in which diminished endothelium-dependant dilator responses occur leading to remodelling in the microcirculation and impaired tissue perfusion in older adults (Ungvari *et al.*, 2018). Understanding the age-related changes in cardiovascular function may lead to the development of therapeutic strategies to reduce morbidity and mortality in the ageing population.

Age is associated with an imbalance in autonomic regulation of the cardiovascular system (Umetani *et al.*, 1998). There are several changes that may occur in the autonomic nervous system due to ageing, such as decline of muscarinic receptor function and density (Brodde *et al.*, 1998), baroreceptor sensitivity impairment, which causes an increase in norepinephrine levels and higher sympathetic muscle nerve activity (Pfeifer *et al.*, 1983). Furthermore, beta-adrenergic modulation of cardiovascular function (Krug *et al.*, 2002), renin angiotensin system activity (Yoon and Choi, 2014) and thermoregulation (Kerckhoffs *et al.*, 1998) have been shown to decline with ageing. Additionally, bradycardia due to the reduction in diastolic depolarization and reduced respiratory sinus arrhythmia have been reported with ageing (Hellman and Stacy, 1976; Opthof, 2000). These complex age-related changes in cardiovascular autonomic function can be easily detected by measuring heart rate variability (HRV).

HRV is a simple, non-invasive measure that has been used to quantify changes in cardiac autonomic control and to predict adverse clinical events (Kleiger *et al.*, 1987). Previous studies have shown that age, sex, health status are important determinants of HRV (Kitney, 1982; Koizumi *et al.*, 1985; Shaffer and Ginsberg, 2017). Age may induce similar changes in HRV as some of the disease states, making it difficult to differentiate between pathological and/or age-related, physiological changes in HRV (Korkushko *et al.*, 1991; Fukusaki *et al.*, 2000). Parameters of HRV are indicators of sympathetic and parasympathetic interaction of the cardiac autonomic system (Moodithaya and Avadhany, 2012). Spectral analysis of HRV examines the

frequency-specific signals of cycle length fluctuations in heart rate and separates series of sequential R wave (RR) intervals into a total of multiple waves with different amplitude and frequencies (Moodithaya and Avadhany, 2012). The low frequency (LF) spectral power reflects mainly baroreceptor and vagal activity during resting conditions (Shaffer *et al.*, 2014; McCraty and Shaffer, 2015). High frequency (HF) spectral power is associated with parasympathetic activity (Moodithaya and Avadhany, 2012; Shaffer and Ginsberg, 2017). The low-to-high frequency ratio (LF/HF) is related to sympathovagal balance (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017).

Sex differences are another well-known prognostic factor that may predict onset of cardiovascular disease, mortality and morbidity (Pereira *et al.*, 2010; Dasinger and Alexander, 2016). Males have earlier onset of cardiovascular diseases such as coronary artery disease compared to females (Berry *et al.*, 2012; Mikkola *et al.*, 2013; Ji *et al.*, 2020). Furthermore, higher resting heart rate is an independent risk factor for heart diseases (Perret-Guillaume *et al.*, 2009; Cooney *et al.*, 2010). It has been found that females do not have an increased risk of cardiovascular diseases compared to males even though their resting heart rate is higher than males (Cordero and Alegria, 2006). Sex differences alongside age are crucial variables affecting overall cardiovascular and autonomic function (Koenig and Thayer, 2016b), but the interaction between age and sex on cardiac autonomic function has not been well reported.

Generally, the age-related decline in autonomic function occurs in both sexes (Yukishita *et al.*, 2010; Almeida-Santos *et al.*, 2016). However, some researchers believe that the influence of sex throughout ageing in HRV is not fully explained (Perseguini *et al.*, 2011; Moodithaya and Avadhany, 2012). Females have lower autonomic modulation compared to males (Nunan *et al.*, 2010b). It has been widely reported in the literature that females tend to have lower RR interval (i.e. higher resting mean heart rate) despite having higher vagal dominance than males (Santos *et al.*, 2013; Koenig and Thayer, 2016b; Shaffer and Ginsberg, 2017). However males have sympathetic outflow dominance compared to females (Koenig and Thayer, 2016b; Shaffer and Ginsberg, 2017). Therefore, it is not unexpected for females to have higher HF power (vagal modulation) compared to males (Koenig and Thayer, 2016b).

Although the age-related influence in autonomic function has been shown previously (Umetani *et al.*, 1998; Felber Dietrich *et al.*, 2006; Zulfiqar *et al.*, 2010), there are limitations in the methodologies. These include a lack of consensus about the assessment techniques and clinical applications (Bravi *et al.*, 2011; Li *et al.*, 2011) as well as inadequate large registries proposed to estimate short term (e.g. five minutes) HRV measures in healthy elderly individuals (Almeida-Santos *et al.*, 2016). Moreover, the inconsistency in recording time (long-term versus short-term), participants' recording status (supine, setting, sleeping or awake) and HRV

parameters (frequency domain and/or time domain) make direct comparison and interpretations difficult (Bonnemeier *et al.*, 2003). A decline in HRV time domain measures with age was reported using a 24-hour recording (Almeida-Santos *et al.*, 2016). More recently, Accardo *et al.* (2020) also confirmed decline in HRV measures with age. Although both studies highlighted the decline in autonomic function with ageing, they used different methodologies, categorisation of age groups, selection of HRV measures and definition of a healthy cohort. Also, using a different assessment tool to HRV to evaluate cardiac autonomic function could add to the complexity of data interpretation. For example, a recent study reported a decline in autonomic function by using heart rate responses to exercise and heart rate recovery (Njemanze *et al.*, 2016).

There are not only inconsistencies in the methodologies but also discrepancies in the results of sex-related changes in HRV measures with ageing. Yeragani *et al.* (1997) concluded that there were no sex-related differences with ageing in HRV measures. In contrast, another study suggests that sex-related differences in time domain HRV measures decline with ageing (Bonnemeier *et al.*, 2003). Moreover, Sinnreich *et al.* (1998) suggested that the decline of HRV measures with ageing occurs earlier in males than females. Similarly, total power decreases with ageing in males whereas HF power declined in females (Felber Dietrich *et al.*, 2006). It has been reported that almost all short-term recordings of time domain and normalised values of LF, HF power and LF/HF ratio were higher in males than females of all age groups in Korean population aged 20-84 years old (Lee *et al.*, 2018). A review of the existing literature suggests that more research is warranted to improve our understanding of how age and sex influence HRV in a healthy population.

It appears that the relationship between HRV and peak exercise cardiac haemodynamic and metabolic variables have not yet been evaluated in a healthy population. However, when HRV was compared between athletes and sedentary individuals, HRV was higher in athletes and related to aerobic fitness (De Meersman, 1993). Thus, HRV could correlate with peak exercise oxygen consumption (VO_2) and cardiac function represented by cardiac power output (CPO). A fundamental question unanswered in literature is whether resting autonomic function represented by HRV could be associated with changes in peak exercise cardiac and metabolic characteristics. Therefore, the relationship between cardiovascular autonomic function and those variables merits further investigation. This information is indeed clinically relevant to enhance the understanding of the strength and the direction of how peak exercise cardiometabolic performance may be associated with baseline autonomic function measures in healthy individuals.

5.3 Aims and Research Hypotheses

The aim of the present study was twofold; firstly, to evaluate the effect of age and sex on HRV measures, cardiac and metabolic function, and secondly to determine the relationship between resting HRV and peak exercise functional capacity and overall cardiac function in healthy individuals.

The following two research hypotheses were tested:

i) There will be a significant influence of age, sex and their interaction on cardiac autonomic function represented by HRV.

ii) There will be a significant correlation between resting HRV and peak exercise oxygen consumption (VO_2) and cardiac power output (CPO).

5.4 Materials and Methods

5.4.1 Study design, setting, and participants

This was a retrospective, single centre, cross-sectional, observational study conducted at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne, UK between January 2018 and July 2019. The study recruited healthy adults. The inclusion criteria were 18 years of age or more, no history of chronic, cardiovascular, pulmonary, or metabolic diseases, and willingness to visit the Clinical Research Facility.

The exclusion criteria were defined as co-existing medical conditions, history of cardiovascular diseases such as cardiomyopathy, myocardial infarction, intermittent claudication, dementia, stroke, mild cognitive impairment, psychiatric disorders, musculoskeletal disease, chronic respiratory illnesses, cancer and/or signs of cardiac ischemia or arrhythmia at rest or during exercise. A total of seventy-five participants were eligible to participate in the present study.

The study was approved by the Research Ethics Committee of the National Health Service, North-East England - Tyne and Wear South, and local Research and Development department (15/NE/0190). All procedures were performed according to the Declaration of Helsinki. Informed written consent was provided by all participants (See appendix A). Study participants were stratified according to age and sex into a younger age group (<40 years old) and older age group (>55 years). The age groups stratification was prespecified as mentioned above due to the retrospective nature of the study design and the limitations of sample size as well as the absence of participants between the age of 41 and 54 years old.

5.4.2 Funding and ethical approval

This study was funded by the UK Medical Research Council Confidence in Concept Scheme grant to D.G.J. (grant no. BH161161). D.G.J. is supported by the UK Research Councils'

Newcastle Centre for Ageing and Vitality (grant no. L016354). The funders of the study had no role in study design or in data collection, analysis, or interpretation. The study was approved by Research Ethics Committee of the National Health Service, North-East England - Tyne & Wear South, and local Research and Development department (15/NE/0190).

5.4.3 Study protocol and measurements

Participants attended the Clinical Research Facility for one visit lasting approximately one and a half hours. All participants were asked to refrain from alcohol- and/or caffeine- containing foods and beverages on the study day and were asked to avoid vigorous exercise 24-hours prior to the study visit. Upon arrival at the laboratory, participants completed a Health-Related Physical Activity Readiness Questionnaire (Riebe *et al.*, 2018) (template is available in appendix B). Whilst seated, participant's blood pressure from the brachial-artery, oxygen saturation, body temperature and heart rate were measured. This was followed by anthropometric measurements, which included standing height and body weight, which were measured using a weighing scale and stadiometer (Seca 769 electric column scale with telescopic measuring rod Seca 220, Seca, Hamburg, Germany). Body mass index (BMI) was calculated using the equation: BMI=body mass (kg)÷stature^2(m^2) whilst body surface area (BSA) was calculated using the equation :(height (cm)*weight (kg)/3600)$^{1/2}$ (Kouno *et al.*, 2003). The daily physical activity, smoking status and alcohol consumption of the participants were assessed by a case report form (template is available in appendix C). They were considered active if they performed ≥ 150 min/week of moderate intensity physical activity as per WHO guidelines (WHO, 2022). Participants then rested in the supine position for five minutes and a 12-lead electrocardiogram was recorded to assess the heart rhythm.

The integrated electrocardiography (ECG) within the TaskForce (CNSystems, Graz, Austria) recorded spectral (frequency) domain HRV measures and RR interval. The HRV measures included: low frequency (LF) region of power analysis, high frequency (HF) power which were given in both normalised (nu) and absolute units (ms^2) and LF/HF ratio. The HF power ranges from 0.15 to 0.4 Hz and LF power ranges from 0.04 to 0.15 Hz (Malik *et al.*, 1996). All of the spectral domain HRV measures were obtained from beat-to-beat variations in RR interval length (RR interval ms^2). HRV measures were recorded and analysed at rest in the supine position over 30 minutes with normal breathing. Spectral domain HRV measures were derived automatically using the autoregressive (AR) spectral estimation method with a sampling frequency of 1000 Hz (Malik *et al.*, 1996; Fortin *et al.*, 1998a).

Blood pressure is regulated through heart rate, stroke volume, peripheral resistance and controlled by the autonomic nervous system (Fortin *et al.*, 1998a). HRV and haemodynamic

(cardiac output (CO) (L/min), cardiac index (CI) (L/min/m^2), heart rate (HR) (beats/min), stroke volume (SV) (ml/beat), stroke volume index (SVI) (ml/beat/m^2) and blood pressure (BP) measurements (mmHg) were collected using the non-invasive impedance cardiography method (TaskForce, CNSystems, Graz, Austria) (Fortin, 2004; Jakovljevic *et al.*, 2014), which provided direct, real time and fully synchronised signals (Gratze, 1998). The TaskForce (CNSystems, Graz, Austria) uses impedance electrodes for cardiac output assessment, which were applied on the neck between both shoulders and the bilateral sides of the thorax. Four-lead electrocardiography electrodes were placed on the chest for HRV measurements, and one finger cuff connected to a forearm unit for continuous blood pressure recording (Fortin, 2004; Jakovljevic *et al.*, 2014). The cardiac power output (CPO) measured in watt (w) is a known measure of overall heart function and was calculated manually as the product of simultaneous recording of blood flow (cardiac output (CO)) and mean arterial blood pressure (MABP) using the following equation: CPO (w) = mean arterial blood pressure (mmHg) x cardiac output (L/min) x K, where K=0.0022 (a conversion factor) (Williams *et al.*, 2001). CPO was indexed to body surface area: CPOI (w/m^2) = CPO/body surface area (Grodin *et al.*, 2015).

Following 30-minute resting measurements in the supine position, all participants completed a progressive cardiopulmonary exercise test using a semi-recumbent cycle ergometer (Corival, Lode, Groningen, Netherlands) with simultaneous gas exchange (metabolic) measurements (Metalyzer 3B, Cortex, Leipzig, Germany). Peak haemodynamic measures were recorded using non-invasive bioreactance (Starling^{TM} SV, Baxter International Inc, USA). The bioreactance method utilises four dual electrodes placed on the back side of the thorax. Participants exercised according to the ramp protocol, maintaining a pedal frequency of 60-70 revolutions per minute. An initial warm up period of two minutes at 10 watts was included in the exercise protocol followed by gradual increments at the rate of 10 watts per minute until participants reached maximal exertion. Cardiac rhythm using 12-lead electrocardiography (Custo, CustoMed, HmbH, Ottobrunn, Germany) and an automated blood pressure measurement (Tango, SunTech Medical; Morrisville, North Carolina, USA) were recorded simultaneously every three minutes. The cardiopulmonary exercise test was terminated when any one of the following criteria were met: 1) upon participant's request, 2) when respiratory exchange ratio (RER) exceeded 1.15 (i.e. universally agreed RER threshold (Issekutz Jr *et al.*, 1962)) , 3) failure to maintain a cadence of 60 revolutions per minute, or 4) failure to increase oxygen consumption although exercise intensity (watts) increased.

5.4.4 Sample size calculation

The power calculation was based on previous literature suggesting a significant difference in RR interval between males and females, as no significant differences in RR interval between

younger and older people has been previously reported (Bonnemeier *et al.*, 2003; Nunan *et al.*, 2010b; Koenig and Thayer, 2016b). RR interval was selected for power calculation as a well-established, time domain measure of HRV. The sample size calculation was derived from G*Power 3.1 software (Faul *et al.*, 2007) retrospectively based on a significant difference in RR interval previously reported between males (827±86) and females (758±74) i.e. 69 ms and standard deviation of the mean difference equal to 12 ms (Bonnemeier *et al.*, 2003). The following settings in G*Power software were indicated: alpha=0.05 (two-tailed); beta=0.70 (power=70%); detectable effect=0.4. It was estimated that a total of 38 participants for each main group was required to detect a significant difference of 69 ms in RR interval between the groups.

5.4.5 Statistical analysis

All statistical analyses were performed using SPSS, Version 27.0 (IBM Corp., Armonk, N.Y., USA). Data were screened for univariate and multivariate outliers using standard z-distribution cut-offs, box plots and Mahalanobis distance test. A total of seven outliers were detected and removed from the analysis. Normality of anthropometric, HRV, haemodynamic and metabolic measurements were assessed using Kolmogorov-Smirnov test and histograms. The majority of HRV, haemodynamic and metabolic measures were normally distributed. Absolute HF power (ms^2), LF power (ms^2) and LF/HF did not meet the assumption of homogeneous variance and normality in both age groups, therefore natural log transformation was performed.

Age and sex differences in demographic and anthropometric variables were assessed by independent samples *t*-test. The Pearson Chi square test was used to assess differences in categorical variables between age groups. Two-way analysis of covariance (ANCOVA) with Bonferroni correction were carried out to determine the main effects of age and sex (and the interaction) on HRV, haemodynamic and metabolic measures using BMI and physical activity as covariates. Physical activity influences cardiac autonomic system and can hence affect HRV (Tornberg *et al.*, 2019). To assess the relationship between HF power (ms^2), RR interval and peak exercise oxygen consumption (VO_2) and peak cardiac power output (CPO), partial correlation was performed using BMI and physical activity as the covariates. Statistical significance was indicated if $p<0.05$. All continuous data were expressed in mean±SD unless otherwise stated.

5.6 Results

5.6.1 Demographic and physical characteristics

Participant's demographic and physical characteristics are presented in Table 5.1. A total of 68 participants (age range: 19-78 years, males, N=40; females, N =28) were included in the study. The younger age group (<40 years old) included 43 participants (age range: 19-39 years, males, N=26; females, N=17) and the older age group (>55 years old) included 25 participants (age range: 57-78 years, males, N=14; females, N=11). Age, height and body mass index were significantly different between the two age groups (p<0.01). Thirty-nine participants (91%) in the younger age group were active, whilst only eleven participants (44%) in the older age group were active. None of the participants reported being smokers.

Table 5.1 Participant demographic and physical characteristics

Variables	**All participants N=68**	**Younger age group (<40 years) N=43**	**Older age group (>55 years) N=25**	***P Value***
Age (years)	40±19	26±6	64±6	**<0.01**
Sex, female N (%)	28 (41)	17 (40)	11(44)	0.71
Height (cm)	171±1	173±1	166±1	**<0.01**
Weight (kg)	74±14	72±16	78±11	0.05
Body mass index (kg/m^2)	25±4	23±4	28±3	**<0.01**
Body surface area (m^2)	1.85±0.21	1.85±0.23	1.86±0.16	0.83
Physical activity, active N (%)	50 (74)	39 (91)	11 (44)	**<0.01**
Alcohol consumption, N (%)	46 (68)	32 (74)	14 (56)	0.11

5.6.2 Resting heart rate variability measures stratified by age and sex

Table 5.2 shows that there was a significant main effect of sex on RR interval, F (1, 64) =7.6, p=0.01. Bonferroni *post hoc* analysis shows that males had significantly higher mean values of RR interval than females (males=1043±165; females=952±128 ms). There was a non-significant main effect of age on RR interval, F (1,64) =1.61, p=0.21, and there was no significant interaction between age and sex in any of the other HRV measures.

Table 5.2 Resting heart rate variability measures stratified by age and sex

Variables	Males N=40		Females N=28		Two-Way ANCOVA *(P* Value)		
	Young N=26	Old N=14	Young N=17	Old N=11	Age	Sex	Interaction
RR interval (ms)	1042±195	1046±95.3	951±145	953±103	0.21	**0.01**	0.72
†LF power (ms^2)	6.39±0.95	6.43±0.58	6.47±0.76	6.55±0.96	0.87	0.43	0.96
†HF power (ms^2)	6.72±0.95	6.55±0.64	6.74±0.86	6.65±1.06	0.63	0.65	0.92
LF power (nu)	44.6±16.3	47.9±14.7	42±17.4	46.5±18.1	0.86	0.68	0.96
HF power (nu)	55.3±16.3	52.0±14.7	57.9±17.4	53.4±18.1	0.86	0.68	0.96
†LF/HF	0.19±0.74	0.02±0.69	0.32±0.76	0.07±0.78	0.97	0.55	0.92

HF, high frequency spectral power, LF, low frequency spectral power, LF/HF, the ratio of low to high frequency spectral power, ms^2 absolute units, nu: normalised units, RR interval, mean time interval between the two consequent heart beats (R waves).
† Natural log transformed data presented.

5.6.3 Peak exercise metabolic and haemodynamic measures stratified by age and sex

The effect of age and sex as well as their interaction was assessed for resting haemodynamic and metabolic measures (see appendix D Table 5.1). Table 5.2 demonstrates that there was a significant effect of age on peak VO_2, F (1, 64) =11.2, $p<0.01$. Bonferroni *post hoc* analysis revealed that the younger age groups had significantly higher peak VO_2 than the older age groups regardless of sex (younger=34±9.88; older=19.2±6.60 ml/kg/min). There was a non-significant effect of sex on peak VO_2, F (1, 64) =1.64, $p=0.20$. There was a significant effect of age on peak exercise work rate (WR) F (1,64) =8.54, $p<0.01$ with the younger age groups demonstrating significantly higher WR than the older age groups regardless of sex (younger=196±63.4; older=112±45.2 watt). There was a non-significant effect of sex on peak exercise mean values of WR, F (1,64) =3.03, $p=0.08$.

Peak exercise haemodynamic variables were significantly influenced by the interaction between by age and sex including peak exercise CI, F (1, 64) =5.34, $p=0.02$. Pairwise comparison has shown that older females have higher CI than older males (older females 13.1±1.67 vs older male 11.5±1.82 L/min/ m^2, $p=0.04$). There was a significant effect of age on peak exercise SV, F (1,64) =6.06, $p=0.02$, with the older age groups had significantly higher SV than the younger age groups regardless of sex (older=164±42.9; younger=134±32.9 ml/beat). There was a non-significant effect of sex on peak exercise SV, F (1,64) =0.04, $p=0.83$.

Table 5.3 Peak exercise metabolic and haemodynamic measures stratified by age and sex

Variables	**Males N=40**		**Females N=28**		**Two-Way ANCOVA (*P* Value)**		
	Young N=26	Old N=14	Young N=17	Old N=11	Age	Sex	Interaction
Metabolic variables							
Oxygen consumption(ml/kg/min)	35.8±10.7	19.42±6.30	31.3±7.98	18.9±7.25	**<0.01**	0.20	0.10
Oxygen consumption (L/min)	2.64±0.77	1.58±0.65	2.06±0.58	1.49±0.43	**<0.01**	0.06	0.12
Respiratory exchange ratio	1.11±0.09	1.07±0.06	1.12±0.09	1.08±0.07	0.06	0.45	0.99
Work rate (Watt)	213±67	113±51.1	172±49.3	108±38.6	**<0.01**	0.08	0.12
Haemodynamic variables							
Cardiac output (L/min)	23.3±4.20	21.3±4.04	21.1±5.9	24.3±4.18	0.82	0.80	**0.04**
Cardiac index (L/min/ m²)	12.1±2.18	11.5±1.82	11.2±1.56	13.1±1.67	0.53	0.35	**0.02**
Cardiac power output (W)	5.37±1.34	5.07±1.80	4.79±1.30	5.43±1.44	0.69	0.74	0.21
Cardiac power output index (w/ m²)	2.82±0.87	2.62±0.97	2.65±0.72	2.89±0.90	0.49	0.95	0.24
Heart rate (beats/min)	221±29.6	200±17.2	220±22	223±19.5	0.34	0.68	0.21
Stroke volume (ml/beat)	139±34.8	161±43.2	126±29	168±44.3	**0.02**	0.83	0.31
Stroke volume Index (ml/beat/ m²)	73.1±18.2	83.9±19.2	74.2±15.8	87.6±17.1	**0.03**	0.60	0.75
Systolic blood pressure (mmHg**)**	182±28.4	176±32.1	161±28.2	179±18.2	0.69	0.14	0.10
Diastolic blood pressure (mmHg)	81.2±12.6	94.2±12.4	81.3±14.7	93.4±7.64	0.19	0.70	0.55
Mean arterial blood pressure (mmHg)	115±9.61	121±16.2	108±15.9	122±8.28	0.25	0.45	0.35

5.6.4. Relationship between heart rate variability and peak exercise metabolic and haemodynamic variables

Table 5.4 details the results of the partial correlations between RR interval and metabolic and haemodynamic variables after controlling for BMI and physical activity. There were significant and positive relationships between RR interval and CPO in young females (r=0.54, p< 0.05) and old females (r=0.81, p<0.01). There was no relationship between CPO and RR in either young or old males. Also, there was no relationship between RR and peak VO_2 in any of groups. Similar to RR interval, Table 5.5 shows that there was a significant and positive relationship between HF power and CPO but only in the young females (r=0.70, p<0.01). There was no relationship between HF power and CPO in any other groups. There was no relationship between HF power and peak VO_2 for any of the groups.

Table 5.4 Partial correlations between RR and metabolic and haemodynamic variables

Variables	**Males N=40**		**Females N=28**	
	RR interval (ms)			
	Young N=26	Old N=14	Young N=17	Old N=11
	r	**r**	**r**	**r**
Cardiac power output (W)	0.10	0.11	0.54*	0.81**
Oxygen consumption (L/min)	0.15	0.02	0.24	-0.16

r correlation coefficient; *p< 0.05; **p<0.01.

Table 5.5 Partial correlations between HF power and metabolic and haemodynamic variables

Variables	**Males N=40**		**Females N=28**	
	†HF power (ms^2)			
	Young N=26	Old N=14	Young N=17	Old N=11
	r	**r**	**r**	**r**
Cardiac power output (W)	-0.24	-0.48	0.70**	0.22
Oxygen consumption (L/min)	0.11	-0.27	-0.31	-0.18

r correlation coefficient; *p< 0.05; **p<0.01. † Natural log transformed data presented.

5.7 Discussion

The aim for the study was to assess the effect of age and sex on cardiovascular autonomic function on HRV, functional capacity and cardiac function. The study also evaluated the relationship between HRV (RR interval and HF power) and peak exercise oxygen consumption (VO_2) and cardiac power output (CPO).

The major findings of this study suggest that there was a significant effect of sex but not age on mean RR interval which indicated that males had significantly higher RR interval than females. There was a non-significant interaction between age and sex in any of the of HRV measures which means that the magnitude of either age or sex does not depend on each other for any of the HRV measures. The novel finding of this study was that RR interval was associated with peak exercise cardiac power output in females regardless of age, whilst HF power was significantly associated with CPO in younger females only. There was no significant relationship between RR interval, HF power and peak VO_2 in any of the age or sex groups.

5.7.1 Heart rate variability: Age and sex interaction

The present study demonstrated that the mean RR interval was not affected by age, although it was significantly influenced by sex since it was higher in males compared to females. These findings are in agreement with previous studies (Bonnemeier *et al.*, 2003; Nunan *et al.*, 2010b), but contrary to those of Zhang (2007) who argued that age had a greater effect on HRV measures than sex. In the present study, the small number in particular the older age group with only four participants >70 years old and no participants ≥80 years old may have masked the effect of age on RR interval. Moreover, the present study findings are in line with the widely reported consensus about differences in resting heart rate between sexes. Males have a higher RR interval despite lower resting heart rate compared to females who tend to have a lower RR interval and higher resting heart rate (Santos *et al.*, 2013; Koenig and Thayer, 2016b; Shaffer and Ginsberg, 2017). Almeida-Santos *et al.* (2016) speculated that the lower global autonomic modulation in females is due to increased sympathetic activity in males potentially related to sex hormones (Du *et al.*, 1994).

Another finding of the present study suggests that none of the HRV indices had a significant interaction between age and sex. This finding was supported by the two previous studies of Moodithaya and Avadhany (2012) and Almeida-Santos *et al.* (2016). The former found a non-significant interaction between age and sex in the same frequency domain HRV indices which were used in the present study. The study assessed resting, supine and short-term (5 minutes) HRV measurements in 267 healthy participants (126 males and 141 females) with an age range

of 6-55 years (Moodithaya and Avadhany, 2012). Whilst the Almeida-Santos *et al.* (2016) evaluated time domain HRV indices which were recorded for 1743 healthy participants (616 males and 1127 females) with an age range of 40-100 years over 24-hour measurement using ambulatory Holter monitoring. However, no specific timing for the start of recording were mentioned which could affect the standardisation between the participants.

Despite the lack of significant interaction between age and sex in these studies, they have reported significant main effects of age and sex on HRV. Moodithaya and Avadhany (2012) studied the effect of age in relation to sex differences in four age groups: children (6-11 year), adolescents (12-19 years), younger adults (20-40 years) and middle-aged adults (41-55years) and found that HF power (ms^2 and n.u), LF power (ms^2) and LF/HF ratio significantly declined with age in both sexes. They observed sex differences in adolescents and adult groups with the LF/HF ratio and LF power (ms^2) being significantly higher in the adolescents and younger adult females compared to males of the same age groups (Moodithaya and Avadhany, 2012). The authors suggested that the limited sex effect on HRV measures in adolescents and young adults may imply the role of the female sex hormones in cardiac autonomic function.

Similarly, Almeida-Santos *et al.* (2016) reported a decline in global autonomic regulation represented by time domain HRV indices specifically standard deviation of normal-to-normal interval (SDNN) and SDNN-index across six decades (40-100 years) in both sexes. Results revealed that the SDNN-index was lower in females than males except parasympathetic activity represented by the root mean square of successive differences between normal heartbeats (RMSSD), which was lower in males than females. The authors disagree with the previous suggestion related to hormonal changes (Moodithaya and Avadhany, 2012) and speculated that the sex differences were not linked to hormonal factors, since they found that HRV measures did not change in the female groups before and after menopausal age. The contradiction between the aforementioned studies and the present study is mostly due to different age categorisation, female underrepresentation the present study and the modest sample size.

5.7.2 Heart rate variability and peak exercise cardiac function

The novel finding of the present study suggests a strong positive relationship between RR interval and peak exercise cardiac power output (CPO) in healthy older females and a moderate positive relationship in healthy younger females. The selection of CPO for the relationship assessment with HRV measures is advantageous because CPO is a direct measure of overall function and pumping capability of the heart and it takes into account all age and sex related changes in cardiac performance (Williams *et al.*, 2001). Also, MABP is the product of cardiac output (CO) and total peripheral resistance (TPR) (MABP= CO x TPR) and CO is the product

of SV and HR (CO= SV x HR) (Williams *et al.*, 2021), thus any changes occurring in these variables will directly affect CPO.

The significant positive relationship between mean RR interval and CPO that was seen in females but not in males could be related to sex hormones. It has been reported that the female heart demonstrates resilience to ageing with no reduction in peak exercise cardiac power output in females compared to males (Goldspink *et al.*, 2009). Also, the fact that particularly older females had the strong relationship between RR interval and CPO could suggest that menopausal transition may not be a substantial factor in determining cardiovascular health (Merz and Cheng, 2016). Although the association between RR interval and CPO has not been studied before in healthy participants, a similar correlation with moderate strength has been reported previously in patients with heart failure (Koshy *et al.*, 2019). The authors suggested that treatment for heart failure may not only improve heart failure symptoms but also improve cardiovascular autonomic function (Koshy *et al.*, 2019). Hence, this present study could adopt a similar concept by understanding the peak exercise cardiac function performance in relation to baseline cardiac autonomic function measures in healthy females.

The present study demonstrated a strong positive relationship between HF power, a vagal index of HRV, and peak exercise CPO in younger females only. Such a relationship was not investigated before in either healthy or clinical populations. The possible explanation for this relationship is that younger females (19-39 years old) may require high CPO during exercise to compensate for the possible estrogen-related increase in vagal tone. This speculation is supported by Liu *et al.* (2003) who found that post-menopausal women who were on estrogen replacement therapy had higher HF power compared to post-menopausal women who did not receive the estrogen replacement therapy. Also, it has been reported in several studies that estrogen facilitates the higher parasympathetic activity in females as compared to males (Kuo *et al.*, 1999; Liu *et al.*, 2003).

5.7.3 Heart rate variability and peak exercise functional capacity

There was no significant relationship between either mean RR interval and HF power with peak VO_2 in the present study. This is an interesting finding as one would expect a positive significant relationship between HRV and functional capacity. In a previous experimental study in animals (i.e. dogs) who suffered from myocardial infarction found that higher functional capacity was associated with better cardiac autonomic function and lower incidence of sudden cardiac death (Hull Jr *et al.*, 1994). The findings of this experimental data would suggest that mechanisms leading to higher oxygen consumption may also result in better autonomic function. The present study does not support such notion in this study cohort.

Although the correlation between peak VO_2 and both RR interval and HF power have not been assessed before in healthy populations stratified by age and sex, other HRV measures in relation to peak VO_2 were evaluated in clinical groups. Interestingly, the German Diabetes study found that lower HRV measures including HF power, LF power and standard deviation of all NN intervals (SDNN) correlated with lower maximal cardiorespiratory fitness in patients with diabetes (Röhling *et al.*, 2017).

5.8 Strengths and limitations of the study

The present study is the first to assess the relationship between HRV and peak VO_2 and CPO in healthy individuals. Also, it is in line with the recent recommendation for the increasing demand and necessity for short-term HRV analysis in various settings to improve prevention and risk stratification (Lee *et al.*, 2018). One merit of short-term HRV analysis is the opportunity to evaluate cardiovascular autonomic regulation under standardised circumstances and another is the ability to obtain immediate test result with less discomfort for participants (Nunan *et al.*, 2010b; Lee *et al.*, 2018).

This study has been conducted in a relatively modest sample size, which limits the statistical power. The study was also limited by using the TaskForce device for HRV analysis, which records only frequency domain measures but not time domain measures, except RR interval. Further adequately powered studies are required to replicate these findings in a larger cohort of healthy individuals. Additionally, older participants (>80 years olds) and/or more age groups categories are needed to address the age-related differences. The number of females and males in this study were non-homogeneous in the younger and older age groups with fewer females compared to males in both age groups. However, it is widely recognised in clinical studies and in health research that females are harder to recruit than males (Uhl *et al.*, 2007). It is unknown whether women in the younger age group had their HRV measures during their menstrual cycle, yet women in the older age group were not using any hormonal replacement therapy. HRV is influenced by menstrual cycle as it has been reported that HF power increased during the follicular phase and LF power increased during the ovulatory phase (Brar *et al.*, 2015; Nagar and Kumar, 2020).

The large standard deviation vzealues for the majority of the HRV measures data indicated large inter-individual variations, which is not uncommon with HRV analysis (Sinnreich *et al.*, 1998). In order to control the issue of large values of standard deviations, future studies need to use frequency and time domain HRV measures and correlate them with haemodynamic and other physiological variables of clinical importance in order to improve knowledge about the influence of age and sex on HRV (Laborde *et al.*, 2017). Finally, future clinical guidelines and recommendations should be proposed to standardise HRV-related methodology to be used in

clinical and research practice considering the current large inconsistency in methodology applied for HRV measurements across different studies.

5.9 Conclusions and clinical implications

In conclusion, mean RR interval was significantly affected by sex only. The awareness of sex differences in HRV values may improve the clinical applications of HRV measures and imply that individual's inclusion criteria should include predetermined cut-off values for HRV based on sex. This will help clinicians as well as researchers to understand the mechanism of sex differences in HRV.

Resting mean RR interval had a significant positive association with overall cardiac function and pumping capabilities at peak exercise in females. This implies that resting HRV could potentially be used as a surrogate of cardiac power. Thus, recognition of this fact and standardisation of the baseline measurement of HRV may remove a significant inconsistency in clinical research studies. Further large-scale studies are warranted to include a wider age range and more age groups that are stratified by sex and health status to define and evaluate the clinical application of HRV measures in the context of prevention and monitoring of cardiovascular disease.

Chapter 6. Heart rate variability and haemodynamic function in individuals with hypertrophic cardiomyopathy

6.1 Abstract

Background and objectives: Heart rate variability (HRV) is an important cardiovascular risk factor to health and disease. The aim of the present study was twofold: (1) To evaluate differences in HRV and haemodynamic function between individuals with hypertrophic cardiomyopathy (HCM), and healthy controls, and (2) to determine the relationship between HRV and haemodynamic variables in individuals with HCM.

Methods: Twenty-eight individuals with HCM (7 females, age 54±15 years old, body mass index: 29±5 kg/m^2) and 28 matched healthy individuals (7 females, age 54±16 years old, body mass index: 29±5 kg/m^2) completed short-term (5-minute) HRV measurements under resting (supine) conditions using bioimpedance technology (TaskForce, CNSystems, Austria). Frequency domain HRV measures including spectral analysis of RR interval (i.e. absolute and normalised low frequency power (LF), high frequency power (HF) and LF/HF ratio) were recorded. In addition, resting non-invasive haemodynamic variables i.e. cardiac output, stroke volume, total peripheral resistance and mean arterial blood pressure were also simultaneously recorded.

Results: After controlling for medication and physical activity, individuals with HCM demonstrated significantly higher vagal activity represented by absolute unit of HF power and LF power compared to healthy controls (HF power, 7.40±2.50 vs. 6.03±1.35 ms^2, p=0.01; LF power 6.86±2.58 vs. 5.93±1.01 ms^2, p=0.01). RR interval significantly reduced in individuals with HCM compared to healthy controls (914±178 vs. 1014±168 ms, p=0.02). Haemodynamic variables were significantly lower in individuals with HCM compared to healthy controls (cardiac index, 2.33±0.42 vs. 3.57±0.82 L/min/m^2, p<0.01; stroke volume index, 33±9 vs. 43±7 ml/beat/ m², p<0.01; and cardiac power output index, 0.02±0.01 vs. 0.04±0.01 W/m^2, p<0.01), whereas total peripheral resistance index was significantly higher in individuals with HCM (3468±1027 vs. 2953±1050 dyn·s·m^2cm^{-5}, p=0.04). In individuals with HCM, there was a significant relationship between HF power and both stroke volume (r= -0.62, p<0.01) and total peripheral resistance (r= 0.51, p<0.01).

Conclusions: Cardiac autonomic and haemodynamic function are altered in individuals with HCM. Vagal activity, represented by HF power, is increased and associated with peripheral resistance in HCM.

Keywords: Hypertrophic cardiomyopathy, heart rate variability, frequency domain, haemodynamic function

6.2 Introduction

The autonomic nervous system (ANS) plays an important role in the pathogenesis of sudden cardiac death (SCD) in hypertrophic cardiomyopathy (HCM) (Ajiki *et al.*, 1993; Fei *et al.*, 1995; Limbruno *et al.*, 1998; Huang *et al.*, 2017). However, the prevalence of autonomic dysfunction in HCM is uncertain (Ommen et al., 2020). Traditionally, it had been thought that individuals with HCM have increased sympathetic activity or hypersensitivity of the myocardium to catecholamines which had been considered as a risk factor for disease progression (Perloff, 1981; Fei *et al.*, 1995; Seggewiss *et al.*, 2009). A down-regulation of beta adrenoreceptors has been noted in individuals with HCM (Lefroy et al., 1993) which is likely due to increased levels of local noradrenaline (Brush et al., 1989) and was shown to be linked with reduced catecholamine reuptake by myocardial sympathetic nerve terminals (Schäfers et al., 1998). Accordingly, it has been hypothesised that there is an increase in cardiac sympathetic activity in individuals with HCM (Mörner *et al.*, 2005). The increase in the adrenergic drive is believed to be a potential stimulus for left ventricular hypertrophy (LVH), increased heart rate and blood pressure, and SCD in HCM (Katarzynska-Szymanska *et al.*, 2013). Therefore, it is important to assess autonomic function in individuals with HCM.

The spectral analysis of heart rate variability (HRV) is a commonly used non-invasive method for assessing autonomic modulation of heart rate (Mörner *et al.*, 2005). The low frequency (LF) spectral power reflects mainly baroreceptor and vagal activity during resting conditions (Shaffer *et al.*, 2014; McCraty and Shaffer, 2015). High frequency (HF) spectral power is associated with parasympathetic activity (Moodithaya and Avadhany, 2012; Shaffer and Ginsberg, 2017). The low-to-high frequency ratio (LF/HF) is related to sympathovagal balance (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017). Analysis of HRV is either performed over short-term recordings (5-60 min) in the clinic or laboratory and is often combined with physiological tests or drug administration or ambulatory long-term recordings (up to 24 hours) (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017). Both methods have been widely used in research and clinical studies as they are non-invasive and efficient at estimating cardiac autonomic function.

The relationship between cardiovascular mortality and ANS imbalance has been thoroughly studied (Lown and Verrier, 1976; Kleiger *et al.*, 1987; Zipes, 1990; Malik *et al.*, 1996; La Rovere *et al.*, 2003; Thayer *et al.*, 2010). Abnormal autonomic modulation, evaluated as alteration in HRV exists in multiple heart conditions and might be attributable to high mortality rate. For example, lower HRV is a risk factor for mortality in individuals with myocardial infarction (MI) and is associated with SCD in chronic heart failure (Kleiger *et al.*, 1987; Malik

et al., 1996; La Rovere *et al.*, 2003). Also, reduced HRV was found in patients with heart failure and dilated cardiomyopathy (Guzzetti et al., 1995; Fauchier et al., 1997).

Assessment of HRV in individuals with HCM has been previously reported (Counihan *et al.*, 1993; Döven *et al.*, 2001). However, different studies have shown wide discrepancies and contradictory results, with evidence of reduction in parasympathetic (PNS) activity in HCM (Ajiki *et al.*, 1993; Counihan *et al.*, 1993; Tanabe *et al.*, 1995; Bonaduce *et al.*, 1997; Limbruno *et al.*, 1998; Döven *et al.*, 2001) or reduction in SNS activity (Fei *et al.*, 1995). Moreover, the lack of consensus of standard methodologies in evaluating HRV in clinical studies is challenging when comparing results between studies (Malik *et al.*, 1996). To date, there is no consensus on the new approaches for the evaluation of HRV such as non-linear dynamic system, short term complexity and entropy and regularity because they are based on complex theoretical and computational frameworks which limit their use in the clinic (Sassi *et al.*, 2015). Consequently, frequency domain and/or time domain analysis remains the method of choice (Sassi *et al.*, 2015).

Other issues related to HRV in HCM which make comparison between studies challenging are the variety of disease severity and possible residual effects of cardiovascular drugs such as beta-blockade (BB) (Mörner *et al.*, 2005).

Evaluation of the existing literature showed that relationships between baseline HRV and haemodynamic measures were not examined previously in individuals with HCM. Instead, a significant number of HRV assessments reported relationships between HRV and echocardiographic and/or clinical characteristics (Mörner *et al.*, 2005). For example, a study reported significant positive correlation between septal thickness and 24-hour recorded standard deviation of all intervals between normal-to-normal beats (NN intervals) (SDNN) (Mörner *et al.*, 2005) in the individuals with HCM. Another study found a significant negative correlation between the exercise induced increase in systolic blood pressure and the 5-minute recorded coefficient of component variance of HF power (CCV_{HF}) in the individuals with HCM (Kawasaki *et al.*, 2008). A fundamental question unanswered in the literature is whether autonomic function represented by HRV could correlate with changes in haemodynamic measures in individuals with HCM. This information is essential and clinically relevant as it has the potential to aid clinicians understanding of the mechanisms of disease progression, haemodynamic instability as well as the effect of treatment.

6.3 Aims and Research Hypotheses

The aim of the present study was two-fold; firstly, to evaluate the differences in resting HRV and haemodynamic measures between individuals with HCM and healthy controls, and

secondly to determine the relationship between resting HRV measures and haemodynamic variables such as stroke volume (SV), cardiac power output (CPO), cardiac output (CO), total peripheral resistance (TPR) and mean arterial blood pressure (MABP) in individuals with HCM.

The following two research hypotheses were tested:

i) There will be a significant difference in resting, five-minute recording of both HRV and haemodynamic measures between individuals with HCM and healthy controls.

ii) There will be a significant relationship between HRV measures and SV, CPO, CO, TPR and MABP in individuals with HCM.

6.4 Materials and Methods

6.4.1 Study design, setting, and participants

This was a prospective, single centre, cross-sectional, observational study conducted at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne, UK between November 2018 and February 2022. This study was a sub-study of the SILICOFCM trial (NCT03832660) (Tafelmeier *et al.*, 2020). Twenty-eight adults (≥18 years old) with HCM were recruited. The mean time since HCM diagnosis was 8±5 years before the study visit.

The inclusion criteria of the individuals with HCM were defined as adults (≥18 years of age) with a confirmed diagnosis of obstructive and/or non-obstructive HCM (i.e., unexplained LVH with either a maximum wall thickness of ≥15 mm or borderline hypertrophy (maximum wall thickness 13–14 mm) on echocardiography and at least one first-degree relative with HCM, agreement to be a participant in the study protocol and ability to provide written informed consent. The exclusion criteria were defined as post septal myectomy or catheter ablation in the three months prior to the study visit, clinical decompensation, defined as New York Heart Association (NYHA) class IV, congestive heart failure symptoms, in the previous three months before the study visit, resting blood pressure greater than 180/100 mm Hg, resting left ventricular outflow tract gradient > 50 mm Hg, left ventricular ejection fraction of less than 50% by echocardiography, renal insufficiency with a glomerular filtration rate of less than 30 mL/min per 1.73m^2, implanted pacemaker or cardio-defibrillator in the last three months before the study visit or scheduled, present or planned pregnancy, life expectancy less than 12 months, severely obese (i.e. body mass index >40 kg/m^2), history or evidence of drug or alcohol abuse within the past 12 months, history of malignancy of any organ system and sustained ventricular tachycardia and atrial fibrillation (AF) or atrial flutter with resting ventricular rate >110 beats per minute.

Potential eligible individuals with HCM were identified by six cardiologists who were members of the research team through cardiology clinics and the North of England Cardiac Family History Service of the Newcastle upon Tyne Hospitals NHS Foundation Trust. Identified individuals with HCM were contacted by a researcher and an information sheet was mailed out upon request.

Age-, sex- and body mass index (BMI)-matched healthy controls were recruited previously for an observational study conducted at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne, UK between January 2018 and July 2019 (15/NE/0190). The inclusion criteria for healthy control participants were 18 years of age or more, no history of chronic, cardiovascular, pulmonary, or metabolic diseases, and willingness to visit the Clinical Research Facility. The exclusion criteria for healthy controls were defined as co-existing medical conditions, history of cardiovascular diseases such as cardiomyopathy, myocardial infarction, intermittent claudication, dementia, stroke, mild cognitive impairment, psychiatric disorders, musculoskeletal disease, chronic respiratory illnesses, cancer and/or signs of cardiac ischemia or arrhythmia at rest or during exercise. All procedures were performed according to the Declaration of Helsinki. Informed written consent was provided by the individuals with HCM and the healthy controls (Templates available in appendix A and E).

6.4.2 Funding and ethical approval

The SILICOFCM trial was approved by the Research Ethics Committee of the National Health Service, North-East England - Tyne & Wear South, and local Research and Development department (18/NE/0318). This project received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no. 777204. The healthy control study was approved by Research Ethics Committee of the National Health Service, North-East England - Tyne & Wear South, and local Research and Development department (15/NE/0190).

6.4.3 Study protocol and measurements

All eligible individuals were asked to refrain from alcohol- and/or caffeine-containing foods and beverages on the day of the study visit and were asked to avoid vigorous exercise 24-hours prior to the study visit. Upon arrival at the Clinical Research Facility, participants were given the opportunity to ask further questions about the study before providing written informed consent and their medical history was reviewed using a Medical History Questionnaire (Riebe *et al.*, 2018) (templates are available in appendix F). The medication list for individuals with HCM was recorded.

Physical activity levels of individuals with HCM and healthy controls were assessed. The daily physical activity, smoking status and alcohol consumption of the healthy participants were assessed by a case report from (template is available in appendix C) and they were considered to be active if they performed ≥ 150 min/week of moderate intensity physical activity (WHO, 2022). The smoking status, alcohol consumption and caffeine intake for individuals with HCM was recorded using a Medical History Questionnaire whilst their physical activity for the past seven days was recorded as steps/day using a pedometer (Omron Health, Japan). Individuals with HCM were considered active if their step count was ≥ 7000 steps/day as evidenced in adults with heart failure (Jordan *et al.*, 2023) .

Whilst seated, participants' blood pressure from the brachial-artery, oxygen saturation, body temperature and heart rate were measured. This was followed by anthropometric measurements, which included standing height and body weight, which were measured using a weighing scale and stadiometer (Seca 769 electric column scale with telescopic measuring rod Seca 220, Seca, Hamburg, Germany). Both individuals with HCM and the healthy controls completed these measures, and both rested in the supine position for five minutes and a 12-lead electrocardiogram was recorded to assess their baseline heart rhythm.

The integrated electrocardiography (ECG) within the TaskForce (CNSystems, Graz, Austria) was used to record spectral (frequency) domain HRV measures and RR interval for both individuals with HCM and the healthy controls. The HRV measures included: low frequency (LF) region of power analysis, high frequency (HF) power (both were given in normalised (nu) and absolute units (ms^2)) and LF/HF ratio. The HF power ranges from 0.15 to 0.4 Hz and LF power ranges from 0.04 to 0.15 Hz (Malik *et al.*, 1996). All spectral domain HRV measures were obtained from beat-to-beat variations in RR interval length (RR interval ms). HRV measures were recorded in a quiet consultation room at ambient temperature for individuals with HCM and healthy controls. participants were requested to lie supine for five minutes with normal breathing. Following resting period, HRV was recorded over five minutes for individuals with HCM and 30 minutes for control participants. Only five minutes of measurement from the 30-minute recording was included in the analysis of control participants to achieve consistency in HRV analysis between HCM and control participants. In order to be consistent in obtaining five minutes from the 30-minute of the HRV recording, three minutes from the start of the recording and two minutes from the end of the recording were excluded and the five minutes data immediately after the excluded first three minutes was considered for the analysis.

Additional HRV measures which were calculated manually were the standard deviation of RR interval (SDRR) in millisecond and the coefficient of component variance of HF power

(CCV_{HF}) as a percentage. SDRR, a time domain HRV variable, was calculated from the RR interval and is a marker of vagal modulation when measured over short-term 5-minute recording (Tanabe *et al.*, 1995; Mazloumi Gavgani *et al.*, 2017). SDRR was calculated in the study instead of SDNN since all beats were included in the analysis without excluding non-sinus beats such as atrial fibrillation.

CCV_{HF} indicates vagal modulation of the heart which is affected by the exposure to drugs, test or disease and is calculated using the following equation: CCV_{HF} (%) = 100*(HF power) $^{1/2}$/ (mean RR interval), and these values correlate linearly to vagal activities (Hayano *et al.*, 1991; Kawasaki, 2005; Arita *et al.*, 2015; Chan *et al.*, 2021).

Blood pressure is regulated by heart rate, stroke volume and peripheral resistance and controlled by the autonomic nervous system (Fortin *et al.*, 1998b). Not only HRV measures but also haemodynamic variables such as CO (L/min), heart rate (HR) (beats/min), SV (ml/beat), stroke volume index (SVI) (ml/beat/m^2), TPR ($dyn \cdot s \cdot cm^{-5}$) and blood pressure (BP) measurements (mmHg)) were collected using non-invasive bioimpedance cardiography method (TaskForce, CNSystems, Graz, Austria) (Fortin, 2004; Jakovljevic *et al.*, 2014), which provided direct, real time and fully synchronised signals (Gratze, 1998).

The TaskForce uses impedance electrodes for cardiac output assessment, which were applied to the participants' neck between both shoulders and the bilateral sides of the thorax. Three-lead electrocardiography electrodes were placed on the chest for HRV measurements, and one finger cuff connected to a forearm unit for continuous blood pressure recordings (Fortin, 2004; Jakovljevic *et al.*, 2014). In addition, cardiac power output (W), which is an integrated measure of overall cardiac function and performance (Grodin *et al.*, 2015) was calculated manually by the equation: CPO (W) = mean arterial blood pressure (mmHg) x cardiac output (L/min) x K, where K=0.0022 (a conversion factor). CPO was indexed to body surface area: CPOI (w/m^2) = CPO/body surface area (Grodin *et al.*, 2015).

6.4.4 Sample size calculation

The power calculation was based on previous literature suggesting a significant difference in RR interval between individuals with HCM and healthy controls. RR interval was selected for the power calculation as a well-established, time domain measure of HRV. The sample size calculation was derived from G*Power 3.1 software (Faul *et al.*, 2007) prospectively based on a significant difference in RR interval previously reported between individuals with HCM (714±96 ms) and healthy controls (829±115 ms), which is 115 ms and a standard deviation of 19 ms (Mörner *et al.*, 2005). The following settings in G*Power software were indicated: alpha=0.05 (two-tailed); beta=0.90 (power=90%); detectable effect=0.31. It was estimated that a total of 55 participants for each group were required to detect a significant difference of 115

ms in RR interval between individuals with HCM and healthy controls. However, due to COVID-19 pandemic, the study was placed on hold from March 2020 until May 2021 and therefore the recruitment was significantly affected and a total of 28 individuals with HCM were recruited by the end of the study. Seventy-five healthy participants were recruited previously but only 28 of them were matched in age-, sex- and BMI-matched with the recruited individuals with HCM.

6.4.5 Statistical analysis

All statistical analyses were performed using SPSS, Version 27.0 (IBM Corp., Armonk, N.Y., USA). Data were screened for univariate and multivariate outliers using standard Z-distribution cut-offs, box plots and Mahalanobis distance test. There were no significant outliers.

Normality of the anthropometric, HRV and haemodynamic measures were assessed using Kolmogorov-Smirnov test and histograms. All anthropometric and haemodynamic were normally distributed. However, all HRV measures were not normally distributed except RR interval, LF (nu) and HF (nu). Therefore, natural log transformation (Ln) was considered for the non-normally distributed HRV measures. After natural log transformation, normality, and homogeneous variance in both individuals with HCM and healthy controls were achieved.

Differences in demographic, physical and clinical characteristics were assessed by independent samples *t*-test for continuous variables and Pearson Chi square for categorical variables. Analysis of covariance (ANCOVA) and the Bonferroni correction were conducted to assess differences in HRV and haemodynamic variables between individuals with HCM and healthy controls with medications and physical activity as covariates. Medications such as beta blockers, vasodilators, negative inotropic and anti-arrhythmic agents are known to alter cardiac autonomic activity (Katzung and Trevor, 2018). Similar to medications, physical activity influences cardiac autonomic system and can hence affect HRV (Tornberg *et al.*, 2019).

Partial correlation was also performed to assess the relationship between HRV and haemodynamic variables using medications and physical activity as the covariates. Statistical significance was indicated if $p<0.05$. All continuous data were expressed in mean±SD unless otherwise stated.

6.6 Results

6.6.1 Demographic, physical and clinical characteristics

Demographic, physical and clinical characteristics for individuals with HCM and healthy controls are presented in Table 6.1. A total of twenty-eight individuals with HCM (54±15 years old; age range: 21-78 years old, males, N=21; females, N =7) were included in the study.

Twenty-eight age-, sex- and BMI–matched healthy control participants (54±16 years old; age range: 20-78 years old, males, N=21; females, N =7) were recruited. The medication list of the individuals with HCM is provided in Table 6.1. Twenty-three (82%) individuals with HCM were on medications, whereas five (18%) patients were not prescribed any medication. Also, fourteen (50%) individuals with HCM were active whilst eighteen (64%) healthy controls were active.

Table 6.1 Demographics, physical and clinical characteristics for individuals with HCM and healthy controls

Variables	**Individuals with HCM N=28**	**Healthy controls N=28**	***P Value***
Age (years)	54±15	54±16	0.90
Sex, female N (%)	7 (25)	7 (25)	1.00
Physical activity, active N (%)	14 (50)	18 (64)	0.28
Height (cm)	173±8	169±9	0.16
Weight (kg)	85±18	83±17	0.68
Body mass index (kg/m^2)	29±5	29±5	0.96
Body surface area (m^2)	2.02±0.2	1.93±0.2	0.15
ICD N (%)	8 (29)	-	-
AF/ Atrial Flutter N (%)	5 (18)	-	-
LVOTO N (%)	1 (4)	-	-
Ischemic cardiomyopathy N (%)	-	-	-
Dilated cardiomyopathy N (%)	-	-	-
Mean time since HCM diagnosis at study visit (years)	8±5	-	-
Medication N (%)			
Beta-adrenergic blockers	17 (61)	-	-
Angiotensin-Converting Enzyme Inhibitors	4 (14)	-	-
Angiotensin II receptor antagonist	4 (14)	-	-
Calcium channel blockers	7 (25)	-	-
Diuretics	6 (21)	-	-
Anti-arrhythmic agents	5 (18)	-	-
No drug therapy	5 (18)	-	-
Lifestyle habits N (%)			
Caffeine	20 (71)	-	-
Alcohol consumption	20 (71)	21(75)	0.76
Smoking	3 (11)	0 (0)	-

AF: atrial fibrillation; HCM hypertrophic cardiomyopathy; ICD: implantable cardioverter defibrillator; LVOTO: left ventricular outflow tract obstruction.

6.6.2 Heart rate variability in individuals with hypertrophic cardiomyopathy and healthy controls

Table 6.2 shows the comparison of all HRV variables between individuals with HCM and healthy controls. RR interval was reduced in the individuals with HCM compared to controls (914±178 vs. 1014±168 ms, p=0.02). All HRV variables which reflect vagal activity were higher in the individuals with HCM versus controls (absolute HF power (7.40±2.50 vs. 6.03±1.35 ms^2, p=0.01), SDRR (4.46±1.43 vs. 3.95±0.60 ms, p=0.01), CCV_{HF} (1.50±1.34 vs. 0.71±0.66 %, p<0.01) and LF power (6.86±2.58 vs. 5.93±1.01 ms^2, p=0.01)). Figure 6.1 demonstrates the differences in RR interval (panel A) and HF power (panel B) between individuals with HCM and the healthy controls.

Table 6.2 Short-term heart rate variability variables in individuals with HCM and healthy controls

Variables	**Individuals with HCM N=28**	**Healthy controls N=28**	***P Value***
Heart rate (beats/min)	76±22	83 ±14	0.84
RR interval (ms)	914±178	1014±168	**0.02**
†SDRR (ms)	4.46±1.43	3.95±0.60	**0.01**
†LF power (ms^2)	6.86±2.58	5.93±1.01	**0.01**
†HF power (ms^2)	7.40±2.50	6.03±1.35	**0.01**
LF power (nu)	38.3±17.1	47.3±19.9	0.98
HF power (nu)	61.6±17.1	52.7±19.9	0.98
†LF/HF	0.51±0.85	0.07±0.94	0.99
†CCV_{HF} (%)	1.50±1.34	0.71±0.66	**<0.01**

CCV_{HF}: coefficient of component variance of high frequency power; HCM: hypertrophic cardiomyopathy; HF: high frequency spectral power; LF: low frequency spectral power; LF/HF: the ratio of low to high frequency spectral power; ms^2: absolute units; nu: normalised units; RR: count number of mean time between r waves; SDRR: standard deviation of RR interval.

† Natural log transformed data presented.

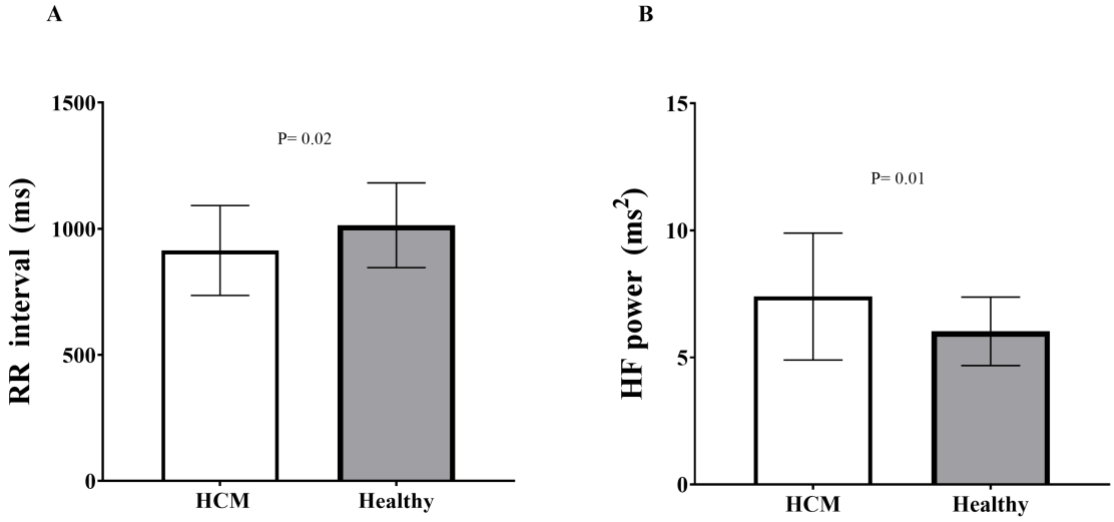

Figure 6.1 Heart rate variability variables in individuals with HCM and healthy controls. (A) RR interval and (B) HF power in individuals with HCM and healthy controls.

6.6.3 Haemodynamic variables in individuals with hypertrophic cardiomyopathy and healthy controls

Differences in haemodynamic variables between individuals with HCM and controls are presented in Table 6.3 after controlling for medication and physical activity. Individuals with HCM had reduced CO (4.6±0.9 vs. 6.8±1.2 L/min, p<0.01), SV (66±17 vs. 82±13 mL/beat, p<0.01) and CPO (0.90±0.22 vs. 1.29±0.37 W, p=0.02) compared to the controls. Indexed values of haemodynamic variables were also significantly reduced in individuals with HCM compared to the controls except TPRI, which was higher in individuals with HCM compared to the controls (Table 6.3). Figure 6.2 represents the differences in SV index (panel A) and TPR index (panel B) between individuals with HCM and healthy controls.

Table 6.3 Haemodynamic variables in individuals with HCM and healthy controls

Variables	**Individuals with HCM N=28**	**Healthy Controls N=28**	***P Value***
Cardiac output (L/min)	4.6±0.9	6.8±1.7	**<0.01**
Cardiac index (L/min/ m²)	2.33±0.42	3.57±0.82	**<0.01**
Heart rate (beats/min)	76±22	83 ±14	0.84
Stroke volume (ml/beat)	66±17	82 ±13	**<0.01**
Stroke volume Index (ml/beat/m^2)	33±9	43±7	**<0.01**
Systolic blood pressure (mmHg**)**	119±14	119±18	0.58
Diastolic blood pressure (mmHg)	76 ±10	73 ±13	0.06
Mean arterial blood pressure (mmHg)	89±12	86±13	0.06
Total peripheral resistance ($dyn{\cdot}s{\cdot}cm^{-5}$)	1724±465	1512±461	0.08
Total peripheral resistance index ($dyn{\cdot}s{\cdot}m^2cm^{-5}$)	3468±1027	2953±1050	**0.04**
Cardiac power output (W)	0.90±0.22	1.29±0.37	**0.02**
Cardiac power output index (W/m^2)	0.44±0.08	0.66±0.16	**<0.01**

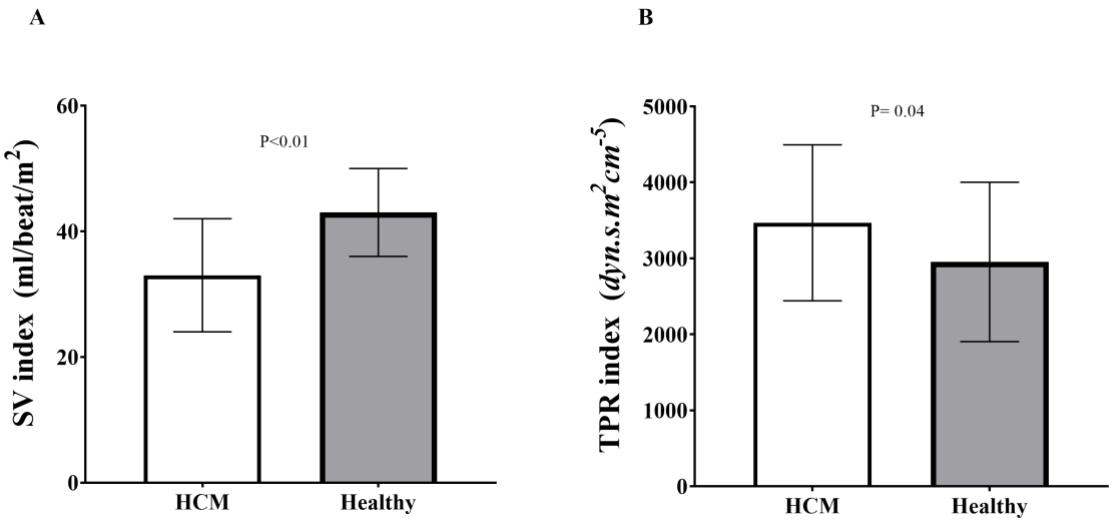

Figure 6.2 Haemodynamic variables in individuals with HCM and healthy controls. (A) Stroke volume (SV) index and (B) Total peripheral resistance (TPR) index in individuals with HCM and healthy controls.

6.6.3 Relationship between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy

Table 6.4 details the results of the partial correlations between HRV and haemodynamic variables after controlling for medications and physical activity. All HRV indices which reflect vagal tone had a negative and moderate relationship with SV; HF power (r= -0.62, p<0.01), LF power (r= -0.58, p<0.01), SDRR (r= -0.62, p<0.01) and CCV_{HF} (r=-0.66, p<0.01). In contrast, there was a positive and moderate relationship between vagal indices of HRV and both TPR; (LF power (r=0.58, p<0.01), HF power (r= 0.58, p<0.01) CCV_{HF} (r= 0.56, p<0.01)) and MABP; (LF power (r=0.50, p<0.01), HF power (r=0.51, p<0.01), SDRR (r=0.49, p<0.05) and CCV_{HF} (r= 0.53, p<0.01)). There was no relationship between all the vagal indices of HRV and CPO and CO.

Table 6.4 Partial correlations between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy

Variables	Stroke volume	Cardiac power output	Cardiac output	Total peripheral resistance	Mean arterial blood pressure
	r	r	r	r	r
RR interval (ms)	0.53**	-0.61**	-0.42*	-0.05	-0.38
†LF power (ms^2)	-0.58**	0.20	-0.12	0.58**	0.50**
†HF power (ms^2)	-0.62**	0.19	-0.13	0.58**	0.51**
†SDRR (ms)	-0.62**	0.25	-0.05	0.51**	0.49*
†CCV_{HF} (%)	-0.66**	0.27	-0.10	0.56**	0.53**

r correlation coefficient; *p< 0.05; **p<0.01. LF nu, HF nu and LF/HF did not have any significant relationships with haemodynamic variables.

† Natural log transformed data presented.

6.7 Discussion

The aim of this study was to assess the cardiovascular autonomic function in individuals with HCM compared to age-, sex- and BMI-matched healthy controls and to evaluate the relationships between HRV measures and haemodynamic function in individuals with HCM.

The major findings suggest that vagal activity indexed by HF power, LF power, SDRR and CCV_{HF} were significantly higher in individuals with HCM versus healthy controls. In contrast, RR interval was significantly reduced in individuals with HCM compared to healthy controls. Haemodynamic variables such as cardiac power output and stroke volume were also significantly reduced in individuals with HCM compared to healthy controls. The current study is the first to investigate the association between HRV and haemodynamic function in individuals with HCM, with findings suggesting that vagal indices of HRV had a significant negative moderate relationship with stroke volume and a significant positive relationship with MABP and TPR.

6.7.1 Heart rate variability in individuals with hypertrophic cardiomyopathy

Available clinical studies on the assessment of HRV in individuals with HCM demonstrate conflicting findings in the literature with evidence of dysfunction in both sympathetic and parasympathetic activities (Ajiki *et al.*, 1993; Counihan *et al.*, 1993; Fei *et al.*, 1995; Tanabe *et al.*, 1995; Bonaduce *et al.*, 1997; Limbruno *et al.*, 1998; Döven *et al.*, 2001; Katarzynska-Szymanska *et al.*, 2013). The conflicting results are due to several issues which make comparison between HRV studies in individuals with HCM difficult. Firstly, the lack of standard methodologies for HRV recording time, participants' measurement position and parameters used among studies testing HRV in individuals with HCM (Malik *et al.*, 1996). Secondly, the variety of disease severity in patients (i.e. comparison of patients with high risk of SCD and low risk of SCD; comparison of patients with obstructive and non-obstructive HCM; comparison of patients with dilated cardiomyopathy and HCM) also add to differences in HRV findings (Mörner *et al.*, 2005). Finally, the possible residual effect of cardioactive drugs such as beta blockers could modify the response to autonomic system and thus HRV (Mörner *et al.*, 2005).

6.7.2 Comparison with previous studies

Despite the wide discrepancy in the results of HRV studies in individuals with HCM, the most frequent findings are the reduction in parasympathetic (PNS) activity and increased sympathetic (SNS) activity (Counihan *et al.*, 1993; Bonaduce *et al.*, 1997; Döven *et al.*, 2001). In contrast,

the present study showed that the short-term recording of all vagal indices of HRV (HF power, LF power, SDRR and CCV_{HF}) were significantly higher in the individuals with HCM compared to healthy controls.

In partial agreement with the present study, Katarzynska-Szymanska (2013) and colleagues evaluated frequency domain HRV for 51 individuals with HCM and 14 age-and sex-matched healthy controls in the supine resting position over 30 minutes, and no significant differences in HRV measures were found between the two groups (Katarzynska-Szymanska *et al.,* 2013). Although the Katarzynska-Szymanska *et al.* (2013) study recruited a higher number of individuals with HCM than the present study and used similar methodologies and inclusion criteria to the current study, there were no significant differences in all spectral components of HRV between individuals with HCM and healthy controls. This is possibly due to the method of HRV assessment, as they used finger pressure wave form approach which is dependent on photoplethysmography (PPG) whereas the present study used the Taskforce device which uses ECG to detect HRV. ECG is the gold standard method to detect HRV accurately as it directly records the electrical activity of the heart whereas PPG records HRV indirectly by detecting blood volume and is sensitive to body motion artefacts (Jeyhani *et al.*, 2015; Jan *et al.*, 2019).

Another study reported short-term HRV in obstructive and non-obstructive individuals with HCM and found that resting LF and HF power were higher in non-obstructive individuals with HCM compared to obstructive individuals with HCM (Limbruno *et al.,* 1998). These findings could be comparable with the present study findings and could indicate that the presence of LVOT obstruction may contribute to the increase in the SNS activity in individuals with HCM. It was not possible to compare obstructive to non-obstructive individuals with HCM in the present study due to the small number of obstructive individuals with HCM (n=1).

In contrast to this study's findings, Döven and collaborators (2001) found that time domain measures representing vagal activity (i.e. SDNN, root mean-squared successive difference (RMSSD) and percentage of cycles differing from the preceding one by more than 50 ms (PNN50%)) were significantly reduced in individuals with HCM compared to healthy controls (Döven *et al.*, 2001). Unlike the present study, the findings of Döven *et al.* (2001) were based on 24 hour recorded time and they did not present the differences between both groups in the mean RR interval. Additionally, two studies reported a reduction in parasympathetic activity in the individuals with HCM, which was apparent only during night-time (Ajiki *et al.*, 1993; Tanabe *et al.*, 1995). Both studies recorded frequency domain HRV measures over 24 hours, yet the former showed lower PNS activity (log HF power) and increased SNS activity (log LF/HF) in individuals with HCM and individuals with dilated cardiomyopathy (DCM) compared to healthy controls (Ajiki *et al.*, 1993). The latter reported decreased PNS (CCV_{HF},

HFnu, SDRR) and higher SNS (LFnu, LF/HF) in hospitalised patients with advanced HCM compared to healthy controls (Tanabe *et al.,* 1995). The possible reasons for the disparity in the findings between the present study and the aforementioned ones are possibly related to firstly, the recording time of the HRV measures since recording of HRV over 24 hours constitutes major fluctuations that may not reflect changes occurring in the short term and may bring about the possibility to study circadian rhythm (Counihan *et al.,* 1993). Secondly, variety of disease severity between studies (HCM, advanced hospitalised HCM and DCM) and finally, the inconsistent use of LF power versus LF/HF as an index of SNS activity between studies. Moreover, in (Ajiki *et al.*, 1993), a wider band width of LF and HF power (0.00 to 0.15 Hz for LF power and 0.15 to 0.50 Hz for HF power) was used, making results incomparable to this present study.

Taking into account the aforementioned reasons for disparity, it should be emphasised that frequency domain measures of HRV aid more accurate assessment of the direction and magnitude of changes in autonomic balance than is likely with time domain analysis (Pumprla *et al.,* 2002). When evaluating short- term recordings of HRV, only spectral analysis has been recommended but there is still no consensus when short-term or long-term HRV recording should be applied (Malik *et al.,* 1996). However, most recent studies have recommended five minute recordings where possible to enable comparison between clinical studies (Laborde *et al.,* 2017). Short-term recordings are advantageous due to being performed under standard laboratories circumstances, whilst long-term recordings may have the possibility to assess circadian pattern (Mörner *et al.,* 2005). Indeed the 24-hour recorded frequency domain measures and their corresponding explanation might be questionable since they may hide details about autonomic modulation of RR intervals (Schwartz, 1990). This is because the physiologic mechanisms accounted for heart rate modulation are not static over a 24-hour period (Bittencourt *et al.,* 2005). For example, the increase in SNS reported in the individuals with HCM compared to healthy controls was questionable when frequency domain HRV measures were analysed over 24-hour period (Fei *et al.*, 1995).

6.7.3 Possible mechanisms underlying the high vagal modulation in the present study

The increase in vagal activity seen in individuals with HCM in the present study could be attributed to the imbalance/dysfunction between sympathetic (SNS) and parasympathetic (PNS) arms of the autonomic nervous system. Under normal circumstances, there is a precise interaction/cross talk between these two systems to maintain bodily functions. Indeed, parasympathetic activity can be directly modulated by the sympathetic nerve terminals and vice versa (Ondicova and Mravec, 2010). Therefore, it can be speculated that a possible reason for

increased PNS activity in individuals with HCM might be a regulatory/compensatory feedback due to either chronic increase in the SNS activity as reported in previous studies (Perloff, 1981) or reduction in SNS activity as reported in (Limbruno *et al.*, 1998) triggering an increase in PNS activity. Indeed, this can be explained by the supernormal systolic function that the individuals with HCM in the present study had i.e, ejection fraction (EF= 63±9.78 %) which is supported by Fei *et al.* (1995) who speculated that supernormal ejection fraction in individuals with HCM may activate a feedback mechanism leading to activation of vagal afferents in the ventricles which contribute to the lower SNS and high PNS activity in individuals with HCM.
However, the extent of sympathetic modulation in the present study could not be measured because HRV variables were not directed to measure SNS activity due to short-term recording of resting LF power, which mainly reflects baroreceptor activity (McCraty and Shaffer, 2015). LF power is primarily the product of PNS (Reyes del Paso *et al.*, 2013) and/or baroreceptor activity (Goldstein *et al.*, 2011). This is because LF power spectrum ranges from 0.04 to 0.15 Hz but the SNS activity does not produce rhythm above 0.1Hz (Shaffer and Ginsberg, 2017). Therefore, the significantly higher LF power in individuals with HCM may possibly confirm the higher vagal tone compared to healthy controls in this present study.
Another possible reason for the higher vagal tone in individuals with HCM in the present study is the prescription of cardiovascular drugs such as beta blockers (BB), vasodilators, negative inotropic and anti-arrhythmic agents. Those medications are known to affect autonomic activity (Houston and Stevens, 2014; Katzung and Trevor, 2018). Therefore, adjustment for medications was performed in this study using ANCOVA and CCV_{HF}. The influence of beta blockers on the autonomic function in heart failure and after myocardial infarction has been extensively studied, since it has a direct influence on beta-adrenoreceptors with inhibition of sympathetic overactivity and reduction in heart rate which was reported (Keeley *et al.*, 1996; Aronson and Burger, 2001). Furthermore, mortality reduction due to beta blockade was confirmed in individuas with heart failure in several large trials such as COPERNICUS, CIBIS and MERIT-HF (Eichhorn and Bristow, 2001; Lechat *et al.*, 2001; Deedwania *et al.*, 2005) respectively. Although the effect of beta blockers on the autonomic function of other cardiovascular diseases was also reported, a limited number of studies evaluated the effect of beta blockers on the cardiovascular autonomic function of individuals with HCM treated or not treated with beta blockers. For example, a previous study reported higher HRV parameters reflecting vagal tone in individuals with HCM treated with beta blockers compared with non-treated individuals with HCM (Mörner *et al.*, 2005). Another medication in line with the increase in vagal indices of HRV is antiarrhythmic drugs. This influnce has been reported for some antiarrhythmic drugs in individulas with non ischemic DCM (Kurtoglu *et al.*, 2014). Other

medications (Calcium channel blockers (CCB), Angiotensin-Converting Enzyme Inhibitors (ACEI), Angiotensin II receptor antagonists (ARBs), and Diuretics), influnce HRV in a number of cardiovascular diseases such as heart failure, ischemic cardiomyopathy and hypertension (Tomiyama *et al.*, 1998; Tomiyama *et al.*, 1999; Özdemir *et al.*, 2007). However, clinical data on their influnce on HRV in the individuals with HCM are limited (Houston and Stevens, 2014). In addition to medications, physical activity is also a well-known modulator of autnomic function by increasing parasympathatic tone in both healthy populations (Soares-Miranda *et al.*, 2014) and in individuals with cardiovascular diseases (Pearson and Smart, 2018). In the present study, vagal indices of HRV were still significantly higher in the individuals with HCM than heathy controls even after adjustment for physical activity.

Additionally, the possibility of higher vagal estimate in individuals with HCM in the present study could be because of the effect of long-term dual chamber pacing (DDD) pacing. In a study that examined the effect of DDD on HRV measures in individuals with obstructive HCM pre-implantation and one year post implantation, there was a significant increase in vagally mediated time domain HRV measures after implantation (Simantirakis *et al.*, 1999). In the present study, eight individuals with HCM had an ICD (seven on DDD pacing and one on ventricular pacing mode (VVI)). To test the effect of vagally mediated DDD pacing on the individuals with HCM for the present study, the individuals with HCM who had DDD were excluded from the analysis. However, the results were not altered and the vagally mediated frequency domain HRV analysis were still significantly higher in individuals with HCM compared to healthy controls (see Table 6.1 in appendix G).

6.7.3 Relationship between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy

The novel finding of the present study was that vagally mediated HRV indices had significant relationships with SV, TPR and MABP in individuals with HCM. The relationship between vagal indices of HRV and SV was negative but positive with TPR and MABP. These findings are likely linked together as the autonomic nervous system plays an essential role in maintaining cardiovascular health by regulating blood pressure and blood flow (Charkoudian and Rabbitts, 2009). MABP is the product of CO and TPR (MABP= CO x TPR) and CO is the product of SV and HR (CO= SV x HR) (Williams *et al.*, 2021). The role of the vagal nerve in regulating HR, SV and CO has been extensively studied (Levy, 1997). Normally, the vagal nerve mediates inhibitory responses in the heart and therefore it decreases CO, SV and HR (Kawasaki and Sugihara, 2014). This effect was observed in individuals with HCM in the present study as the

higher vagal tone was related to a significant reduction in SV, whilst interestingly and paradoxically increases in both MABP and TPR was observed.

An explanation for this paradox between vagal enhancement of higher MABP and TPR in individuals with HCM could possibly be due to the activation of the *Renin-Angiotensin-Aldosterone System* (RAAS). The RAAS is mediated by the kidneys and is vital for controlling blood pressure (Sparks *et al.*, 2014). In conditions of hypoperfusion manifested by low CO and low SV, as shown in present study, renin hormone synthesis is activated to compensate for blood loss (Gargiulo *et al.*, 2015). Renin metabolises angiotensinogen, releasing angiotensin I (AngI), which is subsequently converted into angiotensin II (AngII) via angiotensin converting enzyme (ACE). Angiotensin II is a potent vasoconstrictor, which causes increases in both MABP and TPR, hence maintaining blood flow (Ames *et al.*, 2019). In this present study, both resting SV and CO were significantly reduced in individuals with HCM compared to healthy controls. The reduction in SV was significantly related to the higher vagal tone, which indirectly stimulates the RAAS as a compensatory mechanism against low CO. Accordingly, in this present study the significant positive relationship between TPR, MABP and vagal activity is likely due to the indirect activation of SNS through the activation of RAAS, which is stimulated by the significant reduction in SV and CO in individuals with HCM. This sympathetic activation which caused a further stimulation of vagal activity might prevent haemodynamic collapse (Ames *et al.*, 2019). The correlation analysis between HRV and haemodynamic variables in healthy controls is available in appendix G Table 6.2).

Another possible reason for RAAS stimulation in the individuals with HCM is the presence of ACE polymorphism which is associated with high level of serum ACE (Yuan *et al.*, 2017). However, in the present study neither the activity of RAAS was measured, nor the genetic screening of ACE polymorphism was performed.

Based on the present study findings, the significant relationship between HRV and haemodynamic variables would suggest that treatment for individuals with HCM may not only improve symptoms related to the disease progression but also improve cardiac autonomic function.

6.8 Strengths and limitations of the study

The current investigation is the first to investigate the relationships between HRV and haemodynamic variables in individuals with HCM. It would have been beneficial if the present study finding, i.e. higher vagal activity was associated with higher MABP and TPR at rest, could have been evaluated during exercise. This would have helped our understanding of the mechanisms attributed to the known abnormal vasodilatory response during exercise for individuals with HCM (Olivotto *et al.*, 1999).

The study findings were based on five minute recording time, which also supports the increasing demand and necessity for short-term HRV analysis in various clinical applications (Lee *et al.*, 2018). Although it was previously suggested that 24 hour recordings were the gold standard recording time for clinical HRV assessment (Shaffer and Ginsberg, 2017), spectral analysis of HRV over a 24 hour period is limited by unstable oscillations of the RR intervals (Fei *et al.*, 1995). Therefore, a significant number of studies which involve 24 hour assessment of spectral measures of HRV analyse data over a shorter period of time such as 2-5 minutes (Fei *et al.*, 1995; Mörner *et al.*, 2005). This approach may partially reduce the effect of instability of the data and make values of the 24 hour recorded HRV data directly related to a stable short-term recording (Fei *et al.*, 1994).

The study was also limited by the sample size, which limits the statistical power. Moreover, the TaskForce device for HRV analysis records only frequency domain measures but not time domain measures except RR interval. The findings of this study were based on recording on resting supine position, which causes a lack of information about sympathetic activity. This is because LF component of HRV does not reflect pure sympathetic activity especially when it is recorded on resting and supine position where there is no sympathetic provocation manoeuvre such as a tilt test. In the present study there were five patients with atrial fibrillation (AF) and a further two diagnosed with an atrial flutter. Those patients were not excluded from the study even though arrythmias can critically affect the outcome of HRV analysis (Nunan *et al.*, 2010a). Excluding these patients would have reduced the power of the study further and resulted in significant selection bias (Malik *et al.*, 1996). To confirm, after excluding individuals with HCM who had AF/atrial flutter from the HRV analysis, the results did not change, i.e. vagal indices of HRV were significantly higher in individuals with HCM versus healthy controls (appendix G Table 6.3).

6.9 Conclusions and clinical implications

Individuals with HCM demonstrated greater variation in vagal activity but reduced haemodynamic function in comparison to healthy controls. Resting short-term frequency domain indices of HRV provide a feasible approach to assess autonomic function in individuals with HCM. Further studies are warranted to investigate the prognostic value of HRV in individuals with HCM. Also, standard methodologies of HRV assessment in individual with HCM are required to have comparable results.

Vagal indices of HRV were significantly correlated with stroke volume, total peripheral resistance and mean arterial blood pressure in the individuals with HCM. This finding is clinically relevant since it could aid in the prevention of HCM progression and improve

understanding of the disease pathophysiology. Further studies are required to replicate the study findings in a larger cohort of individuals with HCM.

Chapter 7. Effect of a novel lifestyle intervention on heart rate variability and cardio-metabolic function in individuals with hypertrophic cardiomyopathy

7.1 Abstract

Background and objectives: A limited number of studies investigated the effects of lifestyle interventions on heart rate variability (HRV) in hypertrophic cardiomyopathy (HCM). This study evaluated the effect of a novel lifestyle intervention incorporating physical activity and dietary nitrate supplementation on HRV, haemodynamic and exercise performance in individuals with HCM.

Methods: Twenty-eight individuals with HCM were recruited into a single-centre pilot study (N=7 females, age 54±15 years) and randomised into either the intervention (N=20) or control (N=8) group. Participants completed short-term (5-minute) non-invasive HRV measurements under resting (supine) conditions TaskForce (CNSystems, Graz, Austria). Frequency-domain HRV measures including spectral analysis of RR interval (i.e., absolute, and normalised low frequency power (LF), high frequency power (HF) and LF/HF ratio) were recorded. Non-invasive haemodynamic variables at rest and peak exercise were recorded using bioreactance technology (Starling^{TM} SV, Baxter International Inc, USA). Participants in the intervention group were requested to consume 6 mmol of nitrate daily (concentrated beetroot juice) which was provided to them and increase and maintain daily physical activity by ≥2000 steps/day above baseline for 16 weeks. Participants in the control group were requested to maintain their usual lifestyle and monitored daily step counts. Daily physical activity step counts were evaluated objectively using pedometers (Omron Health, Japan). All participants were monitored weekly via telephone calls and average daily activity levels were recorded using diaries.

Results: Participants in the intervention group significantly increased their average physical activity by 3029 steps/day from baseline to post-intervention (i.e., 6234±2389 vs. 9263± 3341 steps/day, $p<0.01$). In comparison, the control group maintained their usual physical activity (7559±3257 vs. 7604± 2901 steps/day, $p=0.96$). There was a significant increase in post-intervention HF power (7.54±2.14 vs. 8.78±1.60 ms^2, $p<0.01$) and LF power (6.89±2.33 vs. 8.17±1.55, $p<0.01$) respectively in the intervention but not in the control group. Resting and peak mean arterial blood pressure (MABP) in the intervention group significantly reduced in the intervention group (108±6 vs. 102±7 mmHg, $p<0.01$; and 126±11 vs. 116±9 mmHg, $p<0.01$). There was no difference in peak oxygen consumption from baseline to post intervention in either the intervention (18.2±4.20 vs 19.8±2.64 ml/kg/min, $p=0.07$) or control group (19.2±10.0 vs 20.1±9.54 ml/kg/min, $p=0.54$).

Conclusions: A novel lifestyle intervention incorporating physical activity and dietary nitrate supplementation led to a significant increase in daily physical activity and improved

parasympathetic activity and resting and peak mean arterial blood pressure in individuals with HCM.

Keywords: Hypertrophic cardiomyopathy, nitrate, lifestyle, haemodynamic function, heart rate variability

7.2 Introduction

Hypertrophic cardiomyopathy (HCM) is a multifactorial disease associated with a wide range of abnormalities such as autonomic dysfunction, haemodynamic instability and reduced functional capacity (Ommen *et al.*, 2020). Autonomic dysfunction in HCM is characterised by abnormal exercise vasodilation which has been reported in nearly 25% of individuals with HCM (Olivotto *et al.*, 1999; Patel *et al.*, 2014a). Heart rate variability (HRV) which refers to the variation between consecutive heart beats of cardiac rhythm is a non-invasive tool to measure cardiac autonomic modulation in healthy and diseased populations (Vanderlei *et al.*, 2009). Due to the hereditary and heterogeneity nature of HCM, available treatment strategies are based mainly on symptoms alleviation and attenuation of the disease progression (Maron *et al.*, 2022a).

Lifestyle modifications are recommended for the prevention and treatment of cardiovascular diseases (CVD), especially appropriate eating habits and increasing physical activity (PA) (Rippe, 2019). Indeed, lifestyle interventions incorporating PA and inorganic dietary nitrate (NO_3-) supplementation have been assessed individually and separately and have shown improvements of signs and symptoms in individuals with heart failure (HF) (Pearson *et al.*, 2017; Coggan *et al.*, 2018; Okwose *et al.*, 2019). However, in individuals with HCM, the data regarding lifestyle interventions are limited (Ommen *et al.*, 2020).

Long-term PA can directly improve cardiac autonomic function, cardiometabolic variables and exercise performance in individual with HF (Tian and Meng, 2019). Meta-analyses have demonstrated that long-term exercise training improved parasympathetic tone and reduced sympathetic tone indexed by HRV measures in individuals with HF (Pearson and Smart, 2018). However, the effect of chronic physical activity on HRV measures as primary or secondary outcomes has not previously been evaluated in individuals with HCM.

Not only HRV but also cardiometabolic variables and exercise performance have improved following long-term exercise training in individuals with HF. Increasing daily physical activity in individuals with chronic HF led to improved functional capacity represented by peak exercise oxygen consumption (VO_2) and stroke volume (Okwose *et al.*, 2019). In individuals with HCM, there has been only one randomised controlled trial (RESET-HCM) to date that has assessed the effect of moderate-intensity exercise training on the change in peak exercise oxygen consumption (VO_2) as a primary outcome and the authors reported a significant increase in peak VO_2 after 16 weeks of training (Saberi *et al.*, 2017). Although the study by Saberi *et al.* (2017) supported the implementation of moderate intensity exercise as a non-surgical intervention to improve peak VO_2 in individuals with HCM, uncertainties regarding the safety and intensity of

PA still exists in the recent American and European guidelines for HCM (Pelliccia *et al.*, 2019; Ommen *et al.*, 2020).

The intake of food and beverages with a rich source of nitrate (NO_3-) such as beetroot juice (BRJ) can be therapeutic for cardiovascular health as it increases nitric oxide (NO) bioavailability (Lundberg *et al.*, 2008). The therapeutic role of dietary inorganic nitrate (NO_3-) supplementation is based on previous knowledge about the vasodilatory properties of NO and its ability to control blood flow, involved in platelet adhesions, angiogenesis, cellular oxygen consumption, muscular performance, immunity regulations and control of inflammation signalling pathways (Kelm, 1999). Studies have suggested that the intake of BRJ for four to six days could be a strategy to increase the bioavailability of NO and achieve the aforementioned physiological benefits in individuals with increased risk of CVD (Rammos *et al.*, 2014; Raubenheimer *et al.*, 2017). Yet, the effect of long-term dietary nitrate rich BRJ on HRV measures has not been previously evaluated in individuals with either HCM or HF. Furthermore, the effect of dietary nitrate on cardiometabolic function and exercise performance has been evaluated in individuals with HF but not in individuals with HCM. For example, an acute dose of BRJ (11 mmol NO_3-) has led to an increase in submaximal exercise VO_2 in individuals with HF with reduced ejection fraction (HFrEF) (Coggan *et al.*, 2018). Also, the acute consumption of BRJ (12.9 mmol NO_3-) has led to improvements in peak VO_2 and cardiac output in individuals with HF with preserved ejection fraction (HFpEF) (Zamani *et al.*, 2015).

Despite the potential beneficial effects of PA and inorganic dietary nitrate on cardiac autonomic, haemodynamic, and metabolic variables, their combined effects as a lifestyle intervention have not been previously evaluated in individuals with HCM. Also, the available studies evaluating either PA or dietary nitrate supplementation have not targeted HRV as a primary outcome.

7.3 Aims and Research Hypotheses

The primary aim of the present study was to evaluate the effect of the novel lifestyle intervention incorporating PA and dietary nitrate supplementation on cardiac autonomic function represented by HRV. The secondary aim was to assess the effect of the novel lifestyle intervention on haemodynamic and cardiopulmonary exercise testing cardiometabolic variables in individuals with HCM.

The following two research hypotheses were tested:

I. The lifestyle intervention will lead to significant improvements in HRV measures in individuals with HCM.

II. The lifestyle intervention will lead to a significant change in resting and peak exercise cardiometabolic variables in individuals with HCM.

7.4 Materials and Methods

7.4.1 Study design, participants, and recruitment

This was a prospective, single centre, cross-sectional, observational, pilot study conducted at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne, UK between November 2018 and February 2022. This was a sub-study of the SILICOFCM trial (NCT03832660) (Tafelmeier *et al.*, 2020). One hundred and twelve individuals with HCM were screened to determine their eligibility. Twenty-eight adults (≥18 years old) diagnosed with HCM who were not participating in any drug trial, or a regular exercise regimen were recruited and enrolled. All recruited participants were randomized into either intervention or usual standard care (control) group in approximately 2:1 ratio. The inclusion and exclusion criteria are shown in Table 7.1.

Table 7.1 The inclusion and exclusion criteria of the study

Inclusion criteria	Exclusion criteria
Adults (≥18 years old)	Post septal myectomy or catheter ablation in the 3 months prior to the study
Confirmed diagnosis of HCM (unexplained LVH with either a maximum wall thickness of ≥15 mm or borderline hypertrophy (maximum wall thickness 13–14 mm on ECHO and at least one first-degree relative with HCM)	Clinical decompensation (congestive heart failure), i.e. (NYHA) class IV 3 months prior to the study
Agreement to participate in the study protocol	Resting BP >180/100 mmHg
Ability to provide written consent	Resting LVOT gradient > 50mmHg
	LVEF<50% by ECHO
	Renal insufficiency; GFR<30 ml/min/1.73m^2
	Implanted pacemaker or ICD in the last 3 months prior the study or scheduled
	Present or planned pregnancy
	Life expectancy <12 months
	BMI>40 kg/m^2
	History or evidence of drug or alcohol abuse within the past 12 months
	History of malignancy of any organ system
	Sustained VT and AF/flutter with resting VR>110 bpm
	Hypotensive response to exercise testing (≥20 mmHg reduction of SBP from baseline or an initial increase in SBP followed by a decrease of SBP≥20 mmHg
	A history of exercise induced syncope or ventricular arrhythmias
	Inability to exercise due to orthopaedic or other non-cardiovascular limitations

AF: atrial fibrillation; BP: Blood pressure; BMI: body mass index; ECHO: echocardiography; GFR: glomerular filtration rate; HCM hypertrophic cardiomyopathy; ICD: implantable cardioverter defibrillator; LVOT: left ventricular outflow tract; LVH: left ventricular hypertrophy; LVEF: left ventricular ejection fraction; NYHA: New York Heart Association; SBP: systolic blood pressure; VR: ventricular rate; VT: ventricular tachycardia.

Potential eligible individuals with HCM were identified by six cardiologists who were members of the research team through cardiology clinics and the North of England Cardiac Family History Service of the Newcastle upon Tyne Hospitals NHS Foundation Trust. Identified individuals with HCM were contacted by a researcher and a participant information sheet (Appendix H) was mailed out upon request. Informed written consent was provided by the individuals with HCM during the first visit (Appendix E). All procedures were performed according to the Declaration of Helsinki.

7.4.2 Randomisation

After the baseline visit, eligible participants with signed consent were randomly assigned to either the intervention or usual standard care (control) group in a 2:1 ratio to maintain concealment and reduce selection bias. Randomisation was based on sex, age and left ventricular outflow tract gradient, as per the published protocol (Tafelmeier *et al.*, 2020), using

minimisation to maintain balance between the two intervention components. Randomisation numbers were software-generated and assigned in a strict sequence.

7.4.3 Ethical approval and Funding

The SILICOFCM trial was approved by the Research Ethics Committee of the National Health Service, North-East England - Tyne & Wear South, and local Research and Development Department (18/NE/0318). This project received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no. 777204.

7.4.4 Intervention

The lifestyle intervention consisted of two parts: PA and dietary supplementation with inorganic nitrate (NO3−). PA involved a home-based exercise regimen aimed at increasing daily PA by at least 2000 steps/day from baseline for 7 days/week for a total of 16 weeks.

Participants were instructed to walk for approximately 30 minutes either continuously or intermittently into several bouts (i.e. 3x10 min) and use standardised Borg scale (Borg, 1982) to rate their perceived excretion aiming for achieving the levels between 11-13 (easy-light-to somewhat hard). All participants were given a pedometer (Omron Health, Japan) and asked to complete a daily physical activity diary which was reviewed and discussed during weekly telephone calls by a researcher (Template available in appendix I). All participants were also provided with an activity planner sheet to keep a log and track of their PA (Template available in appendix J).

The second part of the lifestyle intervention was a dietary supplementation with inorganic nitrate, which was given in a form of concentrated nitrate-rich BRJ. A single dose of the nitrate-rich BRJ (NO3−, BEET IT Sport, James White Drinks Ltd., Ipswich, UK) contains 6 mmol of NO3− in a 70 ml bottle. Participants in the intervention group were instructed to consume BRJ each morning with breakfast for 16-weeks. Adherence to both components of the intervention were tracked by completion of activity logs, weekly telephone follow-ups, number of returned BRJ bottles, pedometers, and self-reported diaries.

7.4.5 Usual standard care (control)

Participants in the control group did not receive any of the lifestyle intervention (i.e., increased daily steps and dietary nitrate). Similar to the intervention group, the control group were also provided with pedometer, activity planner sheet and weekly telephone calls with a researcher but were asked not to change their PA and dietary habits during the study.

7.4.6 Study visits and measurements

All eligible individuals attended the Clinical Research Facility for two visits: one at baseline and one at follow up (16-weeks). All participants were asked to avoid vigorous exercise 24-hours prior to the study visits. Upon arrival for the baseline visit, participants were given the opportunity to ask further questions about the study before providing written informed consent. During both visits, the medical history of the participant was reviewed using a Medical History Questionnaire and Physical Activity Readiness Questionnaire (Riebe *et al.*, 2018) (Appendix B and F) and medication list was recorded.

Whilst seated, participants' blood pressure from the brachial-artery, oxygen saturation, body temperature and heart rate were measured (Welch Allyn, USA). This was followed by anthropometric measurements, which included standing height and body weight, which were measured using a weighing scale and stadiometer (Seca 769 electric column scale with telescopic measuring rod Seca 220, Seca, Hamburg, Germany). A 12-lead electrocardiogram (ECG) was also recorded for both visits in the rested and supine position for five minutes to assess the heart rhythm for participants.

7.4.6.1 Resting heart rate variability measurements

The integrated ECG within the TaskForce (CNSystems, Graz, Austria) device was used to record spectral (frequency) domain HRV measures and RR interval for all participants. HRV measures were recorded at rest in the supine position for five minutes with normal breathing in a quiet consultation room at ambient temperature. The HRV measures included: low frequency (LF) region of power analysis, high frequency (HF) power (both were given in normalised (nu) and absolute units (ms^2)) and LF/HF ratio. The HF power ranges from 0.15 to 0.4 Hz and LF power ranges from 0.04 to 0.15 Hz (Malik *et al.*, 1996). All spectral domain HRV measures were obtained from beat-to-beat variations in RR interval length (RR interval ms). The standard deviation of RR interval (SDRR) in millisecond and the coefficient of component variance of HF power (CCV_{HF}) as a percentage were calculated manually. SDRR, a time domain HRV variable, was calculated from the RR interval and is a marker of vagal modulation when measured over short-term (5-minute) recording (Tanabe *et al.*, 1995; Mazloumi Gavgani *et al.*, 2017). SDRR has the advantage of calculating all beats without excluding non-sinus beats such as atrial fibrillation.

CCV_{HF} corresponds to the changes in vagal modulation of the heart despite the influence of drugs, imaging and/or laboratory test or disease and is calculated using the following equation: CCV_{HF} (%) = 100*(HF power) $^{1/2}$/ (mean RR interval) (Hayano *et al.*, 1991; Kawasaki, 2005; Arita *et al.*, 2015; Chan *et al.*, 2021).

7.4.6.2 Resting and peak exercise haemodynamic measurements

Non-invasive haemodynamic variables were measured at rest and at peak cardiopulmonary exercise using non-invasive bioreactance technology (Starling^{TM} SV, Baxter International Inc, USA). The bioreactance method utilises four dual electrodes placed on the back side of the upper and lower thorax. Haemodynamic variables included cardiac output (CO), cardiac index (CI), stroke volume (SV), stroke volume index (SVI), heart rate (HR) and blood pressure measurements were recorded directly from the bioreactance technology. However, cardiac power output (CPO), cardiac power output index (CPOI), total peripheral resistance (TPR), and total peripheral resistance index (TPRI) were calculated manually using the following equations. CPO is an integrated measure of overall cardiac function and performance (Grodin *et al.*, 2015). CPO (W) = mean arterial blood pressure (mmHg) x cardiac output (L/min) x K, where K=0.0022 (a conversion factor); CPOI (w/m^2) = CPO/body surface area (Grodin *et al.*, 2015). TPR was estimated as the ratio between mean arterial pressure and cardiac output and, according to convention, was multiplied by a factor of 80 to convert units to dynes per second per centimetre to the fifth power and then it was indexed by dividing the values of TPR by body surface area (Jakovljevic *et al.*, 2017).

7.4.6.3 Cardiopulmonary exercise test and metabolic measurements

At both visits, all participants completed a progressive exercise test using a semi-recumbent cycle ergometer (Corival, Lode, Groningen, Netherlands) with simultaneous gas exchange measurements (Cortex metalyzer 3B, Cortex, Leipzig, Germany). Participants were asked to wear a face mask to collect and record metabolic measures including respiratory exchange ratio (RER), work rate (WR), VO_2 and anaerobic threshold (AT). All participants were rested for two minutes where resting haemodynamic measurements were recorded. An initial warm up period of one minute at 0 watts was included in the exercise protocol followed by gradual increments at the rate of 10 watts per minute starting from 30 watts until maximum excretion achieved. Whilst exercising, participants were asked to maintain a pedal frequency between 60-70 revolutions per minute and measurements were recorded from the 12-lead ECG (Custo, CustoMed, Germany) along with an automated blood pressure (SunTech Tango, SunTech Medical, Inc., Morrisville, USA). The Borg scale from 0 to 10 was used to evaluate the rate of perceived excretion (RPE) (Borg, 1982). A consultant cardiologist was present throughout the cardiopulmonary exercise test, which was terminated when any one of the following criteria were met: 1) upon participant's request, 2) when respiratory exchange ratio (RER) exceeded 1.15 (i.e. universally agreed RER threshold (Issekutz Jr *et al.*, 1962)), 3) failure to maintain a cadence of 60 revolutions per minute, or 4) failure to increase oxygen consumption although

exercise intensity (watts) increased or 5) symptoms warranting termination such as chest pain and dizziness.

7.4.7 Sample size calculation

The power calculation was based on previous literature suggesting a significant difference in RR interval in individuals with HF who underwent exercise intervention, since the effect of exercise intervention on HRV measures in individuals with HCM is unknown. RR interval was selected for the power calculation as a well-established, time domain measure of HRV. The sample size calculation was derived from G*Power 3.1 software (Faul *et al.*, 2007) prospectively based on a significant difference in the mean RR interval previously reported in individuals with HF following 24-weeks of exercise training (989±148 ms vs. 1088±156 ms), which is 99 ms and a standard deviation of 8 ms (Ricca-Mallada *et al.*, 2012). The following settings in G*Power 3.1 software were indicated: alpha=0.05 (two-tailed); beta=0.90 (power=90%); detectable effect=0.3. It was estimated that a total of 56 participants were required to detect a significant difference of 99 ms in RR interval following the intervention in individuals with HCM assuming the same effect of exercise on HF in the Ricca-Mallada *et al.* (2012) study. However, due to the COVID-19 pandemic, the study was placed on hold from March 2020 until May 2021 and therefore the recruitment was significantly affected and a total of 28 individuals with HCM were recruited by the end of the study.

7.4.8 Statistical analysis

All statistical analyses were performed using SPSS, Version 27.0 (IBM Corp., Armonk, N.Y., USA). Data were screened for univariate and multivariate outliers using standard Z-distribution cut-offs, box plots and Mahalanobis distance test. There were no significant outliers.

Normality of the anthropometric, HRV, metabolic, haemodynamic, and step counts measures were assessed using Kolmogorov-Smirnov test and histograms. All data were normally distributed except for the following HRV variables: LF (ms^2), HF (ms^2), LF/HF, SDRR and $CCV_{HF.}$ Therefore, natural log transformation (Ln) was considered for the non-normally distributed HRV measures. After natural log transformation, normality and homogeneity of variance were achieved.

Baseline demographic, physical and clinical characteristics were assessed by independent samples *t*-test and by Pearson Chi square test for the categorical variables. Paired samples t-test was employed to evaluate the within-group time differences (baseline-follow-up) in step counts. A change score for HRV, metabolic and haemodynamic variables was calculated as the mean difference between the baseline visit and follow-up visit and then an analysis of covariance (ANCOVA) followed by Bonferroni correction was used to assess between groups

change score differences using medications as the covariate. Repeated measures analysis of covariance (RM ANCOVA) was employed to assess within group differences at 16-week compared to baseline values. Medications such as beta blockers, vasodilators, negative inotropic and anti-arrhythmic agents are known to alter cardiac autonomic activity (Katzung and Trevor, 2018). Therefore, a composite covariate variable of the medications was developed and included in the ANCOVA and RM ANCOVA analyses. Statistical significance was indicated if $p<0.05$. All continuous data were expressed in mean±SD unless otherwise stated. Drop outs and missing values were dealt with as described by Bland (2015).

7.5 Results

7.5.1 Acceptability and adaptability

One hundred and twelve individuals with HCM were screened with twenty-eight participants successfully recruited (Figure 7.1). The intervention was considered acceptable as the required number of participants recruited completed the follow up visit (completion rate is 79 %; n= 22). A total of five participants from the intervention group and one participant from the control group did not complete the follow-up visit and the reasons were due to the study being suspended due to COVID-19, intolerance to the BRJ and loss of contact after baseline visit (Figure 7.1). There were two participants from the intervention group who did not complete exercise testing during the follow up visit because one developed atrial fibrillation and the other developed atrial flutter, whilst one participant from the control group did not complete exercise testing during both visits due to a technical error at baseline related to ICD fitting so that exercise testing was excluded at follow-up (Figure 7.1). Adherence to BRJ among the intervention group was 95%, as one participant did not tolerate BRJ, whilst adherence to PA among the intervention group was 80%, as four participants did not sustain the required minimum targets of 2000 steps above baseline due to lack of motivation. There were no adverse events reported by the participants in the intervention and the control groups. Although two participants developed atrial fibrillation and an atrial flutter during the follow-up visit, these could be related to HCM progression.

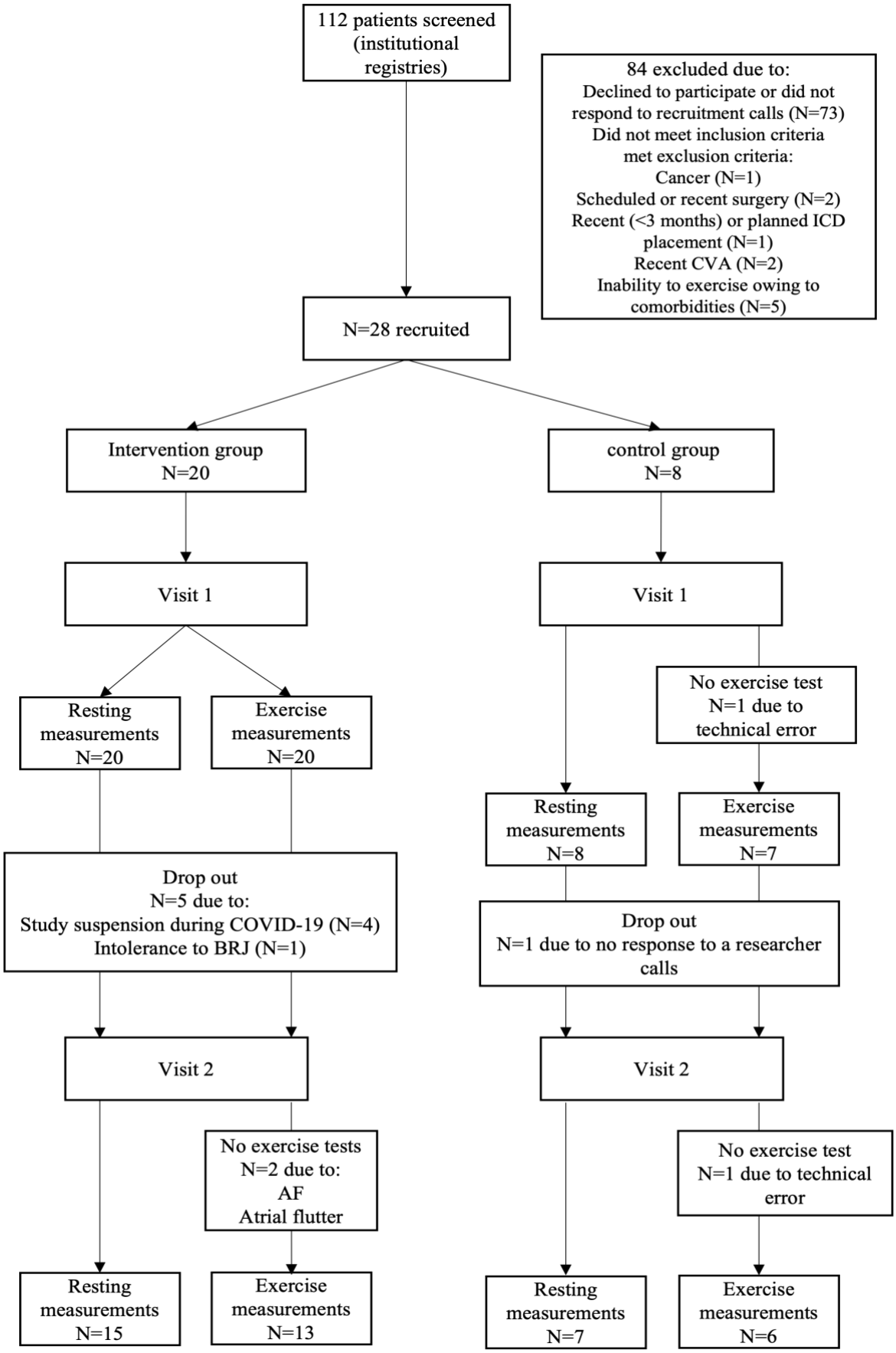

Figure 7.1 Flow diagram showing recruitment into the lifestyle intervention study. AF: atrial fibrillation; BRJ: beet root juice; CVA: cerebrovascular accident; ICD: implantable cardioverter defibrillator

7.5.2 Demographic, physical and clinical characteristics

Twenty-eight individuals with HCM were randomised into either the intervention group n=20 (55±14 years old; age range: 32-77 years old, males, N=16; females, N =4) or control group n=8 (52±18 years old; age range: 21-78 years old, males, N=5; females, N =3). Demographic and clinical characteristics of the recruited study participants are presented in Table 7.2.

Table 7.2 Baseline demographics, physical and clinical characteristics for intervention versus control groups

Variables	**Intervention N=20**	**Control N=8**	***P Value***
Age (years)	55±14	52±18	0.56
Sex, female N (%)	4 (20)	3 (38)	0.33
Height (cm)	174±8.83	172±7.62	0.62
Weight (kg)	85.3±16.2	87.9±23.4	0.73
Body mass index (kg/m^2)	28.3±3.40	29.8±8.04	0.48
Body surface area (m²)	2.02±0.23	2.03±0.26	0.92
ICD N (%)	5 (25)	3 (38)	0.51
AF/ Atrial Flutter N (%)	5 (25)	0	0.12
LVOTO N (%)	1 (5)	0	-
Left Ventricular Ejection Fraction (%)	63±11	64±6	0.76
Ischemic cardiomyopathy N (%)	0	0	-
Dilated cardiomyopathy N (%)	0	0	
Mean time since HCM diagnosis at baseline study visit (years)	8±4	8±5	0.64
Medication N (%)			
Beta-adrenergic blocker	14 (70)	3(38)	0.11
Angiotensin-Converting Enzyme Inhibitors	2 (10)	2 (25)	0.30
Angiotensin II receptor antagonist	4 (20)	0 (0)	0.17
Calcium channel blocker	3 (15)	4 (50)	0.05
Diuretics	5 (25)	1 (13)	0.46
Anti-arrhythmic agents	3 (15)	2 (25)	0.53
No drug therapy	4 (20)	1 (13)	0.64
Comorbidities N (%)			
Diabetes	6 (30)	0	0.08
Thyroid disease	0	3 (38)	**<0.01**
Dyslipidaemia	5 (25)	2 (25)	1
Lifestyle habits N (%)			
Caffeine	14 (70)	6 (75)	0.79
Alcohol consumption	14 (70)	6 (75)	0.79
Smoking	2 (10)	1 (13)	0.84

AF: atrial fibrillation; HCM hypertrophic cardiomyopathy; ICD: implantable cardioverter defibrillator; LVOTO: left ventricular outflow tract obstruction.

Participants in the intervention group significantly increased their average PA by 3029 steps/day post intervention (6234±2389 vs 9263± 3341 steps/day, p<0.01) compared to the control group (7559±3257 vs 7604± 2901 steps/day, p=0.96) who maintained their PA throughout the study (Figure 7.2).

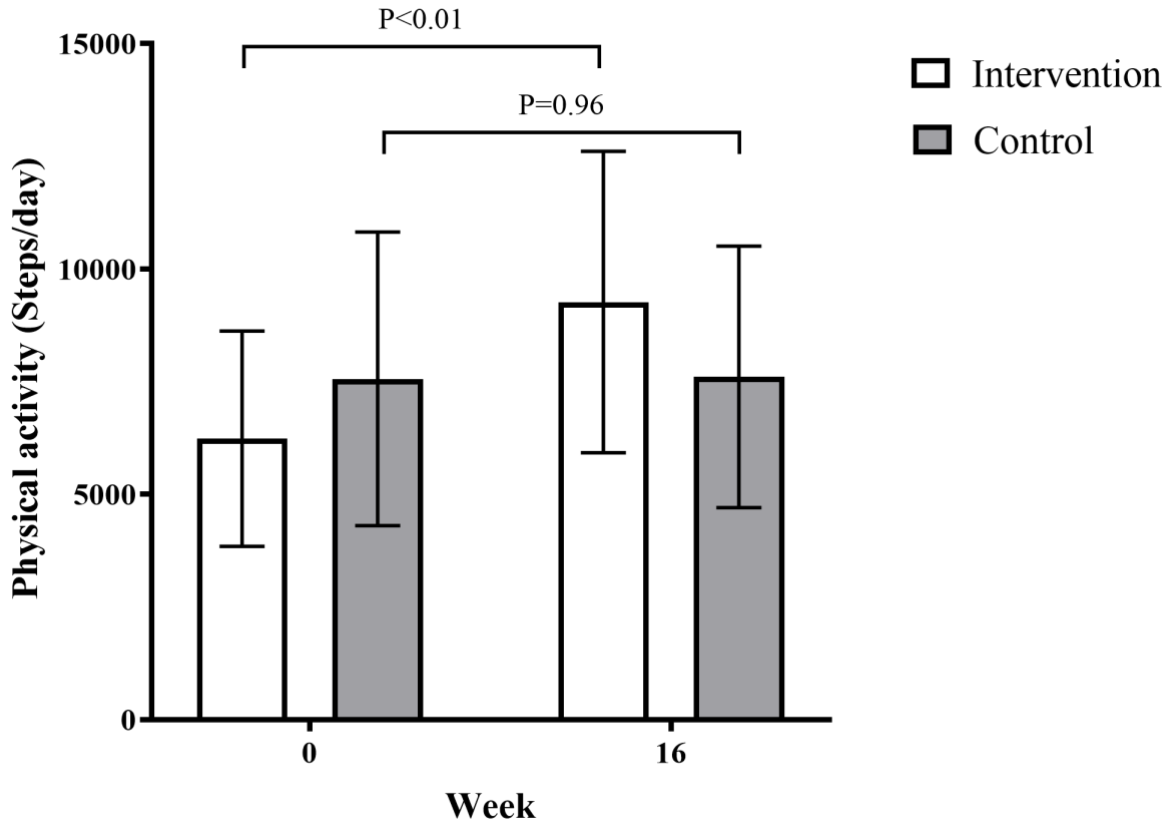

Figure 7.2 The average steps achieved at baseline and at the end of lifestyle intervention for the intervention and control groups.

7.5.3 Heart rate variability in the intervention and control groups

Table 7.3 displays the observed values of all HRV variables between the intervention and control groups across baseline and follow-up. Following the intervention, vagal indices of HRV measures were significantly higher in the intervention group compared to baseline. Absolute HF power, LF power and CCV_{HF} were increased by 16%, 19% and 37% respectively from baseline to follow-up in the intervention group (HF power (7.54±2.14 vs. 8.78±1.60 ms^2, p<0.01); LF power (6.89±2.33 vs. 8.17±1.55 ms^2, p<0.01) and CCV_{HF} (1.58±1.17 vs. 2.17±0.82 %, p<0.01). There were no significant changes in any of the HRV measures in control group at follow-up compared to baseline.

Mean change in SDRR and absolute LF power were significantly higher in the intervention group compared to the control group (SDRR =0.54±1.59 vs. 0.42±0.68 ms, p=0.04) and (LF power= 1.28±2.01 vs. 0.27±1.35 ms^2 p=0.03), respectively.

Table 7.3 Short-term heart rate variability measures at baseline and follow-up for the intervention and control groups

Variables	**Intervention**		**Control**		
	Baseline N=20	Follow-up N=15	Baseline N=8	Follow-up N=7	***P Value****
Heart rate (beats/min)	67±10	67±8	69±11	66±6	0.12
RR interval (ms)	902±166	889±165	942±215	805±236	0.27
†SDRR (ms)	4.54±1.37	5.08±0.96	4.26±1.68	4.68±1.21	**0.04***
†HF power (ms^2)	7.54±2.14	8.78±1.60‡	7.05±3.39	7.64±2.56	0.16
†LF power (ms^2)	6.89±2.33	8.17±1.55‡	6.78±3.31	7.06±2.43	**0.03***
LF power (nu)	36.3±17.3	36.7±14.7	43.4±16.4	34.9±16.9	**0.03***
HF power (nu)	63.6±17.3	63.2±14.7	56.5±16.4	65.0±16.9	**0.03***
†LF/HF	0.61±0.89	0.36±0.78	0.24±0.74	0.52±0.87	0.08
†CCV_{HF} (%)	1.58±1.17	2.17±0.82‡	1.54±1.84	1.77±1.46	0.18

CCV_{HF}: coefficient of component variance of high frequency power, HCM: hypertrophic cardiomyopathy, HF: high frequency spectral power, LF: low frequency spectral power, LF/HF: the ratio of low to high frequency spectral power, ms^2: absolute units, nu: normalised units, RR: count number of mean time between r waves SDRR: standard deviation of RR interval.

† Natural log transformed data presented. *P value of mean change from baseline between intervention and control groups. ‡ P<0.01 at follow-up compared to baseline.

7.5.4 Haemodynamic variables in the intervention and control groups

All resting cardiac haemodynamic variables for the intervention and control groups across baseline and follow-up are shown in Table 7.4. Mean arterial blood pressure (MABP) and diastolic blood pressure (DBP) were significantly reduced in the intervention group at follow-up compared to baseline (MABP= 108±6 vs. 102±7 mmHg, $p<0.01$ and DBP=88±8 vs. 82±7 mmHg, $p<0.01$). There were no significant changes in the haemodynamic variables in the control group at follow-up compared to baseline except for CPO, which was significantly reduced by 55% at follow-up (0.56±0.24 vs. 0.25±0.11 W, $p<0.01$). There were no significant between group haemodynamic changes in mean change scores.

Table 7.4 Resting haemodynamic variables at baseline and week 16 for intervention versus control groups.

Variables	**Intervention**		**Control**		
	Baseline N=20	Follow-up N=15	Baseline N=8	Follow-up N=7	***P Value****
Cardiac output (L/min)	4.48±0.93	4.83±1.16	4.32±1.92	3.91±1.55	0.56
Cardiac index (L/min/ m²)	2.19±0.54	2.30±0.47	2.33±0.77	1.98±0.66	0.98
Heart rate (beats/min)	67±10	67±8	69±11	66±6	0.12
Stroke volume (ml/beat)	63±20	73±22	68±29	60±20	0.73
Stroke volume Index (ml/beat/m^2)	36±11	35±9	34±12	30±8	0.96
Systolic blood pressure (mmHg)	142±11	138±13	141±27	150±29	0.51
Diastolic blood pressure (mmHg)	88±8	82±7‡	84±7	86±5	0.42
Mean arterial blood pressure (mmHg)	108±6	102±7‡	103±13	107±12	0.64
Total peripheral resistance ($dyn{\cdot}s{\cdot}cm^{-5}$)	2189±495	1939±777	2164±748	2558±1161	0.24
Total peripheral resistance index ($dyn{\cdot}s{\cdot}m^2cm^{-5}$)	1093±244	994±503	1092±490	1292±725	0.20
Cardiac power output (W)	0.97±0.21	1.10±0.25	0.56±0.24	0.25±0.11‡	0.27
Cardiac power output index (W/m^2)	0.44±0.13	0.50±0.11	0.47±0.17	0.44±0.15	0.98

*P value of mean change from baseline between intervention and control groups. ‡P<0.01 at follow-up compared to baseline.

7.5.5 Peak exercise cardio-metabolic variables in the intervention and control groups

Table 7.5 demonstrates peak exercise measurements for the intervention and control groups across baseline and follow-up. Oxygen consumption was significantly reduced by 15% at anaerobic threshold (AT) (13±3.33 vs. 11±1.70 ml/kg/min, p=0.04) in the intervention group at follow-up compared to baseline. Also, peak exercise SVI (74±26 vs. 61±16 ml/beat/m^2, p=0.01), DBP (98±11 vs. 84±10 mmHg, p<0.01*)*, MABP *(*126±11 vs. 116±9 mmHg, p<0.01*)*; CPO (4.39±1.83 vs. 3.59±0.97 W, p=0.03) and CPOI (2.14±0.84 vs. 1.77±0.36 W/m^2, p=0.03) were significantly reduced by 18%, 14%, 8%, 18% and 17% respectively in the intervention group at follow-up compared to baseline. There were no significant changes in the peak exercise metabolic and haemodynamic variables in the control group at follow-up compared to baseline. When comparing the mean change in peak exercise cardio-metabolic variables between intervention and control groups, peak mean change of SV was significantly higher (-13±50 vs. -24±36 ml/beat, p=0.04) in the intervention group compared to the control group.

Table 7.5 Peak exercise metabolic and haemodynamic variables at baseline and follow-up for the intervention and control groups.

Variables	**Intervention**		**Control**		
	Baseline N=20	Follow-up N=13	Baseline N=8	Follow-up N=6	***P Value****
Metabolic					
Respiratory exchange ratio	1.06±0.11	1.10±0.04	1.04±0.12	1.05±0.07	0.50
Work rate (Watt)	112±39.8	124±22.6	148±51.1	139±58.3	0.96
Oxygen consumption(ml/kg/min)	18.2±4.20	19.8±2.64	19.2±10	20.1±9.54	0.48
Oxygen consumption (L/min)	1.52±0.40	1.61±0.27	1.61±0.93	1.63±0.97	0.83
Anaerobic threshold (ml/kg/min)	13±3.33	11±1.70†	12±2.30	12±2.03	0.42
Haemodynamic					
Cardiac output (L/min)	16±6	15±4	13±6	11±4	0.06
Cardiac index (L/min/ m²)	8.14±3.59	7.56±1.37	6.93±2.18	5.51±1.70	0.06
Heart rate (beats/min)	118±27	130±20	123±35	113±31	0.50
Stroke volume (ml/beat)	139±51	126±41	112±32	88±13	**0.04***
Stroke volume Index (ml/beat/m^2)	74±26	61±16‡	60±15	45±4	0.09
Systolic blood pressure (mmHg)	184±20	179±23	186±22	181±21	0.83
Diastolic blood pressure (mmHg)	98±11	84±10‡	90±14	93±1	0.85
Mean arterial blood pressure (mmHg)	126±11	116±9‡	122±9	122±8	0.99
Total peripheral resistance ($dyn{\cdot}s{\cdot}cm^{-5}$)	925±1004	632±146	839±330	999±451	0.08
Total peripheral resistance index ($dyn{\cdot}s{\cdot}m^2cm^{-5}$)	495±627	321±93	421±205	506±291	0.11
Cardiac power output (W)	4.39±1.83	3.59±0.97 †	3.57±1.36	3.03±1.13	0.09
Cardiac power output index (W/m^2)	2.14±0.84	1.77±0.36 †	1.72±0.62	1.46±0.50	0.08

*P value of mean change from baseline between intervention and control groups. ‡P≤0.01 and †P<0.05 at follow-up compared to baseline.

7.6 Discussion

The aim of this pilot study was to assess the effect of a novel lifestyle intervention on resting cardiovascular autonomic function, resting haemodynamic and peak exercise cardiometabolic variables in individuals with HCM. The major findings suggest that vagal activity indexed by HF power, LF power and CCV_{HF} was significantly increased in the intervention group at follow-up. Resting DBP and MABP were significantly reduced in the intervention group compared to baseline. Individuals in the intervention group demonstrated lower oxygen

consumption at anaerobic threshold, and lower stroke volume index, mean arterial blood pressure and cardiac power index at follow-up. These findings are important to add further evidence that lifestyle interventions are feasible and acceptable and could present a cost-effective and adjunctive approach to pharmacological therapy with minimal behavioural alterations involving telephone follow-ups and without relying on exercise equipment in a clinical setting.

7.6.1 Effect of the novel lifestyle intervention on heart rate variability in individuals with hypertrophic cardiomyopathy

Available clinical studies on the assessment of lifestyle intervention on autonomic function in individuals with HCM is lacking. This study demonstrated that a 16-week lifestyle intervention increased the vagal indices of HRV (HF (ms^2), LF (ms^2) and CCV_{HF}) in the intervention group but not in the control. The only study that has investigated the effect of combined exercise and dietary nitrate beetroot juice supplementation was conducted in individuals with hypertension (HTN) and HF (Shaltout *et al.*, 2017). The findings of Shaltout and collaborators (2017) were in contrast to the present study findings as they did not find any significant difference in vagal indices of HRV (SDRR and root mean square of successive differences between normal heartbeats (RMSSD) either within the intervention group or between intervention and placebo control groups after 6 weeks of the intervention. The possible explanations for the contrasting findings might be due to different disease state, different protocol as in their study the exercise was centre-based and performed only three sessions per week for 6 weeks, nitrate was given acutely before each exercise session and HRV was recorded over 24-hours via holter monitor. Available studies in the literature have not reported the combined effect of PA and dietary nitrate on HRV in HCM but rather reported either the influence of exercise alone or dietary nitrate alone on HRV in different patient clinical populations.

Several studies have been conducted in individuals with HF and have reported that long-term exercise training increased vagal indices of time and frequency domain HRV which was recorded over short-term and long-term (Kiilavuori *et al.*, 1995; Cider *et al.*, 1997; Malfatto *et al.*, 2002; Selig *et al.*, 2004; Yeh *et al.*, 2008; Mello *et al.*, 2012; Murad *et al.*, 2012; Ricca-Mallada *et al.*, 2012; Krishna *et al.*, 2014; Piotrowicz *et al.*, 2016; Ricca-Mallada *et al.*, 2017). In agreement to the current study findings, the short-term recordings of HF power (ms^2) and LF power (ms^2) were significantly increased in the training group of CHF at week 24 of supervised aerobic training program for 3-days/week compared to baseline (Ricca-Mallada *et al.*, 2012). Also, Murad *et al.* (2012) showed that the mean change value of short-term recorded SDNN was significantly increased in individuals with HF who were on 16-weeks of aerobic

training programme compared to a non-exercised group after adjusting for beta blocker drugs. The finding of Murad *et al.* (2012) is similar to the current study finding in which mean change value of SDRR was significantly higher in the intervention group of HCM but not in the control group regardless of medications. Moreover, studies have shown an increase in short-term recorded vagal indices of time and frequency domain HRV measures reporting values of HF nu in Selig *et al.* (2004); Mello *et al.* (2012); Krishna *et al.* (2014) or of RMSSD in Selig *et al.* (2004); Murad *et al.* (2012); Ricca-Mallada *et al.* (2017)) following long-term exercise in individuals with CHF. Accordingly, the findings of previous work in HF are in favour of long-term exercise therapy as it yielded predominance of vagal indices of HRV.

The exact mechanisms by which long-term exercise training improves HRV by increasing the parasympathetic nervous system (PNS) activity in individuals with HF are still not fully understood (Pearson and Smart, 2018). Nitric oxide release and suppression of angiotensin II could be potential mechanisms of vagal predominance, and both are related to endothelial function (Murad *et al.*, 2012; Pearson *et al.*, 2017). Another mechanism could be that exercise training improves inflammation in individuals with HF (Smart and Steele, 2011), since it has been shown that HRV is inversely correlated with inflammatory markers (Haensel *et al.*, 2008; Papaioannou *et al.*, 2013). Considering follow-up data of HF (ms^2), LF (ms^2), CCV_{HF} and SDRR in the intervention group, the current study speculates similar mechanisms are responsible in individuals with HCM.

The improvement of autonomic control which has been seen in the current study might not only be due to PA but might also be attributable to inorganic dietary nitrate intake. However, the effect of inorganic NO_3^- supplementation on autonomic function in individuals with HCM has not been evaluated yet. Nitric oxide has an essential role in regulating autonomic function by inhibiting sympathetic nervous system (SNS) activity and/or increase PNS (Chowdhary *et al.*, 2000; Buch *et al.*, 2004). Few studies have reported that exogenous source of nitrate, i.e. dietary rich nitrate or nitrate supplementation has the capability to inhibit central SNS activity by crossing the blood-brain barriers and increase NO bioavailability in healthy adults (Seiler *et al.*, 2007; Pereira *et al.*, 2013; Notay *et al.*, 2017). Available studies have reported that BRJ has a beneficial influence on ANS activity in healthy population. Benjamim *et al.* (2021) has shown that the acute consumption of 600 mg of beetroot extract was capable to intensify the return of vagal heart rate control during recovery after exercise in healthy participants indicated by the short-term recorded of HF power (ms^2), SDNN and RMSSD. Also, Bond *et al.* (2014) found that the acute ingestion of 70 ml of BRJ increased the short-term recorded SDNN before and during PA in a healthy population. However, a recent study in hypertensive women has shown that the acute intake of 35 ml BRJ accompanied with aerobic exercise did not change the short-

term recorded time or frequency domain HRV (Carrijo *et al.*, 2021). Also, a 7-day treatment with glyceryl trinitrate, NO donor, for stable individuals with HF did not increase long-term recorded time or frequency domain of HRV measures (Buch *et al.*, 2004). In an animal model (dog) and in humans with coronary slow flow, a diet containing high amounts of NO_3- has been associated with an improvement in HRV measures (Markos *et al.*, 2001; Pekdemir *et al.*, 2004). Accordingly, in the lack of a comparable study, the current study findings are the first to report the effect of dietary nitrate on HRV in individuals with HCM.

7.6.2 Effect of the novel lifestyle intervention on cardio-metabolic function and peak exercise performance in individuals with hypertrophic cardiomyopathy

This study demonstrates that following the 16-week of lifestyle intervention resting and peak exercise MABP and DBP were significantly reduced compared to baseline in the intervention group. At follow-up, peak exercise SVI, CPO, CPOI and oxygen consumption at AT were significantly reduced in the intervention group compared to baseline. In individuals with HCM, the first and only randomised clinical trial that implemented home-based exercise intervention was on 136 individuals with HCM which reported significant and (6%) increase in the mean change of peak exercise VO_2 in the exercise group compared to the control group after 16 weeks of moderate intensity aerobic exercise (Saberi *et al.*, 2017). Although the current study showed a non-significant change in peak VO_2 between intervention and control groups, the mean change values in peak VO_2 had a tendency towards an increase with a (9%) absolute change compared to the control group (5%) at follow-up. It can be speculated that if the current study had a similar sample size of Saberi *et al.* (2017), the finding of peak VO_2 would have been significant since it had a positive trend in the intervention group.

Evidence of the combined effect of PA and dietary nitrate rich BRJ on cardiometabolic measures and exercise performance in individuals with HCM are lacking. However, there are numerous studies reporting the influence of long-term exercise alone on haemodynamic status in individuals with HF, coronary heart disease, diabetes and HTN as it can reduce heart rate, blood pressure and increase arterial compliance (Neves *et al.*, 2009; Mourot *et al.*, 2010; Ghashghaei *et al.*, 2012; Stojanovic *et al.*, 2022). Based on those known beneficial effects of long-term exercise on haemodynamic function, Shaltout *et al.* (2017) evaluated the effect of dietary nitrate when added to moderate intensity supervised exercise versus exercise with placebo in individuals with HFpEF and in individuals with HTN, they reported no additional benefits of dietary nitrate on cardiometabolic variables for both populations. For example, resting SBP was significantly reduced within both groups in HFpEF (exercise with BRJ group and exercise combined with placebo at week-4 of intervention). Also at peak exercise, none of

the haemodynamic or metabolic variables were significantly changed either within or between groups after the intervention. Shaltout *et al.* (2017) findings and the current study findings are incomparable as they have different populations, hypotheses and design, aiming at testing whether dietary nitrate supplementation would have additive or synergistic effect to exercise considering that exercise on its own has the beneficial effects on cardio-metabolic function.

Previous studies have examined the effect of either exercise only or dietary nitrate consumption only on haemodynamic and exercise performance variables in HF. For example and in contrast to the current study findings, the Active-at-Home-HF intervention study found that peak exercise SV, SVI, CO, and VO_2 were increased after 12 weeks of PA compared to baseline in individuals with chronic HFrEF (Okwose *et al.*, 2019). Like the Okwose *et al.* (2019) group findings, the number of steps/days was increased significantly at follow-up in this study. The disagreement between the two studies in haemodynamic findings is possibly due to disparity in the etiology and clinical characteristics of disease as in the current study, individuals with HCM were characterised by preserved ejection fraction (EF) i.e. (EF=63%) whilst Okwose *et al.* (2019) evaluated individuals with chronic HFrEF i.e. (EF=31%). In addition to unavailable evidence evaluating of long-term PA in HCM, most available trials in HF assessing the effect of PA on peak exercise performance focused only on functional capacity VO_2. For example, long-term exercise significantly increased peak VO_2 (ml/kg/min) in the individuals with HFpEF at follow-up compared to baseline (Kitzman *et al.*, 2010; Edelmann *et al.*, 2011; Smart *et al.*, 2012; Brubaker *et al.*, 2020). However, Edelmann *et al.* (2011) also reported a significant increase in peak exercise WR and oxygen consumption at anaerobic threshold after intervention. The possible reasons for the disparity between the current study and the aforementioned studies could be due to different patient populations, duration of exercise therapy, sample size and having dietary NO_3^- supplementation as combination with exercise in the current study.

The effect of dietary nitrate on cardiometabolic variables and exercise performance was also evaluated in individuals with CVD but not in HCM. For example, Eggebeen *et al.* (2016) compared the acute effect of daily dose of nitrate rich BRJ over one week to the single acute dose in individuals with HFpEF and reported a significant reduction only in in resting SBP in both the acute single doses and the one week daily dose with no significant changes in resting or peak exercise VO_2, HR, DBP. Despite that the current study showed none significant 3% reduction in SBP in the intervention individuals with HCM, the finding is incomparable to that of Eggebeen *et al.* (2016) due to different design as dietary nitrate was given for a shorter period of time relative to the current study. In agreement to the current study findings, Zamani *et al.* (2015), reported a significant reduction in peak TPR in individuals with HFpEF who

underwent exercise test with acute single dose of BRJ (12.9 mmol NO_3^-). In the current study, the peak TPR showed a trend towards reduction by 32% in the intervention group at follow-up compared to baseline. Individuals with non-ischemic dilated cardiomyopathy who consumed acute dose of 140 ml of nitrate rich BRJ (12.9mmol NO3) had lower MABP, SBP and DBP compared to placebo though changes did not reach statistical significance (Kerley *et al.*, 2016). Accordingly, further studies are needed in individuals with HCM to evaluate the effect of a lifestyle intervention on cardiometabolic variables and exercise performance.

7.7 Strengths and limitations of the study

This is the first pilot study aiming to evaluate the effect of a novel lifestyle intervention incorporating PA and dietary nitrate on autonomic function, cardiometabolic function and peak exercise performance in individuals with HCM. It is important to note that this is the first study also to evaluate the effect of the lifestyle intervention on HRV as primary outcome in individuals with HCM since the analysis of HRV as the main outcome is lacking in the literature (Carrijo *et al.*, 2021). The findings of the current study support the recent American Heart Association (AHA) and American College of Cardiology (ACC) guidelines which recommend an active lifestyle for the most individuals with HCM (Ommen *et al.*, 2020). Another strength of the current study was the duration of the intervention which lasted for 16-weeks whilst most studies using either exercise or dietary nitrate as intervention have been conducted over shorter periods. The use of step counts as a marker of PA in this study is in line with recent recommendations of adopting daily step counts to monitor and achieve physical activity goals (Kraus *et al.*, 2019).

The following limitations exist in the current study. Firstly, the study was underpowered which limits generalisability of findings. However, this was a pilot study with a preliminary finding that warrants further investigations in larger clinical trials. Furthermore, only seven females were recruited into the study limiting the generalisability of the findings with regards to sex, yet it is well known and unresolved issue since 2008 that females have been under-represented in cardiovascular medicine trials (Mayor *et al.*, 2022). Also, it is unclear whether the observed effects in this study were due to the action of PA or BRJ or both. It is worthwhile to evaluate the effect of PA alone, dietary nitrate supplementation alone and their combination in a single study to determine which component have additive or synergistic effects on autonomic, cardiometabolic functions and exercise performance in individuals with HCM. Measurements of plasma nitrate levels should be considered in future studies to ensure NO bioavailability and the subsequent response to the nitrate intake.

7.8 Conclusions and clinical implications

The present study demonstrated that the novel lifestyle intervention incorporating physical activity and dietary nitrate improved cardiac autonomic function by enhancing vagal indices of HRV and improved resting MABP. The present study results are of clinical importance for the management of individuals with HCM. The lifestyle intervention used in this study may provide a feasible and cost-effective adjunct to pharmacological and/or centre-based rehabilitation programme and could therefore reduce the healthcare burden of the disease. However, further studies are warranted in a larger sample size with equal sex representation to support the preliminary findings from the present study.

Chapter 8. General discussion and future research

8.1 General discussion

Heart rate variability (HRV) is a non-invasive measure of autonomic regulation of the cardiovascular system (Malik *et al.*, 1996). HRV has been widely used as an innovative screening and prognostic tool in medicine including cardiology, psychology, sport and exercise (Laborde *et al.*, 2022). Reduced HRV, particularly vagal indices of HRV measures has been reported as an independent predictor of cardiac mortality and potentially life-threatening arrhythmia in individuals with heart disease (Kleiger *et al.*, 1987; La Rovere *et al.*, 2003) as well as in the general population (Tsuji *et al.*, 1996). The present thesis has shown that HRV studies may enhance our understanding of the physiological phenomena, disease mechanism and influence of therapy. These aspects were investigated in the present thesis with the following aims: 1) determine the impact of age and sex on HRV in healthy individuals, 2) compare HRV between individuals with hypertrophic cardiomyopathy (HCM) and healthy controls, and 3) evaluate the effect of a novel lifestyle intervention on HRV in individuals with HCM.

8.2 The effect of age and sex on heart rate variability in healthy individuals

The present thesis has shown that RR interval is influenced by sex but not age. This is consistent with previous studies which have reported the effect of sex on HRV measures (Nunan *et al.*, 2010b; Koenig and Thayer, 2016a). This finding confirms that HRV data obtained from men and women should not be treated equally and that these differences should be emphasised in research studies and clinical settings (Koenig and Thayer, 2016a). The difference in HRV measures between sexes in the present thesis was limited to RR interval but not other HRV measures and this is possibly due to the modest sample size with fewer females recruited compared to males. The present thesis also reports association between peak exercise cardiac power output (CPO), as a measure of overall cardiac function, and resting RR interval in females regardless of age. Similar correlations between CPO and RR interval were previously reported in individuals with heart failure regardless of their sex and age (Koshy *et al.*, 2019). Accordingly, the reported influence of sex but not age on RR interval and the observed correlation between RR interval and CPO only in females may suggest the role of sex hormones on autonomic and cardiac functions.

8.2.1 Clinical implications

Reporting sex differences in HRV studies may be an insightful approach in understanding the major health disparities between both sexes especially regarding the risk of developing cardiovascular diseases. Also, the correlation between RR interval and CPO in healthy

individuals may imply that treatment may not only improve signs and symptoms of a disease but also autonomic function.

8.3 Heart rate variability in individuals with hypertrophic cardiomyopathy

The present thesis showed that individuals with HCM had higher baseline vagal indices of HRV compared with healthy individuals. It was hypothesised that the high vagal indices of HRV in individuals with HCM may be due to the imbalance between sympathetic and parasympathetic arms of the autonomic nervous system (Perloff, 1981; Limbruno *et al.*, 1998). This may have been due to the preserved systolic function in individuals with HCM which triggers a feedback mechanism to activate vagal afferents in the ventricles (Fei *et al.*, 1995). We have also demonstrated a positive correlation between vagal indices of HRV (high frequency power (HF) and low frequency power (LF)) and mean arterial blood pressure and total peripheral resistance in individuals with HCM which could be due to the activation of the *Renin-Angiotensin-Aldosterone System* (RAAS). If such relationship exists in individuals with HCM at peak exercise, it would have aided our understanding of the abnormal vasodilatory response during exercise.

8.3.1 Clinical implications

HRV has the potential to be a feasible tool that could be integrated during the routine clinical baseline assessment in diagnosed individuals with HCM as it may help clinicians to understand the pathophysiology, enhance risk stratification and prognosis. The correlation between HRV and haemodynamic variables in individuals with HCM may imply that treatment of HCM could improve autonomic and cardiac functions.

8.4 Effect of a novel lifestyle intervention on heart rate variability in individuals with hypertrophic cardiomyopathy

The present thesis has provided an evaluation of a personalised, home-based, novel lifestyle intervention incorporating physical activity (PA) and inorganic dietary nitrate supplementation with beetroot juice (BRJ). The intervention led to improvements in vagal indices of HRV measures in individuals with HCM at follow up visit compared with baseline. This was the first pilot study, which combined PA and dietary nitrate consumption using BRJ as a possible potential adjunct in HCM management. The results of the present pilot study support development of future randomized controlled clinical trials to evaluate the effectiveness of integration PA and nitrate rich BRJ intervention in HCM.

8.4.1 Clinical implications

The present pilot study has demonstrated the feasibility of a 16-week lifestyle intervention program incorporating PA and dietary nitrate rich BRJ in individuals with HCM with no major adverse events recorded. Although more confirmatory future studies are needed, the preliminary findings of the present pilot study may encourage clinicians to discuss the potential benefits of integrating tailored physical activity programme for individuals with HCM to promote a healthy and active lifestyle. The findings of HRV measures may encourage clinicians to target HRV as an effective measure of cardiovascular autonomic function response to a therapy. The lifestyle intervention proposed in the present thesis could improve and shape the cardiology service in the NHS, leading to reduce a financial burden and reduction in hospital admissions as a complication of HCM progression if the future trials confirm its effectiveness.

8.5 Conclusion

Overall, the current HRV-related literature has been limited by a lack of standard methodology of recording, analysis and interpretation of HRV measures. The present thesis has addressed these limitations by using a consistent and standard approach for monitoring HRV measures including frequency domain measures and two-time domain measures. In the present thesis, an adjustment for important confounding factors was also considered as previous evidence has been repeatedly recommended to control for covariates that influence data interpretation (Laborde *et al.*, 2017; Shaffer and Ginsberg, 2017). Failing to adjust for confounding factors as seen in some of the reported studies has prevented comparability between studies and generalisability of findings. The present thesis has also filled a major gap in HRV-related research in terms of using HRV to characterise pathophysiology in individuals with HCM and to explore new modalities for the management of HCM.

8.6 Future research directions and recommendations

Future research directions may involve:

- Inclusion of more time domain variables to comprehensively understand and correlate findings of frequency domain HRV measures with time domain measures.
- Findings of the present thesis studies warrant further investigation in a larger sample size with equal sex representation to support generalisability of the findings.
- Repeating the lifestyle intervention pilot study in a larger randomised controlled trial, with subgroup analysis, and considering 24-hours recording of HRV throughout the intervention period to assess ability of HRV measures to predict prognosis of adverse events in individuals with HCM.

- The nature of the relationship between HRV and cardiometabolic variables in healthy and in individuals with HCM was beyond the present thesis aims. However, future research direction should include simultaneous measurements of HRV and cardiometabolic data at peak exercise, considering regression analysis to explore whether HRV measures may predict changes in peak exercise cardiometabolic variables in healthy individuals or in individuals with HCM. An investigation of such relationship in individuals with HCM may help us understand the mechanism of exercise intolerance.

Based on the results of the studies contained in the present thesis, the following two recommendations can be drawn:

(i) assessment of short-term (5-minute) HRV to be included as a routine clinical assessment to improve understanding of the autonomic function regulation in individuals with HCM.

(ii) Future HRV-related studies in health or disease should report HRV values in relation to sex. Indeed, an evaluation of how sex-related differences in HRV in individuals with HCM can be translated into the pathophysiology of the disease should be investigated.

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Appendix A

Consent form for healthy participants

Written Consent Form / Potential Participant

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.
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

- ③ 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.
- ③ 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.
- ③ 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.
- ③ 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.
- ③ I know that my personal data can be transmitted to research purposes **as part of the project only and under anonymized manner**.
- ③ 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.
- ③ I am informed that the liability of the project management covers the improbable project caused damage that I may suffer.
- ③ 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.
- ③ 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:**

- x **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.** ☐
- x **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.** ☐
- x **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.

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

Appendix B

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 _____

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

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: _____

Date _____ Name _____ Designation _____

Signature _____

Potts Print (UK). Mar 2015 NUTH406

To be completed by investigator:

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

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 NUTH406

Appendix C

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) **LABORATORY TESTS**

Clinical Laboratory tests performed?	☐ **No (comment below)** ☐ **Yes,** Complete below **Comment *:** _____
Date of Sample:	__ __ / __ __ __ / __ __ __ __ **(DD / MMM / YYYY)**
Time of Sample	_____:_____ **HH:MM**

<INSERT ASSESSMENT> Laboratory Parameter	Value	Unit (site to pre-complete prior to the finalization of the template)	If parameter indicated as out of normal range on report, please check if clinically significant:
HbA1C			☐ No ☐ Yes
Glucose			☐ No ☐ Yes
AST/GOT			☐ No ☐ Yes
ALT/GTP			☐ No ☐ Yes
Cholesterol			☐ No ☐ Yes
HDL-C			☐ No ☐ Yes
LDL-C			☐ No ☐ Yes
Triglycerides			☐ No ☐ Yes
Creatinine			☐ No ☐ Yes
Bilirubin			☐ No ☐ Yes
C-reactive protein			☐ No ☐ Yes
Fibrinogen			☐ No ☐ Yes
			☐ No ☐ Yes

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	**Frequency**	**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**

Smoked for ___ ___ months/years

Date when smoking ceased: __ __ / __ __ __ / __ __ __ __
(DD / MMM / YYYY)

☐ **Former smoker**

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 sugar consumption (based on American Heart Association (AHA)
We provide as an Annex a table for calculating the sugar consumption

Excess Sugar Consumption ☐	Moderate Sugar Consumption ☐	Low Sugar Consuption ☐
> 6 teaspoons/day	3-6 teaspoons/day	<3 teaspoons/day

VISIT 1 (SCREENING) **DIET & EXERCISE HABITS**

Participant's Vitamins, Minerals or other dietary supplements

Vitamins	Minerals or dietary supplements	Diet medication	No dietary supplements
☐	☐	☐	☐

Participant's Diet History

Are you?

☐ **Lactose intolerant**	☐ **Gluten intolerant,**	☐ **Vegetarian,**	☐ **Vegan**	☐ **None**

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 vigorousintensity 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 moderateintensity 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 D

Supplementary table for chapter five

Table 5.1. Resting metabolic and haemodynamic measures stratified by age and sex

Variables	**Males N=40**		**Females N=28**		**Two-Way ANCOVA (*P* Value)**		
	Young N=26	Old N=14	Young N=17	Old N=11	Age	Sex	Interaction
Metabolic variables							
Oxygen consumption(ml/kg/min)	3.73±1.34	2.85±0.53	3.58±1.12	2.72±0.64	0.13	0.30	0.74
Oxygen consumption (L/min)	0.25±0.06	0.22±0.05	0.22±0.05	0.21±0.02	0.77	0.18	0.87
Respiratory exchange ratio	0.87±0.05	0.86±0.05	0.80±0.14	0.84±0.06	0.48	0.53	0.19
Haemodynamic variables							
Cardiac output (L/min)	6.03±1.38	4.01±0.49	6.07±1.38	4.50±1.36	**<0.01**	0.49	0.44
Cardiac index (L/min/ m²)	3.18±0.79	2.11±0.35	3.46±1.04	2.53±0.67	**<0.01**	0.24	0.52
Cardiac power output (W)	1.15±0.26	0.75±0.14	1.07±0.31	0.88±0.30	**<0.01**	0.73	0.18
Cardiac power output index (w/ m²)	0.58±0.13	0.39±0.08	0.60±0.13	0.49±0.16	**<0.01**	0.20	0.20
Heart rate (beats/min)	60.3±11.9	59.1±5.22	63.5±10.1	62.2±10	**0.01**	**0.04**	0.55
Stroke volume (ml/beat)	101±22.7	68.3±9.69	95.9±16.5	73.5±19.4	**0.01**	0.45	0.08
Stroke volume Index (ml/beat/ m²)	52.3±11.9	36.1±6.73	57.2±9.43	40.9±10.9	**0.02**	0.41	0.40
Systolic blood pressure (mmHg)	117±20.5	114±14.8	101±10.8	124±13.6	0.78	0.67	**<0.01**
Diastolic blood pressure (mmHg)	70.5±9.76	71.07±8.69	63.4±7.69	73.8±10	0.85	0.83	0.10
Mean arterial blood pressure (mmHg)	85.8±12.8	83.8±11.6	75.1±8.79	87.9±7.42	0.63	0.48	**0.03**

Appendix E

Consent form for hypertrophic cardiomyopathy participants

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 F

Medical History 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

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 OK / Not OK

with specific attention to uniformity of breath sounds in all areas (absence of rales and wheezes) *Comment:* _____

Palpation of cardiac apical impulse OK / Not OK

point of maximal impulse *Comment:* _____

Auscultation of the heart OK / Not OK

with specific attention to murmurs, gallops, clicks and rubs. *Comment:* _____

Evaluation of the abdomen OK / Not OK

Bowel sounds, masses, visceromegaly, tenderness. *Comment:* _____

Evaluation of lower extremities OK / Not OK

Oedema and presence of arterial pulse. *Comment:* _____

Inspection of the skin OK / Not OK

focus on lower extremities in people with diabetes. *Comment:* _____

Neurologic function OK / Not OK

Reflexes *Comment:* _____

Any orthopedic or medical condition YES / NO

that would limit exercise. *Comment:* _____

Ventricular tachycardia OK / Not OK

Comment: _____

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

ST elevation (+1.0 mm) **OK / Not OK**
in leads without diagnostic Q-waves *Comment:*_____
(other than V_1 or aVR)

ST or QRS changes OK / Not OK

such as excessive ST suppression >2mm horizontal or down sloping depression *Comment:*_____

Arrhythmias other than: OK / Not OK

sustained ventricular tachycardia, including *Comment:*_____

_____.

multiple PVCs, triplets of PVCs, supraventricular tachycardia, heart block or bradyarrhythmias.

Cleared to start exercise test YES / NO

Competed by: _____ Date _____

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 _____

Potts Print (UK). Mar 2015 NUTH406

Appendix G

Supplementary tables for chapter six

Table 6.1 Heart rate variability measures in the individuals with HCM without implantable cardioverter-defibrillator and healthy controls.

Variables	HCM no ICD N=20	Healthy controls N=28	*P Value*
Heart rate (beats/min)	77±23	83±13	0.94
RR interval (ms)	912±195	1014±168	**0.03**
†SDRR (ms)	4.74±1.10	3.96±0.60	**<0.01**
†LF power (ms^2)	7.40±1.86	5.93±1.01	**<0.01**
†HF power (ms^2)	7.74±2.09	6.03±1.36	**<0.01**
LF power (nu)	42.4±17.8	47.2±19.9	0.95
HF power (nu)	57.5±17.8	52.7±19.9	0.95
†LF/HF	0.31±0.86	0.07±0.94	0.94
†CCV_{HF} (%)	1.68±1.14	0.71±0.66	**<0.01**

CCV_{HF}: coefficient of component variance of high frequency power; HCM: hypertrophic cardiomyopathy; HF: high frequency spectral power; ICD: implantable cardioverter-defibrillator; LF: low frequency spectral power; LF/HF: the ratio of low to high frequency spectral power; ms^2: absolute units; nu: normalised units; RR: count number of mean time between r waves; SDRR: standard deviation of RR interval.
† Natural log transformed data presented.

Table 6.2 Relationship between heart rate variability measures and haemodynamic variables in healthy controls

Variables	Stroke volume	Cardiac power output	Cardiac output	Total peripheral resistance	Mean arterial blood pressure
	r	r	r	r	r
RR interval (ms)	0.12	-0.21	0	0.10	- 0.43*
†LF power (ms^2)	0.18	0.21	0.37	- 0.42*	-0.18
†HF power (ms^2)	0.11	- 0.10	0.20	- 0.35	- 0.39*
†SDRR (ms)	0.31	0.26	0.48**	- 0.39*	- 0.23
†CCV_{HF} (%)	0.08	0.02	0.21	- 0.39*	- 0.29

CCV_{HF}: coefficient of component variance of high frequency power; HF: high frequency spectral power; LF: low frequency spectral power; ms^2: absolute units; nu: normalised units; RR: count number of mean time between r waves; SDRR: standard deviation of RR interval.
† Natural log transformed data presented.

Table 6.3 Heart rate variability measures in individuals with hypertrophic cardiomyopathy without atrial fibrillation/ flutter

Variables	**HCM no AF N=23**	**Healthy controls N=28**	***P Value***
Heart rate (beats/min)	73±22	83±13	0.73
RR interval (ms)	929±183	1014±168	0.05
†SDRR (ms)	4.30±1.41	3.95±0.60	**<0.01**
†LF power (ms^2)	6.76±2.49	5.93±1.01	**<0.01**
†HF power (ms^2)	7.17±2.48	6.03±1.35	**0.01**
LF power (nu)	41.1±17.2	47.2±19.9	0.92
HF power (nu)	58.8±17.2	52.7±19.9	0.92
†LF/HF	0.36±0.82	0.06±0.94	0.92
†CCV_{HF} (%)	1.37±1.33	0.71±0.66	**<0.01**

AF: atrial fibrillation; CCV_{HF}: coefficient of component variance of high frequency power; HF: high frequency spectral power; LF: low frequency spectral power; ms^2: absolute units; nu: normalised units; RR: count number of mean time between r waves; SDRR: standard deviation of RR interval.

† Natural log transformed data presented.

Appendix H

Participant 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

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 I

Weekly telephone calls 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 J

Activity planner

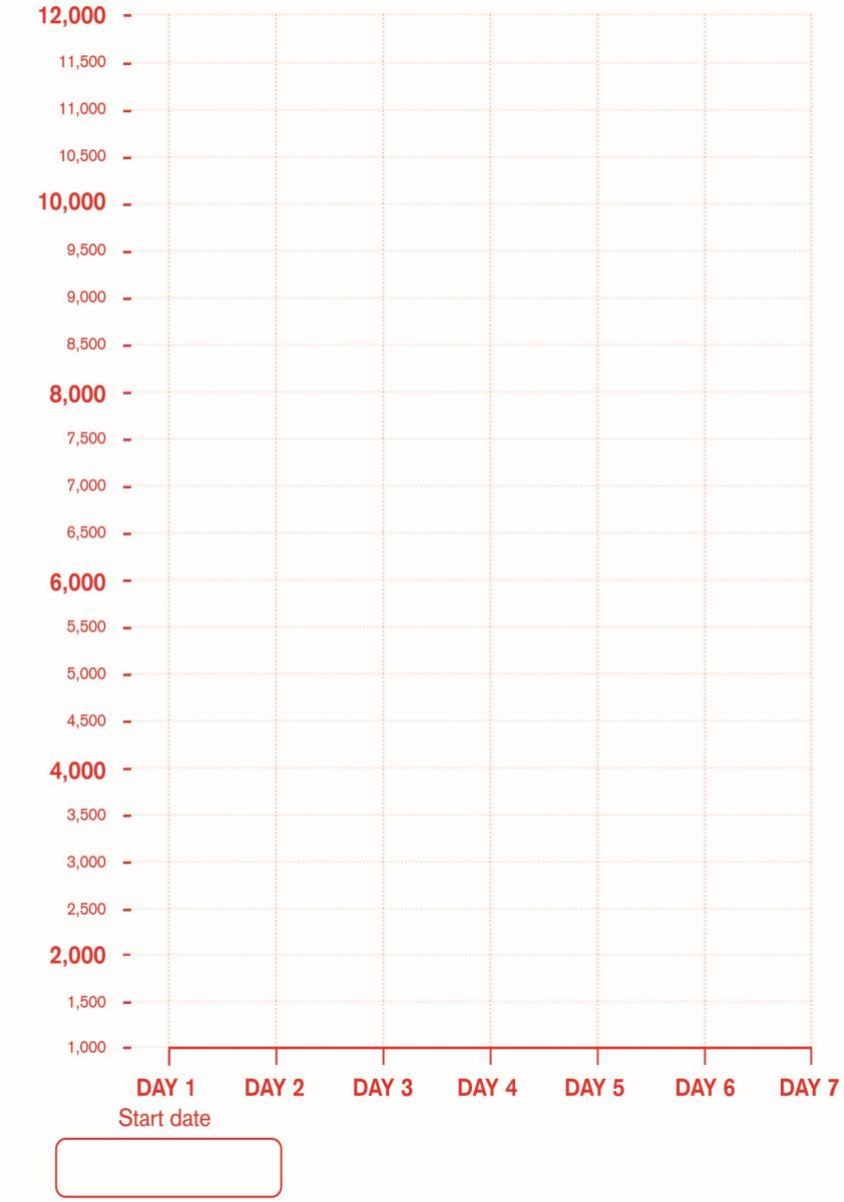
Activity Planner

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			

Activity Tracker

DAILY STEPS



Start date

Appendix K

Accepted Manuscript

Heart rate variability and haemodynamic function in individuals with hypertrophic cardiomyopathy

AI. Alyahya[1, 2, 3], SJ. Charman[1, 2], NC. Okwose[1, 2, 4], AS. Fuller[1, 2], C. Eggett[1, 2], P. Luke[1, 2], K. Bailey[2], GA. MacGowan[2, 5], DG. Jakovljevic[1, 2, 4]

[1]Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, UK

[2]Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

[3]Department of Cardiac Technology, College of Applied Medical Sciences, Imam Abdulrahman Bin Faisal University, 2835 King Faisal Street, Dammam, Kingdom of Saudi Arabia

[4]Faculty Research Centre (CSELS), Institute for Health and Wellbeing, Faculty of Health and Life Sciences, Coventry University, and University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK

[5]Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK

Short title: Autonomic function and haemodynamics in hypertrophic cardiomyopathy

Corresponding Author: Professor Djordje Jakovljevic (djordje.jakovljevic@newcastle.ac.uk), Translational and Clinical Research Institute, Faculty of Medical Sciences, Newcastle University, UK.

Abstract

Objectives: Heart rate variability (HRV) is a measure of cardiac autonomic function. This study: (1) evaluated the differences in HRV and haemodynamic function between individuals with hypertrophic cardiomyopathy (HCM) and healthy controls, and (2) determined the relationship between HRV and haemodynamic variables in individuals with HCM.

Methods: Twenty-eight individuals with HCM (n=7, females; age 54±15 years; body mass index: 29±5 kg/m^2) and 28 matched healthy individuals (n=7 females; age 54±16 years; body mass index: 29±5 kg/m^2) completed 5-minute HRV and haemodynamic measurements under resting (supine) conditions using bioimpedance technology. Frequency domain HRV measures (absolute and normalised low frequency power (LF), high frequency power (HF) and LF/HF ratio) and RR interval were recorded.

Results: Individuals with HCM demonstrated higher vagal activity (i.e., absolute unit of HF power (7.40±2.50 vs. 6.03±1.35 ms^2, p=0.01) but lower RR interval (914±178 vs. 1014±168 ms, p=0.03) compared to controls. Stroke volume (SV) index and cardiac index were lower in HCM compared to healthy individuals (SV, 33±9 vs. 43±7 ml/beat/m², p<0.01; cardiac index,2.33±0.42 vs. 3.57±0.82 L/min/m^2, p<0.01), but total peripheral resistance (TPR) was higher in HCM (3468±1027 vs. 2953±1050 dyn·s·m^2cm^{-5}, p=0.03). HF power was significantly related to SV (r=-0.46, p<0.01) and TPR (r=0.28, p<0.05) in HCM.

Conclusions: Short-term frequency domain indices of HRV provide a feasible approach to assess autonomic function in individuals with HCM. Vagal activity, represented by HF power, is increased, and associated with peripheral resistance in individuals with HCM.

Keywords: Hypertrophic cardiomyopathy, heart rate variability, frequency domain, haemodynamic function, autonomic function

Introduction

The autonomic nervous system (ANS) plays an important role in the pathogenesis of sudden cardiac death (SCD) in hypertrophic cardiomyopathy (HCM) (Ajiki *et al.*, 1993; Fei *et al.*, 1995; Limbruno *et al.*, 1998; Huang *et al.*, 2017). Assessment of heart rate variability (HRV) which indicates cardiac ANS interplay and function has been previously reported in individuals with HCM (Counihan *et al.*, 1993; Döven *et al.*, 2001). It has been previously reported that individuals with HCM have increased sympathetic (SNS) activity and/or hypersensitivity of the myocardium to catecholamines (Perloff, 1981; Ajiki *et al.*, 1993; Tanabe *et al.*, 1995; Seggewiss *et al.*, 2009). The increase in the adrenergic drive is believed to be a potential stimulus for left ventricular hypertrophy (LVH), increased heart rate and blood pressure, and SCD in HCM (Katarzynska-Szymanska *et al.*, 2013). There is also evidence to show reduction in parasympathetic (PNS) activity in HCM (Ajiki *et al.*, 1993; Counihan *et al.*, 1993; Tanabe *et al.*, 1995; Bonaduce *et al.*, 1997; Limbruno *et al.*, 1998; Döven *et al.*, 2001) or reduction in SNS activity (Fei *et al.*, 1995).

In HCM, disease severity and possible residual effects of cardiovascular drugs such as beta-blockade (BB) can make interpretation of HRV challenging (Mörner *et al.*, 2005). However, spectral analysis of HRV is a commonly used non-invasive method for assessing autonomic modulation of the heart (Mörner *et al.*, 2005). It evaluates HRV in low and high frequency bands. The low frequency (LF) spectral power reflects baroreceptor and vagal activity but not sympathetic activity during resting conditions (Shaffer *et al.*, 2014; McCraty and Shaffer, 2015). High frequency (HF) spectral power is associated with parasympathetic activity (Moodithaya and Avadhany, 2012; Shaffer

and Ginsberg, 2017). The low-to-high frequency ratio (LF/HF) measures sympathovagal balance (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017).

The relationships between HRV and haemodynamic function have not been assessed previously in individuals with HCM. Instead, a significant number of HRV studies reported relationships between HRV and echocardiographic (i.e., left ventricular and left atrial dimensions) and/or clinical characteristics (Fei *et al.*, 1995; Mörner *et al.*, 2005). A fundamental question unanswered in literature is whether there is an association between cardiac autonomic function represented by HRV and haemodynamic function in the individuals with HCM.

Therefore, the aim of the present study was two-fold; firstly, to evaluate the differences in resting HRV and haemodynamic measures between individuals with HCM and healthy controls, and secondly to determine the relationship between resting HRV measures and haemodynamic variables such as stroke volume (SV), cardiac power output (CPO), cardiac output (CO), total peripheral resistance (TPR) and mean arterial blood pressure (MABP) in individuals with HCM.

Materials and Methods

Study design, setting, and participants

This was a prospective, single centre, cross-sectional, observational study conducted at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne, UK between November 2018 and February 2022. This study was a sub-study of the SILICOFCM trial (NCT03832660) (Tafelmeier *et al.*, 2020). Twenty-eight adults (≥18 years old) with HCM were recruited. The mean time since HCM diagnosis was 8±5 years before the study visit.

The inclusion criteria of the individuals with HCM were defined as adults (≥18 years of age) with a confirmed diagnosis of obstructive and/or non-obstructive HCM (i.e., unexplained LVH with either a maximum wall thickness of ≥15 mm or borderline hypertrophy (maximum wall thickness 13–14 mm) on echocardiography and at least one first-degree relative with HCM, agreement to be a participant in the study protocol and ability to provide written consent. The exclusion criteria were defined as post septal myectomy or catheter ablation in the three months prior to the study visit, clinical decompensation, defined as New York Heart Association (NYHA) class IV, congestive heart failure symptoms, in the previous three months before the study visit, resting blood pressure greater than 180/100 mm Hg, resting left ventricular outflow tract gradient > 50 mm Hg, left ventricular ejection fraction of less than 50% by echocardiography, renal insufficiency with an glomerular filtration rate of less than 30 mL/min per 1.73m^2, implanted pacemaker or cardio-defibrillator in the last three months before the study visit or scheduled, present or planned pregnancy, life expectancy less than 12 months, severely obese (i.e. body mass index >40 kg/m^2), history or evidence of drug or alcohol abuse within the past 12 months, history of malignancy of any organ system and sustained ventricular tachycardia and atrial fibrillation (AF) or atrial flutter with resting ventricular rate >110 beats per minute.

Potential eligible individuals with HCM were identified through cardiology clinics and the North of England Cardiac Family History Service of the Newcastle upon Tyne Hospitals NHS Foundation Trust by six cardiologists who were members of the research team. Identified individuals with HCM were contacted by a researcher and an information sheet was mailed out upon request.

Data for age-, sex- and body mass index (BMI)-matched healthy controls were taken from the previously observational 'healthy ageing' study conducted at the Clinical Research Facility of the Royal Victoria Infirmary, Newcastle upon Tyne, UK between January 2018 and July 2019. The inclusion criteria for healthy control participants were ≥18 years of age, no history of chronic, cardiovascular, pulmonary, or metabolic diseases, and willingness to visit the Clinical Research Facility. The exclusion criteria for healthy controls were defined as co-existing medical conditions, history of cardiovascular diseases such as cardiomyopathy, myocardial infarction, intermittent claudication, dementia, stroke, mild cognitive impairment, psychiatric disorders, musculoskeletal disease, chronic respiratory illnesses, cancer and/or signs of cardiac ischemia or arrhythmia at rest or during exercise. All procedures were performed according to the Declaration of Helsinki. Informed written consent was provided by all participants.

Study protocol and measurements

All eligible individuals were asked to refrain from alcohol- and/or caffeine-containing foods and beverages on the day of the study visit and were asked to avoid vigorous exercise 24-hours prior to the study visit. Upon arrival at the Clinical Research Facility, participants were given the opportunity to ask further questions about the study before providing written informed consent and their medical history was reviewed.

The integrated electrocardiography (ECG) within the TaskForce (CNSystems, Graz, Austria) was used to record spectral (frequency) domain HRV measures and RR interval for HCM and healthy controls using autoregressive (AR) method with a sampling frequency of 1000 Hz (Malik *et al.*, 1996; Fortin *et al.*, 1998b; Gratze, 1998). The HRV measures included: low frequency (LF) and high frequency (HF) power (both were given in normalised (nu) and absolute units (ms^2)) and LF/HF ratio. The LF power ranges from

0.04 to 0.15 Hz and HF power ranges from 0.15 to 0.4 Hz (Malik *et al.*, 1996). All spectral domain HRV measures were obtained from beat-to-beat variations in RR interval length (RR interval ms). HRV measures were recorded in a quiet consultation room at ambient temperature. Participants were requested to lie supine for five minutes with normal breathing. Following this resting period, five-minute HRV recording was obtained for individuals with HCM and healthy controls.

Additional HRV measures which were calculated manually were the standard deviation of RR interval (SDRR) and the coefficient of component variance of HF power (CCV_{HF}). SDRR, a time domain HRV variable, was calculated from the RR interval and is a marker of vagal modulation when measured over short-term 5-minute recording (Tanabe *et al.*, 1995; Mazloumi Gavgani *et al.*, 2017).

CCV_{HF} indicates vagal modulation of the heart which is affected by the exposure to drugs, clinical procedure or disease and is calculated using the following equation: CCV_{HF} (%) = 100*(HF power) $^{1/2}$/ (mean RR interval), and these values correlate linearly to vagal activities (Hayano *et al.*, 1991; Kawasaki, 2005; Arita *et al.*, 2015; Chan *et al.*, 2021).

Haemodynamic variables such as cardiac output, cardiac index, heart rate, stroke volume, stroke volume index, total peripheral resistance, total peripheral resistance index and blood pressure measurements were also evaluated using the TaskForce (CNSystems, Graz, Austria). In addition, cardiac power output (CPO) (W), which is an integrated measure of overall cardiac function and performance (Grodin *et al.*, 2015) was calculated manually by the equation: CPO (W) = mean arterial blood pressure (mmHg) x cardiac output (L/min) x K, where K=0.0022 (a conversion factor). CPO was indexed to body surface area: CPOI (w/m^2) = CPO/body surface area (Grodin *et al.*, 2015).

Statistical analysis

All statistical analyses were performed using SPSS, Version 27.0 (IBM Corp., Armonk, N.Y., USA). Data were screened for univariate and multivariate outliers using standard Z-distribution cut-offs, box plots and Mahalanobis distance test. There were no significant outliers.

Normality of the anthropometric, HRV and haemodynamic measures were assessed using Kolmogorov-Smirnov test and histograms. All anthropometric and haemodynamic were normally distributed. However, all HRV measures except RR interval, LF (nu) and HF (nu) were not normally distributed and subsequently underwent natural log transformation (Ln). After natural log transformation, normality, and homogenous variance in both individuals with HCM and healthy controls were achieved.

Differences in demographic, physical and clinical characteristics were assessed by independent samples *t*-test. Analysis of covariance (ANCOVA) and the Bonferroni correction were conducted to assess differences in HRV and haemodynamic variables between individuals with HCM and healthy controls using medications as covariate. Partial correlations were also performed to assess the relationship between HRV and haemodynamic variables using medications as the covariate. Statistical significance was indicated if $p<0.05$. All continuous data were expressed in mean±SD unless otherwise stated.

Results

Demographic, physical and clinical characteristics

Demographic, physical and clinical characteristics for individuals with HCM and healthy controls are presented in Table 1. A total of twenty-eight individuals with HCM (54±15 years old; age range: 21-78 years, males, N=21; females, N =7) were included in the

study. Twenty-eight age-, sex- and BMI–matched healthy control participants (54±16 years old; age range: 20-78 years, males, N=21; females, N =7) were recruited. The medication list of the individuals with HCM is provided in Table 1. Twenty-three (82%) individuals with HCM were on medications, whereas five (18%) patients were not prescribed any medication.

Heart rate variability in individuals with hypertrophic cardiomyopathy and healthy controls

Table 2 shows the comparison of all HRV variables between individuals with HCM and controls. RR interval was reduced in the individuals with HCM compared to controls (914±178 vs. 1014±168 ms, p=0.03). Whilst all HRV variables which reflect vagal activity were higher in the individuals with HCM versus controls (absolute HF power (7.40±2.50 vs. 6.03±1.35 ms^2, p=0.01), SDRR (4.46±1.43 vs. 3.95±0.60 ms, p=0.01), CCV_{HF} (1.50±1.34 vs. 0.71±0.66 %, p<0.01) and LF power (6.86±2.58 vs. 5.93±1.01 ms^2, p=0.01)). Figure 1 demonstrates the differences in RR interval (panel A) and HF power (panel B) between individuals with HCM and the healthy controls.

Haemodynamic variables in individuals with hypertrophic cardiomyopathy and healthy controls

Differences in haemodynamic variables between individuals with HCM and healthy controls are presented in Table 3. After controlling for medication, individuals with HCM had a reduced cardiac output (4.6±0.9 vs. 6.8±1.2 L/min, p<0.01), stroke volume (66±17 vs. 82±13 mL/beat, p<0.01) and cardiac power output (0.90±0.22 vs. 1.29±0.37 W, p=0.02) compared to the controls. Indexed values of haemodynamic variables were also reduced, except TPRI, in the individuals with HCM compared to the controls (Table 3). Figure 2 represents the differences in SV index (33±9 vs. 43±7 ml/beat/m^2, p<0.01; panel

A) and TPR index (4368±1027 vs. 2953±1050, p=0.03; panel B) between individuals with HCM and controls.

Relationship between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy

Table 4 details the results of the partial correlations between HRV and haemodynamic variables. After controlling for medications, all HRV indices which reflect vagal tone had a negative and moderate relationship with SV; HF power (r= -0.46, p<0.01), LF power (r= -0.44, p<0.01), SDRR (r= -0.45, p<0.01) and CCV_{HF} (r=-0.52, p<0.01). In contrast, there was a positive but weak relationship between vagal indices of HRV and both TPR; LF (r=0.29, p<0.05), HF (r= 0.28, p<0.05) CCV_{HF} (r= 0.28, p<0.05) and MABP; LF (r=0.31, p<0.05), SDRR (r=0.28, p<0.05) CCV_{HF} (r= 0.28, p<0.05). There was no relationship between all the HRV variables and CPO and CO.

Discussion

The major findings suggest that vagal activity represented by HF power, LF power, SDRR and CCV_{HF} was significantly higher in individuals with HCM versus healthy controls. In contrast, RR interval was significantly reduced in individuals with HCM compared to healthy controls. Haemodynamic variables such as cardiac power output and stroke volume were also significantly reduced in individuals with HCM compared to healthy controls. The current study is the first to investigate the association between HRV and haemodynamic function in individuals with HCM, with findings suggesting that vagal indices of HRV had a significant negative moderate relationship with stroke volume and a significant positive relationship with MABP and TPR in individuals with HCM.

residual effect of cardioactive drugs such as beta blockers which could modify the response to autonomic system and thus HRV (Mörner *et al.*, 2005).

Comparison with previous studies

Despite the wide discrepancy in the results of HRV studies in the individuals with HCM, the most frequent findings are the reduction in parasympathetic (PNS) activity and increased sympathetic (SNS) activity (Counihan *et al.*, 1993; Bonaduce *et al.*, 1997; Döven *et al.*, 2001). In contrast, the present study showed that the short-term recording of all vagal indices of HRV (HF power, LF power, SDRR and CCV_{HF}) were significantly higher in the individuals with HCM compared to healthy controls.

In agreement with the present study, Katarzynska-Szymanska and colleagues found no significant differences in HRV measures between the HCM and healthy control groups (Katarzynska-Szymanska *et al.*, 2013). Although the study recruited a higher number of individuals (51 participants) with HCM, there were no differences in all spectral

components of HRV between individuals with HCM and controls. ECG, as used in the present study, is the gold standard method to detect HRV accurately as it directly records the electrical activity of the heart whereas photoplethysmography (PPG), which was used by Katarzynska-Szymanska and colleagues, records HRV indirectly by detecting blood volume and it is sensitive to body motion artefacts (Jeyhani *et al.*, 2015; Jan *et al.*, 2019). Another study reported short-term HRV in obstructive and non-obstructive individuals with HCM and found that resting LF and HF power were higher in non-obstructive individuals with HCM compared to obstructive individuals with HCM (Limbruno *et al.*, 1998). These findings could be comparable with the present study findings and could indicate that the presence of LVOT obstruction may contribute to the increase in the SNS activity in the individuals with HCM. It was not possible to compare obstructive to non-obstructive individuals with HCM in the present study due to the small number of individuals with obstructive HCM (N=1).

In contrast to this study findings, Döven and collaborators (Döven *et al.*, 2001) found that time domain measures representing vagal activity (i.e. SDNN, root mean-squared successive difference (RMSSD) and percentage of cycles differing from the preceding one by more than 50ms (PNN50%)) were significantly reduced in individuals with HCM compared to healthy controls. Unlike the present study, the findings of (Döven *et al.*, 2001) were based on 24-hour recording time. Additionally, two studies have previously reported a reduction in parasympathetic activity in individuals with HCM, which were apparent only during night-time (Ajiki *et al.*, 1993; Tanabe *et al.*, 1995). Both studies recorded frequency domain HRV measures over 24-hours, yet the former showed lower PNS activity (log HF power) and increased SNS activity (log LF/HF) in individuals with HCM and individuals with dilated cardiomyopathy (DCM) compared to healthy controls

(Ajiki *et al.*, 1993). The latter reported decreased PNS (CCV_{HF}, HFnu, SDRR) and higher SNS (LFnu, LF/HF) in hospitalised patients with advanced HCM compared to healthy controls (Tanabe *et al.*, 1995). The possible reasons for the disparity in the findings between the present study and the aforementioned ones are related to firstly, the recording time of the HRV; over 24 hours constitutes major fluctuations that may not reflect changes occurring in the short-term and may bring about the possibility to study circadian rhythm (Counihan *et al.*, 1993). Secondly, variety of disease severity between studies (HCM, advanced hospitalised HCM and DCM) and finally, the inconsistent use of LF power versus LF/HF as an index of SNS activity between studies. Moreover, in (Ajiki *et al.*, 1993), a wider band width of LF and HF power (0.00 to 0.15 Hz for LF power and 0.15 to 0.50 Hz for HF power) was used, making results incomparable to this study.

Taking into account the aforementioned reasons for disparity, it should be emphasised that frequency domain measures of HRV aid more accurate assessment of the direction and magnitude of changes in autonomic balance than is likely with time domain analysis (Pumprla *et al.*, 2002). The current study recorded frequency domain measures of HRV over short-term which is the most recommended HRV recording time for clinical studies (Laborde *et al.*, 2017).

Possible mechanisms underlying the high vagal modulation in the present study

The increase in vagal activity seen in individuals with HCM in the present study could be attributed to the imbalance/dysfunction between sympathetic (SNS) and parasympathetic (PNS) arms of the autonomic nervous system. Under normal conditions, there is a precise interaction/cross talk between these two systems to maintain bodily functions. Parasympathetic activity can be modulated by the sympathetic nerve terminals and vice versa (Ondicova and Mravec, 2010). Therefore, it could be speculated that increased PNS

activity in individuals with HCM might be a regulatory/compensatory feedback due to either chronic increase (Perloff, 1981) or reduction in SNS activity (Limbruno *et al.*, 1998) triggering an alternate change in PNS activity. However, the extent of sympathetic modulation in the present study could not be measured due to short-term recording of HRV variables and the nature of spectrum ranges of LF power (Shaffer and Ginsberg, 2017). Therefore, the significantly higher LF power in individuals with HCM may possibly confirm the higher vagal tone compared to healthy controls in this study.

Also, a possible reason for high vagal in the individuals HCM in the present study could be attributed to the effect of long-term dual chamber pacing (DDD) pacing. In a previous study that examined the effect of DDD on HRV measures in individuals with obstructive HCM pre-implantation and one year post implantation, there was a significant increase in vagally mediated time domain HRV measures after implantation (Simantirakis *et al.*, 1999). In the present study, eight individuals with HCM had an ICD (seven on DDD pacing and one on ventricular pacing mode (VVI)). However, excluding these individuals did not alter the present results (Supplementary 1 table 1).

Relationship between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy

The novel finding of the present study was that vagally mediated HRV indices (HF (ms^2), LF (ms^2), SDRR and CCV_{HF}) had significant relationships with SV, TPR and MABP in individuals with HCM. The relationship between vagal indices of HRV and SV was negative but positive with TPR and MABP. These findings are likely linked together as the autonomic nervous system play an essential role in maintaining cardiovascular health by regulating blood pressure and blood flow (Charkoudian and Rabbitts, 2009). MABP is the product of CO and TPR (MABP= CO x TPR) and CO is the product of SV and HR

(CO= SV x HR) (Williams *et al.*, 2021). The role of the vagal nerve in regulating HR, SV and CO has been extensively studied (Levy, 1997). Normally, the vagal nerve mediates inhibitory responses in the heart and therefore it decreases CO, SV and HR (Kawasaki and Sugihara, 2014). This effect was observed in the individuals with HCM in the present study as the higher vagal tone was related to a significant reduction in SV, whilst paradoxically, increases in both MABP and TPR were observed. Evaluating these relationships is clinically relevant as it has the potential to aid clinicians understanding of the mechanisms of disease progression, haemodynamic instability as well as the effect of treatment.

An explanation for this paradox between vagal enhancement of higher MABP and TPR in individuals with HCM could possibly be due to the activation of the *Renin-Angiotensin-Aldosterone System* (RAAS). The RAAS is mediated by the kidneys and is vital for controlling blood pressure (Sparks *et al.*, 2014). In this present study, both resting SV and CO were significantly reduced in individuals with HCM compared to the healthy controls which could trigger the activation of RAAS. This sympathetic activation which caused a further stimulation of vagal activity might prevent haemodynamic collapse (Ames *et al.*, 2019). The correlation analysis between HRV and haemodynamic variables in healthy controls is available in the supplementary 1 table 2).

Another possible reason for RAAS stimulation in the individuals with HCM is the presence of ACE polymorphism which is associated with high level of serum ACE (Yuan *et al.*, 2017). However, in the present study neither the activity of RAAS was measured, nor the genetic screening of ACE polymorphism was performed.

Based on the present study findings, the significant relationship between HRV and haemodynamic variables would suggest that treatment for individuals with HCM may not

only improve symptoms related to the disease progression but also improve cardiac autonomic function.

Strengths and limitations of the study

The current investigation is the first to investigate the relationships between HRV and haemodynamic variables in individuals with HCM. The study findings were based on five minute recording time, which also supports the increasing demand and necessity for short-term HRV analysis in various clinical applications (Lee *et al.*, 2018). Although it was previously suggested that 24-hour recordings were the gold standard recording time for clinical HRV assessment (Shaffer and Ginsberg, 2017) but spectral analysis of HRV over a 24-hour period is limited by unstable oscillations of the RR intervals (Fei *et al.*, 1995). Therefore, a significant number of studies which involve 24-hour assessments of spectral measures of HRV analyse data over a shorter period of time such as 2-5 minutes (Fei *et al.*, 1995; Mörner *et al.*, 2005). This approach may partially reduce the effect of instability of the data and make values of the 24-hour recorded HRV data directly related to a stable short-term recording (Fei *et al.*, 1994). In the current study, both frequency domain HRV measures (LF power, HF power in absolute and normalised units and the LF/HF) and time domain HRV measures (RR interval and SDRR) were used.

This study was not without limitations. Firstly, present study finding, i.e. higher vagal activity was associated with higher MABP and TPR at rest, could not have been evaluated during exercise. This would have improved understanding of the mechanisms attributed to the known abnormal vasodilatory response during exercise for individuals with HCM (Olivotto *et al.*, 1999). The present study did not measure spectral power of blood pressure (BP) variability, which is well known to be influenced by the autonomic nervous system (Parati *et al.*, 2018; Elgendi *et al.*, 2019; Abdulhameed *et al.*, 2020; Karavaev *et*

al., 2021). It has been found that the LF power component of HRV and BP variability were coherent and correlated in healthy participants indicating the process of autonomic control of blood circulation (Karavaev *et al.*, 2021). Therefore, measuring spectral power of BP variability along with HRV would have aided our understanding of the correlation that we found between HRV measures and both TPR and MABP in individuals with HCM. It may have expanded the possibility of assessing autonomic dysfunction in this clinical population. Also, the present study did not assess the complex nonlinear measures of HRV, which would have been advantageous in understanding diseases complexity over traditional time and frequency domain measures (Sharif *et al.*, 2016; Valenza *et al.*, 2017; Skazkina *et al.*, 2020).

The study was also limited by the relatively modest sample size, which may limit generalisability of findings. The findings of this study were based on recording on resting supine position, which causes a lack of information about sympathetic activity. This is because LF component of HRV does not reflect pure sympathetic activity especially when it is recorded on resting and supine position where there is no sympathetic provocation manoeuvre such as a tilt test. In the present study there were five patients with atrial fibrillation and a further two with atrial flutter. Inclusion of these patients did not affect the results in the present study (Supplementary 1 table 3) even though arrhythmia have been reported to affect the outcome of HRV analysis (Nunan *et al.*, 2010a). Moreover, excluding these patients would have reduced the power of the study further and resulted in significant selection bias (Malik *et al.*, 1996).

Conclusions and clinical implications

Individuals with HCM demonstrated greater variation in vagal activity but reduced haemodynamic function in comparison to healthy controls. Resting short-term frequency

domain indices of HRV provide a feasible approach to assess the relationship between autonomic function and haemodynamic activity in individuals with HCM This finding is clinically relevant since it could aid in the prevention of HCM progression and improve understanding of the disease pathophysiology. Further studies are warranted to investigate the prognostic value of HRV in HCM.

Declarations

Funding and ethical approval

The study was approved by the Research Ethics Committee of the National Health Service, North-East England - Tyne & Wear South, and local Research and Development department (18/NE/0318). This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under Grant Agreement no. 777204. Miss AI. Alyahya is supported by the Saudi Arabia Government and Imam Abdulrahman Bin Faisal University via an international doctoral scholarship. The views expressed are those of the authors. The funders of the study had no role in study design or in data collection, analysis, or interpretation.

Availability of data and materials

The data sets used and/or analysed during the current study are available from the corresponding author on a reasonable request.

Consent for publication

The manuscript does not contain any individual personal data in any form. All authors reviewed the final version of the manuscript and agree with the submission.

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Conflict of interest

All the authors declare no conflict of interest.

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Figure and table legends

Table 1: Demographics, physical and clinical characteristics for individuals with HCM and healthy controls.

Abbreviations: AF: atrial fibrillation; HCM hypertrophic cardiomyopathy; ICD: implantable cardioverter defibrillator; LVOT:O left ventricular outflow tract obstruction

Table 2: Short-term heart rate variability variables in individuals with HCM and healthy controls.

Abbreviations: CCV_{HF}: coefficient of component variance of high frequency power; HCM: hypertrophic cardiomyopathy; HF: high frequency spectral power; LF: low frequency spectral power; LF/HF: the ratio of low to high frequency spectral power; ms^2: absolute units; nu: normalised units; RR: count number of mean time between r waves; SDRR: standard deviation of RR interval.

† Natural log transformed data presented.

Table 3: Haemodynamic variables in individuals with HCM and healthy controls.

Table 4: **Partial correlations between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy.**

r correlation coefficient; *p< 0.05; **p<0.01. LF nu, HF nu and LF/HF did not have any significant relationships with haemodynamic variables. † Natural log transformed data presented.

Figure 1: Heart rate variability variables in individuals with HCM and healthy controls. (A) RR interval and (B) HF power in individuals with HCM and healthy controls.

Figure 2: Haemodynamic variables in individuals with HCM and healthy controls. (A) Stroke volume (SV) index and (B) Total peripheral resistance (TPR) index in individuals with HCM and healthy controls.

Supplementary 1 Table 1: Heart rate variability measures in the individuals with HCM without implantable cardioverter-defibrillator and healthy control.

† Natural log transformed data presented. ICD: implantable cardioverter-defibrillator.

Supplementary 1 Table 2: Relationship between heart rate variability measures and haemodynamic variables in healthy controls.

† Natural log transformed data presented.

Supplementary 1Table 3: Heart rate variability measures in individuals with hypertrophic cardiomyopathy without atrial fibrillation/ flutter.

† Natural log transformed data presented. AF: atrial fibrillation.

Table 1 Demographics, physical and clinical characteristics for individuals with HCM and healthy controls

Variables	**Individuals with HCM N=28**	**Healthy controls N=28**	***P Value***
Age (years)	54±15	54±16	0.90
Sex, female N (%)	7 (25)	7 (25)	1
Height (cm)	173±8	169±9	0.16
Weight (kg)	85±18	83±17	0.68
Body mass index (kg/m^2)	29±5	29±5	0.96
Body surface area (m^2)	2.02±0.2	1.93±0.2	0.15
ICD N (%)	8 (29)	-	-
AF/ Atrial Flutter N (%)	5 (18)	-	-
LVOTO N (%)	1 (4)	-	-
Ischemic cardiomyopathy N (%)	-	-	-
Dilated cardiomyopathy N (%)	-	-	-
Mean time since HCM diagnosis at study visit (years)	8±5	-	-
Medication N (%)			
Beta-adrenergic blockers	17 (61)	-	-
Angiotensin-Converting Enzyme Inhibitors	4 (14)	-	-
Angiotensin II receptor antagonist	4 (14)	-	-
Calcium channel blockers	7 (25)	-	-
Diuretics	6 (21)	-	-
Anti-arrhythmic agents	5 (18)	-	-
No drug therapy	5 (18)	-	-

Abbreviations: AF: atrial fibrillation; HCM hypertrophic cardiomyopathy; ICD: implantable cardioverter defibrillator; LVOTO left ventricular outflow tract obstruction

Table 2 Short-term heart rate variability variables in individuals with HCM and healthy controls

Variables	**Individuals with HCM N=28**	**Healthy controls N=28**	***P Value***
Heart rate (beats/min)	76±22	83 ±14	0.86
RR interval (ms)	914±178	1014±168	**0.03**
†SDRR (ms)	4.46±1.43	3.95±0.60	**0.01**
†LF power (ms^2)	6.86±2.58	5.93±1.01	**0.01**
†HF power (ms^2)	7.40±2.50	6.03±1.35	**0.01**
LF power (nu)	38.3±17.1	47.3±19.9	0.97
HF power (nu)	61.6±17.1	52.7±19.9	0.97
†LF/HF	0.51±0.85	0.07±0.94	0.99
†CCV_{HF} (%)	1.50±1.34	0.71±0.66	**<0.01**

Abbreviations: CCV_{HF}: coefficient of component variance of high frequency power; HCM: hypertrophic cardiomyopathy; HF: high frequency spectral power; LF: low frequency spectral power; LF/HF: the ratio of low to high frequency spectral power; ms^2: absolute units; nu: normalised units; RR: count number of mean time between r waves; SDRR: standard deviation of RR interval.

† Natural log transformed data presented.

Table 3 Haemodynamic variables in individuals with HCM and healthy controls

Variables	**Individuals with HCM N=28**	**Healthy Controls N=28**	***P Value***
Cardiac output (L/min)	4.6±0.9	6.8±1.7	**<0.01**
Cardiac index (L/min/ m²)	2.33±0.42	3.57±0.82	**<0.01**
Heart rate (beats/min)	76±22	83 ±14	0.86
Stroke volume (ml/beat)	66±17	82 ±13	**<0.01**
Stroke volume Index (ml/beat/m^2)	33±9	43±7	**<0.01**
Systolic blood pressure (mmHg)	119±14	119±18	0.55
Diastolic blood pressure (mmHg)	76 ±10	73 ±13	0.05
Mean arterial blood pressure (mmHg)	89±12	86±13	0.05
Total peripheral resistance ($dyn{\cdot}s{\cdot}cm^{-5}$)	1724±465	1512±461	0.08
Total peripheral resistance index ($dyn{\cdot}s{\cdot}m^2cm^{-5}$)	3468±1027	2953±1050	**0.03**
Cardiac power output (W)	0.90±0.22	1.29±0.37	**0.02**
Cardiac power output index (W/m^2)	0.44±0.08	0.66±0.16	0.07

Table 4 Partial correlations between heart rate variability and haemodynamic variables in individuals with hypertrophic cardiomyopathy

Variables	Stroke volume	Cardiac power output	Cardiac output	Total peripheral resistance	Mean arterial blood pressure
	r	r	r	r	r
RR interval (ms)	0.43**	-0.21	0.01	-0.06	-0.44
†LF power (ms^2)	-0.44**	0.04	-0.10	0.29*	0.31*
†HF power (ms^2)	-0.46**	-0.03	-0.12	0.28*	0.21
†SDRR (ms)	-0.45**	0.09	-0.02	0.26	0.28*
†CCV_{HF} (%)	-0.52**	0.00	-0.12	0.28*	0.28*

r correlation coefficient; *p< 0.05; **p<0.01. LF nu, HF nu and LF/HF did not have any significant relationships with haemodynamic variables. † Natural log transformed data presented. ms^2: absolute units; nu: normalised units.

Figure 1

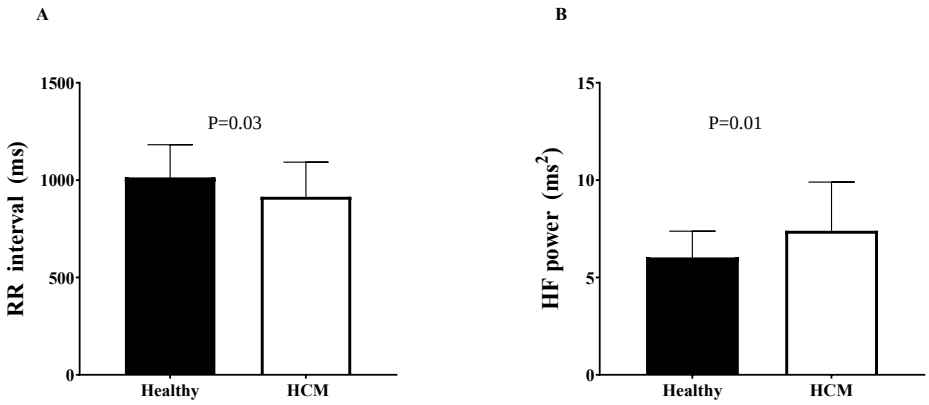
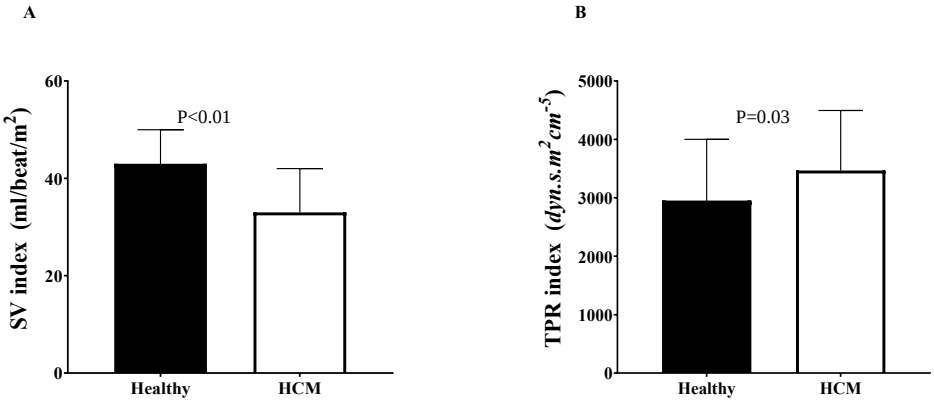


Figure 2



Supplemantary 1

Table 1 Heart rate variability measures in the individuals with HCM without implantable cardioverter-defibrillator and healthy control

Variables	HCM no ICD N=20	Healthy controls N=28	P Value
Heart rate (beats/min)	77±23	83±13	0.95
RR interval (ms)	912±195	1014±168	**0.02**
†SDRR (ms)	4.74±1.10	3.96±0.60	**<0.01**
†LF power (ms^2)	7.40±1.86	5.93±1.01	**<0.01**
†HF power (ms^2)	7.74±2.09	6.03±1.36	**<0.01**
LF power (nu)	42.4±17.8	47.2±19.9	0.96
HF power (nu)	57.5±17.8	52.7±19.9	0.96
†LF/HF	0.31±0.86	0.07±0.94	0.95
†CCV_{HF} (%)	1.68±1.14	0.71±0.66	**<0.01**

† Natural log transformed data presented. ICD: implantable cardioverter-defibrillator.

Table 2 Relationship between heart rate variability measures and haemodynamic variables in healthy controls

Variables	Stroke volume	Cardiac power output	Cardiac output	Total peripheral resistance	Mean arterial blood pressure
	r	***r***	***r***	***r***	***r***
RR interval (ms)	0.19	-0.31	- 0.16	0.13	- 0.44*
†LF power (ms^2)	0.10	0.15	0.22	- 0.43*	-0.18
†HF power (ms^2)	0.11	- 0.10	0.10	- 0.41*	- 0.35
†SDRR (ms)	0.33	0.29	0.51**	- 0.43*	- 0.25
†CCV_{HF} (%)	0.07	- 0.06	0.06	- 0.41*	- 0.24

† Natural log transformed data presented. r correlation coefficient; *p< 0.05; **p<0.01.

Table 3 Heart rate variability measures in individuals with hypertrophic cardiomyopathy without atrial fibrillation/ flutter

Variables	**HCM no AF N=23**	**Healthy controls N=28**	***P Value***
Heart rate (beats/min)	73±22	83±13	0.74
RR interval (ms)	929±183	1014±168	**0.04**
†SDRR (ms)	4.30±1.41	3.95±0.60	**<0.01**
†LF power (ms^2)	6.76±2.49	5.93±1.01	**<0.01**
†HF power (ms^2)	7.17±2.48	6.03±1.35	**0.01**
LF power (nu)	41.1±17.2	47.2±19.9	0.96
HF power (nu)	58.8±17.2	52.7±19.9	0.96
†LF/HF	0.36±0.82	0.06±0.94	0.96
†CCV_{HF} (%)	1.37±1.33	0.71±0.66	**<0.01**

† Natural log transformed data presented. AF: atrial fibrillation.