THE ATHLETIC HEART PHENOTYPE IN ELITE FEMALE FOOTBALLERS

ALEAH BINTI MOHAMMAD

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Doctor of Philosophy

2022

Director of Studies

Professor Keith George Ph.D., FACSM, FECSS, FBASES

Pro-Vice Chancellor for Research and Knowledge Exchange

Liverpool John Moores University

Second Supervisor

Dr David Oxborough Ph.D., MSc, BHSc, FBSE

Reader in Cardiovascular Physiology

Clinical Cardiac Physiology and Fellow of the British Society of Echocardiography

Liverpool John Moores University

Third Supervisor

Late Professor John Somauroo

Professor of Sports Cardiology, Liverpool John Moores University

& Consultant Cardiologist, Countess of Chester Hospital, Chester

Acknowledgements

First and foremost, all praises to Allah, the Almighty, for his showers of blessings throughout my research work to complete the research successfully. Undertaking this PhD has been a truly life-changing experience for me, and it would not have been possible to do without the support and guidance that I received from many people.

I am extremely thankful to my supervisors, Prof Keith George for his tremendous advice, continuous support, and patience during my PhD study. Keith's immense knowledge and plentiful experience encouraged me all the time during my academic research and daily life. Without his guidance and constant feedback, this PhD would not have been achievable. I would also like to thank my other supervisor's Dr David Oxborough and late Prof John Somauroo for their technical support and sacrifices during our numerous wonderful field trips. Thank you for being a fantastic support network during my studies. It was a great privilege and honor to work and study under their supervision.

To my family, this thesis is dedicated to my husband, Izzady who has been by my side throughout this PhD, living every single minute of it, and without him, I would not have had the courage to embark on this journey in the first place. To my wonderful loving children (Imran, Hannah and Muhammad) I am forever indebted for all their support and encouragement, making it possible for me to complete what I started. Without their tremendous understanding and patience in the past few years, it would be impossible for me to complete my study.

I would like to express my endless gratitude to my parents Mr Mohammad and Mrs Rashidah, and my sisters, for their love, prayers, caring and always believing in me to follow my dreams, helping in whatever way they could during this challenging period.

I would like to thank all the LJMU RISES student and staff members. Their kind help and support have made my study and life in the UK a wonderful time. I am also grateful to the funding received through MARA Malaysia, to undertake my PhD. I would like to say thanks to my friends and PhD colleagues, Dr Nur Azyan, Dr Cindy, Dr Emyra, Ernie, Siti Titin, Eileen and my SRP girls for their constant encouragement.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly. God bless you all.

Abstract

Elite athletes can present with electrical (ECG) as well as structural and functional (echocardiography) changes to the heart, compared to healthy controls, referred to as the athlete's heart (AH). In the setting of cardiac pre-participation screening (PPS), both ECG and echocardiographic AH data are valuable to aid differential diagnosis of AH from various cardiac pathologies that increase the risk of sudden cardiac death (SCD). At present, there are limited data describing the AH in elite female footballers to feed into PPS. This thesis presents four studies whose aims were to, (i) critically evaluate the 12-lead ECG in elite female professional footballers, (ii) assess the effect of performing ECG assessments across a competitive season in elite professional female footballers, (iii) document resting cardiac structure and function in elite professional female footballers, and (iv) determine the variability in resting ECG as well as cardiac structure and function across the menstrual cycle in a healthy cohort of young women.

The first study employed a cross-sectional descriptive design with all testing and data collection occurring in one session. 12-lead ECGs were recorded in eighty-one elite female footballers. ECGs were interpreted using 3 published criteria used in PPS. The key findings were that training-related ECG changes were very common in elite female footballers and that the "Seattle" and "International" ECG Criteria significantly reduced the number of ECG false-positives, from 16.2% to 0%. In the second study, a prospective repeated-measures design was employed with ECG data acquired in thirteen elite female football players, at three different time points (pre-season, mid-season, and end-season) across a single competitive season. The key finding was that ECG data in elite female footballers were largely stable across

a competitive season. Consequently, the time point of PPS within a competitive season is unlikely to alter clinical decision making in PPS. The third study employed a cross-sectional design to characterize cardiac structure and function in seventy-nine elite female footballers using standard and novel echocardiography. The key findings were that absolute data for a range of LV and RV chamber size indices as well as LV wall thicknesses and global measures of LV function were greater in elite female footballers than in sedentary controls. This data will contribute to our population specific knowledge of the AH and support PPS in elite female footballers. In the fourth study, a repeated-measures design was employed that assessed resting ECG as well as cardiac structure, and function across three different time points of the menstrual cycle in seventeen healthy, eumenorrheic, female participants. The key findings were that there were no major differences in ECG data as well as cardiac structural and functional indices observed across 3 phases of the menstrual cycles. These data will help inform testing plans and data analysis in cardiac PPS in elite female athletes.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF TERMS AND ABBREVIATIONS

A = Peak Late Diastolic Flow Velocity

AF = Atrial Fibrillation

AH = Athlete's Heart

AHA = American Heart Association

ANOVA = Analysis of Variance

ARVC = Arrhythmogenic Right Ventricular Cardiomyopathy

ASE = American Society of Echocardiography

BA = Black Athlete

BP = Blood Pressure

BPM = Beats per Minute

BSA = Body Surface Area

CO = Cardiac Output

CRY = Cardiac Risk in the Young

DBP = Diastolic Blood Pressure

DCM = Dilated Cardiomyopathy

E- Peak Early Diastolic Flow Velocity

E` = Early Diastolic Annular Peak Velocity

E: A = Ratio of Peak Early and Late Diastolic Flow Velocity

E: E` = Ratio of Peak Early Diastolic Flow velocity and Peak Early Diastolic Mitral Annular Velocity

ECG = Electrocardiogram

Echo = Echocardiogram

EF = Ejection Fraction

ESC = European Society of Cardiology

ET = Endurance Trained Athlete

FA = The Football Association

FAC = Fractional Area Change

FIFA = The Fédération Internationale de Football Association (FIFA)

F-MARC = FIFA Medical Assessment and Research Centre

FSH = Follicle Stimulating Hormone

GnRH = Gonadotropin Releasing Hormone

HCM = Hypertrophic Cardiomyopathy

HR = Heart Rate

IOC = International Olympic Committee

IVCD = Intraventricular Conduction Delay

IVCT = Isovolumic Contraction Time

IVRT = Isovolumic Relaxation Time

IVSWTd = Diastolic Inter-ventricular Septal Wall Thickness

IVSs = Systolic Inter-ventricular Septal Wall Thickness

LA = Left Atrium

LAD = Left Atrial Dimension

LBBB = Left Bundle Branch Block

LH = Luteinizing Hormone

LQTS = Long QT Syndrome

LV = Left Ventricle

LVEDV = Left Ventricular End Diastolic Volume

LVEF = Left Ventricular Ejection Fraction

LVIDd = Left Ventricular Internal Diameter at End-diastole

LVIDs = Left Ventricular Internal Diameter at End-systole

LVIDd/2*WT = LVIDd Divided by 2 Times Posterior Wall Thickness

LVH = Left Ventricular Hypertrophy

LVM = Left Ventricular Mass

LVWT = Left Ventricle Wall Thickness

MaxWT = Maximum Wall Thickness

MeanWT = Mean Wall Thickness

MH = Morganroth's Hypothesis

MI = Myocardial Infarction

MST = Myocardial Speckle Tracking

N = Number

NA = Non-Applicable

P = Statistical probability (e.g., *P*<.05)

PASP = Pulmonary Artery Systolic Pressure

PLAX = Parasternal Long Axis

PPS = Pre-participation Screening

PSAX = Parasternal Short Axis

PVC = Premature Ventricular Contraction

PW = Pulsed Wave

PWTd = Posterior Wall Thickness at End-diastole

PWTs = Posterior Wall Thickness at End-systole

QTc = Corrected QT Interval

RBBB = Right Bundle Branch Block

ROI = Region of Interest

RT = Resistance Trained Athlete

RV = Right Ventricle

RVEDD = RV End-diastolic Diameter

RVEDV = RV End-diastolic Volume

RVD area = Right Ventricular End-diastolic Area

RVD1 = Right Ventricular Dimension at Basal Inflow

RVD2 = Right Ventricular Dimension at Mid-Cavitary Diameter Inflow

RVD3 = Right Ventricular Dimension at Base to Apex RV Length Inflow

RVFAC = Fractional Area Change

RVH = Right Ventricular Hypertrophy

RVL = Right Ventricular Length

RVOT = Right Ventricular Outflow Tract

RVOT1 = RV Outflow Tract Diameter at the Sub-pulmonary Region

RVOT2 = RV Outflow Tract Diameter at the Pulmonic Valve Annulus

RVOT-PLAX = RV Outflow Tract Diameter in the Parasternal Long-axis View

RV A' - Peak Velocity of Atrial Diastolic Annular Motion in RV

RV E'- Peak Velocity of Early Diastolic Annular Motion in RV

RV S' = Peak Velocity of Systolic Diastolic Annular Motion in RV

RV WT = RV Wall Thickness

RWT = Relative Wall Thickness

S' = Peak Velocity of Systolic Diastolic Mitral Annular Motion

SADS = Sudden Arrhythmic Death Syndrome

SBP = Systolic Blood Pressure

SCD = Sudden Cardiac Death

SD = Standard Deviation

SQTS = Short QT Syndrome

SR = Strain Rate

SRA' = Strain Rate during Atrial Ventricular Diastole

SRE’ = Strain Rate during Early Ventricular Diastole

SRS’ = Strain Rate during Ventricular Systole

STE = Speckle Tracking Echocardiography

SV = Stroke Volume

TAPSE = Tricuspid Annular Plane Systolic Excursion

TDI = Tissue Doppler Imaging

TTE = Trans-thoracic Echocardiography

TFC = Task Force Criteria

TWI = T-wave Inversion

UEFA = The Union of European Football Associations

UK = United Kingdom

USA = United States of America

WA = White Athlete

WHO = World Health Organisation

WPW = Wolff–Parkinson–White syndrome

WT = Wall Thickness

ε = Strain

2D = Two-dimensional

= Significant Difference Between Groups or Times

CHAPTER 1:

Introduction

1.0 **INTRODUCTION**

Football is the one of the fastest growing team sports in the world and there are more than 30 million women in the world participating (FIFA, 2014). Substantial growth in participation has led to the increasing numbers of medical events and the potential for serious cardiac events during football matches due to the presence of "silent" underlying cardiovascular diseases (Higgins and Andino, 2013). Because of numerous case studies of sudden cardiac death (SCD) associated with football exposure, a range of national and international football governing bodies, including FIFA, UEFA and the FA, have mandated the application of athlete pre-participation screening (PPS) with electrocardiography (ECG) and echocardiography prior to competition (Kramer et al., 2015).

The ability to differentiate "silent" cardiovascular diseases in footballers during PPS depends on a range of key factors, not least of which is knowledge of the normal cardiac phenotype (electrical activity, structure and function) in any given footballer group. As well as performance and skeletal muscle adaptation in elite footballers, the cardiovascular system adapts to the hemodynamic challenge imposed by training and performance. Training is associated with electrical, morphological and functional changes in the heart; referred to as the "athlete's heart" (AH) (George et al., 2011; 2012). Thus, for PPS to provide clear and valid differential diagnoses between the AH and pathology it must incorporate specific knowledge of the upper limits of the AH phenotype (Wasfy et al., 2015).

For any PPS to be undertaken in elite female footballers required an in-depth knowledge of the electrical, structural and functional presentation of the AH in that population. Clear and substantial normative data in this group is not currently available (George et al., 2011).

When interrogating the data available to describe the AH several issues become apparent and relevant. The male AH is well documented (Utomi et al., 2013) with much less focus on females (Rawlins et al., 2009) using either ECG (electrical activity) or echocardiography (structure and function). With respect to ECG data, we do not have a good database on training related adaptations to the ECG in elite female footballers. In addition, how the use of different ECG interpretation criteria impact on positive and negative outcomes (specifically false negative are greater concerns) in elite female footballers is not known and this would help focus the choice of criteria to be employed. Of practical importance, the impact of when PPS might occur within any given season and how this might mediate PPS outcome is not known and requires immediate study. Furthermore, our knowledge of the structure and function of the elite female footballers is even more restricted when wishing to understand the adaptation to training of the right ventricle (RV) or atria, which are clearly implicated in some disease states and are important to the differential diagnostic process. As technological advancements permit a more detailed and comprehensive assessment of the AH, it is pertinent to evaluate the AH phenotype of female athletes in detail using technologies such as tissue-Doppler and strain imaging. PPS in female athletes can occur at any point of the menstrual cycle. Given that variations in sex-steroid hormones across the menstrual cycle could impact the cardiovascular system it is sensible to determine if ECG and

echocardiographical indices assessed at different menstrual cycle phases differ in a clinically significant fashion.

As a sub-group of female athletes, elite female footballers are interesting as less AH data is available, yet they must submit to PPS as determined by their governing body. Consequently, elite female footballers are a vital group to study. To the best of my knowledge, we propose novel studies to characterise cardiac electrical activity, structure and function in elite female footballers. I will employ 12-Lead electrocardiography, standard and novel echocardiography and where appropriate I will compare elite footballer data to a sedentary age-matched control group. I seek to document the impact of different stages of the competitive season and international criteria on the elite female footballer's ECG. Finally, there is a paucity of data describing variability in cardiac ECG, structure, and function across different phases of the menstrual cycle. I hope that these studies will contribute useful information to help guide PPS and any relevant clinical decision making.

1.1 Research Aims

I propose four empirical studies, and the primary aim of each study is stated here:

1) Chapter 3: Critically evaluate the 12-lead ECG of the elite female footballer and determine the impact of different ECG interpretation criteria used in PPS.

2) Chapter 4: Assess the effect of competitive season stage on the ECG of elite female footballers.

3) Chapter 5: Determine the cardiac structure and function of elite female footballers using standard and novel echocardiographic techniques in comparison to age-matched healthy controls.

4) Chapter 6: Assess the variability in resting ECG as well as cardiac structure and function across three phases of the menstrual cycle in young healthy women.

Chapter 2:

Literature review

2.0 Introduction

This literature review is divided into five main sections which are; 1) Sudden cardiac death (SCD), 2) Pre-participation screening (PPS), 3) History of female football, 4) The female athlete's heart, and 5) The menstrual cycle and variation in cardiac electrical activity, structure and function. This development and progression of background science and literature seeks to underpin the 4 empirical studies and provide the rationale for the specific aims, objectives and/or hypotheses of the studies.

Since the focus of this research is to accurately and comprehensively define the AH phenotype of elite female footballers the literature review is used to place this knowledge in context. Specifically, we need to understand what the AH "looks like" in these athletes as there are recorded instances of SCD in female footballers and major football governing bodies such as FIFA, UEFA, and the FA mandate that female footballers undertake PPS in an effort to pre-empt SCD. This requires a background of knowledge and research in both SCD and PPS. The third section of the literature review discusses the history and development of research in female footballers to place the growth and development of elite female football in some social and scientific/medical context. The literature review than draws previous content together with a focus on the AH, firstly in males, then generally in female athletes and finally in elite female footballers. This provides the direct rationale for the focus of the empirical data collection in the thesis. Finally, yet importantly, this chapter also discusses the potential impact of the menstrual cycle, and cyclic variation in circulating levels of female sex-steroid

hormones, upon cardiovascular physiology. This data, along, with all the studies of the thesis will provide useful information to feed into and develop PPS in this athlete group.

2.1 Sudden Cardiac Death (SCD)

SCD was defined by The World Health Organization (WHO) as an unexpected cardiovascular death within 1 hour of symptom onset, if witnessed, or within 24 hours of having been observed alive and symptom free if unwitnessed (Church et al., 2004). According to numerous studies (Maron et al., 2009; Holst et al., 2010), SCD is "sport related" when it occurs during, or within 1 hour of, moderate-to high-intensity exercise.

When SCD occurs in young athletes it is nearly always a manifestation of the presence of congenital or hereditary cardiovascular disease(s), such as cardiomyopathies or ion-channelopathies. The word 'young' in this context refers to an individual who is aged < 35 years. SCD events in athletes aged > 35 years are usually due to early onset coronary artery disease (Maron et al., 1980; Maron et al., 1986; Maron, 2003). Studies indicate that 56-80% of SCD in young athletes occurs during or after exercise with the remainder considered non-exertional (i.e. at rest or during sleep; Maron et al., 1980; Maron et al., 2014; Harmon et al., 2015).

SCD in a young athlete (or indeed sudden cardiac arrest with recovery as seen in Christian Eriksen at UEFA 2020 recently) is a devastating tragedy and receives much public and media attention (Harmon et al., 2011). Fortunately, these events are very rare, but the exact prevalence is hotly debated and likely mediated by a significant number of athlete and sport-related characteristics. A stated prevalence of 1–3 per 100 000 athletes (Harmon et al., 2011)

may be too simplistic. For example, the incidence of SCD in Division 1 male basketball athletes in USA colleges was 1 in 5200 athlete per year, with the most common findings at autopsy being autopsy-negative sudden unexplained death in 16 (25%), and definitive evidence for hypertrophic cardiomyopathy in 5 (8%) (Harmon et al., 2014).

Personal or sporting characteristics associated with SCD in young athletes include gender, ethnicity, and sport-type. Numerous studies (Corrado et al., 2003; Marijon et al., 2015) have shown up to a 30-fold increase of SCD in males compared to females (10-75 years). This difference was consistent whether the focus was sports related SCD or non-sports related SCD (Marijon et al., 2015). The greater prevalence of SCD in male athletes is likely a multifactorial issue. A greater predominance of male athletes competing in elite sport, potentially longer history and greater volume of more intensive training, greater intensity of activity during sports, and inherent sex differences in disease substrates (structural and electrophysiological) that act as a trigger for SCD are likely factors (Corrado et al., 2003). Data from Italy suggest SCD rates in females are recognised to be 2-25 times lower than in men (Corrado et al., 2003). This difference in SCD prevalence seems to be apparent in the specific sport of football (Mont et al., 2016) but this does not mean that elite female footballers are afforded complete protection against SCD (Maron et al., 2014). Despite this, a consequence of the greater absolute numbers of male SCD and unbalanced participation data has meant that athletes SCD risk and screening data are largely focussed on men, even though we know from the case studies and research papers that SCD does occur in young female athletes as well. This omission must be addressed.

Whilst high profile SCD in young athletes seem to be associated with team sports, there is no clear connection between types of sports and SCD, as participation rates vary and it is likely

that sporting exposure is also complicated by differences in gender and ethnicity (Mont et al., 2016).

In the UK, available data suggests that SCD may occur in approximately 12 seemingly healthy people aged 35 or under every week (Cardiac Risk in Young, 2015). A female athlete SCD is as devastating as a male athlete SCD. As such, every effort to prevent these events (such as PPS) should be applied to both male and female athletes and at the same time incorporating good science and clinical decision making to prevent any athlete from unnecessary disqualification from any competition.

2.1.1 Causes of SCD

It is widely stated that 80% of non-traumatic sudden deaths in young athletes (under the age of 35) are caused by inherited or congenital structural and functional cardiovascular abnormalities, which provide a substrate for arrhythmias predisposing to SCD (Maron et al., 1986). SCD in individuals with normal hearts who are healthy, free from drug or other environmental stressors is exceptionally rare, but commotio cordis (blunt trauma to the chest wall that results in ventricular fibrillation) has been reported in some SCD registry studies (Sharma et al., 1997; Borjesson and Pellicia, 2009; Corrado et al., 2011). In nearly all other circumstances, SCD is associated (or suspected) with the presence of underlying pathology that interacts with environmental circumstances (including the sport exposure) to produce a cardiovascular "event". Examples of inherited diseases include cardiomyopathies, notably hypertrophic cardiomyopathy (HCM) and arrhymogenic right ventricular cardiomyopathy (ARVC) (Maron, 2003; Maron et al., 1986), ion channelopathies (long and short QT syndrome, Brugada syndrome, Wolff–Parkinson–White syndrome) and Marfan Syndrome (Sharma et al.,

1997; Borjesson and Pellicia, 2009; Corrado et al., 2011). A small number of SCD in young athletes may be due to acquired CV disease such as myocarditis and early onset coronary artery disease (which may of course have a genetic component).

In the UK, the underpinning pathology associated with SCD in various athletic populations has been established in several small "pathology registry" studies (see Figure 2.1; Oxborough et al., 2018; Finocchiaro et al., 2016). Finocchiaro's review detailed hereditary heart muscle and electrical conditions underpinning SCD but also notated some congenital conditions such as anomalous coronary arteries.

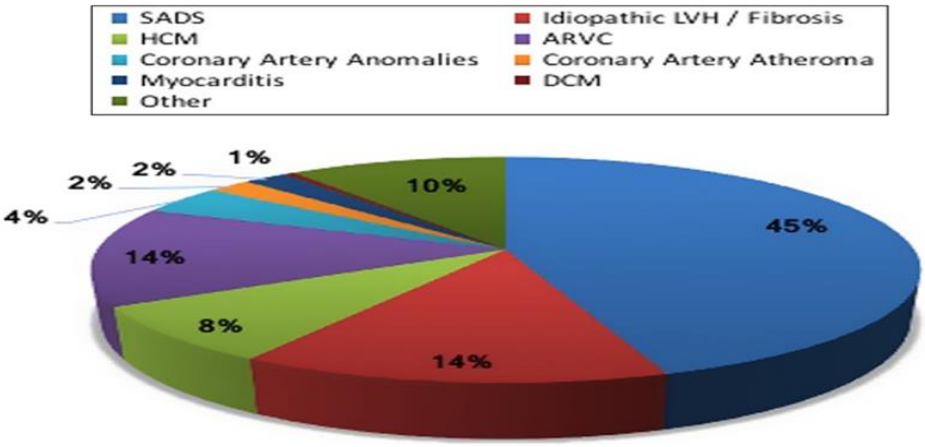


Figure 2.1: Causes of sudden cardiac death in the athletic population in UK registry of young athletes: sudden arrhythmic death syndrome (SADS), Hypertrophic cardiomyopathy (HCM), Left ventricular hypertrophy (LVH), Arrhythmogenic right ventricular cardiomyopathy (ARVC) and Dilated cardiomyopathy (DCM) (adapted from Oxborough et al., 2018)

2.1.1.1 Hypertrophic Cardiomyopathy (HCM)

HCM is an inherited genetic disorder of sarcomeric contractile proteins characterized by inexplicable left ventricular hypertrophy (LVH) and myofibre disarray (Wigle et al., 1995; Maron et al., 2000; Maron, 2002). HCM is a diverse genetic disease and, therefore, presents with a varied phenotypic expression including myocardial hypertrophy (symmetric, asymmetrical, apical), histopathological evidence of myocyte disarray (Varnava et al., 2001), anomalies of the intramyocardial small vessels (Maron et al., 1988), and fibrosis (Sutton et al., 1980; Ferrans et al., 1972). It is likely that this combination of phenotypical factors in HCM may predispose to cardiac arrhythmia during periods of stress, such as exercise, but the exact mechanism by which sarcomere mutations lead to the above-mentioned phenotypical appearance is still not completely understood (Keren et al., 2008). The prevalence of HCM is commonly reported as 1 in 500 persons (0.2%) which was originally based on the CARDIA (Coronary Artery Risk Development in Young Adults) cohort study that used standard echocardiography in 5115 unrelated people who ranged from 23 to 35 years of age (Maron et al., 1995). Prevalence data in different cohorts, countries and ethnicities have not been determined. HCM has been reported as the commonest cause of SCD in young athletes (Maron, 2002), accounting for one-third of fatal deaths (Maron 2002; Vakrou and Abraham, 2014). In contrast, however, in Veneto region of Italy, ARVC has been reported as the leading cause of SCD, possibly because of screening programs successfully detecting HCM (Corrado et al., 1998; Corrado et al., 2005).

Most cases of SCD attributed to HCM have been seen among athletes who were engaged in dynamic, intermittent sports such as basketball and football (Sheppard, 2012). For example, a well-documented case was the SCD of Mark Vivien Foe, an elite Cameroonian footballer,

which occurred during a televised international match and was attributed to ventricular fibrillation due to HCM.

Abnormalities on ECG are present in >90% of patients with HCM, although no specific ECG pattern is pathognomonic. Due to the diverse presentation of the disease, the detection of increased LV wall thickness, which is unexplained by loading conditions, should prompt a systematic search for its underlying cause. In many patients, this work-up should include specialized laboratory testing and, in some circumstances, genetic analysis.

2.1.1.2 Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)

ARVC is a genetic disease that affects desmosome function within the cardiomyocyte characterised by fibro-fatty replacement of myocardial tissue that can lead to ventricular arrhythmias (Basso et al., 1996). It is believed that fibro-fatty replacement disrupts normal electrical conduction, more commonly in the right ventricle, causing epsilon waves, right bundle branch block (RBBB), late potentials, and re-entrant ventricular arrhythmias which may predispose to fatal cardiac arrhythmias (Corrado et al., 1997; Basso and Thiene, 2005). Previous studies (Basso et al., 1996; Nava et al., 2000; Basso et al., 2009; Bauce et al., 2011) have determined that despite having a genetic base for the disease, there is no notable phenotypic expression at birth and ARVC is often diagnosed after maturation when the clinical signs are apparent. Cardiac arrest is often the first presenting manifestation (as with HCM). Sports participation has been shown to increase the risk of SCD by 5-fold in people with ARVC, since acute volume overload and stretching of the RV (Thiene et al., 2007) and sympathetic stimulation during exercise are major triggers of life-threatening arrhythmias (Corrado et al., 2003; Basso et al., 2007). In a 20-year prospective study of young people in the Veneto region

of Italy, ARVC presented in 22% of SCD cases in athletes and in 10% of SCD cases in non-athletes (Corrado et al., 2003).

The prevalence of ARVC is very difficult to determine because of the absence of symptoms in most cases and the late phenotypical presentation. This complexity has also meant there is no single gold standard test for ARVC. The current diagnosis of ARVC is based on the presence of major and minor characteristics set out in a report from a Task Force Criteria (TFC; Marcus et al., 2010). The TFC is a multivariate model that assesses data from ECG, echocardiography, cardiac MRI, and tissue characterisation. Based on TFC, it is required that 2 major criteria or 1 major criterion and 2 minor criteria or 4 minor criteria from different categories in order to give a definitive ARVC diagnosis (see Table 2.1).

Table 2.1: Modified Task Force Criteria 2010 (adapted from Marcus et al., 2010)

	I. Global or regional dysfunction and structural alterations	
	Major	**Minor**
Original task force criteria	• Severe dilatation and reduction of RV ejection fraction with no (or only mild) LV impairment • Localized RV aneurysms (akinetic or dyskinetic areas with diastolic bulging) • Severe segmental dilatation of the RV	• Mild global RV dilatation and/or ejection fraction reduction with normal LV • Mild segmental dilatation of the RV • Regional RV hypokinesia
Revised task force criteria	By 2D echo: • Regional RV akinesia, dyskinesia, or aneurysm • and 1 of the following (end diastole): • — PLAX RVOT ≥32 mm (corrected for body size [PLAX/BSA] ≥19 mm/m2) • — PSAX RVOT ≥36 mm (corrected for body size [PSAX/BSA] ≥21 mm/m2) • — or fractional area change ≤33% By MRI: • Regional RV akinesia or dyskinesia or dyssynchronous RV contraction • and 1 of the following: • — Ratio of RV end-diastolic volume to BSA ≥110 mL/m2 (male) or ≥100 mL/m2 (female)	By RV angiography: • Regional RV akinesia, dyskinesia, or aneurysm By 2D echo: • Regional RV akinesia or dyskinesia • *and* 1 of the following (end diastole): • — PLAX RVOT ≥29 to <32 mm (corrected for body size [PLAX/BSA] ≥16 to <19 mm/m^2) • — PSAX RVOT ≥32 to <36 mm (corrected for body size [PSAX/BSA] ≥18 to <21 mm/m^2) • — *or* fractional area change >33% to ≤40% By MRI: •Regional RV akinesia or dyskinesia or dyssynchronous RV contraction

	• — or RV ejection fraction ≤40%	•and 1 of the following: •—Ratio of RV end-diastolic volume to BSA ≥100 to <110 mL/m2 (male) or ≥90 to <100 mL/m2 (female) • — or RV ejection fraction >40% to ≤45%

	2. Tissue characterization of wall	
	Major	**Minor**
Original task force criteria	Fibrofatty replacement of myocardium on endomyocardial biopsy	
Revised task force criteria	• Residual myocytes <60% by morphometric analysis (or <50% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy	• Residual myocytes 60% to 75% by morphometric analysis (or 50% to 65% if estimated), with fibrous replacement of the RV free wall myocardium in ≥1 sample, with or without fatty replacement of tissue on endomyocardial biopsy
	3. Repolarization abnormalities	
Original task force criteria		Inverted T waves in right precordial leads (V2 and V3) (people age >12 years, in absence of right bundle-branch block)
Revised task force criteria	Inverted T waves in right precordial leads (V_1, V_2, and V_3) or beyond in individuals >14 years of age (in the absence of complete right bundle-branch block QRS ≥120 ms)	• Inverted T waves in leads V1 and V2 in individuals >14 years of age (in the absence of complete right bundle-branch block) or in V4, V5, or V6 • Inverted T waves in leads V1, V2, V3, and V4 in individuals >14 years of age in the presence of complete right bundle-branch block

	4. Depolarization/conduction abnormalities Major	
	Major	**Minor**
Original task force criteria	Epsilon waves or localized prolongation (>110 ms) of the QRS complex in right precordial leads (V_1 to V_3)	Late potentials (SAECG)
Revised task force criteria	Epsilon wave (reproducible low-amplitude signals between end of QRS complex to onset of the T wave) in the right precordial leads (V_1 to V_3)	• Late potentials by SAECG in ≥1 of 3 parameters in the absence of a QRS duration of ≥110 ms on the standard ECG • Filtered QRS duration (fQRS) ≥114 ms • Duration of terminal QRS <40 µV (low-amplitude signal duration) ≥38 ms • Root-mean-square voltage of terminal 40 ms ≤20 µV • Terminal activation duration of QRS ≥55 ms measured from the nadir of the S wave to the end of the QRS, including R', in V1, V2, or V3, in the absence of complete right bundle-branch block.
	5.Arrhythmias	
	Major	**Minor**
Original task force criteria		• Left bundle-branch block-type ventricular tachycardia (sustained and no sustained) (ECG, Holter, exercise)

		• Frequent ventricular extrasystoles (>1000 per 24 hours) (Holter)
Revised task force criteria	Non-sustained or sustained ventricular tachycardia of left bundle-branch morphology with superior axis (negative or indeterminate QRS in leads II, III, and aVF and positive in lead aVL)	Non-sustained or sustained ventricular tachycardia of RV outflow configuration, left bundle-branch block morphology with inferior axis (positive QRS in leads II, III, and aVF and negative in lead aVL) or of unknown axis • > 500 ventricular extrasystoles per 24 hours (Holter)

PLAX indicates parasternal long-axis view; RVOT, RV outflow tract; BSA, body surface area; PSAX, parasternal short-axis view; aVF, augmented voltage unipolar left foot lead; and aVL, augmented voltage unipolar left arm

2.1.1.3 Long QT syndrome

Long-QT syndrome (LQTS) is a genetically mediated condition that potentially predisposes to ventricular arrhythmias and has been associated with SCD without structural heart disease. The incidence of cases of SCD due to LQTS in young athletes is estimated to be from 0.5% to 8% (Puranik et al., 2005; Corrado et al., 2006). The prevalence of LQTS itself, has been estimated to be 1 in 2500 (Moss et al. 1991; Schwartz 1997) and is characterized by delayed repolarization of the myocardium and QT internal prolongation on the ECG (see Figure 2.2). LQTS was recognized, alongside the discovery of various attributable genetic mutations, in the early 1990s (Wang et al., 1996; Wang et al., 1995). Since this breakthrough, mutations in 3 genes (KCNQ1, KCNQ2 and SCN5A) comprise the majority of LQTS cases (80-90%) (Schwartz et al. 1975; Schwartz 1985; Napolitano et al., 2012).

The gene mutations in LQTS prolong the QT interval (>440 ms in men and >460 ms in women) possibly due to an excess of late inward sodium current or a reduced outward potassium current (Moss et al. 1991; Schwartz 1997; Roden 2008). Despite this data, there has been on-going discussion as to what absolute level of QT interval duration constitutes the upper limit of normal in healthy adults as well as elite sportspeople. In general, a heart rate corrected (Bazet; QTc) of >470 ms in males and >480 ms in females requires further investigation (Sharma et al., 2017). A patient with a resting QTc of >500 ms is generally considered at increased clinical risk for a significant arrhythmia (Pellicia et al., 2005).

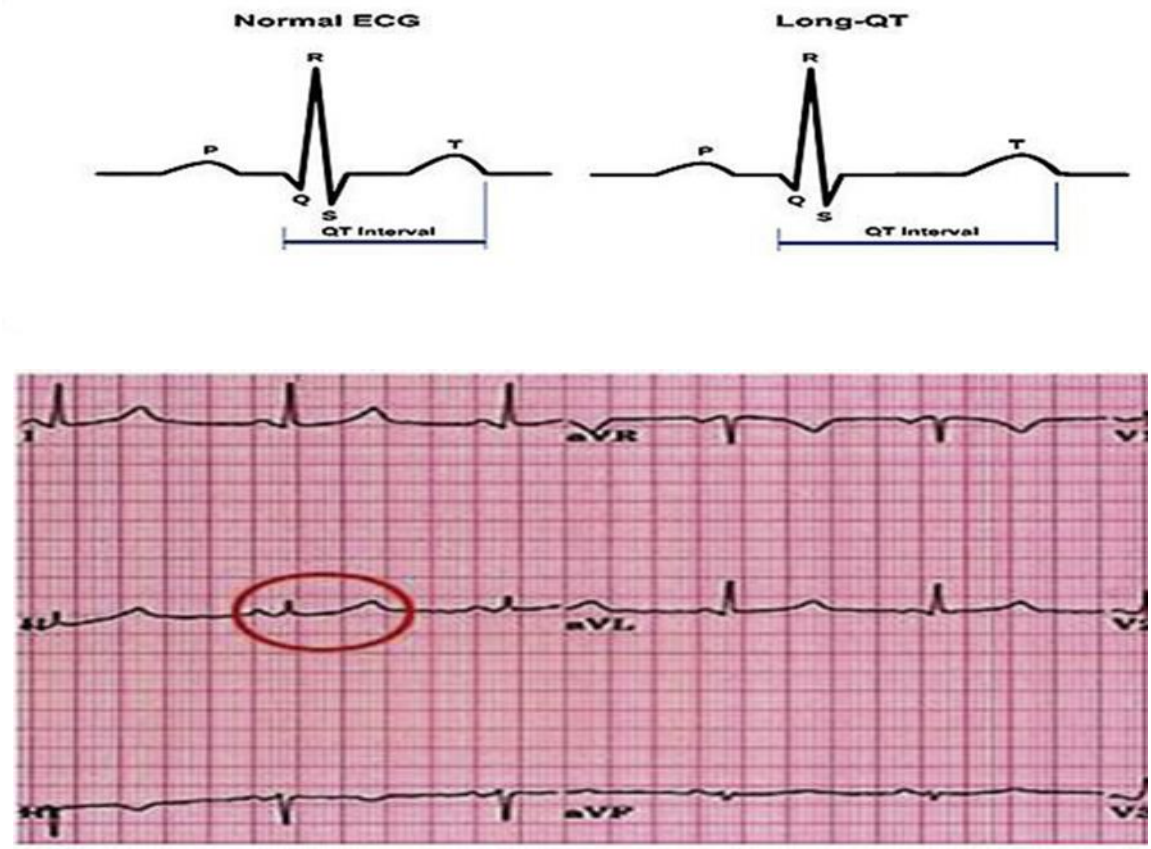


Figure 2.2: A. Normal and prolonged QT interval B. Prolonged QT on sample ECG.

2.1.1.4 Brugada syndrome

Brugada syndrome is characterised by right bundle branch block (RBBB), primarily in leads V1 through V3, with ST-segment elevation (Brugada and Brugada 1992; see Figure 2.3). Brugada syndrome can lead to ventricular fibrillation (VF) and SCD, typically occurring with mild activity or during sleep (Brugada and Brugada 1992; Brugada et al., 2003). Although the disease is recognized worldwide, the prevalence of events seems much higher during sleeping time in some areas, especially in Southeast Asia (Baron et al., 1983; Tungsanga and Sriboonlue, 1993; Nademanee et al., 1997). This ECG phenomenon was identified in the majority of sudden and unexpected death syndrome (SUDS) of patients in Thailand, with 17 out of 27 victims of either aborted sudden death or syncope having a dynamic pattern of ST segment elevation in

precordial leads $V_1$to V_3. Brugada syndrome in these cases was often accompanied by apparent conduction block in the right ventricle (Nademanee et al., 1997) and all were in young men with a strong family history of unexpected and unexplained death.

The diagnosis of patients with Brugada syndrome is complex and still being debated. Brugada and Brugada (1992) reported that the clinical presentation of Brugada syndrome is distinguished by a male predominance (8:1 ratio of male: female) and the appearance of arrhythmic events at an average age of 40 years (range: 1 to 77 years) (Brugada and Brugada 1992; Priori et al., 2000).

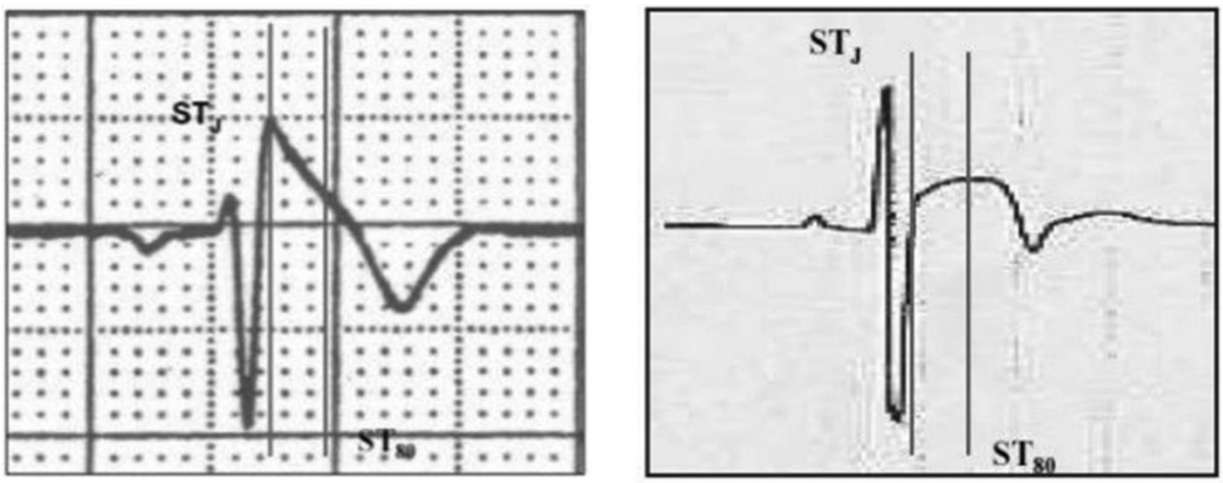


Figure 2.3: Brugada type 1 ECG (left) should be distinguished from early repolarisation with 'convex' ST segment elevation in a trained athlete (right). Vertical lines mark the J-point (STJ) and the point 80 ms after the J-point (ST80), where the amplitudes of the ST segment elevation are calculated. The 'downsloping' ST segment elevation in Brugada pattern is characterised by a STJ/ST80 ratio >1. Early repolarisation patterns in an athlete show an initial 'upsloping' ST segment elevation with STJ/ST80 ratio <1.

The prevalence of Brugada Syndrome remains to be fully determined. The frequency of incidents has ranged from 5 to 66 per 10 000 and a few candidate genes have been considered as plausible. So far, the syndrome has been linked to mutations in *SCN5A*, the gene encoding for the α and β subunits of the calcium channel (Brugada et al., 2003; Antzelevitch et al., 2007) and the gene that encodes glycerol-3-phosphate dehydrogenase 1-like enzyme (GPD1L) (London et al., 2007). Although a clear relation between exercise and SCD in Brugada Syndrome patients has not been fully recognized, the restriction of athletes from participation in sports with low static and dynamic intensity seems advisable (Zipes et al., 2005).

2.1.1.5 Wolf-Parkinson-White syndrome

Wolff–Parkinson–White syndrome (WPW) syndrome is a pre-excitation electrical disorder of the cardiac conduction system, where an abnormality of supplementary electrical pathways can lead to re-entrant supraventricular tachyarrhythmias, which may trigger VF (Sharma et al., 1997; Basilico 1999; see Figure 2.4). WPW syndrome can be inherited (Timmermans et al., 1995). Normally the atria and ventricle are isolated chambers with electrical conductance only linked through a single passage, via the AV node. A pre-excitation pattern is caused by an additional electrical connection (accessory pathway) between the atria and ventricles that predisposes to arrhythmia (Rao et al., 2013). This anomaly presents itself on the ECG as a shortened PR interval (<120 ms) (Surawicz et al., 2009) and a delta wave overlapping the initial portion of the QRS complex (Drezner et al., 2013). The prevalence of pre-excitation in athletes is estimated to be 0.1–0.3%, parallel to that of the general population (Timmermans et al., 1995). It is estimated that WPW occurs in approximately 1:1000 of the athletic population (Pelliccia et al., 2007), and has been shown to account for 1% of SCD (Maron et al., 2009)

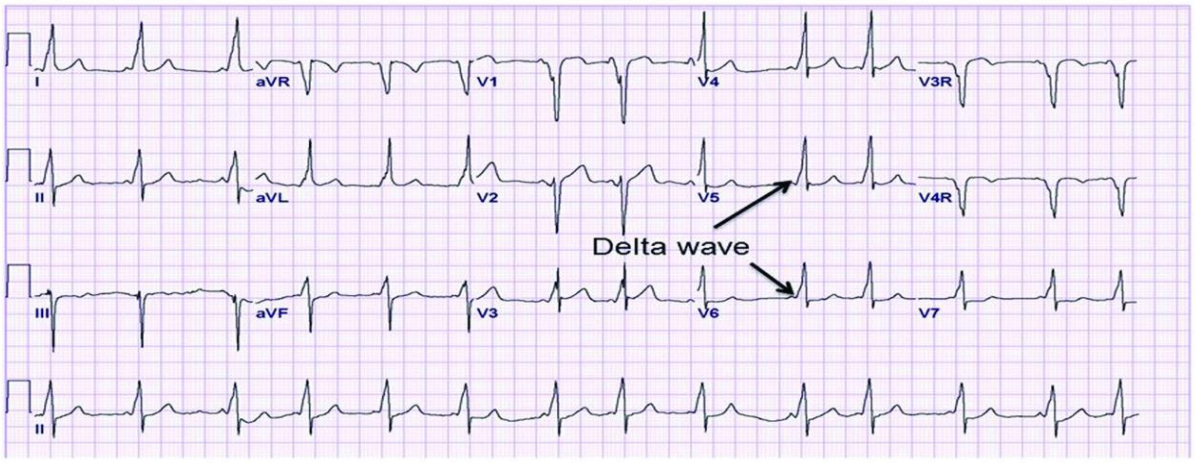


Figure 2.4: ECG demonstrating the classic findings of Wolf-Parkinson-White pattern with a short PR interval (<120 ms), delta wave (slurred QRS upstroke) and prolonged QRS (>120 ms). (Sharma et al., 2017)

As is obvious from the above example, the development of electrocardiogram (ECG) criteria (Sharma et al., 2017) in PPS, has contributed to a detailed understanding of cardiac electrical activity in highly trained athletes with a focus of reducing the false positive rates without compromising the sensitivity (Hyde et al., 2019).

2.2 Pre-participation screening (PPS)

2.2.1 History

The fact that SCD occurs at all in athletes, and that the diseases associated with a SCD risk can be identified and to some extent treated (or at least the risk reduced), underlines the role and potential importance of a systematic (PPS) scheme (Bohm et al., 2013). The American College of Cardiology (ACC), the American Heart Association (AHA) and the European Society of Cardiology (ESC) recommend screening for occult cardiovascular disease before athletic participation, but their screening protocols are not entirely aligned (Baggish and Thompson 2009). Since 1996, the proposal by AHA is a PPS that includes a focused personal history (assessed by means of a health questionnaire particularly concerned with cardiac symptoms and family history of cardiac disease) and a clinical examination (Maron et al., 2007). The AHA approach does not advocate the inclusion of a resting 12-lead ECG, reflecting that the cost effectiveness and high false-positive rate are the primary reasons for this viewpoint (Myerburg and Vetter, 2007).

Contrary to what happens in the USA and other countries, cardiac PPS in Italy has been mandatory for every athlete since 1982 (Pelliccia and Maron, 1995). It consists of a medical history, physical examination, spirometry, urine analysis plus basal and post-step test 12-lead ECG (Italian Ministry of Health, 1982). The addition of the ECG to the PPS protocol has increased both the sensitivity and specificity of the screening process (Corrado et al., 2004). It helps the identification of cardiomyopathies (identified as key pathologies responsible for an increased risk of SCD in young athletes in Section 2.1), in that 95% of individuals with HCM, and 80% of individuals with ARVC present with electrocardiographic abnormalities (Marcus, 2002; Maron 2002). A 12-lead ECG is also a powerful tool to identify ionchannelopathies

although the diagnosis can be challenging. Despite this background knowledge, there remains an on-going debate about if, and how, to perform PPS as screening does not pick up all hereditary and congenital CV diseases with 100% sensitivity and specificity. What remains is a mixed picture of PPS that ranges from legally mandated requirements in Italy (Corrado et al., 2006; Pelliccia et al., 2000), through limited technical approaches in the USA (Maron et al., 2007) to the UK where no state sponsored PPS exist and other organizations like Cardiac Risk in the Young fill the void and provide charity supported PPS (establishing a cardiac screening program for young individuals in 1997; Cardiac Risk in the Young, 2015). Layered on top of this national/international sports governing bodies, including IOC, FIFA, UEFA, and the FA, have mandated the application of athlete pre-participation screening (PPS) with electrocardiography (ECG) and echocardiography prior to national and international competitions (Kramer et al., 2015).

2.2.2 Efficacy

Accurate and evidence-based evaluation of a 12-lead ECG in any given athlete, and appropriate subsequent action, has the potential to increase efficacy, accuracy, and cost-effectiveness of PPS (Corrado et al., 2008; Corrado and Mckenna, 2007; Drezner, 2008; Myerburg and Vetter, 2007; Papadakis et al., 2008). According to McGrew (2003), the purpose of PPS is to provide medical clearance for participation in sport through routine systematic evaluations intended to identify pre-existing cardiovascular pathology or abnormality and thereby reduce the potential for adverse events and loss of life. It is clear from both case-series and case study data that the identification of cardiac disorders in an athlete can prevent SCD (Basavarajaiah et al., 2007), although there is still continuing debate on this issue as

running a randomized control trial is virtually impossible for practical and ethical reasons (George et al., 2012).

The efficacy of ECG as a diagnostic tool during PPS has been supported in one of the largest and most significant retrospective studies to date (Corrado et al., 2006). This study in Italy, where PPS is legally mandated, demonstrated that the annual incidence of SCD in young (12-35 years) competitive athletes decreased by 89% in Veneto region of Italy from 1979 to 2004 after the introduction of PPS. Specifically, SCD prevalence was 3.6 per 100000 athletes per year during the pre-PPS period (before 1979). Once PPS, with ECG, was started there was a slow decline in SCD prevalence year-on-year to a low of 0.4 per 100000 athletes per year in 2004. Over the same period the incidence of SCD among the unscreened non-athletic population did not change significantly at around 1.0 per 100000 per year (see Figure 2.5)

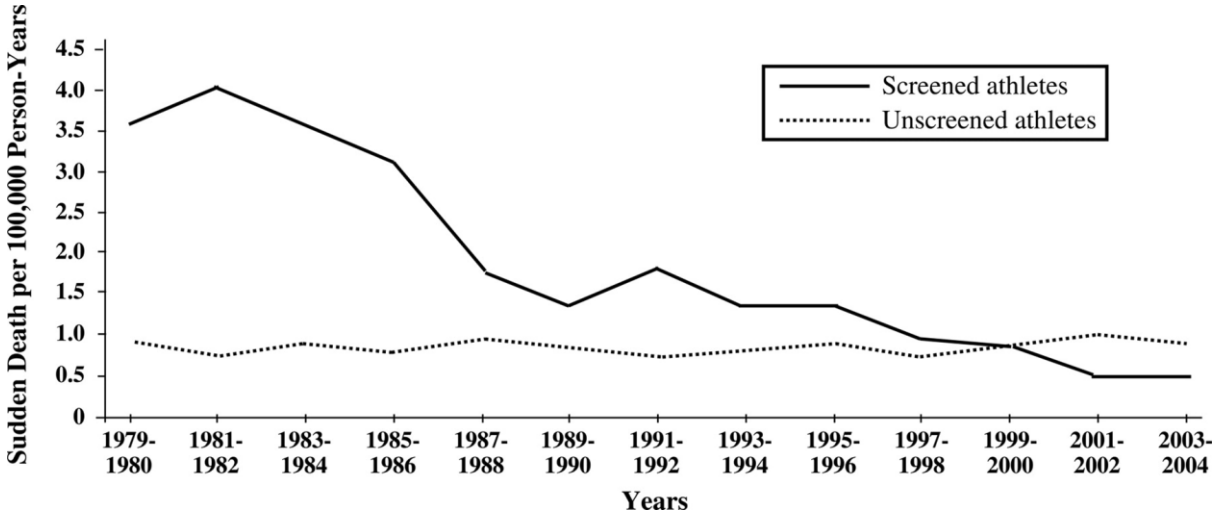


Figure 2.5: Annual Incidence Rates of Sudden Cardiovascular Death in Screened Competitive Athletes and Unscreened Non-athletes Aged 12 to 35 Years in the Veneto Region of Italy (1979-2004) (adopted from Corrado et al., 2006).

Corrado et al., (1998; 2006) confirmed the efficacy of ECG in their PPS for identifying cases of HCM and postulated this helped reduce the prevalence of SCD. This also saw HCM replaced by ARVC as the most common cause of SCD in young athletes. During the Italian study 879 athletes (2.0%) were disqualified from competition due to cardiovascular causes; 455 (1.04%) in the early screening period and 424 (0.96%) in the late screening period. The proportion of athletes who were disqualified for cardiomyopathies increased from 20 (4.4%) of 455 in the early screening period to 40 (9.4%) of 424 in the late screening period ($p = 0.005$). Interestingly, no deaths occurred during long-term follow-up among athletes with HCM who were disqualified from competitive sports, suggesting that screening may have prevented significant SCD related loss of life (Wasfy et al., 2016). The strength and impact of the Italian data led to the International Olympic Committee (IOC) recommending that ECG be included in the PPS of Olympic athletes in 2004 (Lausanne Recommendations, 2004). The US Olympic Committee continue to follow the 1996/2007 AHA PPS recommendations (Maron et al., 1996; Maron et al., 2007) which does not include routine ECG screening.

Other studies provide both supporting and contrasting data to the Italian model. A range of studies that have screened athletes from various sporting disciplines have confirmed the efficacy of resting 12-lead ECG in addition to personal/family history and physical examination in identifying disease and SCD risk. Specifically, this was the case within a cohort elite junior athletes and physically active schoolchildren (Wilson et al., 2007). This study demonstrated that 12-lead ECG was essential when screening for diseases that have potential to cause SCD, as the personal symptoms/family history data alone were inadequate in disease identification.

In 1997, a mandatory PPS program was implemented in Israel (Israel Sport Law 5748-1988 and The Sport Medical Tests Regulations 5757-1997). The obligatory screening, mandated PPS for all athletes, that included a medical questionnaire, physical examination, resting ECG and Bruce-protocol exercise testing on a yearly basis (Israel Ministry of Health Athlete Pre-Participation Medical Screening Guidelines: Ministry of Health website). Only certified physicians who had participated in a specialized accreditation course could perform this screening. A retrospective observational study of SCD from 1985 to 2009, determined if ECG mandated in the PPS resulted in fewer SCD. Mandatory ECG screening of athletes had no apparent effect on the risk for cardiac arrest (Steinvil et al., 2011). However, it is fair to suggest that certain methodological limitations could hinder the apparent strength of their conclusions; for example, the number of SCD was derived only from 2 Israel newspaper articles and not from a national (prospective) registry. Primarily, both the number of cardiac events and the population of competitive athletes at risk were only roughly estimated.

In 1996, the AHA recommended a 12-element preparticipation screening history and physical exam for high school athletes (Maron et al., 1996). An update in 2007 recommended no substantive changes but raised concerns regarding adding an ECG to the screening process, and did not recommend its use (Maron et al., 2007). The AHA continues to support a less specific method of screening and could ultimately endanger athletes at risk of SCD (Wilson et al., 2008). It is interesting to note that many professional sports in the USA do indeed use an ECG in PPS. In summary, no study monitoring SCD has shown that PPS via personal symptom and family history questionnaire and physical examination alone can detect the majority of athletes at risk of SCD (Drezner and Khan, 2008).

2.2.3 Current guidelines and Consensus

After consideration of the available evidence around the globe, in 2004 and 2005, the IOC Medical Commission and ESC, respectively, recommended a screening program for young athletes based on the 12-lead ECG in addition to the physical exam (Corrado et al., 2005; Bille et al., 2006).

2.2.3.1 ECG Criteria

Despite the adoption of ECG in PPS in some places, there has been widespread concern about the false positive rates, largely because of the broad spectrum of physiological ECG adaptations to exercise training that mirror those observed in cardiovascular pathologies. To provide clear professional guidelines for ECG interpretation in athletes, the 2010 European Society of Cardiology (ESC) recommendations for "ECG interpretation in Athletes" (see Table 2.2) attempted to facilitate the differentiation between physiological ECG patterns (likely training-related; Group 1) and those indicative of cardiac disease (likely non-training related; Group 2) (Corrado et al., 2010). This sought to make differential diagnosis much simpler and systematic.

Table 2.2: ECG Criteria in Athletes

Group 1: Common training-related ECG changes	**Group 2: Uncommon likely not training-related ECG changes**
Sinus bradycardia	T-wave inversion
First-degree Av block	ST-segment depression
Incomplete RBBB	Pathological Q-waves
Early repolarization	Left atrial enlargement
Isolated QRS voltage criteria for left ventricular hypertrophy	Left-axis deviation/left anterior hemiblock
	Right-axis deviation/left posterior hemiblock
	Right ventricular hypertrophy
	Ventricular pre-excitation
	Complete LBBB or RBBB
	Long or short-QT interval
	Brugada-like early repolarization

European Society of Cardiology recommendations (Corrado et al; 2010)

Although the IOC (Ljungqvist et al., 2009) endorsed the ESC ECG criteria in their PPS program, high false positive rates continued to be reported with a worry over subsequent costs (and psychological stress) generated by additional investigations to confirm or refute cardiac disease (Corrado et al., 2011; Papadakis and Sharma, 2009). The 2010 ESC recommendations for ECG interpretation in athletes have been associated with a false positive rate ranging from 9% to 22% (Corrado et al., 2006; Corrado et al., 2010; Brosnan et al., 2013; Sheikh et al., 2014) and as a consequence athlete PPS ECG criterion have continued to be revisited and updated.

The Seattle criteria were the result of consensus reached among a group of experts in cardiology and sports medicine who gathered for the first time in February 2015 in Seattle USA (see Table 2.3). The Seattle Criteria significantly improved the specificity of ECG screening criteria by having fewer abnormal variants than ESC criteria, including benign repolarization anomalies associated with black ethnicity and giving less conservative limits for an abnormal QT interval (Drezner et al., 2013; Sheikh et al., 2014). Despite these alterations there was no change in sensitivity of the criteria (Wilson et al., 2008).

Table 2.3: Seattle Criteria for Abnormal ECG findings in athletes that would suggest further investigation.

Abnormal Finding
T wave inversion >1 mm in 2 or more leads
ST-segment depression
Complete Left bundle brunch block (LBBB)
Intraventricular conduction delay at any QRS >140 ms
Left axis deviation -30° to -90°
Left atrial enlargement

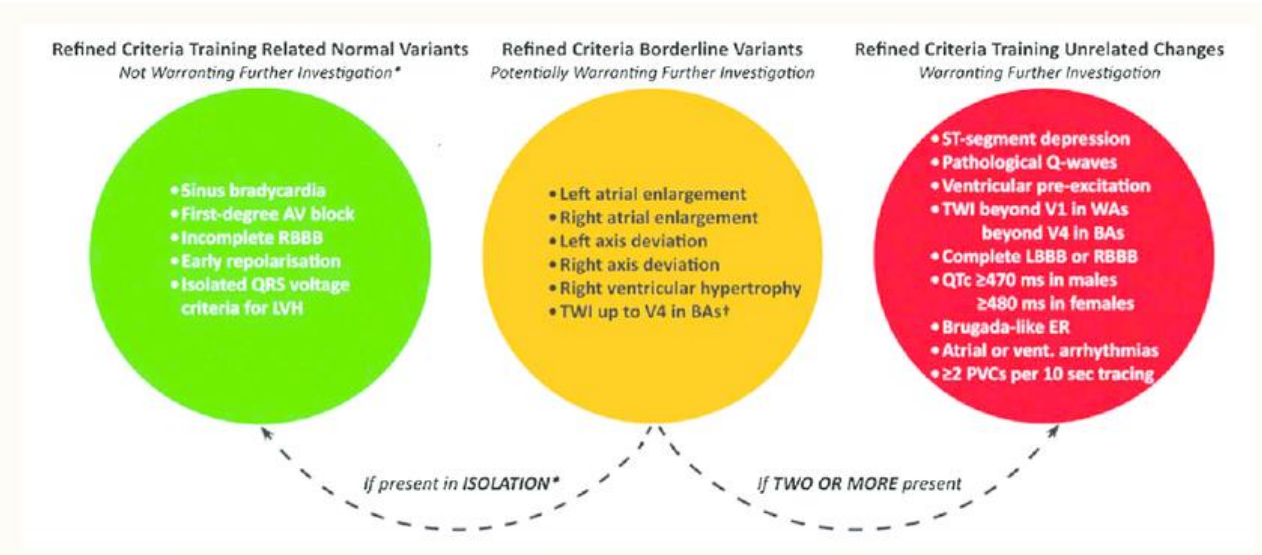
Right ventricular hypertrophy pattern
Right atrial enlargement
Ventricular pre-excitation
Long QT interval QTc >470 ms (male)/ QTc > 480 ms (female) QTc
Short QT interval QTc <320 ms
Brugada-like ECG pattern
Atrial tachyarrhythmias
Premature ventricular contractions
Ventricular arrhythmias

WA – "white" athletes; BA – "black" athletes (Drezner et al., 2013)

Sheikh et al. (2014) published a new 'Refined Criteria' for ECG interpretation, based upon their experience of screening 4297 white and 1208 black elite athletes, and 103 athletes diagnosed with HCM using both the ESC recommendations and the Seattle Criteria. The findings of this study demonstrated that the "Refined Criteria" had a lower false-positive rate in both white and black athletes (6.1% and 15.8%) as compared to the ESC criteria (26.5% and 59.9%) and Seattle criteria (7.9% and 20.7%) (Sheikh et al., 2014). The Refined criteria are described in Figure 2.6. Borderline findings (yellow) do not constitute findings that require further follow-up if found in isolation and include 1) left atrial enlargement (LAE), 2) right atrial enlargement (RAE), 3) left axis deviation (LAD), 4) right axis deviation (RAD) and 5) Sokolow-Lyon voltage criteria for right ventricular hypertrophy (RVH) (Sheikh et al., 2014). In line with the Seattle Criteria, a corrected (Bazett‟s formula) QT interval (QTc) ≥470 ms in males and ≥480 ms in females, and T-wave inversion preceded by convex ST-segment elevation in leads V1-V4 in asymptomatic Black athletes do not require further investigation. Riding et al., (2014) compared the 3 ECG criteria in a large cohort (2491) of male Arabic, Black

and Caucasian athletes undergoing PPS. Application of the ESC, Seattle and Refined criteria led to abnormal ECG rates of 22.3%, 11.6% and 5.3%, respectively, all with 100% sensitivity for the pathological conditions detected when undertaking PPS. Both Riding et al., (2014) and Sheikh et al., (2014) demonstrated a lower false positive rate with the Refined Criteria compared to ESC and Seattle without losing diagnostic specificity.

Figure 2.6: The "Refined" ECG Criteria for athlete PPS (Sheikh et al., 2014).

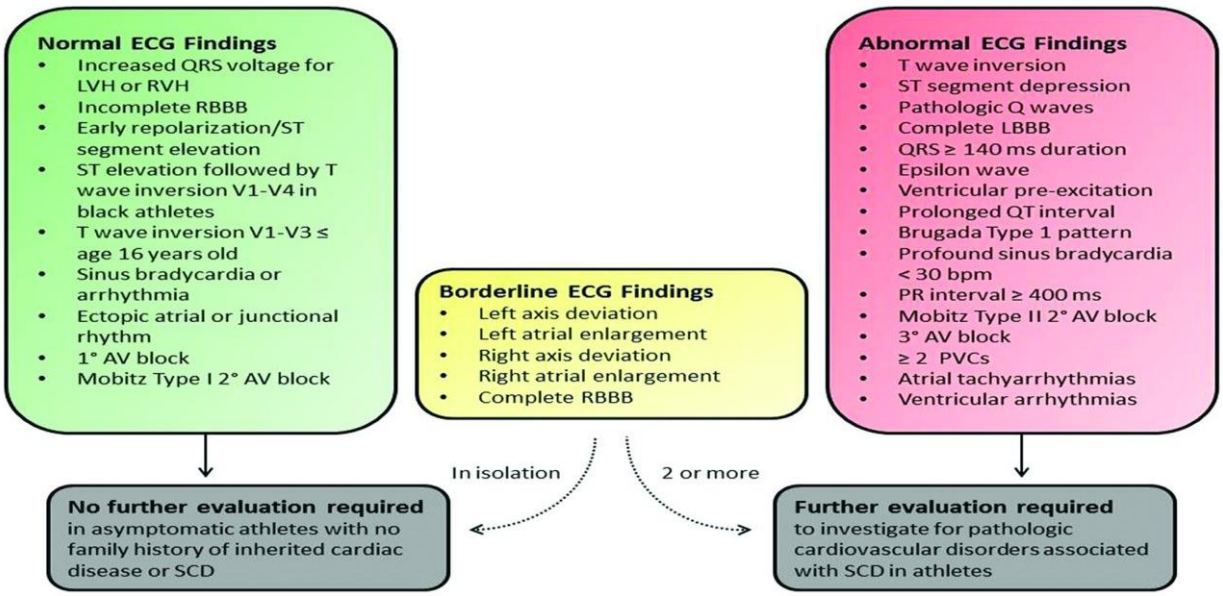


International Consensus Standards for Electrocardiographic Interpretation in Athletes AV-atrioventricular block; LBBB - left bundle branch block; LVH - left ventricular hypertrophy; RBBB - right bundle branch block; PVC - premature ventricular contraction.

In 2017, the International Criteria adopted the "Refined Criteria" and was released by the same expert panel used for the Seattle Criteria with a goal to further improve accuracy based on new and emerging research (Drezner et al., 2017; Sharma et al., 2017) and to surpass preceding guidelines as the current standard for ECG interpretation in athletes. In developing this progressive improvement in ECG criteria, the ECG of athletes of different ethnicity,

gender and intensity of sports participation have been of great value (Drezner et al., 2017). This contemporary revision of ECG criteria has enhanced specificity without compromising sensitivity for the ECG to detect pathological conditions associated with SCD (Zorzi et al., 2018; Hyde et al., 2019). The updated criteria (see Figure 2.7) further define and provides a clear link between specific ECG abnormalities and the recommended next steps for secondary testing (Hyde et al., 2019). In a cohort study (Malhotra et al., 2020) of 11168 adolescent soccer players from the UK, the total number of athletes with an abnormal ECG was reduced 57% by moving from the Seattle Criteria (4.3%) to the International Criteria (1.8%). The International Criteria for ECG interpretation improved specificity and significantly reduced the total false-positive ECG rates compared to the Seattle Criteria without compromising sensitivity (Hyde et al., 2019).

Figure 2.7: International guidelines for electrocardiographic interpretation in athletes (Sharma et al., 2017).



International Consensus Standards for Electrocardiographic Interpretation in Athletes AV-atrioventricular block; LBBB - left bundle branch block; LVH - left ventricular hypertrophy;

RBBB - right bundle branch block; RVH - right ventricular hypertrophy; PVC - premature ventricular contraction; SCD - sudden cardiac death.

2.2.3.2 Sex-based differences in PPS

Sex-based differences in the electrical activity of the heart have been noted for nearly 100 years, for example, women tend to have longer QT intervals than men (Bazett, 1920). In large heterogenous male and female non-athletic populations (with no overt cardiovascular disease) Mason et al., (2007) (n=80,000) and Ramirez et al., (2011) (n=30,000), found no significant differences were observed in heart rate, QRS-axis, QRS duration, QTc interval and PR interval between men and women. Mandic et al., (2009) assessed the effect of sex on the ECG of 658 college athletes (54% male athletes) and noted a larger QRS duration, PR interval, Q wave duration and J point amplitude in male athletes, while female athletes had higher QTc intervals. Although women have a, potentially proarrhythmic, longer QTc than men, women are less likely than men to suffer SCD in their reproductive years (Kannel et a., 1998). Wasfy et al., (2015), in a small cohort of 330 competitive rowers (56% male), observed that the ECG in male athletes had more frequent early repolarisation patterns and more isolated QRS voltage criteria for LVH compared to female athletes. They also noted a similar prevalence of sinus bradycardia and incomplete RBBB pattern, which has implications for PPS. A recent study by Bessem et al., (2017) in 1436 athletes (72% were male) noted that male athletes had significantly more sinus bradycardia, longer QRS duration, and more isolated QRS voltage criteria for LVH, whereas female athletes had a significantly higher resting heart rate. Corici et al., (2018) added that sinus arrhythmia was more common in female athletes.

Although there have been studies that have described the ECG in elite male footballers (Somauroo et al., 2001; Malhotra et al., 2018), there are very few data available that describes the ECG of elite female footballers, even though elite female footballers must undergo PPS. Recent data from Churchill et al., (2020) reported that normal training-related ECG changes are highly prevalent in USA male football players but are less common among female athletes. This study noted that female footballers had a shorter PR intervals (154[149-159] ms) compared with male footballer's PR intervals (166[162-171] ms; P<0.01), but longer corrected QT intervals (415[411-419] ms) vs (403[399-407] ms; P<0.01). The limited dataset presented in female footballers drives the rationale for the study of the ECG phenotype in elite female footballers and the impact of different criteria for ECG interpretation in athlete screening. Understanding the normal limits of the ECG in elite female footballers is vital since FIFA/UEFA have mandated screening in elite female footballers using the ESC model (screening health questionnaire, a 12-lead ECG, and an echocardiogram). Ultimately, the results will facilitate cardiologists in the interpretation of ECG in female footballers. How this data impacts upon PPS in female footballers and whether it leads to an increase in specificity and a reduced false positive rate requires further investigation.

A final point regarding PPS is the varied use of cardiac imaging to directly estimate structure and function of the heart. This is relevant to the study of the AH and to rationale for the descriptive study of cardiac structure and function in Chapter 5 of this thesis. Different countries, often within Europe, apply different regulations regarding the methods of PPS. There are several sporting organisations that advocate echocardiography as a primary investigation (Corrado et al., 2011; Mont et al., 2016). Specific sporting/screening

organisation will decide whether athletes require a routine transthoracic echocardiogram (TTE) as a first line investigation within the standard screening protocol (Mont et al., 2016; Oxborough et al., 2018). The utilisation of the echocardiogram within PPS is challenged, predominantly because of the increased cost and the need for qualified personnel to conduct the test. Nevertheless, FIFA is one of the sporting bodies, which mandates all players competing in one of their affiliated competitions to undergo screening with an echocardiogram (Dvorak et al., 2009). This, obviously, includes female footballers. The reasoning for this was to target their pre-competition medical assessment (PCMA) to be all encompassing, achieving maximal probability to detect SCD risk factors. Citing a low pre-test probability of exercise induced myocardial ischemia, FIFA also advocated the use of ECG exercise testing, via bicycle ergometer; to establish a truly comprehensive screening process. Regardless of this approach, a zero risk at PPS is likely unattainable. Indeed, following the initial application of these tests at the 2006 FIFA World Cup, the use of the ECG exercise test was questioned and subsequently removed from the screening protocol of the authors (Thünenkötter et al., 2009). Echocardiography does remain as a mandatory inclusion in FIFA PCMA screening.

2.3 Female Football

2.3.1 History and Development

Female participation in Olympic Games sport has increased over time, and women now participate in almost equal numbers as men, including team sports like football (International Olympic Committee, 2016). The first recorded UK women's football match took place in Scotland at the end of nineteenth century, and in 1894, the British Ladies Football league was

founded (Williams and Woodhouse, 1991). Female football then became popular 20 years later during World War I, when games were organised by factory workers in England to raise money for charity (Williamson, 1991). Historically, however, the movement of women and girls into this traditionally male-dominated sport has been challenging, and discourses surrounding the sport, particularly in the UK, are powerful in reproducing a male-preserve (Mangan, 1987; Williamson, 1991; Brus and Trangbaek, 2004).

In 2003, football surpassed netball as the most popular female team sport in the UK for girls with recent estimations suggesting that 1.6 million play regularly (Randhawa, 2003). Today, female football is one of the fastest growing team sports globally, with 30 million participants (FIFA, 2014). The number of participants in FIFA Women's World Cup qualifiers has almost tripled from 45 teams in 1991 to 128 in 2015, while the number of qualification matches has risen from 110 to 398 (FIFA, 2013). With the support of its members, FIFA aspired to see the number of female players increase to 45 million by 2019, when France hosted the eighth edition of the FIFA Women's World Cup. Although the popularity of female football is increasing (Olson, 2008) there still exists a large gap in the volume of medical and physiological research supporting female football players (Milanović et al., 2017). Because of the limited research in female football, the female player, and their needs, remains less well understood than the male counterpart.

2.3.2 Physiological profile of the female footballer

The general physical characteristics of female football players have been described in a small number of studies over the past 20-30 years as the popularity of the sport has grown (Tumilty and Darby 1992; Davis and Brewer 1993; Tamer et al., 1997; Todd et al., 2002; Stølen et al.,

2005). This remains at a fraction of the level of research into male footballers. Studies of female footballers have demonstrated that average maximal oxygen uptake (VO_{2max}: 47-58 $mL.min^{-1}.kg^{-1}$), vertical jump performance (31-44 cm) and 20-m sprint time (3.00-3.31 s) vary among players due to position of play and level of competition (Tumilty and Darby, 1992; Siegler et al., 2003; Polman et al., 2004; Krustrup et al., 2005; Stølen et al., 2005; Mohr et al., 2008). Matković et al., (2003) advocated that care is required when making direct comparisons between male and female players, as males tend to have a higher ratio of lean body mass to body fat than female players. Present research considering morphological characteristics of female football players (Can et al., 2004; Ingebrigtsen et al., 2011; Milanovic et al., 2012) concluded that elite female football players were of average height (160 to 169 cm) and weight (52 to 65 kg), which did not differ from the unselected population of healthy women.

Data on physiological and performance parameters associated with game play is growing but in some cases contradictory. Mean heart rate (HR), blood lactate levels and distance covered during game play in elite female players are like those described in males (Reilly and Thomas 1979; Bangsbo et al., 1991; Reilly et al., 1990). Other research has suggested that competitive female matches were categorised by less distance covered (nearly 33% less), although at higher intensity levels (maximum speeds greater than 15 km/h) than typically found in the male game (Krustrup et al., 2005). Some studies (Andersson et al., 2010; Krustrup et al., 2005; Mohr et al., 2008) have reported that female football players typically cover 9000-11000 m during a match. Gabbett and Mulvey (2008) suggested that the level of competition influences this value in female matches and suggested that the training exposure in elite women's football is similar to that in men's football.

2.4 The female athletes' heart

As well as performance and skeletal muscle adaptation to training in elite footballers there are well-recognised electrical, morphological, and functional changes in the heart, referred to as the AH (George et al., 2011; George et al., 2012). Work related to the cardiovascular consequences of female football training and game play (independently or in comparison to men) is limited but if the sport/work exposure of the male and female players is similar, you may expect a comparable qualitative and quantitative cardiovascular adaptation to training in male and female footballers. This would suggest that a female AH should be observed as frequently in elite female footballers compared to male footballers. Understanding the physiological adaptation of the AH has become an important part of monitoring player health and is required to support PPS in females as well as males (George et al., 2011).

There remains a need to describe in detail the AH in elite female athletes, and footballers specifically, that will allow differentiation from pathological adaptation that can predispose to SCD events (Finocchiaro and Sharma, 2016). A recent meta-analysis has pointed to a limited evidence base regarding the female AH in a broad range of sports, including football (Utomi, et al., 2013). The FA report in 2004 stated that 2.6 million women aged 16 and over play football in England. This means the imperative to develop this scientific information is clear. It also means that the absolute number of SCD cases related to football participation is expected to be higher as compared to less popular sporting disciplines (Corrado et al., 2003; Shurlock, 2009) as despite a lower prevalence of SCD in females they are not immune from such events (Corrado et al., 2003). Consequently, understanding the upper normal limits of the female AH in elite footballers is important to aid PPS and the differential diagnosis of

physiological or pathological adaptation. It is pertinent to describe the cardiovascular phenotype associated with the AH in elite athletes, including female footballers.

Although research over the last 50 years has extensively described the phenotype (electrical conduction, structure and function) of the male AH, limited data is available for the female AH (Pelliccia et al., 1995). Some female athletes have been included in largely male populations, but data has rarely been sex disaggregated (Fagard, 2003). Here, we will document the available evidence in relation to the female AH that can be broken down into an electrical (ECG) focus or a structural/functional (largely echocardiography) focus before a specific focus on the AH in elite female footballers.

2.4.1 The Electrical (ECG) Phenotype in the Female AH

As discussed to some extent in the previous PPS sub-section, ECG modifications in athletes due to long-term physical trainings are common and are considered as a fundamental component of the AH (Sharma et al., 2002; Fagard, 2003; Barbier et al., 2006; Maron and Pelliccia, 2006). In general, long term high-intensity endurance exercise is associated with ECG alterations including resting bradycardia, repolarisation changes and increased voltages suggestive of left ventricular hypertrophy (LVH) (Pluim et al., 2000; Pelliccia et al., 2002; Whyte et al., 2004; Pelliccia et al., 2008).

Latest research from the USA (Churchill et al., 2020), detailed normal training–related and some limited abnormal ECG data in male and female footballers, using the International Recommendation (Sharma et al., 2017). They demonstrated that female footballers (n=122) were more likely to have abnormal ECG patterns (14 of 122[11%]) compared to male football

players (0 of 116; P<0.01). It is largely accounted for by abnormal T-wave inversions (9 of 14 [69%]), and additional abnormal ECG findings included septal Q waves (2 of 14 [14.3%]), inferior ST-segment depressions (1 of 14 [7.1%]), and a mildly prolonged corrected QT interval (481 ms; 1 of 14 [7.1%]).

It is crucial to have a substantial database of the ECG phenotype in elite female footballers, as well as assessing these data against various international criteria. This information could be used by clinicians, physicians, sporting organizations and allied health professionals who work with elite professional footballers.

2.4.2 The Cardiac Structural and Functional Phenotype in the Female AH

2.4.2.1 Left ventricle (LV) structure and function

A large body of evidence suggests a range of structural and functional characteristics of the AH that are mediated by multiple factors in any individual (Brown et al., 2017). Two key elements that influence or mediate the appearance of the AH are sex and sporting (training) discipline (Brown et al. 2017). This does suggest that population-specific data in elite female footballers is both needed and potentially different from males and females engaging in other sports.

Initially it is valuable to look at the broader AH literature before focussing on an elite female footballer population. Athletes participating in cycling, rowing, canoeing, and swimming are associated with the biggest left ventricular (LV) cavity size and mass in both male and female athletes (Pelliccia et al., 1991; Gajda et al., 2019), with bigger absolute data in the male athletes. Data that directly compares LV structure and function in elite male and female

athletes is rare, but Pelliccia et al., (1996) observed 23% lower LV wall thickness (WT) and 11% smaller LV cavity size in female compared to male athletes with similar age and training intensity. In this study, no female had an LVWT >12 mm. Sharma et al., (2002) reported similar findings in adolescent athletes (11% lower LVWT and 6% smaller LV size in female athletes). Almost 5% of male athletes had an LVWT ≥12 mm, but no females had an LVWT >11 mm. Again, consistent with earlier work, recent data from Finocchiaro et al., (2017) noted that no female athlete had an LVWT >12 mm (vs. 2.5% of males) and comparatively few females (7%) had an LV end-diastolic dimension >54 mm (vs. 47% of males). Despite AH parameters being quantitatively smaller in female, other evidence suggests that female athletes present with an enlarged LV cavity dimension (average, +6%), increased WT (average, +14%), larger relative WT (average, +9%), and elevated LV mass normalized to body size (average, +25%) compared to sedentary female controls (Fagard, 2003), documenting that a female AH does exist. In summary, data suggests similar qualitative changes in LV structure and function with athletic training in male and females' athletes, but that a quantitative sex-differences may still exist (George et al., 2011; Oxborough et al., 2012).

Data pertaining to LV structure in female footballers is limited. Randers et al., (2013) examined the intermittent exercise performance and cardiovascular health profile in elite female football players in comparison to untrained young women. They observed that cardiac ventricular dimensions were larger in female footballers compared to untrained women, as shown by a higher left ventricular diastolic diameter (51 ± 3 vs. 45 ± 4 mm; $P < 0.001$). Churchill et al., (2020) reported limited pathologic findings, meeting the criteria for sport restriction or further investigation, but noted some individual data that exceeded normal ranges for structural cardiac parameters (1 athlete had an LV-end- diastolic dimension more than 60 mm; 61 mm), suggestive of exercise-induced remodelling.

Mean wall thickness was greater in male athletes compared to female football players (10.0 [9.8-10.3] vs 8.9 [8.7-9.1] mm; P < .001).

George et al., (1999) pointed out that although LV mass data are larger in female athletes than controls, resting diastolic and systolic functional indices (apart from stroke volume) were within normal limits in female athletes and not different to controls. Randers et al., (2013) reported that left ventricular diastolic function was better in elite female footballers compared to untrained women, as measured by the E/A ratio (2.4 ± 0.6 vs. 1.7 ± 0.4; P < 0.001; d = 1.25) and by a lower A (0.43 ± 0.09 vs. 0.53 ± 0.15 m · s^{-1}; P = 0.026; d = 0.87). They summarised that elite female footballers have a superior cardiovascular health profile compared to untrained controls. Again, Churchill et at., (2020) reported the E/A ratio was higher in female footballers (2.5[1.3-5.6] vs 2.3[1.3-6.0]; P<0.001) than their male counterparts, but no comparison was made to untrained controls.

2.4.2.2 Right ventricle (RV) structure and function

Zaidi et al., (2013) demonstrated that 61% of male and 46% of female athletes exhibited RV dimensions that fulfil the minor diagnostic criterion for ARVC. Assessment of the RV is, therefore, a vital component of the PPS process in athletes, as it is vital to differentiate the AH from ARVC (La Gerche et al., 2010; see Table 2.1; Marcus et al., 2010). Randers et al., (2013) reported that RV diastolic diameter was larger in elite female footballers compared to untrained women (31 ± 5 vs. 26 ± 5 mm; P = 0.010) and RV systolic function, determined by TAPSE, was higher in elite female footballers than untrained women (28 ± 5 vs. 22 ± 3 mm; P < 0.001). Interestingly, increased RV basal diameter data are less common in female

footballers than male footballers (16 of 118 [14%] vs. (60 of 112 [54%]; P<0.001) (Churchill et al., 2020,).

At present, there are limited data defining the normal RV echocardiogram in elite female footballers, and more data is needed in extended echocardiographic modalities as well as focussed data for the left and right atria.

2.5 Menstrual cycle

Current PPS directives in female athletes takes no account of the menstrual cycle phase or health (or oral contraceptive use) when planning and timetabling events. This may be a relevant consideration given the cardiovascular impacts of female sex-steroid hormone levels, that vary throughout the menstrual cycle. Consequently, we briefly review basic menstrual cycle physiology here and assess the rationale for a study of cardiac electrical activity, structure and function that may vary with a change in menstrual cycle phase.

2.5.1 Basic regulatory physiology of the menstrual cycle

The physiology of the menstrual cycle is a complex, coordinated sequence of events involving the hypothalamus, anterior pituitary, ovaries, and endometrium (Janse de Jonge, 2003; Martin and Elliott-Sale, 2016). Hormones are secreted in a negative and positive feedback manner to control the menstrual cycle. Figure 2.8 gives a general overview of the key regulatory factors in the menstrual cycle.

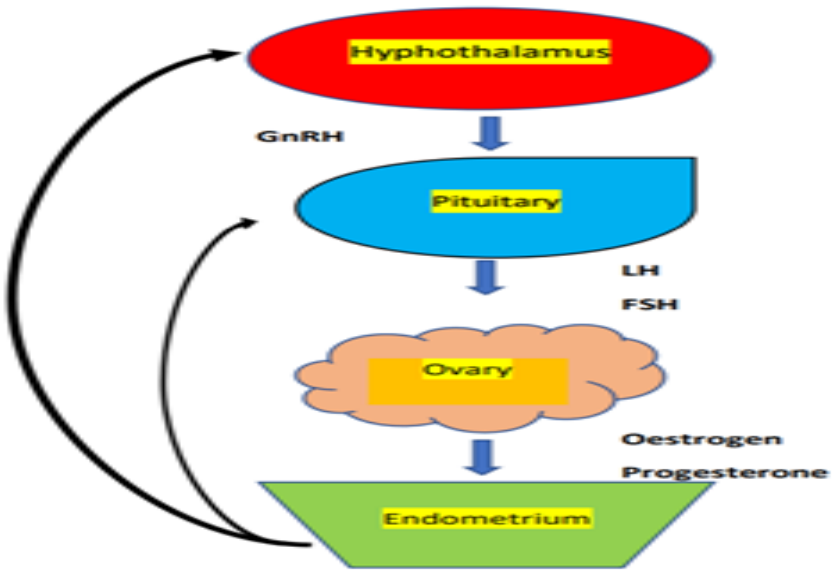


Figure 2.8: Schematic representation of the hypothalamus-pituitary endocrine axis. Gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH)

Hormone secretion begins in the hypothalamus where gonadotropin releasing hormone (GnRH), is secreted in a pulsatile manner at around one pulse per hour. GnRH initiates the start of the follicular phase of the menstrual cycle as it stimulates the anterior pituitary to secrete both follicle stimulating hormone (FSH) and luteinising hormone (LH). FSH and LH have a direct impact on the ovaries, which results in the production of oestrogens, progesterone, and inhibin as well as the maturation of the follicle (Speroff and Fritz, 2005; Barbieri, 2014). In the middle of the menstrual cycle when the follicle has matured, oestrogen concentrations reach a threshold and a surge in LH concentration occurs. The developing follicle releases an oocyte into the fallopian tube, a process called ovulation, and this oocyte is now known as an ovum (Silberstein and Merriam, 2000; Pan and Li, 2019). After the release of the ovum, the granulosa cells of the ovulated follicle form the corpus luteum, which becomes a source of progesterone secretion (Silberstein and Merriam, 2000). Progesterone

secretions target the hypothalamus to reduce the frequency of the GnRH pulse. Both progesterone and oestrogen target the uterus in the modification and development of the endometrium (Figure 2.8). The withdrawal of progesterone following ineffective fertilization of the ovum in the womb at the end of the luteal phase results in a spasm of the spiral arteries within the developed endometrium and as a result menstrual bleeding (menses) happens (Lenton et al., 1984; Buffenstein et al., 1995; Silberstein and Merriam, 2000). The length of the menstrual cycle differs between individuals, but normally last between 21 to 35 days, with an average span of 28 days (Reed et al., 2018). The concentrations of reproductive hormones throughout a typical menstrual cycle are presented in Figure 2.9

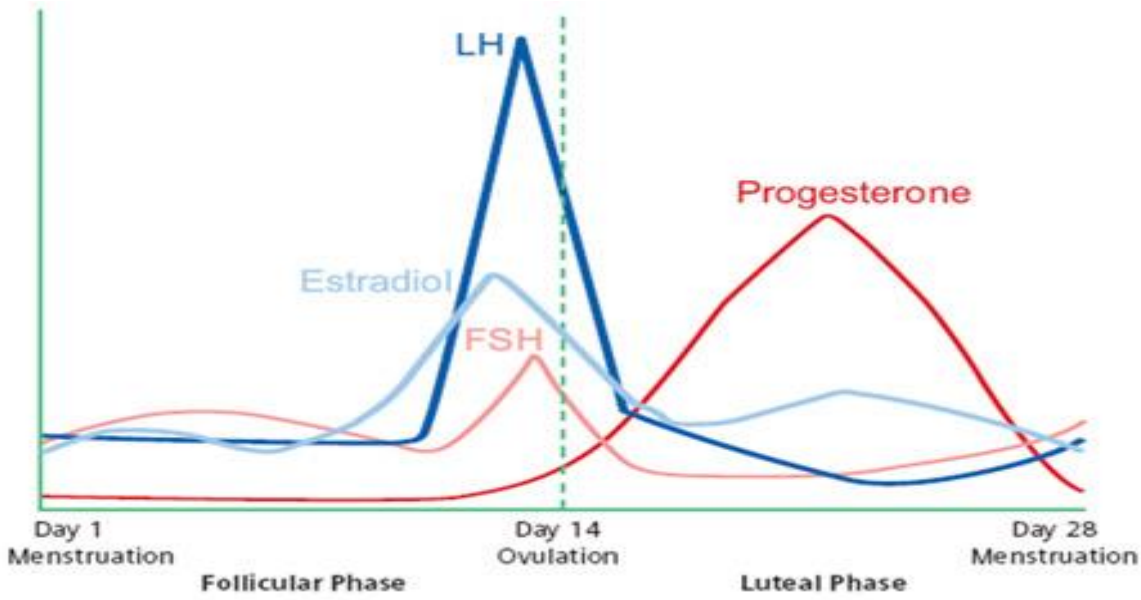


Figure 2.9: Schematic changes in the plasma concentrations of FSH, LH, Oestradiol and Progesterone during a 'standard' menstrual cycle. Follicle Stimulating Hormone (FSH, mIU/ml), Luteinizing Hormone (LH, mIU/ml), Oestrogen (µg/ml) and Progesterone (ng/ml).

With specific emphasis on the variation of sex-steroid hormones during the menstrual cycle (as seen in Figure 2.9), oestradiol concentrations peak before ovulation and sharply decline in the early luteal phase before a more modest rise in the mid-luteal phase following the formation of the corpus luteum (Ferin, 1999; Stricker, 2006). Progesterone concentrations remain low throughout the FP and OP, increasing during LP (Silberstein and Merriam, 2000) again due to release from the corpus luteum.

2.5.2 The impact of the menstrual cycle phase on cardiovascular physiology

The intersex differences in body size, body composition, and hormonal profile present challenges in PPS (Rowland and Roti, 2010). One challenging factor in the PPS of female athletes is whether consideration should be given to the phase of the menstrual cycle when PPS take place (and/or presence of menstrual cycle dysfunction; use of oral contraceptives). Fluctuations in sex-steroid hormones throughout the menstrual cycle (as described above) have been linked to changes in both central and peripheral cardiovascular factors including blood volume and total peripheral resistance. The lack of data describing normal biological variation in cardiac electrical activity, structure, and function in female athletes during menstrual cycle, presents a challenge to clinicians (Datson et al., 2014; Bruinvels et al., 2016). To date, available evidence related to ECG and echocardiography assessments across the menstrual cycle is limited and confounding (McKinley et al., 2009). Fuenmayor et al., (2000) observed a significant difference in mitral valve E/A ratio between FP and LP. Conversely, George et al., (2000) reported no meaningful differences in functional parameters between these time points. Burke et al., (1997), Hulot et al., (2003) and Khan et al., (2016) reported that there were no significant changes in ECG across the menstrual cycle. Rosano et al.,

(1996), however, conducted a study on 26 premenopausal women and noted a correlation between plasma progesterone concentration and the number and duration of episodes of supraventricular tachycardia during the FP and LP of the menstrual cycle. Nonetheless, this is an uncommon finding in young, healthy females and may be of limited relevance in athlete cardiac PPS. Nevertheless, the increasing amount of scientific work surrounding female footballer, gender-specific aspects of physiology, and the impact of hormonal variations during the menstrual cycle upon cardiovascular physiology requires more attention (Martınez-Lagunas et al., 2014).

This review has defined, described and critically appraised the complex, controversial and highly important topics of the AH, SCD and PPS in the light of developing databases, evolving techniques and awareness of key issues in elite female athletes. The main issue of concern in this thesis is the development of new data, in elite female footballers, related to the AH. The rationale for this is the development of information to support/guide PPS assessment in elite female footballers.

Chapter 3

This Chapter is redacted due to publication

Barbara Morrison MSc, Aleah Mohammad (MPhil), David Oxborough PhD, John Somauroo BMedSci, Sarah Lindsay (MD), Aimee L Drane (PhD), Rob Shave PhD and Keith George PhD (2021): The 12-lead electrocardiogram of the elite female footballer as defined by different interpretation criteria across the competitive season, European Journal of Sport Science, August 2021;1-9.

DOI: 10.1080/17461391.2021.1966103

Chapter 4

Seasonal variation of the electrocardiogram in elite female footballers

4.1 Introduction

Sudden cardiac death (SCD) is a tragic event that may be especially impactful in football due to high participation rates and the global nature of the sport (Corrado et al., 2005). Incidents of SCD affects players, coaches, fans, and receives substantial media attention. In most cases, SCD occurs in people with underlying cardiac pathology (Maron et al., 1996) but in many instances the first evidence of the presence of any disease is when the SCD event occurs. To prevent SCD, and reduce the risks of cardiac pathology, many sports have adopted cardiac PPS as a systematic process to identify individuals with cardiac pathology and at an elevated risk of SCD (Shmied and Borjesson, 2014). To improve prevention of football related SCD, the Medical Assessment and Research Centre of FIFA have mandated comprehensive pre-competition medical screening in professional footballers (Thünenkötter et al., 2010). Other football governing bodies such as UEFA and the FA now include PPS as a standard medical tool (Dvorak et al., 2004).

PPS involves several assessment tools and processes (health questionnaire, assessment of personal and family history of disease, physical exam etc.) but the "European Model" of PPS, adopted by FIFA and UEFA, includes a resting 12-lead ECG (Mont et al., 2016). The presence of a 12-lead ECG within PPS has been hotly debated within the clinical and scientific literature (Drezner et al., 2012; Pelliccia et al., 1991; Corrado et al. 2010). The 12-lead ECG is, however, viewed by many sports organizations as vital when screening for disease as the assessment of personal symptoms/family history data alone has been determined as inadequate for the identification of common inherited cardiac pathologies associated with SCD (Wilson et al., 2007). The ECG is now largely accepted as a vital component of PPS, particularly in its ability

to diagnose cardiomyopathies and ion-channelopathies, the commonest causes of SCD in young athletes (Sharma et al., 1997). The acceptance of ECG is also growing due to the recent developments in the assessment criteria for athlete ECGs with the recent International Criteria significantly reducing false positive rates (Sharma et al., 2017; Riding et al., 2017; Drezner et al., 2017). Specifically, we documented a reduction in false positive rates for ECGs in elite female footballers from 16.2% (ESC Criteria) to 0% (International Criteria) in the previous Chapter and thus the current study will utilise the International Criteria.

One specific issue that has received scant attention in the PPS literature is "when" the screening events should take place. It is clear from cross-sectional and longitudinal research that cardiovascular adaptation to training can occur quickly (George et al., 2011). Data assessing changes in cardiac structure and function across training cycles demonstrated increased LV wall thickness in cyclists during a competitive season with small decreases in LV function observed in the off-season (Fagard et al., 1983). Similarly, Weiner and Baggish, (2012) noted changes in LV mass, volumes as well as thickening of the LV walls with changes in training exposure in rowers. Data in male football players have demonstrated increased LV mass during periods of high training volume (D'Ascenzi et al., 2015; Cabanelas et al., 2013). Whether changes in cardiac structure and function that occur with short-term variations in game and training load (and thus hemodynamic challenge) also results in changes in the athlete's ECG is not as clear. This data is vital to inform PPS outcomes. Data relating to cardiac adaptation over competitive seasons in female athletes are relatively limited with no data describing changes in 12-lead ECG in repeated assessments at times of different training volume exposure (acute or accumulated) in elite female footballers. Therefore, the aim of this study was to assess 12-lead ECG data in elite female footballers at different stages of the

competitive season. This data could then feed into sex-specific guidance for PPS in elite female footballers.

4.2 Methods

4.2.1 Study population and design

Thirteen elite female footballers were recruited after approval from the Ethics Committee of Liverpool John Moores University (IRAS169429). Players were recruited through direct contact from the supervisory team with a single professional football club, as part of the need to complete PPS as per FA and FIFA guidelines (including a resting 12-lead ECG). The elite female footballers were aged 26±4 years (range 16-35) and provided written informed consent to participate in the study.

The footballers had all been participating in elite or international level training and game play, with structured training as defined by the club, for a minimum of 6 months. The exclusion criteria included athletes who were currently experiencing symptoms suggestive of cardiovascular diseases or had an early family history of SCD as well as those athletes that had undergone significant restriction (>4 weeks loss of exposure) of training and game play in the last 6 months due to injury. All athletes presented either at Liverpool John Moores University or at their own club facilities for testing.

PPS screening data were acquired prospectively in a repeated measures design. Specifically, resting 12-lead ECG were collected during three separate testing sessions at the following time points across the 2018/2019 season; 1) pre-season (PRE) defined as the beginning of preparation training before the start of pre-season training and just after player return from off-season; 2) mid-season (MID) defined as an assessment that occurred after 50% of

competitive league games had been completed, and; 3) end-season (END) defined as an assessment after the completion of competitive league matches.

Training and game exposure/load varied in the 4 weeks prior to PRE, MID and END testing points. At the start of pre-season training the athletes, (PRE) players' self-reported activity that was highly variable and lacking in detail related to intensity, duration, frequency and thus volume. Players then went into pre-season training and were taking part in 4 field training sessions (endurance, skills and football specific training) each of 60 minutes duration, 2 gym sessions (strength and conditioning) each of 60 minutes duration. During a typical in-season week, athletes were taking part in 5 field sessions each of 60 minutes duration and 2 gym sessions each of 90 minutes duration as well as 1 competitive match per week. This weekly exposure did not change significantly for the cohort between MID and END but clearly END represented a longitudinal accumulation of more training/game exposure.

Match exposure differed across participants related to selection and/or substitution in game. Those not selected to play in any given fixture performed an extra training (field) session lasting up to 60 minutes. All athletes refrained from exercise training or recreational activity for at least 6 hours prior to each data collection session. Alcohol and caffeine consumption were restricted for the 12 hours prior to testing. Every effort was made to timetable repeat tests at the same time of day (morning or afternoon) but we did not control testing within menstrual cycle phase or oral contraceptive use of the athletes across screening sessions.

4.2.2 Procedures

After a detailed explanation of the testing protocol and at each testing point, the athletes completed a medical questionnaire to document any cardiovascular signs and symptoms as well as any data related to an early family history of SCD.

A standard anthropometric assessment included height (Seca 217, Hannover, Germany) and body mass (Seca supra 719, Hannover, Germany) measurements with body surface area (BSA) calculated (Mosteller, 1987). Blood Pressure (BP) was measured with an automated sphygmomanometer (Dinamap 300, GE Medical systems, USA) from the upper left arm after 5 min of seated rest.

A standard 12-lead ECG was attained using commercially available equipment (CardioExpress SL6, Spacelabs Healthcare, Washington US). Each ECG was interpreted using current International recommendations for ECG interpretation in athletes (Sharma et al., 2017; see Figure 2.1 and Chapter 2).

4.2.3 Data Analysis

All continuous ECG data were presented as mean ± SD, with ranges. Statistical analyses were performed using commercially available software package SPSS Version 23.0 for Windows (SPSS, Illinois, USA). Variables were analysed using repetitive ANOVA with post-hoc Bonferroni assessment. $P<0.05$ was considered statistically significant. All clinical data (ECG Criteria) were determined as absent or present as assessed by an experienced consultant sports cardiologist (Professor John Somauroo). If indicated by questionnaire or ECG, follow-up testing was organised and evaluated by the consultant cardiologist as required. At the end of testing all participants were deemed free of cardiac pathology and included in the study.

4.3 Results

There were no significant differences ($P>0.05$) in height, weight, BSA, systolic blood pressure and average training hours per week across the testing sessions (Table 4.1). There was a

significant difference in diastolic blood pressure (P<0.01) across the testing period. Specifically, diastolic blood pressure was lower at PRE compared to MID and END. The number of training days was significantly different across testing (P<0.01) with PRE and END lower than MID (Table 4.1).

Table 4.1: Participant's demographic, blood pressure and training data

	PRE **Mean ± SD** **(Range) [n]**	**MID** **Mean ± SD** **(Range) [n]**	**END** **Mean ± SD** **(Range) [n]**
Age (yrs)	25±4 (16-35) [13]	25±4 (16-35) [13]	25±4 (16-35) [13]
Height (m)	170±6 (156-179) [13]	170±6 (156-179) [13]	170±6 (156-179) [13]
Weight (kg)	61±9 (43-77) [13]	63±5 (51-75) [13]	64±6 (54-75) [13]
BSA (m^2)	1.69±0.15 (1.43-1.92) [13]	1.72±0.09 (1.53-1.90)	1.73±0.10 (1.53-1.90) [13]
Systolic BP (mmHg)	109±12 (88-126) [13]	117±11 (103-135) [13]	123±13 (93-142) [13]
Diastolic BP (mmHg)	60±7 # (48-71) [13]	67±5 (55-73) [13]	67±7 (48-74) [13]
Average Training (Sessions per Week)	6±1 (6-7) [13]	8±2 (6-9) [13]	6±0 (6) [13]
Average Training (Hours per Week)	18±6 (12-30) [13]	18±1 (16-18) [13]	16±1 (16-18) [13]

BSA- Body Surface Area; BP- Blood Pressure; Pre- Pre-season; Mid- Mid-season, End- End season. # PRE lower than MID and END (P<0.01).

There was no significant difference in heart rate and other quantitative ECG data across testing sessions (P>0.05; Table 4.2).

Table 4.2: Quantitative ECG data at three seasonal assessment points

	PRE **Mean ± SD** **(Range) [n]**	**MID** **Mean ± SD** **(Range) [n]**	**END** **Mean ± SD** **(Range) [n]**
Heart rate (beats.min^{-1})	54±7 (43-61) [13]	53±10 (40-71) [13]	57±9 (49-71) [13]
P Duration (ms)	100±8 (90-112) [13]	99±10 (76-118) [13]	99±19 (80-154) [13]
PR Interval (ms)	164±29 (128-186) [13]	149±18 (120-178) [13]	157±37 (118-266) [13]
QRS Duration (ms)	87±6 (82-96) [13]	89±6 (80-98) [13]	89±6 (82-94)[13]
QT Interval (ms)	444±29 (402-468) [13]	436±25 (398-488) [13]	[14]432±22 (390-468) [13]
QTc (Bazett) (ms)	420±22 (386-459) [13]	407±27 (375-459) [13]	420±24 (373-457) [13]
P Axis (^{o}) (degree)	35±35 (-10-79) [13]	39±36 (24-108) [13]	29±70 (-114-79) [13]
QRS Axis (degree)	66±12 (31-96)[13]	67±17 (39-99) [13]	47±51 (-45-129) [13]
T Axis (degree)	29±9 (12-46) [13]	31±13 (2-53) [13]	21±62 (-85-164) [13]
Voltage: RV1 + SV5 (mV)	2.1±0.7 (0.1-0.3) [13]	2.3±0.7 (0.1-0.3) [13]	1.8±0.6 (0.1-0.3) [13]
Voltage: SV1 +RV5 (mV)	2.0±0.6 (1.3.-2.9) [13]	2.0±0.5 (1.4-3.0) [13]	2.0±0.6 (1.1-3.2) [13]

BP- Blood Pressure; R- R wave height; S – wave depth; V1- Chest lead 1; V5- Chest lead 5

The presence (or absence) of training related changes in the athlete ECGs at all testing points are detailed in Table 4.3. Early repolarisation and sinus bradycardia were the most common training related changes, and these were largely consistent across PRE, MID and END. First degree atrioventricular block was observed in one athlete at both PRE and END assessments although this was not apparent in this athlete at MID assessment.

Table 4.3: Training related changes in the athlete ECG at three different times of the season - International standard ECG criteria interpretation in athletes (Sharma et al., 2017).

	Training-Related ECG Findings n (%)		
	PRE	MID	END
Increased QRS voltage	0	0	0
Incomplete right bundle branch block	0	0	0
Early repolarisation	11(85%)	11(85%)	11(85%)
Black athlete repolarisation variant	0	0	0
Juvenile T wave pattern	0	0	0
Sinus bradycardia	10 (77%)	10 (77%)	7(54%)
Sinus arrhythmia	0	0	0
Ectopic atrial rhythm	0	0	0
Junctional escape rhythm	0	0	0
1° atrioventricular block	1(8%)	0	1(8%)
Mobitz type 1 (Wenkebach) 2° atrioventricular	0	0	0

Although there was one example of a borderline ECG finding (right axis deviation; Table 4.4) this was observed only at the END testing session. All ECG recordings were considered normal across the season, with no abnormal findings at any point (Table 4.5), using current International Criteria guidelines for the athlete (Sharma et al., 2017).

Table 4.4: Borderline ECG findings at three different times of the season - International standard ECG criteria interpretation in athletes (Sharma et al., 2017)

	Borderline ECG Findings n (%),		
	PRE	MID	END
Left axis deviation	0	0	0
Left atrial enlargement	0	0	0

Right axis deviation	0	0	1(8%)
Complete right bundle branch block	0	0	0

Table 4.5: Abnormal ECG findings at three different times of the season - International

	Abnormal ECG Findings (n)		
	PRE	**MID**	**END**
T wave Inversion	0	0	0
ST segment depression	0	0	0
Pathological Q waves	0	0	0
Complete left bundle branch block	0	0	0
Profound non-specific intraventricular conduction delay	0	0	0
Epsilon wave	0	0	0
Ventricular pre-excitation	0	0	0
Prolonged QT interval	0	0	0
Brugada type 1 pattern	0	0	0
Profound sinus bradycardia	0	0	0
Profound 1° atrioventricular block	0	0	0
Mobitz type II 2° atrioventricular block	0	0	0
3° atrioventricular block	0	0	0
Atrial tachyarrhythmias	0	0	0
Premature ventricular contractions	0	0	0
Ventricular arrhythmias	0	0	0

standard ECG criteria interpretation in athletes (Sharma et al., 2017).

4.4 Discussion

The main findings of this study were; (1) there were only small changes in participant demographics across testing time points, (2) there were no changes in continuous ECG data across testing time points, and (3) there were only small changes in "training-related" and "borderline" ECG with no presentation of "abnormal" ECG patterns at any point during repeated testing across a competitive season in elite female footballers. Taken together these data suggesting that moving the time of PPS across a competitive season in elite female footballers is unlikely to alter any relevant clinical outcome.

The key finding of the study was the lack of significant change in the ECG of elite female footballers assessed at three time points in a competitive season. This type of ECG data has not been studied in elite male or female footballers previously so direct data comparisons are not possible. The lack of change in ECG parameters is, however, somewhat at odds with a database suggested seasonal changes in measures of LV structure and function in athletes (Fagard et al., 1983; Weiner and Baggish, 2012; D'Ascenzi et al., 2015; Cabanelas et al., 2013). This past data suggests the heart of highly trained athletes is still malleable to training load changes and would raise the issue of the potential for the ECG to be mediated in the same way. The lack of change in all continuous ECG data as well as incidence of training-related ECG patterns in this cohort suggests one of two things; (1) that the athletes ECG is not as sensitive to training load as measures of LV structure and function, or (2) the three assessment points employed in the current study did not represent large changes in training and match-play exposure.

Whilst we made the a-priori assumption that the assessment at PRE would come after a relatively "inactive" off-season period this is not well documented and may not be the case in most elite professional female footballers and the "old" notion of an off-season "rest or recovery period", with limited training, may not be apparent to the same extent in the current professional football world. We were not able to collect accurate training and physical activity data for players in the off-season but although the PRE session came after an "off-season" the players adjusted to a highly structured training demand with few problems. There were few training exposure differences noted between MID and END other than a slight rise in number of training sessions per week at MID compared to END although total hours training exposure was quite consistent between assessment times. Consequently, it may be that in the modern age of elite, professional female footballers training exposure is likely maintained almost continuously and thus acute changes in load due to small changes in exercise frequency, intensity and volume do not represent a large enough alteration in physiological stimuli to mediate changes in the ECG.

Whilst the cohort ECG data was consistent, it is useful to briefly discuss the most common training related changes in the ECG as well as the small number of individual changes in "training-related" and "borderline" ECG patterns. By using current International ECG interpretation guidelines for the athlete (Sharma et al., 2017) we identified 11/13 athletes (85%) had a consistent finding of early repolarisation (an elevated ST segment). This is similar to previous study by Drezner et al., (2013) and suggests that early repolarisation is a common finding in young, healthy, competitive athletes that is a direct result of exercise training (Noseworthy et al., 2011). Similarly, in a recent study by Churchill et al., (2021), early repolarisation was a very common finding reported in 35/122 (29%) of USA females footballers. We cannot determine from this, or previous, work whether exercise-related early

repolarisation may be an electrical phenomenon that is or is not developed in parallel with structural myocardial remodelling.

Further comments from Aagard et al., (2016) stated that most asymptomatic athletes with early repolarisation, even those with inferolateral J waves followed by a horizontal or downsloping ST segment, are at low risk of SCD.

Resting sinus bradycardia was the next most common training related ECG pattern (10/13, 77%) in PRE. This prevalence is common in trained athletes (Sharma et al., 1999; Boyett et al., 2013). Maron and Pelliccia, (2006) reported that a reduced heart rate in athletes at rest has been largely ascribed to an increase in vagal tone. Interestingly despite a similar cohort heart rate at the END season assessment the prevalence of sinus bradycardia had dropped (7/13, 54%). Whether this reflects a subtle lack of control of activity prior to the assessment of ECG at END or potentially a cumulating fatigue or over-reaching, which can alter sympatho-vagal balance (Driscoll and Dicicco, 2000), is not possible to determine. Only 1 athlete (8%) presented with a 1° atrioventricular block at PRE and END although this was not present at MID. The PR interval > 200 (PRE and END) for this individual athlete is likely, at least partially, associated with high vagal tone as the heart rates at PRE (43 bpm) and END (40bpm) were lower than at MID (49 bpm).

The latest ECG criteria include a borderline category comprising axis deviation, voltage criteria for atrial enlargement, and complete right bundle branch block. Athletes with more than one of these abnormalities require further investigation. In our cohort only one athlete had right axis deviation at END. The presence of axis deviation or voltage criteria for atrial enlargement on the 12-lead ECG rarely predicts pathology in young athletes (Gati et al., 2013). Exclusion of these abnormalities from the abnormal category in asymptomatic athletes without a family

history of premature cardiac disease, or abnormal physical findings is appropriate in the absence of other signs, symptoms, or ECG changes.

The only other measured variable to change over the season was a small but significant increase in diastolic blood pressure at MID and END compared to PRE ($P<0.01$). We can only speculate why this might have occurred but it is unlikely to be mediated by alterations in vagal tone with training accumulation, as this is not supported by cohort resting HR data.

4.5 Study implications, limitations, and future research

The major practical implication from this study, gathered in a small cohort of elite professional female footballers, is that PPS could occur at any time during the competitive season without altering clinical decision making. It is reassuring that few borderline and no abnormal ECG patterns were present in this cohort and the absence of abnormal patterns were consistent. The common appearance of sinus bradycardia and early repolarisation is, perhaps, predictable given past work in male and female athletes (Drezner et al., 2013) but is useful to inform PPS in elite female footballers.

There are several factors that may limit the external validity of our data. Firstly, the sample size was small, and a larger study of female footballers would be informative. The ethnicity of the group was uniform, and we know ethnicity can affect ECG outcomes in male and female athletes (Wilson et al., 2012; Sharma et al., 1999). It would be interesting to make more ECG observations across one or maybe multiple seasons and potentially collect data during periods of injury to assess the impact of acute detaining. We did not control for menstrual cycle phase or oral contraceptive use and this might be informative in future research. Finally,

more detailed, and individual assessment of training and game exposure should be used rather than more "blunt" assessments of hours and days employed in this study.

Whilst we have provided novel insight into the ECG of elite female footballers, from the perspective of informing the approach to, and content of, PPS it would now seem sensible to study cardiac structure and function in these athletes, using standard and novel echocardiography including assessment of all chambers as well as regional and global data. This would add further specific data to inform cardiac PPS in elite female footballers. This is the focus for data presented in the next chapter.

4.6 Conclusions

There was no significant difference in continuous ECG data measured at 3 time points across a competitive season. Only small changes were noted in the presence of "training related" and "borderline" ECG patterns with no "abnormal" ECG observed at across testing points. Taken together there was no clinically relevant difference in the ECG of a small cohort of elite female footballers assessed at 3 time points across a competitive season (PRE, MID, END). These findings suggest that PPS could occur at any point of the athlete's competitive season with limited impact upon clinical outcome, although future studies should confirm this finding in bigger and more diverse athlete groups.

Chapter 5:

Cardiac structure and function in elite female footballers: What are the upper limits of adaptation to long-term physiological sport-specific training?

5.1 Introduction

In 2014 a FIFA survey indicated that there were more than 30 million women participating in football worldwide (FIFA, 2014). The Women's Football World Cup, run by FIFA, is the largest sporting event in the world for women. In England, in 2008 Sport England's "Active People Survey" estimated that 260,000 women and 1.1 million girls played football in England (Active people survey, 2008). Football is clearly a mass participation sport for women. One consequence of increasing participation rates has been a professionalization of the game at the elite level in many countries around the globe, including the UK. With professionalization came a drive for more co-ordinated scientific, technical, and medical activity to support increasing training loads and playing demands in elite female footballers.

Many physical and physiological adaptations are likely to be associated with the greater training loads associated with preparation for elite level football competition. One element of this adaptation occurs in the cardiovascular system and has been labelled the AH (George *et al.,* 2011). Defining the nature of any physiological cardiac adaptation to training is essential for many reasons, not least of which is the desire to differentiate the AH, adequately and rapidly, from any form of pathological adaptation in the heart that may pre-dispose to an increased risk of SCD (Harmon *et al.,* 2014). The incidence of SCD is low in the general population as well as in elite athletes but there is some suggestion that risk rises in the presence of a disease substrate when undertaking moderate to intense exercise (Corrado *et al.,* 2006). Whilst SCD in footballers is uncommon (Harmon *et al.,* 2014), when it does occur, it is a tragedy that can have a powerful effect on those that play and watch sports. Because of the nature of SCD events and the increased risk associated with sports participation; as sports governing body, including the IOC and FIFA, now provide guidance on mandatory pre-

participation cardiac screening (PPS) of players (Dvorak *et al.,* 2006). PPS requires that every player in the Premier League or any UEFA competition must have at least one electrocardiogram (ECG) and echocardiogram in their medical folder (normally at the beginning of their professional playing career). Consequently, knowledge of the upper limits of the structural and functional phenotype of the AH in elite footballers is essential to aid PPS and associated differential diagnostic processes. Whilst PPS was originally adopted in male football, it is now mandatory for elite female footballers in the UK.

The most reported cardiac structural adaptation to training is an increase in LV mass and this has been reported in meta-analyses in male (Pluim *et al.,* 2000; Utomi *et al.,* 2014) and female (Whyte *et al.,* 2004) general athlete populations. What is apparent from the plethora of AH research is that individual cardiac adaptations to training are heterogeneous and depend on a complex interaction of factors including age, body size, sex, ethnicity, training status and sports discipline (Brown *et al.,* 2017). The specific impact of sex has been the focus of some research attention in this field in recent years. Rowland *et al.,* (2010) and Churchill et al., (2020) demonstrated that male athletes were characterised by greater cardiac volume and mass than comparably trained females. Male-female differences in relative wall thickness, however, were not evident suggesting that the qualitative nature of cardiac adaption may be similar in both sexes (Rowland *et al.,* 2010). This work was supported by a recent large sample comparison of mixed groups of male and female athletes (Giraldeau *et al.,* 2015) although it seems that LV wall thickening beyond 11 mm is very rare in female athletes (Whyte *et al.,* 2004).

The assessment of the AH phenotype in elite footballers is largely limited to male players across a broad age range (Pelliccia *et al.,* 1991; Rawlins *et al.,* 2009; Scharf *et al.,* 2010). Overall, this body of work suggests an AH phenotype that includes moderately enlarged chamber dimensions and only small changes in wall thickness (Rawlins *et al.,* 2009). This data is helpful to support PPS in male footballers. A similar database in elite female footballers is not as well developed. Randers *et al.* (2013) examined the cardiovascular health profile in a small (n=27) cohort of elite Danish female football players in comparison to untrained young women. LV dimensions were larger (51 ± 3 vs 45 ± 4 mm; P<0.001) and LV systolic and diastolic functional parameters were improved in elite female football players when compared to untrained women. Recent data from Churchill et al., (2020), in a larger cohort of USA female footballers, reported that female footballers have lower diastolic function compared to male footballers (Peak E' 18 [17-19] vs 21 [20-22] cm/s; P < .001) although no control group was assessed so a "training" impact in the female footballers is unclear. Whilst a useful initial insight this work needs to be developed with assessment of other cardiac chambers and the employment of new imaging technologies such as speckle tracking to assess cardiac mechanics (Oxborough *et al.,* 2016). A recent addition to echocardiographic imaging tools, speckle tracking echocardiography can determine regional and global strain (ε), strain rate (SR) and twist data, and has recently been explored in general AH data (Utomi *et al.,* 2014; Basavarajah *et al.,* 2008; Smiseth *et al.,* 2006). The application of this technique may aid conventional assessment of the AH and contribute to differential diagnostic decision making in cardiac PPS (George *et al.,* 2011).

In summary, we sought to describe the structural and functional phenotype of the AH in elite female footballers and compared their data to that obtained from a sedentary control group

matched for age and sex. We employed standard 2D, Doppler, tissue Doppler as well as longitudinal ε to describe structure and function of the elite female footballer's heart. We hypothesise that the AH in elite female footballers will present with larger chamber dimensions, than healthy controls, but resting cardiac function and mechanics will not be different between groups. This data will contribute to a very small database on the AH phenotype in female footballers that will support sex-specific PPS in elite female footballers.

5.2 Methods

Seventy-nine elite female footballers and thirty sex and age-matched sedentary controls were recruited into the study. Athletes were recruited via Football club and Football Association requirements for cardiac PPS with an experienced sports cardiologist (JS). Controls were recruited by advertisement at the University. All athletes were classified as elite as they performed at national or international level and had been in training with a professional women's football club for a minimum of 6 months. Sedentary controls were undertaking less than 3 hr of physical activity per week in the 6 months prior to assessment and had not been in any formal training program for sports participation in the last 12 months. Other general inclusion criteria included: aged between 16 and 35 years old, not currently taking any medication (except for oral contraceptives) and being free from self-reported cardiovascular disease. General exclusion criteria included a personal or early family history of known cardiovascular disease, current illness and/or prescription medication consumption and periods of recent (last month) injury of greater than 7 days, that would disrupt training and competition play and thus the hemodynamic stress on the heart. The Ethics Committee of

Liverpool John Moores University (IRAS169429) granted approval for this research and all participants provided written informed consent.

5.2.1Design and Procedures

This study employed a cross-sectional design where all participants presented at a testing location (players club or University cardiovascular labs) for data collection at a single time point. Participants were requested to have eaten their normal meal and taken adequate hydration (breakfast or lunch) prior to morning or afternoon testing sessions. We requested all participants to attend PPS having avoided alcohol and caffeinated drink intake as well as strenuous exercise for 12 hours prior to the examination. We could not control time-of-day for all screening tests as these were arranged by the club to fit with training and or other elements of their professional contract (travel, game play etc.), but this reflects an element of ecological validity to the testing process. Prior to the single testing session, all participants were provided with health questionnaire to detail cardiovascular symptoms, family history of sudden cardiac death and any other cardiovascular disease history.

All participants had body mass assessed using standard scales (Seca 217, Hannover, Germany) and height measured using a stadiometer (Seca Supra 719, Hannaover, Germany). Body surface area (BSA) was calculated by using a standardised formula (Mosteller, 1987). Systolic and diastolic blood pressure were measured using an automated sphygmomanometer (DINAMAP 300, GE medical system, Milwaukee, Wisconsin, USA). Blood pressure data were recorded as the lowest values from a minimum of two repeat blood pressure assessment after 5 minutes of quiet seated rest.

All participants then undertook a standard resting 12-lead electrocardiogram (CardioExpress SL6, Spacelab Health care, Washington, US). Electrode placement was undertaken by a trained cardiac physiologist. This exam and data analysis were carried out in accordance with the International Criteria (Sharma *et al*., 2017). The ECG was analysed by an experienced sports cardiologist who confirmed, in accordance with International Criteria, the presence or absence of (likely) training, borderline and non-training related ECG changes. This data has been presented in Chapter 2. If either the questionnaire or ECG (following guideline assessment) raised any concerns in relation to the potential presence of cardiovascular disease, a bespoke approach to follow-up studies was undertaken and overseen by an experienced sports cardiologist.

5.2.2 Echocardiography assessment.

A full echocardiographic assessment was undertaken by a single experienced sonographer using a commercially available ultrasound system (Vivid Q, GE Healthcare, Horten, Norway) and a 1.5-4 MHz phased array transducer. All images were acquired with the participant in the left lateral decubitus position and in accordance with American Society of Echocardiography (ASE; Lang *et al.,* 2015). Images were stored in a raw DICOM format and exported to an offline workstation (EchoPac version 6.0, GE Healthcare, Horten, Norway) for subsequent analysis. All data was analysed by the researcher under the supervision of a single accredited sonographer.

5.2.3 Left ventricle

Standard measurements of LV structure and function were made in accordance with ASE guidelines (Lang *et al.,* 2015). LV linear dimensions included LV diameter in systole and diastole (LVDs; LVDd) assessed from the parasternal long-axis view (Figure 4.1). Wall thickness dimensions (WT) were measured from the parasternal short axis view. In order to provide a comprehensive assessment of LV WT, eight measurements were made at basal and mid-levels from the antero-septum, infero-septum, posterior wall and lateral wall (Wigle *et al*., 1985; Figure 5.2). Mean WT (MeanWT) was calculated as an average of all eight measures and the maximum value of WT (MaxWT) was reported. Relative wall thickness (RWT) was determined using the equation: LVDd divided by 2 times the posterior wall thickness (LVDd/2*WT). LV mass was calculated from linear data using the ASE corrected equation (Lang *et al.,* 2015). LV end diastolic volume (LVEDV), LV end systolic volume (LVESV), stroke volume (SV) and ejection fraction (EF) were calculated using the Simpson's biplane method utilising both apical four- and two-chamber orientations (Figure 5.3). Structural data are presented as absolute values and then indexed for individual differences in BSA using an allometric approach (Batterham *et al.,* 1999).

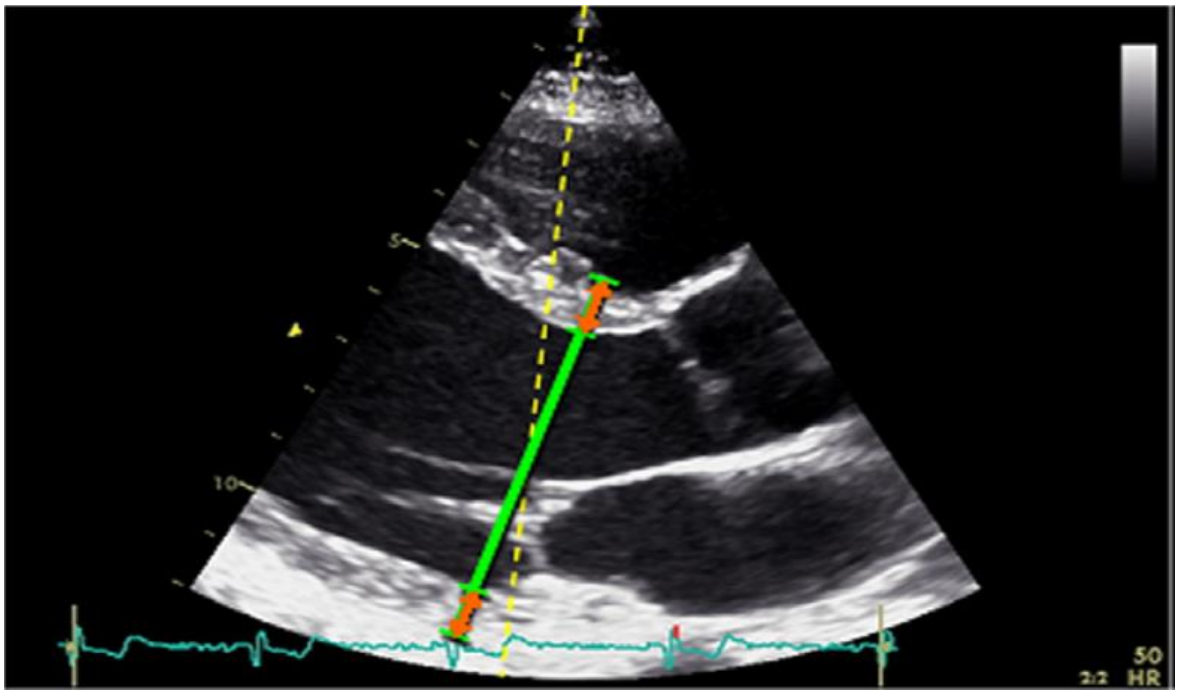


Figure 5.1: Linear method for measurements of LVDd. (Lang et al., 2015)

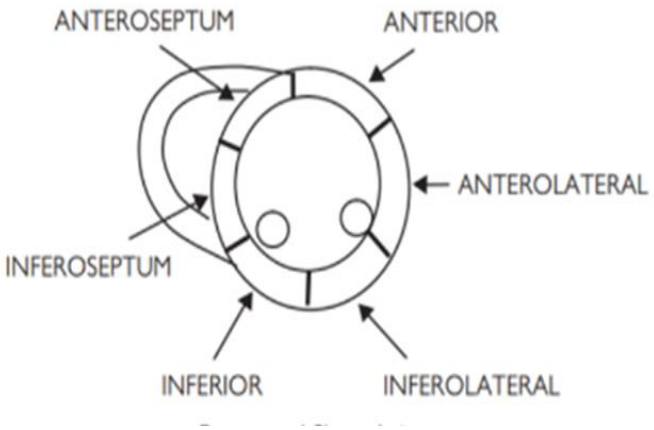


Figure 5.2: Parasternal short axis

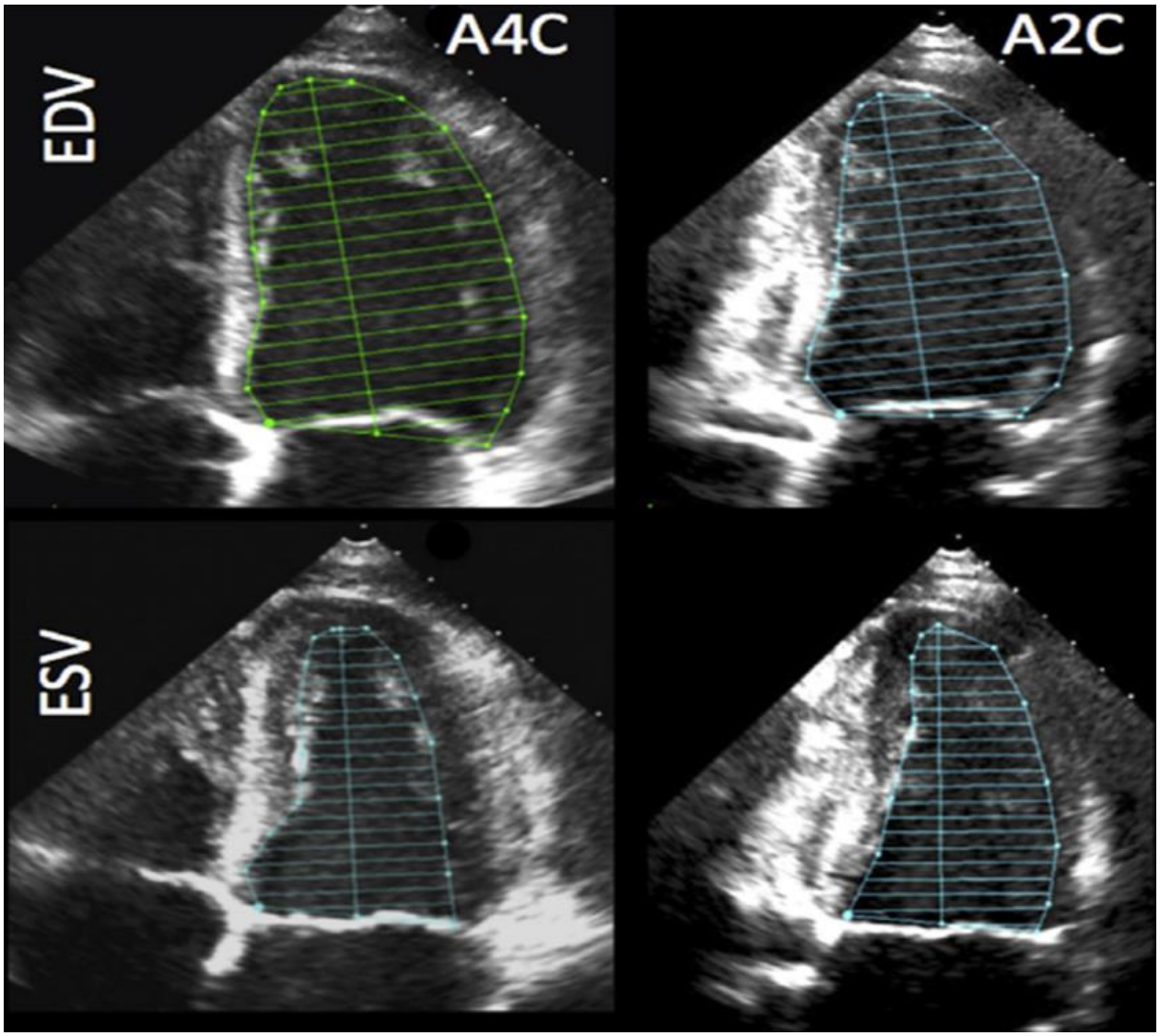


Figure 5.3: LV volume measurement using Biplane Method of Discs (modified Simpson's rule). (Lang et al., 2015)

Doppler and tissue-Doppler (TDI) scanning were also performed using an apical 4-chamber view. A 4 mm sample volume placed at the tips of the mitral valve leaflet determined pulsed-wave Doppler assessment of mitral inflow. This led to the quantification of peak early (E) and late/atrial (A) transmitral diastolic flow velocities.

The ratio E/A was then calculated (Figure 5.4). The deceleration time of the E wave was also determined via standard techniques (Lang et al., 2015). After adjustment of filters and range, the sample volume was placed at the level of the mitral annulus in the septal wall and lateral wall to determine peak tissue velocities in systole (S') as well as early (E') and late/atrial (A') diastole (Figure 5.5). The ratio E/E' was then calculated as an estimate of left atrial driving pressure (Nagueh *et al.,* 1997).

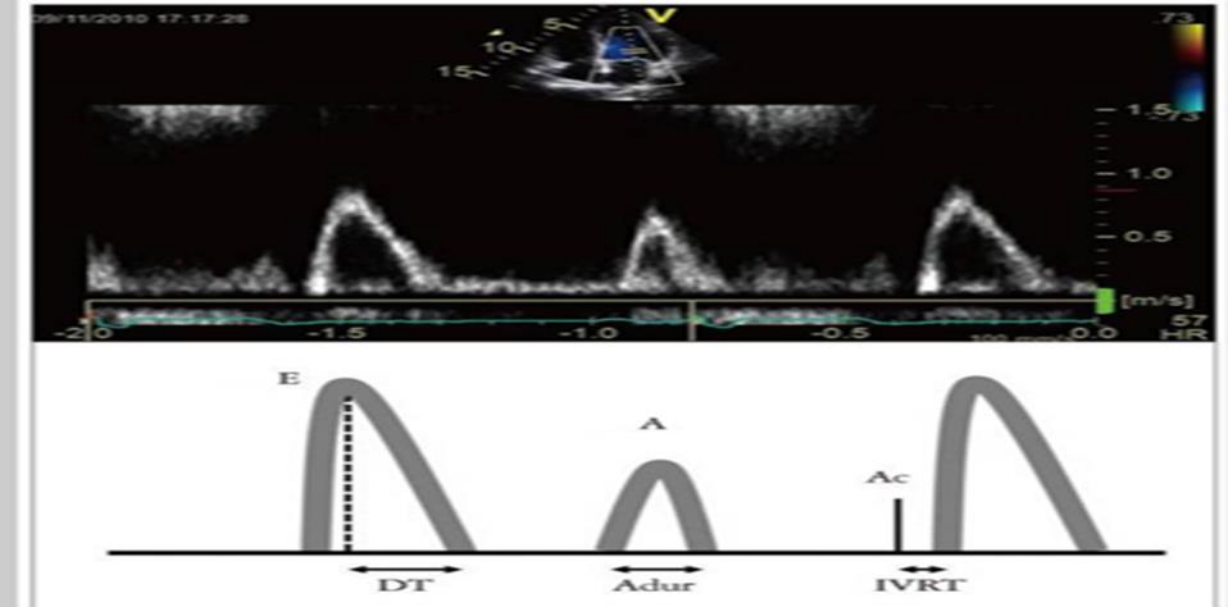


Figure 5.4: Peak mitral inflow velocity during early diastole (E wave), peak mitral inflow velocity at atrial contraction (A wave), mitral deceleration time (DT), duration of A wave (Adur), and interval between aortic valve closure (Ac).

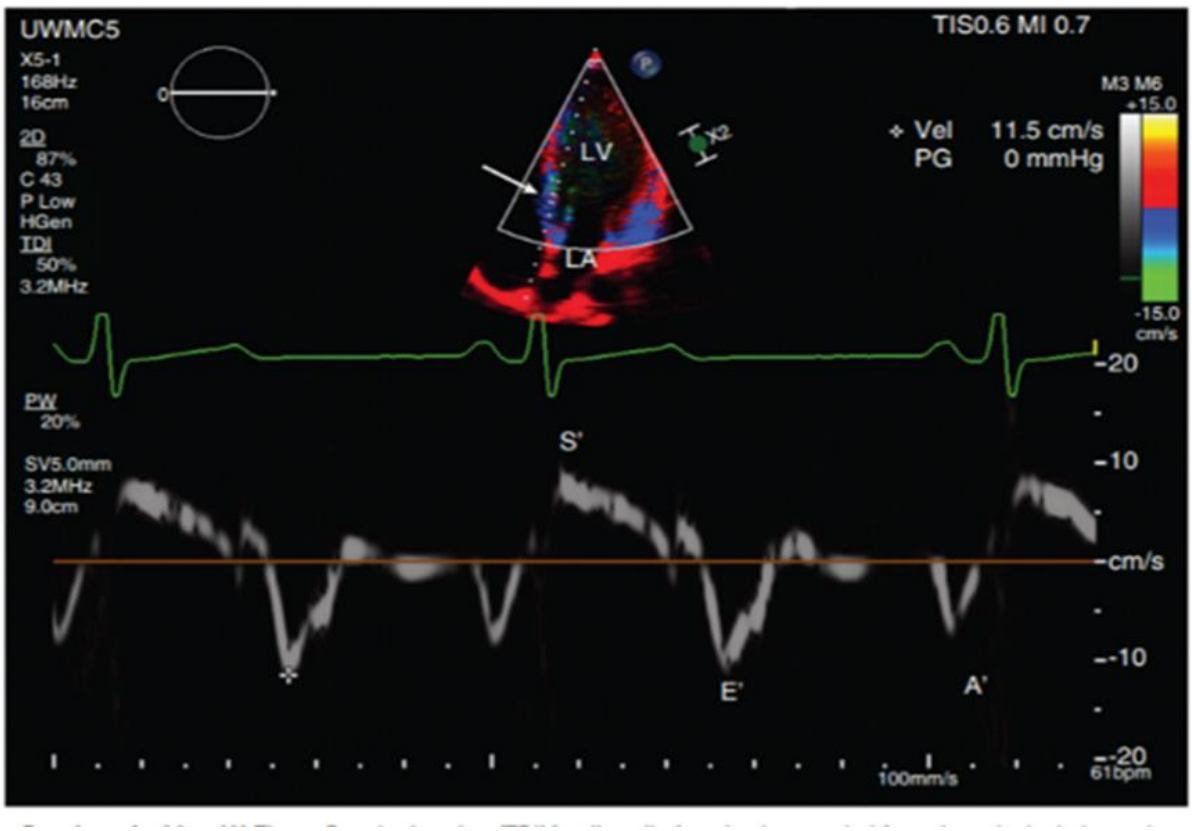


Figure 5.5: Mitral annulus in the septal wall and lateral wall peak tissue velocities in systole (S') as well as early (E') and late/atrial (A') diastole. (Echocardiography textbook).

Images for the assessment of peak longitudinal myocardial strain (ε) and strain rate (SR), via myocardial speckle tracking, were acquired from an apical 4-chamber view. During the offline analysis (EchoPac, version 6.0, GE Healthcare, Horten, Norway) a region of interest was placed around the LV from basal septum through the basal lateral wall ensuring the whole of the myocardium was included (Figure 5.6). Automated ε analysis provided peak ε data for six myocardial segments and an average of these provided a global index of LV longitudinal ε and the same occurred for SR. Peak SR was determined in systole (SRS), early diastole (ESR) and late/atrial diastole (ASR). Time (ms) to peak ε and SR were also determined automatically.

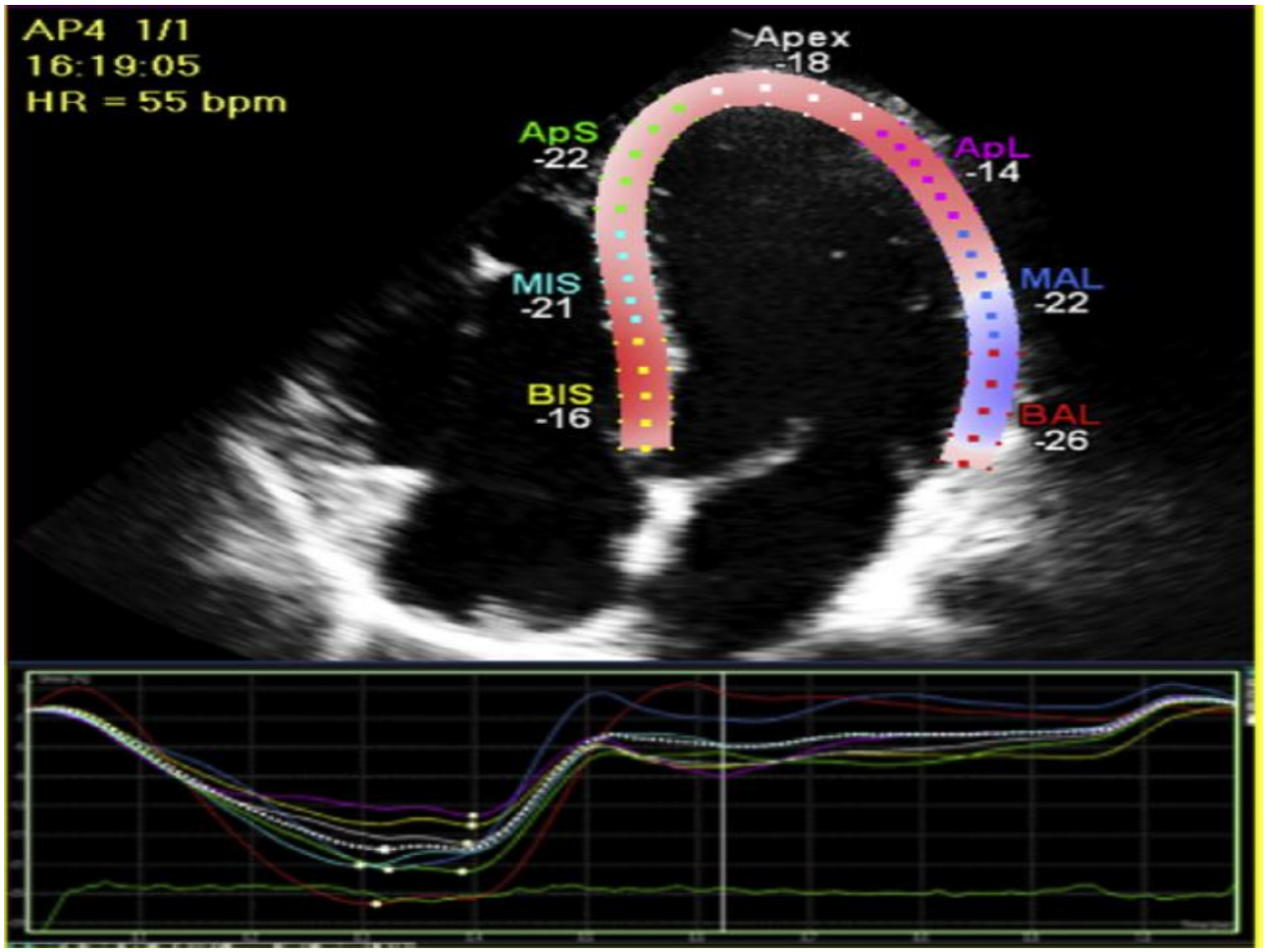


Figure 5.6: Peak values of global longitudinal ℇ were calculated using post-hoc speckle tracking of apical 4 chamber views. (Lang et al., 2015)

5.2.4 Right ventricle

A range of RV dimensions were recorded given the complex anatomy and geometry compared to the LV (Rudski et al., 2010). The RV outflow tract (RVOT) was measured at three places in views from the parasternal long and short axis orientation (RVOTplax, RVOT1 and RVOT2; Figure 5.7 and 5.8).

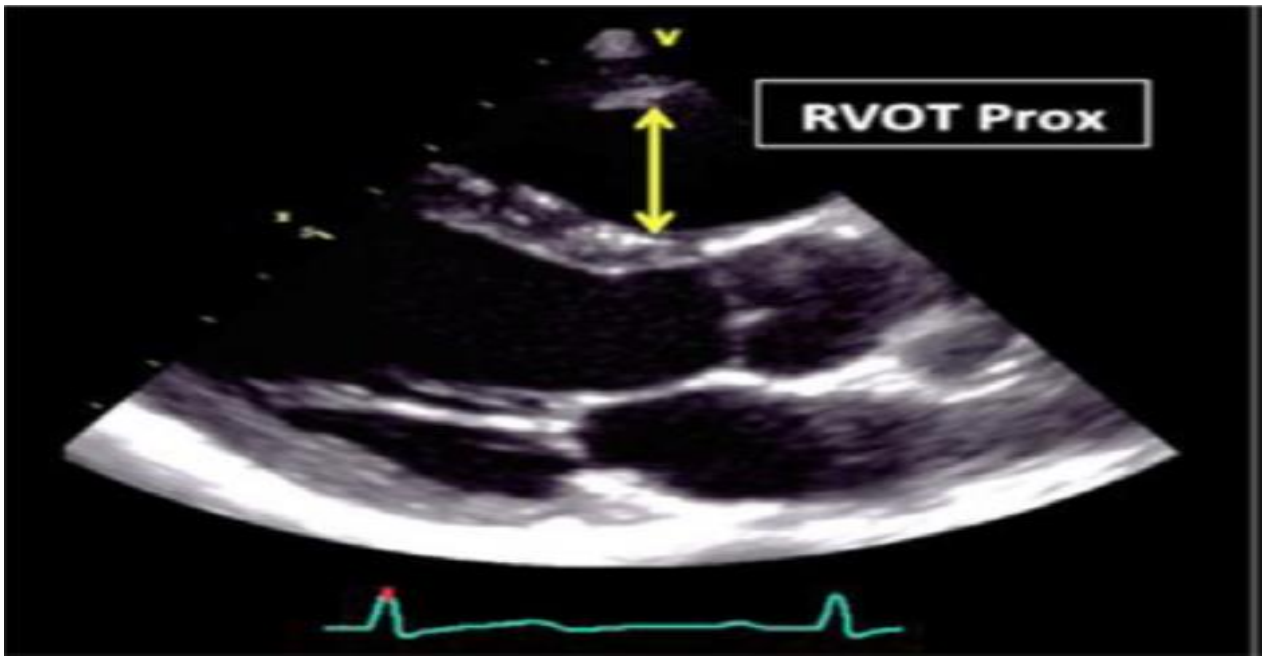


Figure 5.7: RV outflow tract diameter from the parasternal long-axis view (RVOT-PLAX) (Rudski et al., 2010)

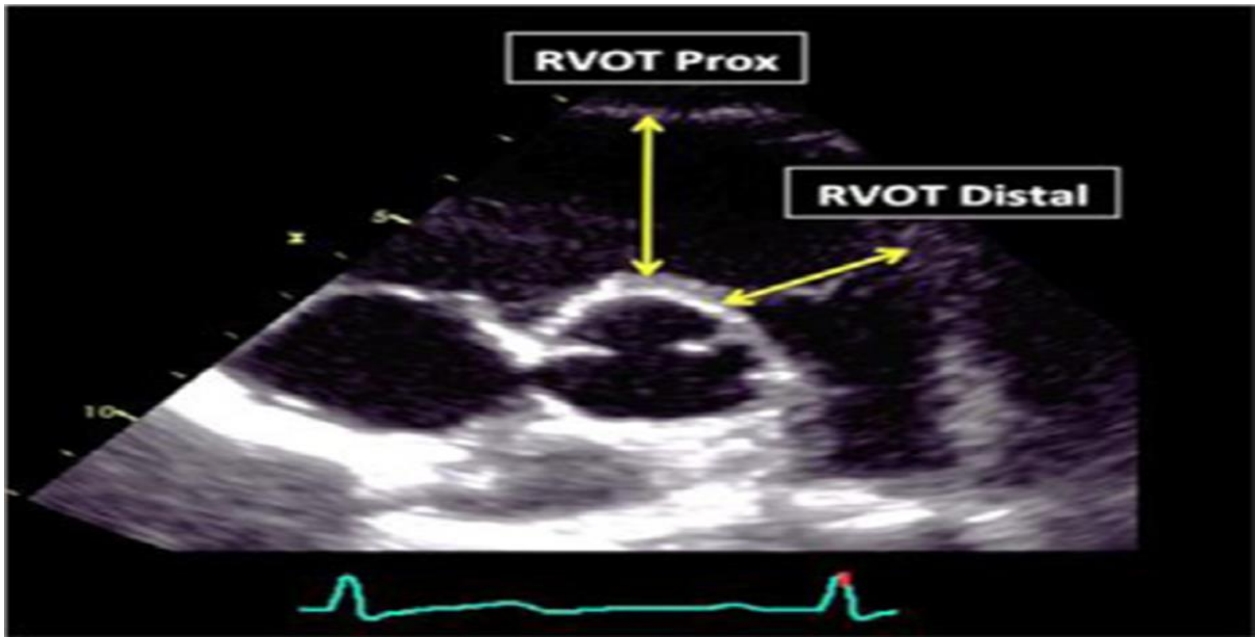


Figure 5.8: RV outflow tract diameter at the short axis region (RVOT1 or RVOT Prox) and RV outflow tract diameter at the pulmonic valve annulus (RVOT2 or RVOT distal). (Rudski et al., 2010)

RV inflow dimensions were assessed at the base (RVD1), mid-cavity (RVD2) and the length from base to apex (RVD3) from a modified apical four-chamber orientation (Figure 5.9).

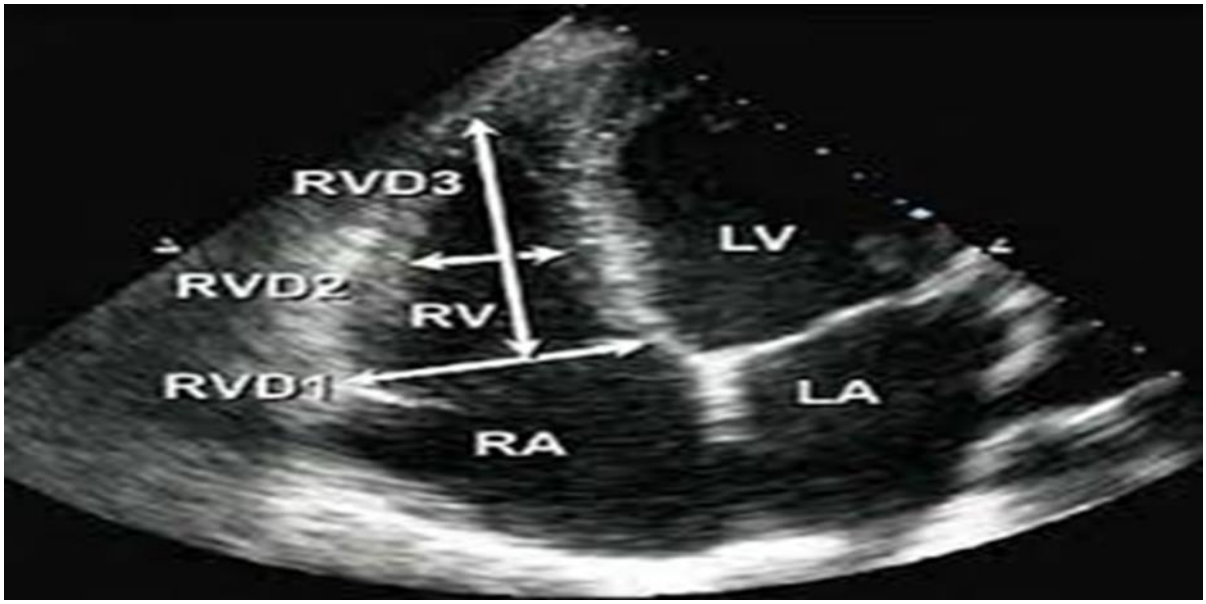


Figure 5.9: Basal RV diameter (RVD1), mid RV diameter (RVD2) and base-to-apex length (RVD3) (Rudski et al., 2010)

RV end-diastolic area (RVDa) and RV end-systolic area (RVSa) were measured from the same adjusted 4-chamber orientation and fractional area change (RVFAC) was calculated: RVFAC (%) = (RVDA – RVSA) / RVDA (Figure 4.10). RV dimensional data were presented as absolute values and then indexed for individual differences in BSA using an allometric approach (Batterham *et al.,* 1999).

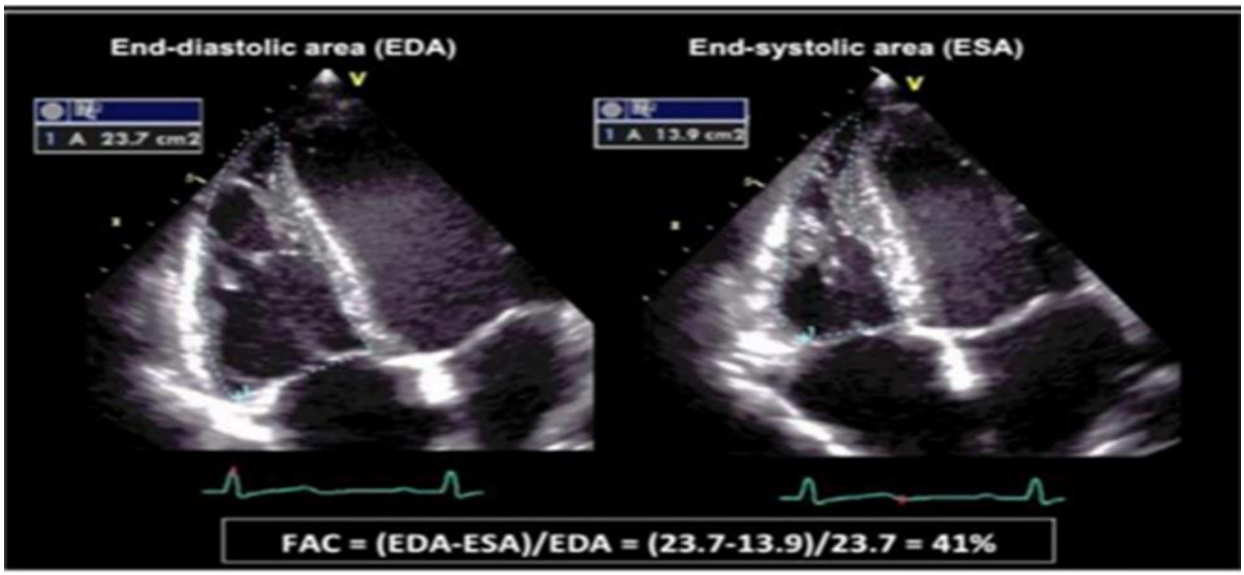


Figure 5.10: RV end-diastolic area (RVDA) and RV end-systolic area (RVSA). (Rudski et al., 2010)

RV longitudinal function was determined using M-Mode via the tricuspid annular plane systolic excursion method (TAPSE; Figure 5.11) and pulsed-wave TDI (Figure 5.12) which permitted measurements of peak RV lateral wall systolic (S'), and early diastole (E') and late diastole(A') myocardial velocities.

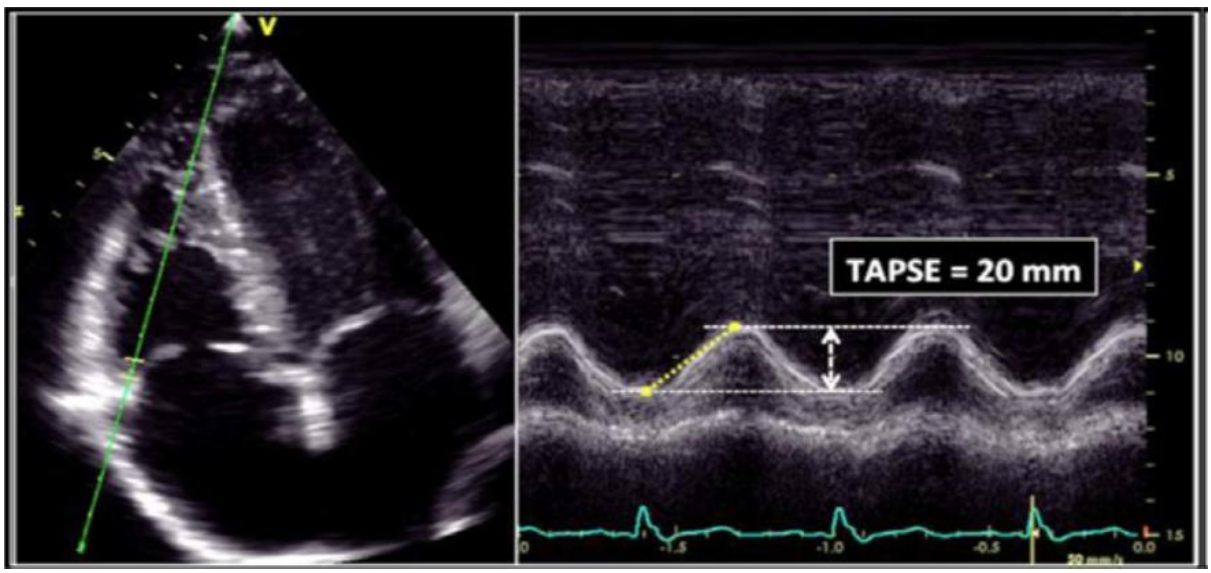


Figure 5.11: Visualisation of the TAPSE assessment. (Rudski et al., 2010)

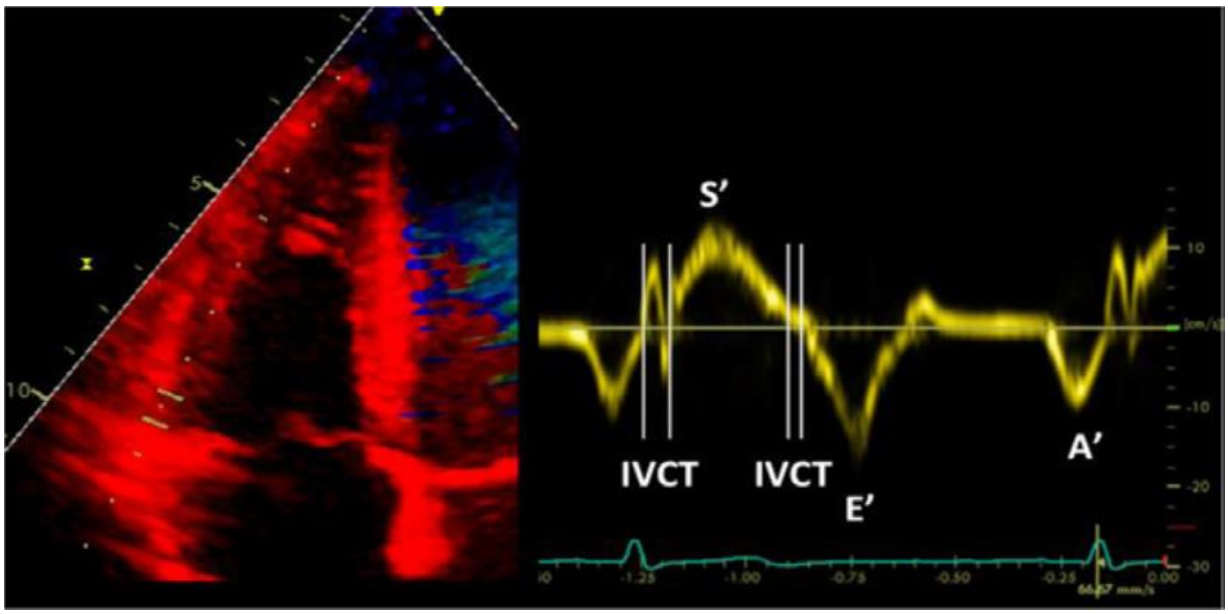


Figure 5.12: Pulse wave TDI of the RV free wall assessment of peak S', E' and A'. (Rudski et al., 2010)

5.2.5 Data Analysis

Data were collected and managed using REDCAP electronic data capture tools hosted at Liverpool John Moores University (Harris et al., 2009). For both cohorts all echocardiographic study data were presented as mean ± SD as well as range. Statistical analysis were accomplished using commercially accessible software package SPSS Version 25.0 for Windows (SPSS, Illinois, USA). All variables were analysed between groups using independent T-tests. A $P<0.05$ was adopted to determine statistical significance.

5.3 Results

5.3.1 Demographic Data

Table 5.1 summarizes the demographic data from both groups. Age was not different between groups (P>0.05). The footballers had significantly lower body mass, resting heart rate as well as systolic and diastolic blood pressure compared with the sedentary control group (P<0.05).

Table 5.1: Participant demographic data in athletes and controls

Variable	Elite Footballers Mean ± SD (Range) [n]	Controls Mean ± SD (Range) [n]
Age(years)	21±4 (14-35) [79]	21±2 (18-22) [29]
Height (m)	1.66±0.06 (1.58-1.79) [79]	1.65±0.06 (1.53-1.78) [30]
Body mass (kg)	63±7 # (52-75) [79]	69±11 (49-94) [30]
Body Surface Area (m^2)	1.7±0.1 (1.5-1.9) [79]	1.8±0.2 (1.5-2.0) [30]
Systolic BP (mmHg)	117±13 # (98-143) [78]	125±10 (100-138) [26]
Diastolic BP (mmHg)	67±8 # (50-85) [78]	73±9 (55-91) [26]
Heart Rate (beats/min)	58±10 # (42-87) [79]	76±14 (49-101) [27]
Training Duration (years)	14±4 (5-25) [79]	NA
Training (days per week)	6±1 (3-7) [79]	NA
Training (hours per week)	14±5 (7-30) [79]	NA

BP – Blood Pressure; NA- Non-Applicable; # significant difference between the groups (P<0.05)

5.3.2 Left Ventricle

Various parameters of LV structure (Table 5.2) and function (Table 5.3) are presented. LV chamber dimensions in diastole and systole were larger in elite female footballers even after adjusting for body size ($P<0.05$). Absolute LVEDV and LVESV were not different between groups, although BSA indexed LVEDV was significantly larger in the elite footballers. Between group differences in individual WT measurements were limited but both MeanWT and MaxWT were significantly greater in elite female footballers before and after body size adjustment ($P<0.05$). RWT was not different between group but both absolute and indexed LV mass was greater in elite female footballers ($P<0.05$).

2D, Doppler, tissue-Doppler (TDI) and speckle-tracking LV functional data are presented in Table 5.3. In systole EF, SV and peak longitudinal Ɛ were higher in the elite female footballers ($P<0.05$). In diastole transmitral E deceleration time was longer in elite female footballers ($P<0.05$). Peak transmitral A velocity as well as peak lateral and average A' tissue velocities were higher in the sedentary control group ($P<0.05$).

Table 5.2: Left ventricular structural data in athletes and controls

Variable	Elite Footballers Mean ± SD (Range) [n]	Controls Mean ± SD (Range) [n]
LVDd (mm)	49±3 **#** (42-58) [78]	45±3 (40-49) [27]
LVDd index (mm/$(m^2)^{0.5}$)	38±2 **#** (33-41) [78]	34±2 (30-37) [30]
LVDs (mm)	33±3 **#** (25-40) [78]	30±3 (26-39) [30]
LVDs index (mm/$(m^2)^{0.5}$)	25±2 **#** (19-31) [78]	23±3 (19-31) [30]
LVEDV (ml)	100±23 (54-160) [79]	94±17 (66-134) [30]
LVEDV index (ml/$(m^2)^{1.5}$)	45±9**#** (25-77) [79]	40±7 (29-59) [30]
LVESV (ml)	38±9 (22-71) [79]	39±9 (27-68) [30]
LVESV index (ml/$(m^2)^{1.5}$)	17±4 (13-34) [79]	17±4 (11-25) [30]
Basal anterior WT (mm)	8±1 **#** (6-9) [78]	6±1 (6-7) [30]
Basal inferior WT (mm)	7±1 (5-9) [78]	7±1 (5-8) [30]
Basal septal WT (mm)	7±1 (5-9) [78]	7±1 (6-7) [30]
Basal posterior WT (mm)	7±1 (5-10) [78]	7±1 (6-8) [30]
Mid anterior WT (mm)	7±1 **#** (6-9) [78]	6±1 (6-7) [30]
Mid inferior WT (mm)	7±1 # (5-10) [78]	6±1 (5-7) [30]
Mid septal WT (mm)	7±1 (5-9) [78]	7±1 (6-8) [30]
Mid posterior WT (mm)	7±1 (5-10) [78]	7±1 (6-8) [30]

MeanWT (mm)	7±1 **#** (6-9) [78]	7±1 (6-7) [30]
MeanWT index (mm/(m2)$^{0.50}$)	6±1**#** (5-7) [78]	5±1 (4-6) [30]
MaxWT (mm)	8±1# (7-10) [79]	7±1 (7-8) [30]
MaxWT index (mm/(m2)$^{0.50}$)	6±1# (5-7) [79]	5±1 (5-6) [30]
Relative Wall Thickness	0.31±0.04 (0.21-0.40) [78]	0.29±0.02 (0.25-0.33) [30]
LV Mass (g)	123±22 **#** (85-164) [78]	89±11 (69-100) [30]
LV Mass index (g/(m^2)$^{2.7}$)	31±5 **#** (22-43) [78]	23±3 (17-29) [30]

LVDd- Left ventricular dimension in diastole; LVDs- Left ventricular dimension in systole; WT – wall thickness, # significant difference between the groups (P<0.05).

Table 5.3: Left ventricular functional data in athletes and controls

Variable	Elite Footballers Mean ± SD (Range) [n]	Controls Mean ± SD (Range) [n]
Systolic indices		
Ejection Fraction (%)	62±5 **#** (51-72) [79]	58±5 (49-68) [30]
Stroke Volume (ml)	63±14 **#** (32-94) [79]	55±11 (38-91) [30]
Medial S' (cm/s)	9±2 (7-15) [77]	10±2 (7-16) [29]
Lateral S' (cm/s)	11±2 (6-16) [77]	12±3 (6-23) [29]
Average S' (cm/s)	10±2 (7-14) [77]	11±2 (8-20) [29]
Peak Longitudinal Ɛ (%)	-20±2 **#** (-16 -25) [79]	-17±1 (-14 -20) [29]
Time to Peak Longitudinal Ɛ (ms)	40±9 (30-70) [79]	40±3 (29-41) [29]
Peak Systolic SR (s^{-1})	-0.98±0.20 (-0.75--1.29)	-0.93±0.10 (-0.65—1.45)
Time to Peak Systolic SR (ms)	190±30 (110-260)	170±20 (100-220)
Diastolic indices		
Transmitral E Velocity (cm/s)	100±20 (54-124) [77]	91±16 (66-127) [29]
Transmitral A Velocity (cm/s)	40±12 **#** (20-80) [77]	51±15 (27-76) [29]
Transmitral E: A Ratio	2.42±0.72 (1.24-4.20) [77]	1.96±0.78 (1.23-4.33) [29]
Transmitral E Deceleration Time (ms)	166±32 **#** (105-220) [75]	133±37 (74-195) [26]
Medial E' (cm/s)	14±3 (6-23) [77]	14±3 (6-18) [29]
Lateral E' (cm/s)	19±4 (13-24) [77]	20±5 (6-28) [29]
Average E' (cm/s)	17±3 (10-22) [77]	17±3 (8-21) [29]
Average E/E'	6±1 (3-9) [77]	6±2 (4-10) [29]
Peak Early diastolic SR (s^{-1})	1.62±0.26 **#** (1.27-2.12)	1.47±0.13 (1.05-1.99)
Time to Peak Early Diastolic SR (ms)	690±120 **#** (520-860)	510±30 (420-650)
Medial A' (cm/s)	7±2 (4-15) [77]	8±2 (3-12) [29]

Lateral A' (cm/s)	7±3 **#** (3-19) [77]	9±4 (4-23) [29]
Average A' (cm/s)	7±2 **#** (4-20) [77]	8±2 (4-13) [29]
Peak Late/Atrial diastolic SR (s^{-1})	0.54±0.11 (0.36-0.97)	0.60±0.07 (0.40-0.89)
Time to Peak Late/Atrial diastolic SR (ms)	620±150 **#** (350-1170)	400±30 (200-740)

S'- Peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; E'- Peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A' - Peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; E- Peak velocity of early diastolic transmitral flow; A- Peak velocity of late transmitral flow; E:A- Ratio of E to A; E:E- Ratio of E to E; ; Ɛ- strain; SR strain rate # significant difference between the groups (P<0.05)

5.3.3 Right Ventricle

Parameters of RV structure (Table 5.4) and function (Table 5.5) are presented. Absolute one-dimensional RV structural data were all greater in the elite female footballers (P<0.05). This difference was only maintained in RVD1 and RVD3 after indexing for body size. Absolute and body size adjusted RV areas were not different between groups. No RV systolic functional index (TAPSE, FAC, S') was different between groups. In diastole RV peak lateral wall E' tissue velocity was significantly lower in elite female footballers (P<0.05).

Table 5.4: Right ventricular structural data in athletes and controls

Variable	Elite Footballers Mean ± SD (range) [n]	Controls Mean ± SD (range) [n]
RVOT PLAX (mm)	28±5 (15-36) [79]	26±4 (20-34) [29]
RVOT PLAX (mm/$(m^2)^{0.5}$)	21±3 (12-29) [79]	20±3 (15-24) [29]
RVOT 1 (mm)	28±4 (19-41) [79]	26±4 (21-34) [29]
RVOT1 (mm/$(m^2)^{0.5}$)	21±3 (15-30) [79]	20±3 (10-18) [29]
RVOT 2 (mm)	22±3 (14-29) [79]	22±3 (11-24) [29]
RVOT2 (mm/$(m^2)^{0.5}$)	17±2 (11-23) [79]	16±2 (11-24) [29]
RVD1 (mm)	38±5 **#** (29-49) [79]	30±4 (24-37) [29]
RVD1 (mm/$(m^2)^{0.5}$)	29±3 **#** (22-34) [79]	23±3 (18-29) [29]
RVD2 (mm)	25±3 (19-32) [79]	24±3 (19-31) [29]
RVD2 (mm/$(m^2)^{0.5}$)	19±3 (14-24) [79]	18±2 (15-21) [29]
RVD3 (mm)	82±7 **#** (67-98) [79]	76±6 (59-90) [29]
RVD3 (mm/$(m^2)^{0.5}$)	63±5 **#** (53-76) [79]	57±4 (47-66) [29]
RV Systolic Area (cm^2)	9±2 (5-14) [62]	10±3 (7-16) [29]
RV Systolic Area (cm^2/m^2)	5±1 (3-8) [62]	6±1 (4-9) [29]
RV Diastolic Area (cm^2)	18±3 (14-28) [62]	19±4 (12-28) [29]
RV Diastolic Area (cm^2/m^2)	11±2 (8-13) [62]	11±2 (7-15) [29]

RVOT – Right ventricular outflow tract; PLAX – parasternal long axis; RV- Right Ventricle; RVD- Right ventricular in diastole; # significant difference between the groups ($P<0.05$)

Table 5.5: Right ventricular functional data in athletes and controls

Variable	Elite Footballers Mean ± SD (range) [n]	Controls Mean ± SD (range) [n]
TAPSE (mm)	23±3 (17-29) [79]	21±4 (12-29) [29]
RVFAC (%)	50±6 (33-59) [62]	45±9 (31-68) [29]
RV S' (cm/s)	14±2 (9-21) [65]	14±3 (9-19) [27]
RV E' (cm/s)	15±3 # (7-21) [65]	17±4 (12-25) [27]
RV A' (cm/s)	10±3 (6-16) [65]	11±3 (6-21) [27]

TAPSE- Tricuspid annular plane systolic excursion; RVFAC- Right ventricular fractional area change; RV S'- Peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; RV E'- Peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; RV A' - Peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler; # significant difference between the groups ($P<0.05$)

5.4 Discussion

The key findings of this study were; (1) elite footballers had lower resting HR and BP as well as a smaller body mass; (2) absolute data for a range of LV and RV chamber size indices as well as LV wall thickness' were greater in elite female footballers; (3) global measures of LV function were greater in footballers; and (4) there were only sporadic differences in Doppler, tissue-Doppler and ε indices of LV and RV function. These findings are important to provide insight into the upper normal limits of cardiac structure and function in elite female footballers that can be considered during population specific cardiac PPS.

5.4.1 General training adaptation

Elite female footballers presented with significantly lower resting heart and resting blood pressure compared to sedentary controls. The lower resting heart rate reflected a relatively high proportion of female footballer's demonstrating resting snus bradycardia (Chapter 2) that is a common adaptation to training (George et al., 2011) that has been reported in previous studies of elite male footballers (Spatoro et al., 1985) and recently reported in elite female footballers (Churchill et at., 2020). A reduction in resting heart rate with training likely reflects an enhanced vagal tone (George et al., 2011). A lower systolic and diastolic blood pressure at rest in the footballers is also a common finding with prolonged periods of training (George et al., 2011) and is a likely consequence of training induced changes in vasculature structure and tone (Baggish and Wood, 2011). The lower body mass in footballers could reflect the adaptation to prolonged periods of training or some level of selection bias. Taken together with reported training data, elite female footballers are exposed to physiological stress that could alter cardiac structure and function. Likewise, the subtle differences in body size between groups emphasise the need to appropriately scale cardiac data to aid interpretation (Batterham et al., 1999).

5.4.2 Left ventricular adaptation

To the best of our knowledge this is the first study to compare the AH in elite UK-based female footballers with sedentary controls using comprehensive echocardiographic imaging. Using 2D, Doppler, tissue-Doppler and strain imaging we present extensive evidence of cardiac adaptation that moves on from the information presented in Danish female footballers (Randers et al., 2013) and provides significant additional insight to a recent study in USA female footballers (Churchill et al., 2020).

5.4.2.1 Left ventricular structure

As expected, elite female footballers presented with higher values for LVDd. This difference persisted after body size scaling, and indexed LVEDV was also higher in the footballers. This data supports the difference in LVDd reported by Randles et al. (2013) although the mean LVDd in the current study was 49 mm compared to 51 mm (Randles et al., 2013). The difference in LVEDV sits alongside data from Churchill et al., (2020) who reported that 77% of their female footballers had higher than normal chamber volume data. The between group differences are comparable to previous studies in general male and female athletic populations (Whyte et al., 2004; Utomi et al., 2014) and previous data in elite male footballers (Somauroo et al., 2012). The LV chamber remodelling is thought to be the consequence of the hemodynamic volume overload stress placed on the heart during sustained period of training in a sport with a significant aerobic component to training and game play (Reilly et al., 2002). Other factors that could influence LV dimensions are the lower heart rate at rest in the female footballers and a likely greater blood volume (George et al., 2011) which would both promote greater diastolic filling and thus a larger LV volume at the end of diastole. The upper limit of LV chamber dimension in our elite female footballers was 58 mm. This is similar to data reported in previous empirical studies (Rawlins et al., 2008) and meta-analyses (Whyte et al., 2004) in general female athletes, and is a useful guide when interpreting cardiac PPS in elite female footballers. It should be noted that the absolute values of LV chamber size varied considerably across individuals (42 – 58 mm) likely reflecting individual differences in body size, training exposure and the interaction between genetics and training exposure, such that some athletes will "respond" more to any given stimulus (Kusunose et al., 2013; Doronina et al., 2018).

Data for LV wall thickness was collected across multiple sites to help develop a more extensive database compared to most of the previous male and female AH literature (Whyte et al., 2004; Utomi et al., 2014). In addition, we reported both mean and maximal LVWT because of their relevance to cardiac PPS. Overall, both absolute and scaled LV wall thicknesses were greater in the elite female footballers when compared to controls and this reached statistical significance for the basal anterior septum and the mid anterior septum. The combination of data also led to significantly greater mean WT and maximal WT in elite female footballers (both absolute and body size scaled). The absolute upper limit in this study was 10 mm for LV WT and is very similar to that reported in a general population of elite female athletes (Whyte et al., 2004) and elite female footballers (Randers et al., 2013; Churchill et al., 2020). This again helps inform cardiac PPS in this specific population group. The differences though are important to reflect upon mechanistically even though in descriptive cross-sectional study we can only speculate at the cause(s). Clearly, a larger body size in female footballers cannot be an explanation. Classic AH theory has suggested that increased LV WT is likely to reflect periods of resistance, strength or power training that place a hemodynamic pressure overload on the LV (Morganroth et al., 1975; George et al., 1991). Two points are important to make; (1) the absolute values of WT reported here are substantially smaller than those reported in some resistance trained male athletes (Morganroth et al., 1975), and (2) the existence of a pressure overload physiological adaptation in the AH has been significantly challenged recently (Utomi et al., 2013) largely due to the limited and highly intermittent nature of this hemodynamic stress that is also modulated by Valsalva (Haykowsky et al., 2000). Indeed, the relative WT data in the current study, reflecting the balance between WT and chamber size was not different between athletes and controls. This suggests a normal LV geometry with a lack of concentric adaptation that seems to support recent data in male athletes (Utomi et

al., 2013; Utomi et al., 2014). Finally, the combination of larger LV chamber and wall dimensions contributed to a significantly greater absolute and scaled LV mass in the elite female footballers. This is a very common feature of the AH (George et al., 2011) and has been reported in multiple studies of general male and female athletes (Whyte et al., 2004; Utomi et al., 2014). Again, the upper limit of LV mass in this study (164 g) is within previously published ranges for elite female athletes (Whyte et al., 2004) and contribute to the knowledge base underpinning cardiac PPS in elite female footballers (Randers et al., 2013; Churchill et al., 2021).

5.4.2.2 Left ventricular function

Randers et al. (2013) noted that global measures of LV function tended to be higher in Danish female football players in comparison to untrained young women. The current study confirmed this with a larger SV in the elite female footballers compared to sedentary controls. This is another common AH finding in general populations of, largely aerobically trained, male and female athletes (Whyte et al., 2004; Utomi et al., 2014). The small but significant difference in the footballer's EF (62 cs. 58%) is not a common finding in studies of the AH at rest (Utomi et al., 2012). This difference is, however, close to the day-to-day variability of this index and may simply reflect great contractile activity during systole in a LV that is receiving greater venous return and thus is relatively "stretched". In line with the Frank-Starling laws of the heart, increased filling will then result in increased contractility (independent of any other internal or external effector of cardiac contractile activity).

In developing this topic and building on the work of Randers' we sought to determine other measures of LV systolic and diastolic function to augment global indices of function like SV and EF. Some of this new data provides indices of regional function in the LV. Tissue-Doppler

derived, peak systolic myocardial velocities at the basal septal wall and the basal lateral wall were not different between groups, whereas peak longitudinal Ɛ was higher in the elite footballers (20 vs. 17%). Recent data from Churchill et al., (2020) reported that diastolic function was normal in all athletes, but female footballers had lower lateral LV early tissue Doppler velocity (E'), (18 [17-19] vs 21 [20-22] cm/s; P < .001) than their male footballers.

The lack of difference in tissue Doppler data during systole between groups is novel in this group of female athletes but does mirror data reported in mixed groups of male athletes (Utomi et al., 2012). Peak global longitudinal Ɛ, that occurs during systole has been reported less frequently but in those few studies to employ this technique small or no differences have been reported in the AH (Utomi et al., 2014; Forsythe, et al., 2018; Beaumont et al., 2017). The fact that a small difference is seen in global longitudinal Ɛ but no change is observed in peak longitudinal tissue velocities, both in systole, is likely a consequence of the former measure reflecting functional change across the length of the septum or lateral wall whilst tissue-Doppler velocities are regional measures limited to a small region of interest in the wall. It is also the case that tissue-Doppler data tends to have greater variability, and thus small differences are associated with low statistical "power" in the data.

The most common index of global diastolic function, the ratio of the peak flow velocities during the early and late phases of the left ventricular filling (E/A ratio), was higher in the elite female footballers (2.42 vs. 1.96). This difference was not significant due to the large SD (large inter-individual variability) in this index. This outcome is commonly seen in the AH literature (George et al., 2013) and supports the recent data presented by Churchill et al., (2020). The lack of significant difference between athletes and control in E/A is often attributed to the fact that this measure occurs at rest where an augmentation in diastolic function is not

required to meet filling or ejections demands. Changes in diastolic function during exercise may be more pertinent to performance and the full realisation of the diastolic capacities of the AH (George et al., 2013). Overall, global (Doppler) and regional (tissue-Doppler) indices of early diastolic function were similar between the two groups. Peak E and E' myocardial velocities were not different between groups.

This would suggest that early relaxation is not augmented at rest. This is like data in other athletes (George et al., 1991; Utomi et al., 2012) although some studies have reported enhanced diastolic function at rest (Pavlik et al., 2013; Kasikcioglu et al., 2006; D'Andrea et al., 2007). The lack of difference in the current study, again, likely reflects the lack of need to augment function at rest. The slightly longer deceleration time (of the E wave) is likely attributable to the lower HR in the elite footballers. The ratio E/E', an index of left atrial driving pressure, was not different between groups and likely contributes to the lack of difference in flow and tissue-Doppler indices in early diastole.

Some sporadic differences were noted between groups in indices of atrial (A, A') flow and tissue velocities. Peak A (flow because of atrial contraction) and peak A' (tissue velocities during atrial contraction) were higher in the controls compared to the elite female footballers. This has been reported in previous AH studies (George et al., 2013) but is not a consistent finding. In essence, it is suggested that higher flow and tissue velocities during atrial contraction in the sedentary controls is seen as a compensatory response to reduced early filling seen in some sedentary group, so as to meet overall filling demands of the LV. As Doppler and tissue-Doppler data do not reflect volumes of flow, this is hard to substantiate. We do know that A and A' increase with heart rate (Giannaki et al., 2008) and thus the higher values in the controls could simply reflect the higher resting heart rate in this group.

5.4.3 Right Ventricular structure

Randers et al., (2013) and Churchill et al., (2020) only reported structural and functional data related to the LV of elite female footballers. Consequently, this chapter builds on available knowledge in this group of athletes. In recent years, more data have been reported referring to the structural and functional adaptation of the RV in the general AH literature. This is because the RV should be involved in any athletic adaptation (acute or chronic) and can be the site for pathology development (George et al., 2011). Because of geometric differences, compared to the LV, and potential challenges in non-invasive imaging, the database of the RV in the AH is smaller than the LV. Despite this there is evidence that the RV chamber is larger in male athletes compared to controls (Metaxas et al., 2000; D'Andrea et al., 2007; D'Andrea et al., 2013; Forsythe et al., 2019) and this has been extended in a limited number of studies in female athletes (Rawlins et al., 2009). The current study adds new and unique data related to RV structure in elite female footballers. Apart from RV areas, where data reflects a smaller sample size and greater variability in measurement, all RV dimensions were significantly higher in the elite footballers, compared to sedentary controls. Some of these differences were maintained after scaling for body size differences. Increased RV dimensions in the athletes (e.g., RVOTPLAX) suggests a proportional enlargement of the RV alongside LV adaptation. This global hypertrophy of both the RV and LV is commonly reported in male athletes (Utomi et al., 2012; Utomi et al., 2013) and likely reflects that both ventricles are subject to a hemodynamic volume overload stimulus during training exposure. Although some suggest the RV may develop, further with training than the LV (La Gerche et al., 2012) this is not obvious in the current data set. The upper limits recorded for absolute RV dimensions again provides insight and information that will be useful in cardiac PPS in this specific group (Oxborough et al., 2012).

5.4.4 Right ventricular function

Assessment of RV function, like structural measurement, is complex and different to the LV as geometry and mechanics are unique, and imaging is more complex. Despite this, elements of RV function can be determined and contribute to a broader understanding of the AH phenotype in these athletes (Oxborough et al., 2012) as well as when attempting to differentiate physiological RV remodelling from diseases such as ARVC (Marcus et al., 2010) that can predispose to SCD. Previous research suggests that a multi-factorial approach to RV functional assessment should include global indices such as TAPSE and RVFAC as well as regional tissue-Doppler myocardial velocities in the free wall (Qasem et al. 2016). In the current study no between group differences were noted for TAPSE, RVFAC and peak S'. This is similar to previous work in general male (Oxborough et al., 2012) and female (Rawlins et al., 2009) athletic populations.

Diastolic data were also similar between groups. Even A and A' were not different despite a higher heart rate in sedentary controls. This suggests that the RV functional data at rest are potentially less sensitive to training stimuli than the LV, although it is not uncommon to see similar peak A' velocities in groups of different training status in the literature (George et al., 2013). Also, of interest, and somewhat counterintuitively, was a lower RV peak E' in the footballers compared to the controls. This did not match data in the LV and is less common than augmented (or no change) in early diastolic function seen in previous athlete-control studies (George et al., 2013). The explanation for these findings is unclear although we are aware that small, negative changes in RV function may occur associated with recent bouts of acute exercise (Lord et al., 2014). This data requires follow-up confirmation and further investigation.

5.5 Implications, limitations and future research

The findings from the current study provide confirmatory as well as new/novel data pertaining to the AH in elite female footballers that may contribute to cardiac PPS, specifically to the differential diagnosis of AH and cardiomyopathies in elite female athletes. The upper limit of structural and functional data presented in 79 elite female footballers is a valuable indicator of the range of physiological adaptation to intensive sport-specific training.

Despite a moderately large sample size of 79, this is still lower than some multi-sports studies in the literature (Rawlins et al., 2008). Thus, the current sample size of elite female footballers represents something of a limitation. Specifically, AH data in elite female footballers of different ages, ethnicities and/or geographic regions will be useful future additions to the literature. Despite novel data on RV structure and function as well as the use of tissue-Doppler and Ɛ imaging, further development or technical insights might be useful, including 3D structural assessment and looking at twist mechanics in the LV. Further assessment of the atria in elite female footballers would provide a more complete cardiac assessment that can feed through to cardiac PPS.

Further investigations are warranted to characterize cardiac function of elite female footballer's during exercise. We must take into consideration as well, that several other factors than the character of sports activity determine the development of the AH and its assessment in PPS, such as hormonal variation in female athletes due to menstrual cycle phase or oral contraceptive use. Understanding cardiac structural and functional variation during different phases of the menstrual cycle could not be deduced in the current study, as

these issues could not practically be controlled during testing. This provides the specific rationale for the research undertaken in the next chapter.

5.6 Conclusions

The findings obtained in the current study suggest that long-term training resulted in myocardial adaptation in both the LV and RV of elite professional female footballers that constitute a specific AH phenotype and upper limits that will feed into cardiac PPS. The structural presentation of the AH in elite female footballers suggests an eccentric remodelling within an overall normal geometry. To the best of our knowledge, this is the first study to comprehensively assess the AH in elite female footballers and thus this work should serve as a springboard for ongoing study in these areas.

Chapter 6

Variability in resting cardiac structure and function across the menstrual cycle

6.1 Introduction

Historically, women's participation in competitive sport has lagged behind that of men with female athletes subjected to a variety of inequitable practices (Costello et al., 2014). Part of this inequality was a long-held belief that competitive sport could negatively affect female reproductive health (De Souza et al., 2010). Whilst there is some concern about the impact of elite athletic participation on menstrual cycle function (O'Donnell et al., 2011), it is now the consensus that women should have the same sporting opportunities as men. Whilst prejudices and problems still exist, there is a narrowing of gender inequality in terms of sports participation, elite competitive opportunities, financial rewards, media coverage and audience make up (Smith et al., 2013; Capranica et al., 2001; 2002; 2013). For example, the 2012 Summer Olympics in London were the first games in which every participating country included a female competitor (Kian et al., 2013).

With growing elite level participation in female sports, such as in women's professional association football, there has been a concomitant development in research and clinical enquiry into relevant topics (Lopez, 1997; Pfister, 2003; Bruinvels et al., 2016). One specific issue of interest is related to the impact of exercise participation on menstrual cycle function (Datson et al., 2014,) and the parallel issue of the effect of menstrual cycle phase or status on athlete performance or physiology (Bailey et al., 2000; Constantini et al., 2005). Interest in the association between menstrual cycle phase and cardiovascular physiology is based on knowledge that there are oestrogen receptors in cardiovascular organs/tissues (Christensen et al., 2012) and that epidemiological data would suggest oestrogen exerts a substantial cardio-protective effect in adult women (Owen et al., 2005; Wake et al., 2009) that is lost after menopause. Consequently, several observational studies have demonstrated that

oestrogen could be involved in the regulation of cardiac structure and function in animal (Gorodeski et al., 1990; Magness & Rosenfeld, 1989; Malhotra et al., 1990) and human models (Garavaglia et al., 1989; Paterni et al., 2014). This interest has, however, not been extended to the potential for menstrual cycle phase to mediate cardiovascular assessments incorporated in cardiac PPS.

Our understanding of the impact of menstrual cycle phase on cardiovascular structure and function is complicated by contradictory data. Fuenmayor et al., (2000) observed a significant increase in the E/A ratio, as an index of LV diastolic function, during the LP of the menstrual cycle in 20 healthy eumennorheic women. In addition, Zengin et al., (2007) reported an increase in peak early diastolic filling flow velocity during the LP in 27 healthy eumenorrheic women. Conversely, George et al., (2000) reported no significant differences in multiple indices of LV function between the FP and LP. Likewise, Hirose et al., (2017) reported no significant differences in either conventional or 2D-STE parameters of diastolic function between the FP and LP. Whilst these data provide a useful initial insight into this topic, early research has been limited by; (1) a focus on just 2 phases of the menstrual cycle (no data associated with ovulation), (2) a focus on LV function with no RV data, and (3) a focus on standard 2D or Doppler functional data (no tissue-Doppler data). Of interest to PPS there is limited data related to the impact of menstrual cycle phase on ECG parameters outside of the clinical setting (Rosano et al., 1996; Burke et al., 1997; Hulot et al., 2003).

Thus, the aim of this study was to: (1) identify the impact of menstrual cycle phase (mid-luteal, ovulation, mid-follicular) on cardiac electrical activity (12 lead ECG) in eumenorrheic, healthy women, and (2) document variability in cardiac structure and function (comprehensive

echocardiographic assessment) across menstrual cycle phases in the same cohort. These data will help inform testing plans and data analysis in cardiac PPS in elite female athletes.

6.2 Methods

6.2.1 Participants and Design

Seventeen healthy, eumenorrheic, female participants volunteered to participate in the study. The mean (± SD) age for the cohort was 20 (± 2) years. Inclusion criteria required all participants to self-report regular, consistent and pain free menstrual cycles for the previous 12 months, no oral contraceptive use in the previous 2 years, no other current prescription drug use, that they were non-smokers, that they had maintained a consistent level of physical activity (<3 hrs per week) for the previous 12 months and that they had no personal or early family history of cardiovascular disease. Participants were provided with full details of all experimental procedures and provided written informed consent to participate in the study. Liverpool John Moores University Ethical Committee approved experimental procedures and protocols for this research.

This study employed a repeated measures design where all participants presented at the Cardiovascular Laboratory for data collection at all-time points. These occurred with a random (menstrual cycle phase) initial test based upon when participants were recruited, followed by the other two test points in time order. Tests took place in; (a) the follicular phase (FP) 1-5 days after cessation of menses when circulating oestrogen and progesterone concentrations are both low (Vollman, 1977; Fehring et al., 2006; Reed and Carr, 2018); (b) during ovulation (OP) 12-14 days after the cessation of menses, when circulating oestrogen

concentration is high and progesterone concentration is low (Su et al., 2017; Hilgers and Bailey, 1980) and finally; (c) in the luteal phase (LP) 21-24 days after the previous menses, when both circulating oestrogen and progesterone concentrations are high (Ecochard et al., 2001; Fraser et al., 2011). These selected time periods have previously been shown to identify time points when oestrogen and progesterone levels vary (Minson et al., 2000; Nielsen et al., 2001; Kim et al., 2012; Hartwich et al., 2013, Bull et al., 2019). The time for testing in the OP was also confirmed using ovulation kits (Clearblue®; SPD Swiss Precision Diagnostics GmbH, Petit Lancy, Switzerland). This product detects the rise in the luteinising hormone (LH) during ovulation.

Participants were requested to have eaten their normal meal prior to testing but to refrain from caffeinated beverages, alcohol consumption and strenuous physical activity for at least 12 hours before any testing. We could not control time-of-day of testing between subjects due to participant availability but within subject, all tests occurred in either morning or afternoon testing slots.

6.2.2 Procedures

At each visit to the laboratory, the same testing procedures were employed in the same test order. Anthropometry: All participants had body mass assessed using standard scales (Seca 217, Hannover, Germany) and height measured using a stadiometer (Seca Supra 719, Hannover, Germany). Body surface area (BSA) was calculated by using a standardised formula (Mosteller, 1987). This facilitated within and between participant data comparison by facilitating the scaling of data for individual differences in body size.

Blood pressure: Systolic and diastolic brachial artery blood pressure were measured using an automated sphygmomanometer (DINAMAP 300, GE medical system, Milwaukee, Wisconsin,

USA). Blood pressure data were recorded as the lowest values from a minimum of two repeat blood pressure assessment after 5 minutes of quiet, seated rest.

Electrocardiography: All participants undertook a standard resting 12-lead electrocardiogram (CardioExpress SL6, Spacelab Health care, Washington, US). Electrode placement was undertaken by a trained cardiac physiologist. Data analysis was carried out in accordance with International Criteria (Sharma et al., 2017) for ECG interpretation. The ECG was analysed by an experienced sports cardiologist who confirmed the presence or absence of (likely) training, borderline, and non-training related ECG changes, as in previous chapters.

Echocardiography: A full echocardiographic assessment was undertaken by a single experienced sonographer using a commercially available ultrasound system (Vivid Q, GE Healthcare, Horten, Norway) and a 1.5-4 MHz phased array transducer. All image acquisition occurred with the participant in the left lateral decubitus position and in accordance with American Society of Echocardiography (ASE; Lang et al., 2015) guidelines. Images were stored in a raw DICOM format and exported to an offline workstation (EchoPac version 6.0, GE Healthcare, Horten, Norway) for subsequent analysis. All data was analysed by the researcher under the supervision of a single experienced sonographer.

Standard measurements of LV structure and function were in accordance with ASE guidelines (Lang et al., 2015) and followed previous protocols detailed in study 3. Strain and strain data were not recorded in this study. Measures of RV structure and function are also adopted the protocols outlined in study 3.

6.2.3 Data Analysis

All data were managed using REDCAP electronic data capture tools hosted at Liverpool John Moores University (Harris et al., 2009). Cohort data are presented as mean ± SD, as well as ranges. Analysis of data between menstrual cycle phases was undertaken using repeated-measures ANOVAs and pairwise Bonferroni post-hoc testing where a significant main effect for cycle phase was present. Statistical analysis was completed using a commercially available software package, SPSS Version 25.0 for Windows (IBM Corporation, Somers, NY, USA). For all analyses, a probability value <0.05 was considered statistically significant.

6.3 Results

6.3.1 Anthropometric and Blood Pressure Data

There were no significant differences ($P>0.05$) in height, weight, BSA and BP across the three menstrual phases (Table 6.1)

Table 6.1: Cohort anthropometric and cardiovascular data across menstrual cycle phases

Variable	FP Mean ± SD (Range) [n]	OP Mean ± SD (Range) [n]	LP Mean ± SD (Range) [n]
Height (m)	1.66±0.08 (1.53 - 1.78) [17]	1.66±0.08 (1.53 - 1.78) [17]	1.66±0.08 (1.53 - 1.78) [17]
Body mass (kg)	70±12 (49 - 94) [17]	70±12 (49 - 94) [17]	70±12 (49 - 94) [17]
BSA (m^2)	1.8±0.2 (1.5-2.0) [17]	1.8±0.2 (1.5-2.0) [17]	1.8±0.2 (1.5-2.0) [17]
Heart Rate (beats/min)	76±13 (50 - 99) [17]	76±13 (52 - 102) [17]	75±13 (49 - 101) [17]
Systolic BP (mmHg)	122±10 (100-137) [13]	120±6 (110-132) [13]	123± 12 (108-150) [13]
Diastolic BP (mmHg)	71±8 (55-85) [13]	69±9 (55-82) [13]	68±9 (56-85) [13]

FP – Follicular phase; OP –Ovulation phase; LP – Luteal phase; BSA–Body Surface Area; BP-Blood Pressure

ECG data, for continuous variables, are detailed in Table 6.2. There was no significant main effect of menstrual cycle phase on this data and all durations, axes and amplitudes were within normal limits. ECG data pertinent to the International Criteria are contained in Tables 6.3 and 6.4. Early repolarisation and sinus bradycardia were the most common "training related" changes and were relatively consistent across FP, OP and LP (Table 6.3). Sinus arrhythmia was observed in one participant at the OP assessment but was not apparent at either the FP or LP assessment. There were no borderline or abnormal findings in any participant during any phase of the menstrual cycle.

Table 6.2: Cohort ECG data, continuous variables, across three menstrual cycle phases

Variable	FP Mean ± SD (Range) [n]	OP Mean ± SD (Range) [n]	LP Mean ± SD (Range) [n]
Heart rate (beats.min^{-1})	73±17 (38-107) [17]	74±17 (44-103) [17]	76±17 (44-101) [17]
P Duration (ms)	95±26 (0-118) [17]	98±9 (83-112) [17]	97±14 (74-110) [17]
PR Interval (ms)	147±42 (0-194) [17]	149±21 (97-180) [17]	149±24 (98-182) [17]
QRS Duration (ms)	87±8 (74-105) [17]	87±8 (73-105) [17]	89±10 (71-110) [17]
QT Interval (ms)	383±47 (324-526) [17]	380±51 (312-516) [17]	378±44 (325-484) [17]
QT Corrected (Bazett) (ms)	414±14 (400-451) [17]	417±15 (399-454) [17]	411±22 (343-454) [17]
P Axis (°)	40±27 (-19-77) [17]	38±32 (-27-74) [17]	34±42 (-90-76) [17]
QRS Axis (°)	68±20 (26-97) [17]	65±25 (26-98) [17]	67±21 (31-99) [17]
T Axis (°)	38±21 (-19-68) [17]	37±21 (-20-70) [17]	39±20 (-19-66) [17]
Voltage: RV_1 + SV_5 (mV)	2.3±0.8 (1.0-3.0) [17]	2.3±0.8 (1.0-4.0) [17]	2.4±0.7 (1.0-4.0) [17]
Voltage: SV_1 +RV_5 (mV)	9.9±4.0 (5.0-17.0) [17]	10.0±3.6 (5.0-18.0) [17]	9.5±4.0 (5.0-15.0) [17]

P- Duration; PR- Interval; QRS- Duration; QT- Interval; QT- Corrected; P, QRS, T – Axis; R - R wave; S – wave; V1- Chest lead 1; V5- Chest lead 5

Table 6.3: Prevalence of training-related ECG changes in the cohort across the menstrual cycle – International standard ECG criteria interpretation (Sharma et al., 2017).

	Training-Related ECG Findings		
	FP	OP	LP
Increased QRS voltage	0	0	0
Incomplete right bundle branch block	0	0	0
Early repolarisation	1(6%)	1(6%)	1(6%)
Black athlete repolarisation variant	0	0	0
Juvenile T wave pattern	0	0	0
Sinus bradycardia	4(24%)	5(29%)	4(24%)
Sinus arrhythmia	0	1(6%)	0
Ectopic atrial rhythm	0	0	0
Junctional escape rhythm	0	0	0
1° atrioventricular block	0	0	0
Mobitz type 1 (Wenkebach) 2° atrioventricular	0	0	0

Table 6.4: Prevalence of borderline and abnormal ECG findings across the menstrual cycle - International standard ECG criteria interpretation (Sharma et al., 2017).

	Borderline ECG Findings		
	FP	**OP**	**LP**
Left axis deviation	0	0	0
Left atrial enlargement	0	0	0
Right axis deviation	0	0	0
Complete right bundle branch block	0	0	0

Table 6.5: Prevalence of abnormal non-training-related ECG changes in the cohort across the menstrual cycle – International standard ECG criteria interpretation (Sharma et al., 2017).

	Abnormal ECG Findings		
	FP	OP	LP
T wave Inversion	0	0	0
ST segment depression	0	0	0
Pathological Q waves	0	0	0
Complete left bundle branch block	0	0	0
Profound non-specific intraventricular conduction delay	0	0	0
Epsilon wave	0	0	0
Ventricular pre-excitation	0	0	0
Prolonged QT interval	0	0	0
Brugada type 1 pattern	0	0	0
Profound sinus bradycardia	0	0	0
Profound 1° atrioventricular block	0	0	0
Mobitz type II 2° atrioventricular block	0	0	0
3° atrioventricular block	0	0	0
Atrial tachyarrhythmias	0	0	0
Premature ventricular contractions	0	0	0
Ventricular arrhythmias	0	0	0

6.3.2 Echocardiography: Left Ventricle

Numerous parameters of LV structure are presented in Table 6.6. LV chamber dimensions in diastole and systole were marginally larger in OP and LP compared to FP, even after adjusting for body size (P<0.0.5). Whilst there was a significant main effect for menstrual cycle phase for the measurement of mean wall thickness (with values in OP higher than both FP and LP) the absolute difference is very small and is lost when data is appropriately rounded up (decimal places). Absolute and indexed LV mass was higher in the OP phase compared to FP and LP (P<0.05).

Table 6.6: Left ventricular structural data across the menstrual cycle

Variable	FP Mean ± SD (Range) [n]	OP Mean ± SD (Range) [n]	LP Mean ± SD (Range) [n]
LVDd (mm)	44±2 **#** (40-48) [17]	45±2 (42 - 50) [17]	46±2 (42 - 49) [17]
LVDd index (mm/$(m^2)^{0.5}$)	33±2 **#** (30-38) [17]	34±2 (31-39) [17]	34±2 (31-38) [17]
LVDs (mm)	29±4 **#** (23-38) [17]	30±4 (24 - 39) [17]	30±4 (26-39) [17]
LVDs index (mm/$(m^2)^{0.5}$)	21±3 **#** (16-30) [17]	22±3 (17-30) [17]	22±3 (19-31) [17]
LVEDV (ml)	101±16 (75-130) [17]	101±17 (31-62) [17]	100±18 (73-139) [17]
LVEDV index (ml/$(m^2)^{1.5}$)	42±6 (33-57) [17]	42±7 (32-59) [17]	42±7 (30-59) [17]

LVESV (ml)	41±8 (30-63) [17]	41±9 (31-62) [17]	41±10 (27-68) [17]
LVESV index (ml/(m^2)$^{1.5}$)	17±3 (13-23) [17]	17±3 (12-23) [17]	17±4 (11-24) [17]
Basal anterior WT (mm)	6±1 (6-7) [17]	6±1 (6-7) [17]	6±1 (6-7) [17]
Basal inferior WT (mm)	7±1 (5-7) [17]	7±1 (6-7) [17]	7±1 (5-7) [17]
Basal septal WT (mm)	7±1 (6-7) [17]	7±1 (6-8) [17]	7±1 (6-7) [17]
Basal posterior WT (mm)	7±1 (6-8) [17]	7±1 (6-8) [17]	7±1 (6-8) [17]
Mid anterior WT (mm)	7±1 (6-7) [17]	7±1 (6-8) [17]	7±1 (6-7) [17]

LVDd- Left ventricular dimension in diastole; LVDs- Left ventricular dimension in systole; LVEDV; Left ventricular end-diastolic volume; LVESV; Left ventricular End-Systolic Volume WT – wall thickness, # significant difference between the groups (P<0.05).

Table 6.7: Left Ventricular functional data across the menstrual cycle

Variable	**FP** **Mean ± SD** **(Range) [n]**	**OP** **Mean ± SD** **(Range) [n]**	**LP** **Mean ± SD** **(Range) [n]**
Systolic Indices			
Ejection Fraction (%)	59±5 (52-69) [17]	59±5 (51-68) [17]	58±5 (49-68) [17]
Stroke Volume (ml)	59±11 (41-90) [17]	60±12 (41-91) [17]	58±12 (38-91) [17]
Medial wall peak S' tissue velocity(cm/s)	9±2 **#** (6-15) [17]	10±2 (7-15) [17]	10±2 (7-16) [17]
Lateral wall peak S' tissue velocity (cm/s)	12±3 (7-22) [17]	13±3 (8-21) [17]	13±3 (8-23) [17]

Average S' (cm/s)	8±2 **#** (6-11) [17]	11±2 (8-18) [17]	11±3 (8-20) [17]
Diastolic Indices			
Transmitral peak E flow velocity (cm/s)	0.9±0.2 (0.7-1.2) [17]	1.0±0.2 (0.8-1.2) [17]	1.0±0.2 (0.7-1.2) [17]
Transmitral peak A flow velocity (cm/s)	0.5±0.1 (0.3-0.8) [17]	0.5±0.1 (0.3-0.7) [17]	0.5±0.1 (0.3-0.8) [17]
Transmitral E: A Ratio	2.0±0.9 (1.3-4.3) [17]	2.0±0.9 (1.3-4.3) [17]	2.0±0.9 (1.3-4.3) [17]
Transmitral E Deceleration Time (ms)	129±38 (76-195) [17]	130±36 (79-193) [17]	130±39 (74-195) [17]
Medial wall peak E' tissue velocity (cm/s)	13±3 **#** (7-17) [17]	13±3 (8-18) [17]	14±3 (6-18) [17]
Lateral wall peak E' tissue velocity (cm/s)	20±4 (10-26) [17]	19±4 (10-27) [17]	21±4 (10-28) [17]
Average E' (cm/s)	16±3 **#** (9-20) [17]	16±3 (9-21) [17}	17±3 (8-21) [17]
Medial wall peak A' tissue velocity (cm/s)	8±2 (5-12) [17]	8±2 (6-13) [17]	8±2 (5-12) [17]
Lateral wall peak A' tissue velocity (cm/s)	8±2 (5-13) [17]	8±2 (5-13) [17]	8±2 (5-13) [17]
Average A' (cm/s)	8±2 (6-11) [17]	8±2 (6-11) [17]	8±2 (6-11) [17]
Average E/E'	6±2 (4-12) [17]	6±2 (4-12) [17]	6±2 (4-13) [17]

S'- systolic velocity; E'- early diastolic velocity; A' – late/atrial diastolic velocity; E- early diastolic transmitral flow; A- late/atrial transmitral flow; # significant difference between the groups ($P<0.05$)

6.3.3 Echocardiography: Right Ventricle

Parameters of RV structure are presented in Table 6.8 with RV functional data in Table 6.9. Absolute RVOT PLAX and RVOT 1 were lower in FP compared to both OP and LP ($P<0.05$). Absolute RVD2 was larger in OP than FP and LP ($P<0.05$), whereas RVD3 was larger in LP compared to OP and FP ($P<0.05$). No RV functional index was significantly mediated by menstrual cycle phase.

Table 6.8: Right ventricular structural data across the menstrual cycle

Variable	FP Mean ± SD (Range) [n]	OP Mean ± SD (Range) [n]	LP Mean ± SD (Range) [n]
RVOT PLAX (mm)	26±4 **#** (20-32) [16]	27±4 (21-33) [16]	27±4 (21-34) [16]
RVOT PLAX (mm/$(m^2)^{0.5}$)	19±3 (15-23) [16]	20±2 (16-24) [16]	15±2 (11-18) [16]
RVOT 1 (mm)	26±4 **#** (20-32) [16]	27±4 (21-33) [16]	27±4 (21-34) [16]
RVOT1 (mm/$(m^2)^{0.5}$)	19±3 (14-24) [16]	20±2 (15-22) [16]	20±3 (15-25) [16]
RVOT 2 (mm)	22±4 (15-33) [16]	23±4 (15-33) [16]	23±4 (15-33) [16]
RVOT2 (mm/$(m^2)^{0.5}$)	16±3 (11-24) [16	17±3 (11-24) [16]	17±3 (11-24) [16)
RVD1 (mm)	30±3 (23-35) [16]	30±4 (21-25) [16]	31±3 (24-37) [16)
RVD1 (mm/$(m^2)^{0.5}$)	23±2 (17-26) [16]	23±3 (17-26) [16]	24±2 (18-27) [16]
RVD2 (mm)	24±3 **#** (19-31) [16]	25±4 (19-33) [16]	24±4 (19-31) [16]

RVD2 (mm/$(m^2)^{0.5}$)	18±3 (15-23) [16]	19±3 (15-24) 16]	18±3 (15-23) [16]
RVD3 (mm)	75±5 **#** (66-82) [16]	75±5 (69-85) [16]	77±5 (69-85) [16]
RVD3 (mm/$(m^2)^{0.5}$)	56±4 (47-63) [16]	57±4 (48-64) [16]	57±4 (49-66) [16]
RV Diastolic Area (cm^2)	19±3 (15-25) [17]	20±3 (14-26) [17]	20±4 (14-25) [17]
RV Diastolic Area (cm^2/m^2)	11±2 (8-14) [17]	11±2 (8-15) [17]	11±2 (8-15) [17]
RV Systolic Area (cm^2)	10±3 (6-15) [17]	10±3 (7-16) [17]	10±3 (7-16) [17]
RV Systolic Area (cm^2/m^2)	6±2 (3-9) [17]	6±2 (4-10) [17]	6±2 (4-10] [17]

RVOT – Right ventricular outflow tract; PLAX – parasternal long axis view; RVD- Right ventricular dimension in diastole; # significant difference between the groups (P<0.05)

Table 6.9: Right ventricular functional data across the menstrual cycle

Variable	**FP** **Mean ± SD** **(Range) [n]**	**OP** **Mean ± SD** **(Range) [n]**	**LP** **Mean ± SD** **(Range) [n]**
TAPSE (mm)	20±4 (12-25) [16]	20±3 (12-25) [16]	21±4 (12-27) [16]
RVFAC (%)	50±11 (30-70) [16]	49±10 (27-65) [17]	48±9 (32-68) [17]
RV S' (cm/s)	13±2 (8-17) [16]	13±3 (8-17) [16]	13±3 (9-18) [16]
RV E' (cm/s)	17±4 (8-17) [16]	17±4 (13-23) [16]	17±4 (13-24) [16]
RV A' (cm/s)	11±3 (6-17) [16]	11±3 (5-17) [16)	11±3 (6-17) [16]

TAPSE- Tricuspid annular plane systolic excursion; FAC- fractional area change; S'- Peak velocity of systolic mitral annular motion as determined by pulsed wave Doppler; E'- Peak velocity of early diastolic mitral annular motion as determined by pulsed wave Doppler; A' - Peak velocity of diastolic mitral annular motion as determined by pulsed wave Doppler;

6.4 Discussion

The key findings from this study were (1) there were no meaningful differences in continuous and International Criteria ECG data across different phases of the menstrual cycle, and (2) whilst there were some significant differences in cohort data for LV/RV structural and functional data across the menstrual cycle, these differences were small and likely within the normal variation of specific indices (and within resolution limits of measurement tools), and suggesting this likely has no significant clinical impact. Overall, performing ECG and echocardiographic screening assessments at different stages of the menstrual cycle will likely have little or no impact on decision making in cardiac PPS.

6.4.1 ECG data

The lack of change in ECG parameters across the menstrual cycle supports two specific studies of QT interval duration that did not vary across menstrual cycle phases (Burke et al., 1997; Hulot et al., 2003). In a clinical setting, Rosano et al., (1996) observed a significant positive correlation between plasma progesterone concentration and the number and duration of episodes of supraventricular tachycardia during the FP and LP of the menstrual cycle, but this is an uncommon finding in young, healthy females and thus of limited relevance in athlete cardiac PPS. It is highly likely that in young, active, healthy women with normal menstrual cycles, the variation in concentrations of circulating female sex hormones has a limited effect on cardiac electrical activity. This means that there is likely a negligible effect of physiological reproductive hormone variation on the ECG component of cardiac PPS in female participants although the current study should be replicated in elite athletes. After repeating this study in an elite athlete female cohort, cardiac PPS could be undertaken at any time point in the

menstrual cycle with no great concern that menstrual cycle phase would mediate the outcome of ECG testing.

Of interest, we observed several normal or likely "training-related" ECG patterns in young, healthy eumenorrheic women who are not highly trained elite athletes. The prevalence was, however, lower than that observed in female athlete groups (Rawlings et al., 2008; Chapter 3). The absence of borderline or abnormal ECG findings in the current cohort reflects a healthy group with no silent or family history of early cardiovascular disease.

6.4.2 LV structure and function

The results of this study demonstrated some small but statistically significant differences in LV structure and function at different phases of the menstrual cycle. Initially, this seems to be in direct opposition to the earlier work of George et al., (2000) and Hirose et al., (2016) who reported no significant variation in global measures of LV structure and function between the FP and LP. These authors suggested that the influence of variations in oestrogen and progesterone on LV structure and function were of minimal significance (George et al., 2000). Differences in sample sizes, development of testing quality and guidelines, the use of various methods to define testing times within the menstrual cycle may partially explain the disparate findings between studies. On reflection, however, the statistically significant differences reported in the current study were of a limited absolute and relative magnitude. Specifically, whilst there is a pattern of larger LV dimension (chamber size) during the OP and LP compared to the FP, it should be noted that these differences, in absolute terms, are small, within the likely daily biological variation of the indices and the measurement error of the tools/protocols, and thus likely of no significant scientific or clinical impact (Lang et al., 2015).

The overall conclusions of the present study are, therefore, largely in line with the previous work of George et al., (2000) and Hirose et al., (2016).

It is important to note that some previous work has reported isolated differences in LV structure and function across the menstrual cycle. Feunmayor et al., (2000) and Zengin et al., (2007) both reported that the mitral E/A ratio was significantly increased during the LP, suggesting enhanced cardiac diastolic function. It was speculated in both studies that the increase in oestrogen levels during the LP could mediate an increase in LV diastolic function though an indirect vasodilator effect on the vascular system although no evidence was produced to support this supposition. We did not observe any change in E/A ratio across the menstrual cycle and it is likely that the high variability of this index could lead to sporadic findings of significance in some studies but not others. It is surprising to note previous work highlighted diastolic functional changes independent of any effect on HR.

Although likely to be physiologically and clinically of little relevance or impact, the small rise in chamber dimensions at OP and LP could be linked to increases in oestrogen that could be associated with an increase in blood volume (Colau et al., 1983; Cardoso et al., 1997). Small differences were also noted in medial wall peak S' and average S' across the menstrual cycle with lower values in the FP. In diastole, we observed a slightly higher peak E' in the OP and LP compared to the FP that may simply reflect the structural variation in LV dimensions. These differences, like those in LV structure, this would not likely impact in any meaningful way on cardiac PPS outcomes.

6.4.3 RV structure and function

In the present study, small variations in RV structure were noted across menstrual cycle phase that mirrored the small changes seen in the LV (minor increase in values in the LP). This data adds to previous LV focussed studies (George et al., 2000; Hirose et al., 2016) and suggests that despite minor variations in mean cohort data this would likely have minimal impact on clinical decision making, especially in the context of a cardiac PPS. In a similar vein to the LV, the minor alterations in RV structure in the LP could be explained by small blood volume shifts due to the influence of estrogen (Colau et al., 1983; Cardoso et al., 1997). This should be confirmed in future studies.

Most previous studies of cardiac function across the menstrual cycle have also focused on the LV (George et al., 2000; Hirose et al., 2016) with only Zengin et al., (2007) documenting measures of RV function in 27 healthy women in the FP and LP. They reported that RV tissue-Doppler-derived peak myocardial velocities during systole and diastole were higher in the LP. This difference may be explained by small differences in sample size, demographics or the development of technical measurement but should be confirmed with further research. The lack of change in RV function across the menstrual cycle is largely in line with small and clinically irrelevant changes in LV function during FP, OP and LP. This would suggest that RV functional indices are not influenced by physiological variation in female reproductive hormones that occur in a normal healthy menstrual cycle. Again, this has important implications for data interpretation and the practicalities of cardiac PPS in females.

6.5 Limitations

A small sample of healthy but relatively inactive females was studied and this data may not to be representative of female athlete groups presenting for cardiac PPS. Future work should look at larger samples of female athletes in different sporting disciplines where menstrual cycle irregularities, due to high level training volumes, have been commonly reported.

A further limitation of this study is that it recruited Caucasian participants only. Given the importance of ethnicity in both female participation in elite sport and as a key issue in cardiac PPS (Sharma et al., 2017), the current work should be developed in diverse ethnic samples.

In this research, an imprecise method of predicting menstrual cycle phase by counting days from the onset of menses was employed and no plasma or whole blood hormone concentrations were measured (Janse de Jonge, 2003). Day counting errors, from researcher or participant, could have affected the results if any miscalculation of dates occurred. From the perspective of technical echocardiographic measurement, whilst images for post-hoc strain analysis were recorded data corruption during storage prevented this analysis. Strain and SR imaging using speckle tracking echocardiography (STE) would provide a more complete and regional description of cardiac mechanics across the menstrual cycle and should be included in future research.

6.6 Conclusions

This study identified; (1) no significant impact of menstrual cycle phase (FP, OP and LP) on 12 lead ECG data in eumenorrheic, healthy women, and (2) only small variations (within day-to-day biological variability and technical resolution limits) in LV/RV structure and function across menstrual cycle phases in the same cohort. These data will help inform testing plans and data analysis in cardiac PPS in elite female athletes. Performing cardiac PPS does not need to be consistently performed in any specific phase of the menstrual cycle if the female participants are young, healthy and eumenorrheic.

CHAPTER 7

GENERAL DISCUSSION

7.1 Summary of the findings

Due to limited literature regarding the AH in elite female footballers, this thesis represents novel data related using comprehensive ECG and echocardiographic imaging. A short summary finding of each study is presented below.

Study 1: I described the 12-lead ECG of elite female football players and then interpreted the data in relation to different criteria used in PPS. Eighty-one elite female footballers (21±4 yrs) completed a medical assessment form, anthropometrics, blood pressure, and a resting 12-lead ECG. Each 12-lead ECG was interpreted in accordance with; 1) European Society of Cardiology (ESC) 2) Seattle, and 3) International Criteria to determine training-related and non-training related ECG patterns. Eighty percent (80%) had training-related ECG patterns. Sinus bradycardia (65%) and early repolarization (42%) were the most common. Using the ESC Criteria, 25% (20/81) of the athletes were considered to have an abnormal ECG, compared to 0% using the Seattle and International Criteria. Training-related ECG changes are very common in elite female footballers and The Seattle and Refined/International ECG Criteria significantly reduced the number of ECG false-positives. Both points are relevant for PPS.

Study 2: I determined the seasonal variation in the 12-lead ECG of elite female footballers. Thirteen elite female footballers (26±4 yrs) had a standard 12-lead ECG interpreted at three different time points (pre-season, mid-season, and end-season) across a single competitive season. Minimal changes were observed in numerical or waveform pattern ECG data across a competitive season in elite female footballers. This suggests that the acute and/or transient changes in (as well as accumulation off) exercise frequency, intensity, and volume across a competitive season in elite female footballers do not represent a large enough physiological

stimuli to mediate changes in the athletes' ECG. The time point of PPS within a competitive season in elite female footballers is unlikely to alter any clinical outcome.

Study 3: I investigated cardiac structure and function in elite female footballers, to determine the sport-specific upper limits of adaptation to long-term training. Seventy-nine elite female footballers (21±4 yrs) and thirty sex and age-matched sedentary controls (22±4 yrs) were recruited. Standard measurements of LV and RV structure and function was made in accordance with ASE guidelines (Lang et al., 2015; Rudski et al., 2010). LV and RV structures tended to be bigger in elite female footballers compared to controls with some functional data higher in the athletes. The AH phenotype upper limits in female footballers will feed into PPS in this group.

Study 4: I assessed cardiac structure and function across the menstrual cycle in healthy females. Seventeen healthy, eumenorrheic, female participants (20 ± 2 yrs) volunteered to participate and completed a medical assessment form, anthropometrics, blood pressure, a resting 12-lead ECG and a standard echocardiographic at three different time points of menstrual cycle: (1) the follicular phase, (2) during ovulation and (3) in the luteal phase. No significant differences in International Criteria ECG data were observed across different phases of the menstrual cycle. Small variations in LV and RV structure and function were detected (within day-to-day biological variability and technical resolution limits) across menstrual cycle phases in the same cohort. These data will inform testing plans and data analysis in cardiac PPS in elite female athletes, but it is likely thar menstrual cycle phase is not an important consideration in the timing of PPS events.

7.2 General issues across studies

The novel findings of this thesis are the predominance of a normal ECG, normal LV/RV geometry and normal LV/RV function (at rest) in elite female footballers. The limited expression of "abnormalities" in the cardiac phenotype of elite female footballers is reassuring from the perspective of PPS and SCD.

Several relevant issues have emerged from the combination of individual studies that are worth raising here before we address the clinical implications, limitations and future research issues that have emerged. The issue of research design (cross-sectional versus prospective or interventional studies) is important to reflect upon. Most of the data feeding into PPS comes from cross-sectional studies of athletes (see first and third empirical chapter) as this represents the reality of PPS assessment in athletes. The upper limit of structural and functional data presented in elite female footballers are valuable indicators of the range of physiological adaptation to intensive training in elite female footballers and so will directly inform PPS. I did use a prospective approach in the seasonal variation of ECG study and the menstrual cycle phase. Additional insights might be gained from looking at cardiac structure and function (echocardiography) across a competitive season, or indeed across years of training accumulation.

We employed current state of the art in technology and assessment criteria to provide new and informative data. New and novelty in echo and ECG techniques are constantly evolving and further studies could provide more insight as technologies develop. WE did not employ 3D echo or MRI in these studies as there are logistical limitations to any work, but this could provide important and novel data, especially in relation to atrial structure and function.

The importance of appropriate scaling of cardiac structure and function is apparent in the final two studies. Outcome data must be accurately and consistently evaluated so interpretation is clear and valid. Any outcome with significant clinical implications, for the athlete and sports cardiologist, should be based on best practice and clear and valid procedures. In the world of sports cardiology, the use and application of scaling of data is not consistent and often poorly interpreted. Any data that informs cardiac screening of female football athletes to identify those at risk of SCD, must scale data on an evidence-base and/or consensus process. This area of study needs more empirical work and expert discussion.

7.3 Clinical Implications

Overall, the results of this thesis provide a useful reassessment of concepts and models in the AH literature, and in elite female footballers specifically. The findings have expanded our understanding of the impact of elite football training, seasonal variation, menstrual cycle and imaging technologies upon ECG and echocardiography data. The results of these empirical studies provide relevant information on the upper limits of cardiac adaptation in elite female footballers that importantly transfer to organising and running PPS in this group:

a) International ECG criteria should be adopted in PPS.

b) Training-related ECG findings in elite female footballers are common and mirror those observed in other male and female athletes.

c) PPS can be undertaken at any stage in the competitive season for elite female footballers without unduly influencing clinical decision making.

d) Resting cardiac structure and function in elite female footballers adhere to current "general athlete" data related to upper normal limitations of phenotypical presentation.

e) It is highly likely that PPS can be undertaken at any point of a normal menstrual cycle, although further studies in athletes are needed for adoption of this suggestion by Football clubs, the FA, UEFA and FIFA.

7.4 Limitations and Future Research

Several possible limitations apply to the present studies and where appropriate, specific future directions are raised.

1. Our small samples may limit the ecological or external validity of the data. With a larger sample, the research will continue to help define the AH of elite female footballers. Larger and more diverse samples (see below) will contribute to the on-going development of ECG criteria in elite female footballer during PPS, and specifically more structural and functional data (via echocardiography) and ECG data will inform the differential diagnosis of AH and cardiomyopathies in elite female athletes.

2. Our samples were largely mono-ethnic (Caucasian) and should be reproduced in different ethnic elite female football groups. Football is multicultural, multi-ethic and is now played all over the globe. Given the importance of ethnicity in mediating SCD as well as female participation in elite sport (Sharma et al., 2017), the current work should be viewed as a useful beginning in this field and sport.

3. Whilst our descriptive studies of the AH in elite female footballers suggest that time of season and time of menstrual cycle may not mediate data collected and interpreted in PPS,

more work in this area of specific organisational guidelines around consistency of screening time, is required. We should look to assess the impact of time of day of testing. Further, we should repeat the menstrual cycle study with athlete groups as well as determine the impact of oral contraceptive use and/or menstrual dysrhythmias.

4. From the perspective of technical echocardiographic measurement, whilst images for post-hoc strain analysis were recorded data corruption during storage prevented this analysis. In future research, Ɛ and SR imaging using speckle tracking echocardiography (STE) should be included to provide a complete and regional description of cardiac mechanics. Despite the novel data on RV structure and function, as well as the use of tissue-Doppler and Ɛ imaging, further development or technical insights might be useful, including 3D structural assessment and looking at twist mechanics in the LV. Further assessment of the atria in elite female footballers would provide a more complete cardiac assessment that can feed through to cardiac PPS. This knowledge will further aid the diagnostic challenges associated with PPS in athletes, and the protection against SCD.

7.5 Conclusions

The research presented in the thesis is the first to characterise the elite female football player's ECG whilst comparing published interpretation criteria. The findings from the seasonal variation of the ECG in elite female footballer's studies are likely to develop our understanding of structure and function of the AH with high dynamic and static components of training among elite female footballers. Further, use of standard and novel echocardiographic techniques provided additional understanding of the normal physiological adaptation of the AH in elite female footballers which will influence decision making in PPS of

female athlete groups. Finally, ECG and echocardiography data did not differ between phases of the menstrual cycle that would impact on PPS.

We have succeeded in providing answers to the aims set out at the beginning of the thesis.

1) Chapter 3: We critically evaluated the 12-lead ECG of the elite female footballer and determined the impact of different ECG interpretation criteria used in PPS.

2) Chapter 4: We assessed the effect of competitive season stage on the ECG of elite female footballers.

3) Chapter 5: We determined the cardiac structure and function of elite female footballers using standard and novel echocardiographic techniques in comparison to age-matched healthy controls.

4) Chapter 6: We assessed the variability in resting ECG as well as cardiac structure and function across three phases of the menstrual cycle in young healthy women.

Chapter 8

References

Aagaard P., Baranowski B., Aziz P. and Phelan D. 2016. Early repolarization in athletes: a review. *Circulation* Arrhythmia Electrophysiology, 9(3): e003577.

Activepeople.sportengland.org.

Anderson, J. B., Grenier, M., Edwards, N. M., Madsen, N. L., Czosek, R. J., Spar, D. S., Barnes, A., Pratt, J., King, E. and Knilans, T. K. 2014. Usefulness of combined history, physical examination, electrocardiogram, and limited echocardiogram in screening adolescent athletes for risk for sudden cardiac death. *American Journal of Cardiology*, 114, 1763–1767.

Antzelevitch, C., Pollevick, G. D., Cordeiro, J. M., Casis, O., Sanguinetti, M. C., Aizawa, Y., Guerchicoff, A., Pfeiffer, R., Oliva, A., Wollnik, B., Gelber, P., Bonaros, E. P., Jr, Burashnikov, E., Wu, Y., Sargent, J. D., Schickel, S., Oberheiden, R., Bhatia, A., Hsu, L. F., Haïssaguerre, M., Wolpert, C. 2007. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation*, *115*(4), 442–449.

Babiker F. A., De Windt L. J., Van Eickels M., Grohe C., Meyer R. and Doevendans P. A., 2002. Estrogenic hormone action in the heart: regulatory network and function. *Cardiovascular Research*, 15; 53(3):709-19.

Baggish A. and Thompson P. D. 2009. Thick hearts, high stakes, great uncertainties: screening athletes for hypertrophic cardiomyopathy. *Circulation*, 95:345-347.

Baggish, A. L. and Wood, M. J. 2011. Athlete's heart and cardiovascular care of the athlete: scientific and clinical update. *Circulation*, 123, 2723–2735.

Bailey, S. P., Zacher, C. M. and Mittleman, K. D. 2000. Effect of menstrual cycle phase on carbohydrate supplementation during prolonged exercise to fatigue. *Journal of Applied Physiology* (1985), 88(2):690-7.

Bangsbo, J., Gollnick, P. D., Graham, T. E. and Saltin, B. 1991. Substrates for muscle glycogen synthesis in recovery from intense exercise in man. *Journal of Applied Physiology*, 434:423-40.

Barbier, J., Ville, N., Kervio, G., Walther, G. and Carre, F. 2006. Sports-specific features of athlete's heart and their relation to echocardiographic parameters. *Herz,* 31: 531–543

Barbieri R. L. 2014. The endocrinology of the menstrual cycle. *Methods in molecular biology (Clifton, N.J.)*, *1154*, 145–169.

Baron, RC, Thacker, SB, Gorelkin, L., Vernon, A. A., Taylor, W. R. and Choi, K. 1983. Sudden death among Southeast Asian refugees. An unexplained nocturnal phenomenon. *JAMA,* 2; 250(21):2947-51.

Basavarajaiah, S., Wilson, M., Whyte, G., Shah, A., Behr, E. and Sharma, S. 2007. Prevalence and significance of an isolated long QT interval in elite athletes. *European Heart Journal*, 28, 2944-9.

Basavarajah, S., Wilson, M., Whyte, G., Shah, A., Mckenna, W. and Sharma, S. 2008. Prevalence of hypertrophic cardiomyopathy in highly trained athletes: relevance to pre-participation screening. *Journal American Collection Cardiology,* 51, 1033-9.

Basilico, F. C. 1999. Cardiovascular disease in athletes. *The American Journal of Sports Medicine*, *27*(1), 108–121.

Basso, C., Corrado, D., Marcus, F. I., Nava, A. and Thiene, G. 2009. Arrhythmogenic right ventricular cardiomyopathy. *The Lancet*, 373, 1289–1300.

Basso, C., Corrado, D. and Thiene, G. 2007. Arrhythmogenic right ventricular cardiomyopathy in athletes: diagnosis, management, and recommendations for sport activity. *Cardiology Clinicians,* 25, 415-422.

Basso, C., Thiene, G., Corrado, D., Angelini, A., Nava, A. and Valente, M. 1996. Arrhythmogenic right ventricular cardiomyopathy dysplasia, dystrophy, or myocarditis? *Circulation,* 94, 983-991

Basso, C. and Thiene, G. 2006. The pathophysiology of myocardial reperfusion: a pathologist's perspective. *Heart (British Cardiac Society)*, *92*(11), 1559–1562.

Batterham, A. M., George, K. P., Whyte G., Sharma S. and McKenna W. 1999. Scaling cardiac structural data by body dimensions: a review of theory, practice, and problems. *International Journal Sports Medicine,* 20(8):495-502.

Bauce, B., Rampazzo, A., Basso, C., Mazzotti, E., Rigato, I., Steriotis A., Beffagna, G., Lorenzon, A., De Bortoli, M., Pilichou, K., Marra, M. P., Corbetti, F., Daliento, L., Iliceto, S., Corrado, D., Thiene, G. and Nava, A. 2000. Clinical phenotype and diagnosis of arrhythmogenic right ventricular cardiomyopathy in pediatric patients carrying desmosomal gene mutations. *Heart Rhythm*, *8*:1686–1695

Bazett, H. C. 1920. The time relations of the blood-pressure changes after excision of the adrenal glands, with some observations on blood volume changes. *Journal of Applied Physiology*; 53: 320–39.

Beaumont, A., Grace, F., Richards, J., Hough, J., Oxborough, D. and Sculthorpe, N. 2017. Left ventricular speckle tracking-derived cardiac strain and cardiac twist mechanics in athletes: a systematic review and meta-analysis of controlled studies. *Sports Medicine*, 47, 1145-1170.

Bille K., Figueiras D., Schamasch P., Kappenberger L., Brenner J. I., Meijboom F. J. and Meijboom E. J. 2006. Sudden cardiac death in athletes: the Lausanne recommendations. *European Journal of Cardiovascular Prevention and Rehabilitation*, 13(6):859-75.

Bohm, M., Borer, J., Ford, I., Gonzalez-Juanatey, J. R., Komajda, M., Lopez-Sendon, J., Reil, J. C., Swedberg, K. and Tavazzi, L. 2013. Heart rate at baseline influences the effect of ivabradine on cardiovascular outcomes in chronic heart failure: analysis from the SHIFT study. *Clinical Research in Cardiology*, 102(1):11-22.

Borjesson, M. and Pelliccia, A. 2009. Incidence and aetiology of sudden cardiac death in young athletes: an international perspective. *British Journal Sports Medicine*, 43(9):644-8.

Brosnan, M., La Gerche, A., Kalman, J., Lo, W., Fallon, K., MacIsaac, A and Prior D. 2014. The Seattle Criteria increase the specificity of preparticipation ECG screening among elite athletes. *British Journal Sports Medicine*, 48(15):1144-50.

Brown, B., Somauroo, J., Green, D. J., Wilson, M., Drezner, J., George, K. and Oxborough, D. 2017. The complex phenotype of the athlete's heart: implications for preparticipation screening. *Exercise and Sport Sciences Reviews*, 45, 96–104.

Boyett, M. R., D'Souza, A., Zhang H., Morris, G. M., Dobrzynski, H. and Monfredi, O. 2013. Viewpoint: is the resting bradycardia in athletes the result of remodelling of the sinoatrial node rather than high vagal tone*? Journal of Applied Physiology* (1985), 114(9):1351-5.

Brugada, P. and Brugada, J. 1992. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome: a multicentre report. *Journal of the American College of Cardiology*; 20, 1391-1396.

Brugada, P., Brugada, R., Mont, L., Rivero, M., Geelen, P and Brugada J. 2003. Natural history of Brugada syndrome: the prognostic value of programmed electrical stimulation of the heart**.** *Journal Cardiovascular Electrophysiology*, 14: 455-457

Bruinvels, G., Burden, R. J., McGregor, A. J., Ackerman, K. E., Dooley, M. and Richards T. 2016. Sport, exercise and the menstrual cycle: where is the research? *British Journal Sports Medicine*.

Brus, A. B. and Trangbæk, E. 2004. Asserting the right to play. Women's Football in Denmark. *Soccer and Society*, *4*(2/3), 95-111.

Bull, J. R., Rowland, S. P., Scherwitzl, E. B., Scherwitzl, R., Danielsson, K. G. and Harper, J. 2019. Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. *NPJ digital medicine*, *2*, 83.

Burke, J. H., Ehlert, F. A., Kruse, J.T., Parker, M. A., Goldberger, J. J., and Kadish. 1997. AH. Gender-specific differences in the QT interval and the effect of autonomic tone and menstrual cycle in healthy adults. *American Journal Cardiology*, Jan 15; 79(2):178-81.

Cabanelas N., Freitas, S. and Gonçalves, L. 2013. Morphological and functional changes in athletes' hearts during the competitive season. *Portuguese Journal of Cardiology*, 32(4):291-6

Can, F., Yilmaz I. and Erden, Z. 2004. Morphological characteristics and performance variables of women soccer players. *Journal of Strength and Conditioning Research*, 18(3):480-5.

Capranica, L. and Aversa, F. 2002. Italian television sport coverage during the 2000 Sydney Olympic Games: A gender perspective. *International Review for the Sociology of Sport*, 37: 337–349.

Capranica, L., Aversa, F. and Franzen, A. 2001. Italian sport newspaper coverage of women's sports during the 1996 Atlanta Olympic Games. *Italian Journal of Sport Sciences*, 2: 30–34.

Capranica, L., Piacentini, M. F., Halson, S., Myburgh, K. H., Ogasawara, E., and Millard-Stafford, M. 2013. The gender gap in sport performance: equity influences equality. *International Journal of Sports Physiology and Performance*, 99-103,

Cardiac Risk at Young, 2015 Cardiac Risk in the Young (CRY). 2015. 12 Young sudden deaths a week [online] Available from: www.c-r-y.org.uk/ Accessed 08/10/2015.

Cardoso, F., Polónia, J., Santos, A., Silva-Carvalho, J., and Ferreira-de-Almeida, J. 1997. Low-dose oral contraceptives and 24-hour ambulatory blood pressure. *International journal of*

gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics, *59*(3), 237–243.

Christensen, A., Bentley, G. E, Cabrera, R., Ortega, H. H., Perfito N., Wu, T. J. and Micevych P. 2012. Hormonal regulation of female reproduction. *Hormone and Metabolic Research,* 44(8):587-91.

Church, T. S., Cheng, Y. J., Earnest, C. P., Barlow, C. E., Gibbons, L. W., Priest, E. L. and Blair S. N. 2004. Exercise capacity and body composition as predictors of mortality among men with diabetes. *Diabetes Care*, 27(1):83-8

Churchill, T. W., Groezinger, E., Loomer, G. and Baggish A. L. 2020. Exercise-induced cardiac remodelling during adolescence. *European Journal of Preventive Cardiology*, 27(19):2148-2150.

Churchill, T. W., Petek, B. J., Wasfy, M. M., Guseh, J. S., Weiner, R. B., Singh, T. K., Schmied, C., O'Malley, H., Chiampas, G., and Baggish, A. L. (2021). Cardiac structure and function in elite female and male soccer players. *JAMA cardiology*, *6*(3), 316–325.

Colau, J. C., de Gennes, J. L., Gheron, G. and Taurelle, R. 1983. Etude de la fonction ovarienne résiduelle sous estroprogestatifs minidosés (Residual ovarian function after minidoses of estroprogestative agents). *Journal de gynecologie, obstetrique et biologie de la reproduction*, *12*(5), 465–471.

Constantini, N. W., Dubnov, G., Lebrun, C. M., 2005. The menstrual cycle and sport performance. *Clinical Sports Medicine*, 24(2):e51–82.

Corîci, O. M., Mirea-Munteanu, O., Donoiu, I., Istrătoaie, O., Corîci, C. A. and Lancău, M. 2018. Gender-related electrocardiographic changes in athletes. *Current Health Science Journal*, 44(1):29-33.

Corrado, D., Basso, C., Pavei, A., Michieli, P., Schiavon, M. and Thiene, G. 2006. Trends in sudden cardiovascular death in young competitive athletes after implementation of a preparticipation screening program. *JAMA*, *296*(13), 1593–1601.

Corrado, D., Basso, C., Rizzoli, G., Schiavon, M. and Thiene, G. 2003. Does sports activity enhance the risk of sudden death in adolescents and young adults? *Journal of the American College of Cardiology*, 42, 1959–1963.

Corrado, D., Basso, C., Schiavon, M., Pelliccia, A. and Thiene, G. 2008. Pre-participation screening of young competitive athletes for prevention of sudden cardiac death. *Journal of the American College of Cardiology,* 52, 1981–1989

Corrado, D., Basso, C., Schiavon, M. and Thiene, G. 1998. Screening for hypertrophic cardiomyopathy in young athletes. *New England Journal of Medicine*, 339, 364-369.

Corrado, D., Basso, C. and Thiene, G. 2011. Comparison of United States and Italian experiences with sudden cardiac deaths in young competitive athletes: are the athletic populations comparable? *American Journal of Cardiology*, 105, 421-422.

Corrado, D., Basso, C., Thiene, G., McKenna, W.J., Davies, M.J., Fontaliran, F., Nava, A., Silvestri, F., Blomstrom-Lundqvist, C., Wlodarska, E.K., Fontaine, G. and Camerini, F. 1997. Spectrum of clinicopathologic manifestations of arrhythmogenic right ventricular

cardiomyopathy/dysplasia: a multicentre study. *Journal of the American College of Cardiology*, 30, 1512-1520.

Corrado, D. and McKenna W. J. 2007. Appropriate interpretation of the athlete's electrocardiogram saves lives as well as money. *European Heart Journal,* 28(16):1920-2.

Corrado, D., Pelliccia, A., Bjørnstad, H. H., Vanhees, L., Biffi, A., Borjesson, M., Panhuyzen-Goedkoop, N., Deligiannis, A., Solberg, E., Dugmore, D., Mellwig, K. P., Assanelli, D., Delise, P., Van-Buuren, F., Anastasakis, A., Heidbuchel, H., Hoffmann, E., Fagard, R., Priori, S. G., Basso, C., Arbustini, E., Blomstrom-Lundqvist, C., McKenna, W. J. and Thiene, G. 2005. Cardiovascular pre-participation screening of young competitive athletes for prevention of sudden death: proposal for a common European protocol - consensus statement of the study group of sport cardiology of the working group of cardiac rehabilitation and exercise physiology and the working group of myocardial and pericardial diseases of the European Society of Cardiology. *European Heart Journal*, 26, 516–524.

Corrado, D., Pelliccia, A., Heidbuchel, H., Sharma, S., Link, M., Basso, C., Biffi, A., Buja, G., Delise, P., Gussac, I., Anastasakis, A., Borjesson, M., Bjørnstad, H. H., Carrè, F., Deligiannis, A., Dugmore, D., Fagard, R., Hoogsteen, J., Mellwig, K. P., Panhuyzen-Goedkoop, N. 2010. Section of sports cardiology, European association of cardiovascular prevention and rehabilitation. 2010. Recommendations for interpretation of 12-lead electrocardiogram in the athlete. *European Heart Journal*, *31*(2), 243–259.

Corrado, D., Schmied, C., Basso, C., Borjesson, M., Schiavon, M., Pelliccia, A., Vanhees, L. and Thiene G. 2011. Risk of sports: do we need a pre-participation screening for competitive and leisure athletes? *Eur Heart J*, 32(8):934-44.

Corrado, D., Thiene, G., Cocco, P. and Frescura, C. 1992. Non-atherosclerotic coronary artery disease and sudden death in the young. *British Heart Journal*, 68, 601-607.

Costello, J.T., Bieuzen, F., Bleakley, C. M., 2014. Where are all the female participants in sports and exercise medicine research? *European Journal Sport Science*, 14(8):847-51.

D'Andrea, A., Caso, P., Salerno, G., Scarafile, R., De Corato, G., Mita, C., Di Salvo, G., Severino, S., Cuomo, S., Liccardo, B., Esposito, N. and Calabrò R. 2007. Left ventricular early myocardial dysfunction after chronic misuse of anabolic androgenic steroids: a Doppler myocardial and strain imaging analysis. *British Journal Sports Medicine,* 41(3):149-55.

D'Andrea, A., Riegler, L., Golia, E., Cocchia, R., Scarafile, R., Salerno, G., Pezzullo, E., Nunziata, L., Citro, R., Cuomo, S., Caso, P., Di Salvo, G., Cittadini, A., Russo, M. G., Calabrò, R. and Bossone, E. 2013. Range of right heart measurements in top-level athletes: the training impact. *International Journal of Cardiology*, 164, 48–57.

D'Ascenzi, F., Pelliccia, A., Alvino, F., Solari, M., Loffreno, A., Cameli, M., Focardi, M., Bonifazi M. and Mondillo S. 2015. Effects of training on left ventricular strain in competitive athletes. *Heart,* 101(22):1834-9.

Datson, N., Hulton, A., Andersson, H., Lewis, T., Weston, M., Drust, B. and Gregson, W. 2014. Applied physiology of female soccer: an update. *Sports medicine (Auckland, N.Z.)*, *44*(9), 1225–1240.

Davis, J. A. and Brewer, J. 1993. Applied physiology of female soccer players. *Sports Medicine Journal,* 16(3):180-9.

Decree of the Italian Ministry of Health, February 18, 1982. Rules concerning the medical protection of athletic activity. Gazzetta Ufficiale della Repubblica Italiana, 5:63.

De Souza, M. J., Toombs, R. J., Scheid, J. L., O'Donnell, E., West, S. L. and Williams, N. I. 2010. High prevalence of subtle and severe menstrual disturbances in exercising women: confirmation using daily hormone measures. *Human reproduction (Oxford, England)*, *25*(2), 491–503.

Drezner, J. A., Ackerman, M. J., Anderson, J., Ashley, E., Asplund, C. A., Baggish, A. L., Börjesson, M., Cannon, B. C., Corrado, D., DiFiori, J. P., Fischbach, P., Froelicher, V., Harmon, K. G., Heidbuchel, H., Marek, J., Owens, D. S., Paul, S., Pelliccia, A., Prutkin, J. M., Salerno, J. C., Wilson, M. G. 2013. Electrocardiographic interpretation in athletes: the 'Seattle criteria'. *British journal of sports medicine*, *47*(3), 122–124.

Drezner, J. A. 2008. A Contemporary approach to the identification of athletes at risk for sudden cardiac death, *Current Opinion in Cardiology*, p 494-501

Drezner, J. A. and Khan, K. 2008. Sudden cardiac death in young athletes. *British Medicine Journal*, 337(7661):a309.

Driscoll, D., and Dicicco, G. 2000. The effects of metronome breathing on the variability of autonomic activity measurements. *Journal of manipulative and physiological therapeutics*, *23*(9), 610–614.

Doronina A., Édes, I. F., Ujvári, A., Kántor, Z., Lakatos, B. K., Tokodi, M., Sydó, N., Kiss, O., Abramov, A., Kovács, A. and Merkely, B. 2018. The female athlete's heart: comparison of cardiac changes induced by different types of exercise training using 3D echocardiography. *Biomed Research International,* 28; 2018:3561962

Dvorak, J., Grimm, K., Schmied, C. and Junge, A. 2009. Development and implementation of a standardized precompetition medical assessment of international elite football players -2006 FIFA World Cup Germany. *Clinical Journal of Sport Medicine*, 19, 316-321.

Dvorak, J., Junge, A., Derman, W, Schwellnus, M. 2011. Injuries and illnesses of football players during the 2010 FIFA World Cup. *British Journal Sports Medicine*, 45(8):626-30.

Ecochard, R., Boehringer, H., Rabilloud, M., and Marret, H. 2001. Chronological aspects of ultrasonic, hormonal, and other indirect indices of ovulation. *BJOG: An International Journal of Obstetrics and Gynaecology*, *108*(8), 822–829.

Fagard R. 2003. Athlete's heart. *Heart (British Cardiac Society)*, *89*(12), 1455–1461.

Fagard, R., Aubert, A., Lysens, R., Staessen, J., Vanhees, L. and Amery, A. 1983. Noninvasive assessment of seasonal variations in cardiac structure and function in cyclists. *Circulation,* 67(4):896-901.

Ferin M. 1999. Clinical review 105: Stress and the reproductive cycle. *The Journal of Clinical Endocrinology and Metabolism*, *84*(6), 1768

Fehring, R. J., Schneider, M. and Raviele, K. 2006. Variability in the phases of the menstrual cycle. *Journal Obstetric Gynecol Neonatal Nursing,* 35(3):376-84.

FIFA, (2014) FIFA Women's Football Survey.
https://www.womeninfootball.co.uk/news/2014/03/07/women-in-football-survey-2014/

Finocchiaro, G., Papadakis, M., Robertus, J. L., Dhutia, H., Steriotis, A. K., Tome, M., Mellor, G., Merghani, A., Malhotra, A., Behr, E., Sharma, S. and Sheppard, M. N. 2016. Etiology of sudden death in sports insights from a United Kingdom regional registry. *Journal of the American College of Cardiology*, 67, 2108–2115.

Finocchiaro, G. and Sharma, S. 2016. Do endurance sports affect female hearts differently to male hearts? *Future Cardiology*, *12*(2), 105–108.

Finocchiaro, G., Papadakis, M., Sharma, S. and Sheppard, M. 2017. Sudden Cardiac Death. *European Heart Journal*, *38*(17), 1280–1282.

Football Association (FA) (2005) 'Football for All', URL (Consulted June 2008): http://www.thefa.com/TheFA/EthicsandSportsEquity/RacialEquality/Postings/2005/10/KickIt Out_WeekofAction.htm

Forsythe, L., MacIver, D. H., Johnson, C., George, K., Somauroo, J., Papadakis, M., Brown, B., Qasem, M. and Oxborough, D. 2018. The relationship between left ventricular structure and function in the elite rugby football league athlete as determined by conventional echocardiography and myocardial strain imaging. *International Journal of Cardiology*, 261, 211–217.

Forsythe, L., Somauroo, J., George, K., Papadakis, M., Brown, B., Qasem, M. and Oxborough, D. 2019. The right heart of the elite senior rugby football league athlete. *Echocardiography*, 36(5):888-896.

Fraser, I. S., Römer, T., Parke, S., Zeun, S., Mellinger, U., Machlitt, A. and Jensen, J. T. 2011. Effective treatment of heavy and/or prolonged menstrual bleeding with an oral contraceptive containing estradiol valerate and dienogest: a randomized, double-blind Phase III trial. *Human reproduction (Oxford, England)*, *26*(10), 2698–2708.

Fuenmayor, A. J., Ramírez, L. and Fuenmayor, A.M. 2000. Left ventricular function and autonomic nervous system balance during two different stages of the menstrual cycle. *International Journal of Cardiology*, *72*(3), 243–246.

Gajda, R., Kowalik, E.,, Rybka S., Rębowska, E., Śmigielski, W., Nowak, M., Kwaśniewska, M., Hoffman, P. and Drygas ,W. 2019. Evaluation of the Heart Function of Swimmers Subjected to Exhaustive Repetitive Endurance Efforts During a 500-km Relay. *Front Physiol*, 22; 10:296.

Gati, S., Chandra, N., Bennett, R. L., Reed, M., Kervio, G., Panoulas, V. F., Ghani, S., Sheikh, N., Zaidi, A., Wilson, M., Wilson, M., Papadakis, M., Carré, F. and Sharma, S. 2013. Increased left ventricular trabeculation in highly trained athletes: do we need more stringent criteria for the diagnosis of left ventricular non-compaction in athletes? *Heart (British Cardiac Society),* 99, 401–8.

George, K. P., Birch, K. M., Jones, B. and Lea, R. 2000. Estrogen variation and resting left ventricular structure and function in young healthy females. *Medicine and Science in Sports and Exercise*, *32*(2), 297–303.

George, K, P., Gates, P. E., Whyte, G., Fenoglio, R. A. and Lea, R. 1999. Echocardiographic examination of cardiac structure and function in elite cross-trained male and female Alpine skiers. *British Journal Sports Medicine,* 33, 93-8.

George, K. P., Spence, A., Naylor, L. H., Whyte, G. P., and Green, D. J. 2011. Cardiac adaptation to acute and chronic participation in endurance sports. *Heart (British Cardiac Society)*, *97*(24), 1999–2004.

George, K. P., Whyte G., Green, D., Oxborough, D., Shave, R, Gaze, D. and Somauroo J. 2012. The endurance athlete's heart: acute stress and chronic adaptation. *British Journal Sports Medicine,* 46

Giannaki, C. D., Oxborough, D. and George, K. P. 2008. Diastolic Doppler flow and tissue Doppler velocities during, and in recovery from, low-intensity supine exercise. *Application Physiology Nutrition Metabolic*, 33(5):896-902.

Giraldeau, G., Kobayashi, Y., Finocchiaro, G., Wheeler, M., Perez, M., Kuznetsova, T., Lord, R., George K. P., Oxborough, D., Schnittger, I., Froelicher, V., Liang, D., Ashley, E and Haddad F. 2015. Gender differences in ventricular remodelling and function in college athletes, insights from lean body mass scaling and deformation imaging. *American Journal Cardiology,* 116(10):1610-6

Gorodesk, G. I., Sheean, L. A and Utian, W. H. 1990. Myometrial oxytocin receptors levels in the pregnant rat are higher in distal than in proximal portions of the horn and correlate with disparate oxytocin responsive myometrial contractility in these segments. *Endocrinology,* 127(3):1136-43.

Harmon, K. G., Asif, I. M., Klossner, D. and Drezner, J. A. 2011. Incidence of sudden cardiac death in national collegiate athletic association athletes. *Circulation,* 123, 1594-600

Harmon, K. G., Drezner, J. A., Wilson, M. G. and Sharma, S. 2014. Incidence of Sudden Cardiac Death in Athletes. *Heart,* 100, 1227-1234.

Harmon, K. G., Zigman, M. and Drezner, J. A. 2015. The effectiveness of screening history, physical exam, and ECG to detect potentially lethal cardiac disorders in athletes: A systematic review/meta-analysis. *Journal of Electrocardiology*, 48, 329–338.

Harris, P. A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N. and Conde, J. G. 2009. Research electronic data capture (REDCap)-A metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, 42, 377–381.

Hartwich, D., Aldred, S., and Fisher, J. P. 2013. Influence of menstrual cycle phase on muscle metaboreflex control of cardiac baroreflex sensitivity, heart rate and blood pressure in humans. *Experimental physiology*, *98*(1), 220–232.

Haykowsky, M. J., Quinney, H. A., Gillis, R. and Thompson, C. R. 2000. Left ventricular morphology in junior and master resistance trained athletes. *Medicine and Science in Sports and exercise*, 32, 349–52.

Higgins, J. P. and Andino, A. 2013. Soccer and sudden cardiac death in young competitive athletes: a *review. Journal Sports Medicine* (Hindawi Publication Corporate), 967183.

Hilgers, T. W. and Bailey, A. J. 1980. Natural family planning. II. Basal body temperature and estimated time of ovulation. *Obstetrics and Gynaecology*, *55*(3), 333–339.

Hirose, K., Daimon, M., Miyazaki, S., Chiang, S. J., Morimoto-Ichikawa, R., Maruyama, M., Kawata, T., Ohmura, H. and Daida, H. 2017. Estrogen variation during the menstrual cycle does not influence left ventricular diastolic function and untwisting rate in premenopausal women. *Journal Cardiology*, 69(1):389-393.

Holst, A. G., Winkel, B. G., Theilade, J., Kristensen, I. B., Thomsen, J. L., Ottesen, G. L., Svendsen, J. H., Haunsø, S., Prescott, E., & Tfelt-Hansen, J. 2010. Incidence and etiology of sports-related sudden cardiac death in Denmark-implications for pre-participation screening. *Heart rhythm*, *7*(10), 1365–1371.

Hulot, J. S., Démolis, J. L., Rivière, R., Strabach, S., Christin-Maitre, S. and Funck-Brentano C. 2003. Influence of endogenous oestrogens on QT interval duration. *European Heart Journal,* 24(18):1663-7.

Hyde, N., Prutkin, J. M. and Drezner, J. A. 2019. Electrocardiogram interpretation in NCAA athletes: comparison of the 'Seattle' and 'international' criteria. *Journal Electrocardiology*, 56: 81- 84.

Ingebrigtsen, J., Dillern, T. and Shalfawi, S. A. 2011. Aerobic capacities and anthropometric characteristics of elite female soccer players. *Journal Strength Conditioning Research,* 25(12):3352-7.

Israel Ministry of Health Athlete Pre-Participation Medical Screening Guidelines: Ministry of Health website http://www.health.gov.il Accessed on September 29,

Israel Sport Law 5748-1988 and The Sport (Medical Tests) Regulations 5757-1997 http://www.nevo.co.il/Law_word/law06/TAK-5828.pdf Accessed September 29, 2010

Janse de Jonge X. A. 2003. Effects of the menstrual cycle on exercise performance. *Sports Medicine (Auckland, N.Z.)*, *33*(11), 833–851.

Kannel, W. B., Wolf, P. A., Benjamin, E. J. and Levy, D. 1998. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: population-based estimates. *American Journal Cardiology*, 82(8A):2N-9N.

Keren, A., Syrris, P. and Mckenna, W. J. 2008. Hypertrophic cardiomyopathy: the genetic determinants of clinical disease expression. *Nature Clinical Practice Cardiovascular Medicine*, 5, 158-168.

Kian, E. M., Bernstein, A, and McGuire, J. S. 2013. A major boost for gender equality or more of the same? The television coverage of female athletes at the 2012 London Olympic Games. *The Journal of Popular Television*, 1(1), 143-149

Kim, A., Deo, S. H., Fisher, J. P. and Fadel, P. J. 2012. Effect of sex and ovarian hormones on carotid baroreflex resetting and function during dynamic exercise in humans. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *112*(8), 1361–1371.

Kramer, C. M., Appelbaum, E., Desai, M, Y., Desvigne-Nickens, P., DiMarco, J. P., Friedrich, M. G., Geller, N., Heckler, S., Ho, C. Y., Jerosch-Herold, M., Ivey, E. A., Keleti, J., Kim, D.Y., Kolm , P., Kwong R. Y., Maron, M. S., Schulz-Menger, J., Piechnik, S., Watkins, H., Weintraub, W. S.,

Wu, P. and Neubauer, S. 2015. Hypertrophic cardiomyopathy registry: the rationale and design of an international, observational study of hypertrophic cardiomyopathy. *American Heart Journal,* 170(2):223-30.

Krustrup, P., Mohr, M., Ellingsgaard, H. and Bangsbo, J. 2005. Physical demands during an elite female soccer game: importance of training status. *Medicine Science Sports Exercise,* 37(7):1242-8

Kusunose, K., Kwon, D. H., Motoki, H., Flamm, S. D. and Marwick, T. H. 2013. Comparison of three-dimensional echocardiographic findings to those of magnetic resonance imaging for determination of left ventricular mass in patients with ischemic and non-ischemic cardiomyopathy. *American Journal Cardiology.*

La Gerche A, Jurcut R, Voigt JU. 2010. Right ventricular function by strain echocardiography. *Current Opinion Cardiology*, 25(5):430-6.

Lang, R. M., Badano, L. P., Mor-Avi, V., Afilalo, J., Armstrong, A., Ernande, L., Flachskampf, F. A., Foster, E., Goldstein, S. A., Kuznetsova, T., Lancellotti, P., Muraru, D., Picard, M. H., Rietzschel, E. R., Rudski, L., Spencer, K. T., Tsang, 221 W. and Voigt, J.-U. 2015. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American society of echocardiography and the European association of cardiovascular imaging. *Journal of the American Society of Echocardiography,* 28, 1–39

Lenton, E. A., Landgren, B. M. and Sexton, L. 1984. Normal variation in the length of the luteal phase of the menstrual cycle: identification of the short luteal phase. *British Journal of Obstetrics and Gynaecology*, *91*(7),

Ljungqvist, A., Jenoure, P. J., Engebretsen, L., Alonso, J. M., Bahr, R., Clough, A. F., De Bondt, G., Dvorak, J., Maloley, R., Matheson. G., Meeuwisse, W., Meijboom, E. J., Mountjoy, M., Pelliccia, A., Schwellnus, M., Sprumont, D., Schamasch, P., Gauthier, J .B and Dubi, C. 2009. The International Olympic Committee (IOC) consensus statement on periodic health evaluation of elite athletes. *Clinical Journal Sport Medicine*, 19(5):347-65.

London, B., Michalec, M., Mehdi, H., Zhu, X., Kerchner, L., Sanyal, S., Viswanathan, P. C., Pfahnl, A. E., Shang, L. L., Madhusudanan, M., Baty, C. J., Lagana, S., Aleong, R., Gutmann, R., Ackerman, M. J., McNamara, D. M., Weiss, R. and Dudley, S. C. Jr. 2007. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. *Circulation, 116*: 2260–2268.

Lord, R., George, K., Somauroo, S. and Oxborough, D. 2014. Reproducibility and feasibility of right ventricular strain and strain rate (SR) as determined by myocardial speckle tracking during high-intensity upright exercise: a comparison with tissue Doppler-derived strain and SR in healthy human hearts. *Echo Research and Practice*, 1, 31-41.

Lopez. 1997. Women on the Ball: Guide to Women's Football. London, Scarlett Press.

Magness, R. R. and Rosenfeld, C. R. 1989. Local and systemic estradiol-17 beta: effects on uterine and systemic vasodilation. *American Journal Physiology*, 256(4 Pt 1):E536-42.

Malhotra, A., Buttrick, P., Scheuer, J. 1990. Effects of sex hormones on development of physiological and pathological cardiac hypertrophy in male and female rats. *American Journal Physiology*, 259(3 Pt 2):H866-71.

Malhotra, A., Dhutia, H., Finocchiaro, G., Gati, S., Beasley, I., Clift, P., Cowie, C., Kenny, A., Mayet, J., Oxborough, D., Patel, K., Pieles, G., Rakhit, D., Ramsdale, D., Shapiro, L., Somauroo, J., Stuart, G., Varnava, A., Walsh, J., Yousef, Z. and Sharma, S. 2018. Outcomes of cardiac screening in adolescent soccer players. *The New England journal of medicine*, *379*(6), 524–534.

Malhotra, A., Dhutia, H., Gati, S., Yeo, T., Dores, H., Bastiaenen, R., Merghani, R., Finocchiaro, G., Sheikh, N. and Steriotis, A. 2016. Prevalence and significance of anterior T wave inversion in young white athletes and non-athletes. *Journal of the American College of Cardiology,* 69, 1–9.

Malhotra, A., Dhutia, H., Yeo, T. J., Finocchiaro, G., Gati, S., Bulleros, P., Fanton, Z., Papatheodorou, E., Miles, C., Keteepe-Arachi, T., Basu, J., Parry-Williams, G., Prakash, K., Gray, B., D'Silva, A., Ensam, B., Behr, E., Tome, M., Papadakis, M. and Sharma, S. 2020. Accuracy of the 2017 international recommendations for clinicians who interpret adolescent athletes' ECGs: a cohort study of 11 168 British white and black soccer players. *British Journal Sports Medicine*.

Mandic, S., Fonda, H., Dewey, F., Le, V. V., Stein, R., Wheeler, M., Ashley, E. A., Myers, J. and Froelicher, V. F. 2010. Effect of gender on computerized electrocardiogram measurements in college athletes. *Journal Physiology Sports Medicine,* 38(2):156-64.

Mandic, S., Tymchak, W., Kim, D., Daub, B., Quinney, H. A., Taylor, D., Al-Kurtass, S. and Haykowsky, M. J. 2009. Effects of aerobic or aerobic and resistance training on cardiorespiratory and skeletal muscle function in heart failure: a randomized controlled pilot trial. *Clinical Rehabilitation*, 23(3):207-16.

Mangan, J.A. and Park, R.J. 1987. From “fair sex” to feminism: sport and the socialization of women in the industrial and post-industrial eras. London: Frank Cass and Company Ltd.

Marijon, E., Uy-Evanado, A., Reinier, K., Teodorescu, C., Narayanan, K., Jouven, X., Gunson, K., Jui, J. and Chugh, S. S. 2015. Sudden cardiac arrest during sports activity in middle age. *Circulation*. 21; 131(16):1384-91.

Marcus, F.I. 2002. Update of arrhythmogenic right ventricular dysplasia. *Cardiac Electrophysiology Review,* 6(1-2), pp. 54-6.

Marcus, F. I., McKenna, W. J., Sherrill, D., Basso, C., Bauce, B., Bluemke, D. A., Calkins, H., Corrado, D., Cox, M. G. P. J., Daubert, J. P., Fontaine, G., Gear, K., Hauer, R., Nava, A., Picard, M. H., Protonotarios, N., Saffitz, J. E., Yoerger Sanborn, D. M., Steinberg, J. S., Tandri, H., Thiene, G., Towbin, J. A., Tsatsopoulou, A., Wichter, T. and Zareba, W. 2010. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/Dysplasia: proposed modification of the Task Force criteria. *Circulation,* 121, 1533–1541.

Maron, B. J. 1986. Structural features of the athlete heart as defined by echocardiography. *Journal of the American College of Cardiology,* 7, 190-203.

Maron, B. J. 2002. Hypertrophic cardiomyopathy. *Circulation*, 106, 2419–2421.

Maron, B. J. 2003. Sudden Death in Young Athletes. *New England Journal of Medicine,* 349, 1064–1075.

Maron, B. J. 2007. Controversies in cardiovascular medicine. Surgical myectomy remains the primary treatment option for severely symptomatic patients with obstructive hypertrophic cardiomyopathy. *Circulation,* 116(2):196-206.

Maron, B. J., Doerer, J. J., Haas, T. S., Tierney, D. M. and Mueller, F. O. 2009. Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980–2006. *Circulation,* 119(8):1085–1092.

Maron, B. J., Gardin, J. M., Flack, J. M., Gidding, S. S., Kurosaki, T. T., and Bild, D. E. 1995. Prevalence of hypertrophic cardiomyopathy in a general population of young adults. Echocardiographic analysis of 4111 subjects in the CARDIA Study. Coronary Artery Risk Development in (Young) Adults. *Circulation*, *92*(4), 785–789.

Maron, B. J., Levine, B. D., Washington, R. L., Baggish, A. L., Kovacs, R. J. and Maron, M. S. 2015. Eligibility and disqualification recommendations for competitive athletes with cardiovascular abnormalities: Task Force 2: pre-participation screening for cardiovascular disease in competitive athletes: a scientific statement from the American Heart Association. *Journal of the American College of Cardiology*, 66, 2356–2361.

Maron, B. J., Ommen, S. R., Semsarian, C., Spirito, P., Olivotto, I. and Maron, M. S. 2014. Hypertrophic cardiomyopathy: present and future, with translation into contemporary cardiovascular medicine. *Journal American College Cardiology,* 64(1):83-99.

Maron, B. J. and Pelliccia, A. 2006. The heart of trained athletes: Cardiac remodelling and the risks of sports, including sudden death. *Circulation,* 114, 1633–1644.

Maron, B., Roberts, W., Mcallister, H., Rosing, D. and Epstein, S. 1980. Sudden death in young athletes. *Circulation,* 62, 218-229.

Maron, B. J., Shen, W. K., Link, M. S., Epstein, A. E., Almquist, A. K., Daubert, J. P., Bardy, G. H., Favale, S., Rea, R. F., Boriani, G., Estes, N. A., and Spirito, P. 2000. Efficacy of implantable cardioverter-defibrillators for the prevention of sudden death in patients with hypertrophic cardiomyopathy. *The New England journal of medicine*, *342*(6), 365–373.

Maron, B. J., Thompson, P. D., Ackerman, M. J., Balady, G., Berger, S., Cohen, D., Dimeff, R., Douglas, P. S., Glover, D. W., Hutter, A. M., Krauss, M. D., Maron, M. S., Mitten, M. J., Roberts, W. O. and Puffer, J. C. 2007. Recommendations and considerations related to preparticipation screening for cardiovascular abnormalities in competitive athletes: 2007 Update: A scientific statement from the American Heart Association council on nutrition, physical activity, and metabolism. *Circulation,* 115, 1643–1655.

Maron, B. J., Wolfson, J. K., Epstein, S. E. and Roberts, W. C. 1987. Morphologic evidence for "small vessel disease" in patients with hypertrophic cardiomyopathy. *Zeitschrift fur Kardiologie*, *76 Suppl 3*, 91–100.

Mason, J., Hancock, E. and Gettes, L. 2007. Recommendations for the standardization and interpretation of the electrocardiogram. *Journal of the American College of Cardiology,* 49(10), 1128–1135

Martin, D. and Elliott-Sale, K.J. 2016. The effects of hormonal contraceptives on determinants of female athlete performance: a mini-review. *Brazilian Journal of Physical Education and Sport*, 30 (4), 1087-96.

Martínez-Lagunas, V., Niessen, M., and Hartmann, U. 2014. Women's football: player characteristics and demands of the game. *Journal of Sport and Health Science, 3*, 258-272.

Matković, B. R., Misigoj-Duraković, M., Matković, B., Janković, S, Ruzić, L., Leko, G. and Kondric, M. 2003. Morphological differences of elite Croatian soccer players according to the team position. *Collection Anthropology,* 1:167-74.

McGrew, C. A. 2003. Sudden cardiac death in competitive athletes. *Journal Orthopaedic Sports Physical Therapy*, 33(10):589-93.

McKinley, P. S., King, A. R., Shapiro, P. A., Slavov, I., Fang, Y., Chen, I. S., Jamner, L. D., and Sloan, R. P. 2009. The impact of menstrual cycle phase on cardiac autonomic regulation. *Journal Psychophysiology*, 46(4), 904–911.

Metaxas, T.I., Sentelidis, T., Kouidi, E.J., Deligiannis, A.P., Dipla, K., Zafeiridis, A. and Koutlianos, N. A. 2000. Cardiorespiratory adaptations in professional and amateur soccer players. *Hung. Rev. Sports Medicine,* 41, 85–94

Milanović, Z., Sporiš, G., James, N., Trajković, N., Ignjatović, A., Sarmento, H., Trecroci, A. and Mendes B. M. B. 2017. Physiological demands, morphological characteristics, physical abilities and injuries of female soccer players. *Journal Human Kinetics*, 28; 60:77-83.

Milanovic, Z., Sporis, G. Pantelic, S., Trajkovic, N. and Aleksandrovic, M. 2012. The effects of physical exercise on reducing body weight and body composition of obese middle-aged people. A systematic review. *Health Medicine,* 6(6):2175-89.

Minson, C. T., Halliwill, J. R., Young, T. M., & Joyner, M. J. 2000. Influence of the menstrual cycle on sympathetic activity, baroreflex sensitivity, and vascular transduction in young women. *Circulation,* 101(8), 862–868.

Mohr, M., Krustrup, P., Andersson, H., Kirkendal, D and Bangsbo, J. 2008. Match activities of elite women soccer players at different performance levels. *Journal Strength Conditioning Research*. 22(2):341–9.

Mont, L., Pelliccia, A., Sharma, S., Biffi, A., Borjesson, M., Terradellas, J. B., Carré, F., Guasch, E., Heidbuchel, H., Gerche, A., Lampert, R., McKenna, W., Papadakis, M., Priori, S. G., Scanavacca, M., Thompson, P., Sticherling, C., Viskin, S., Wilson, M., Corrado, D., Lip, G. Y., Gorenek, B., Lundqvist, C. B., Merkely, B., Hindricks, G., Hernández-Madrid, A., Lane, D., Boriani, G., Narasimhan, C., Marquez, M. F., Haines, D., Mackall, J., Marques-Vidal, P. M., Corra,. U, Halle, M., Tiberi, M., Niebauer, J. and Piepoli M. 2017. Pre-participation cardiovascular evaluation for athletic participants to prevent sudden death: Position paper from the EHRA and the EACPR, branches of the ESC. Endorsed by APHRS, HRS, and SOLAECE. *Europace: European pacing, arrhythmias, and cardiac electrophysiology: Journal of The Working Groups on Cardiac Pacing, Arrhythmias, and Cardiac Cellular Electrophysiology ofTthe European Society of Cardiology*, *19*(1), 139–163.

Morganroth, J., Maron, B. J., Henry, W. L. and Epstein, S. E. 1975. Comparative left ventricular dimensions in trained athletes. *Annals of Internal Medicine*, 82, 521-4

Moss, A. J., Schwartz, P. J., Crampton, R. S., Tzivoni, D., Locati, E. H., MacCluer, J., Hall, W. J., Weitkamp, L., Vincent, G. M. and Garson, A., Jr. 1991. The long QT syndrome. Prospective longitudinal study of 328 families. *Circulation*, *84*(3), 1136–1144.

Mosteller, R. D. 1987. Simplified calculation of body-surface area. *New England Journal Medicine*, 22; 317(17):1098.

Myerburg, R. J. and Vetter, V. L. 2007. Electrocardiograms should be included in preparticipation screening of athletes. *Circulation, 116*: 2616–1626.

Nademanee, K., Veerakul, G., Nimmannit, S., Chaowakul, V., Bhuripanyo, K., Likittanasombat, K., Tunsanga, K., Kuasirikul, S., Malasit, P., Tansupasawadikul, S. and Tatsanavivat, P. 1997. Arrhythmogenic marker for the sudden unexplained death syndrome in Thai men. *Circulation,* 21; 96(8):2595-600.

Nagueh, S. F., Middleton, K. J., Kopelen, H. A., Zoghbi, W. A. and Quiñones, M. A. 1997. Doppler tissue imaging: a non-invasive technique for evaluation of left ventricular relaxation and estimation of filling pressures. *Journal of the American College of Cardiology*, *30*(6), 1527–1533.

Napolitano, C., Bloise, R., Monteforte, N. and Priori, S. G. 2012. Sudden cardiac death and genetic ion channelopathies: long QT, Brugada, short QT, catecholaminergic polymorphic ventricular tachycardia, and idiopathic ventricular fibrillation. *Circulation*, *125*(16), 2027–2034.

Nava, A., Bauce, B., Basso, C., Muriago, M., Rampazzo, A., Villanova, C., Daliento, L., Buja, G., Corrado, D., Danieli, G. A. and Thiene, G. 2000. Clinical profile and long-term follow-up of 37 families with arrhythmogenic right ventricular cardiomyopathy. *Journal of the American College of Cardiology*, *36*(7), 2226–2233.

Nielsen, M. S., Barton, S. D., Hatasaka, H. H., and Stanford, J. B. 2001. Comparison of several one-step home urinary luteinizing hormone detection test kits to OvuQuick. *Fertility and sterility*, *76*(2), 384–387.

Noseworthy, P. A., Tikkanen, J. T., Porthan, K., Oikarinen, L., Pietilä, A., Harald, K., Peloso, G. M., Merchant, F. M., Jula, A., Väänänen, H., Hwang, S. J., O'Donnell, C. J., Salomaa, V., Newton-Cheh, C. and Huikuri, H. V. 2011. The early repolarization pattern in the general population: clinical correlates and heritability. *Journal of the American College of Cardiology*, *57*(22), 2284–2289.

O'Donnell, E., Goodman J. M. and Harvey, P. J. 2011. Clinical review: Cardiovascular consequences of ovarian disruption: a focus on functional hypothalamic amenorrhea in physically active women. *Journal Clinical Endocrinal Metabolic*, 96(12):3638-48.

Olson, J. R. 2008. VO2 peak and running economy in female collegiate soccer players across a competitive season. Master of Science (M.S.) in Exercise and Sport Science, Oregon State University.

Owan, T. E. and Redfield, M. M. 2005. Epidemiology of diastolic heart failure. *Program Cardiovascular,* 47(5):320-32.

Oxborough, D., Augustine, D., Gati, S., George, K., Harkness, A., Mathew, T., Papadakis, M., Ring, L., Robinson, S., Sandoval, J., Sarwar, R., Sharma, S., Sharma, V., Sheikh, N., Somauroo,

J., Stout, M., Willis, J. and Zaidi, A. 2018. A guideline update for the practice of echocardiography in the cardiac screening of sports participants: a joint policy statement from the British Society of Echocardiography and Cardiac Risk in the Young. *Echo Research and Practice*, 5, G1–G10.

Oxborough, D., Birch, K., Shave, R. and. George, K. P. 2010. Exercise-induced cardiac fatigue. A review of the echocardiographic literature. *Echocardiography*, 27(9), 1130-1140.

Oxborough, D., George, K. P. and Birch, K. 2012a. Intra observer reliability of two-dimensional ultrasound derived strain imaging in the assessment of the left ventricle, right ventricle, and left atrium of healthy human hearts. *Echocardiography,* 29(7), 793–802.

Oxborough, D., Heemels, A., Somauroo, J., Mcclean, G., Mistry, P., Lord, R., Utomi, V., Jones, N., Thijssen, D., Sharma, S., Osborne, R., Sculthorpe, N. and George, K. P. 2016. Left and right ventricular longitudinal strain-volume / area relationships in elite athletes. *The International Journal of Cardiovascular Imaging,* 32, 1199–1211.

Oxborough, D., Sharma, S., Shave, R., Whyte, G., Birch, K., Artis, N., Batterham, A. M. and George, K. P. 2012b. The right ventricle of the endurance athlete: the relationship between morphology and deformation. *Journal of the American Society of Echocardiography*, 25(3), 263–71.

Oxborough, D., Shave, R., Warburton, D., Williams, K., Oxborough, A., Charlesworth, S., Foulds, H., Hoffman, M., Birch, K. and George, K. P. 2011. Dilatation and dysfunction of the right ventricle immediately following ultra-endurance exercise: exploratory insights from conventional 2-dimensional and speckle tracking echocardiography. *Circulation,* 4,253-263

Pan, B. and Li, J. 2019. The art of oocyte meiotic arrest regulation. *Reproductive biology and endocrinology*, *17*(1), 8.

Papadakis, M. and Sharma, S. 2009. Electrocardiographic screening in athletes: the time is now for universal screening. *British Journal of Sports Medicine*, 43, 663-668.

Paterni, I., Granchi, C., Katzenellenbogen, J. A., and Minutolo, F. 2014. Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. *Steroids*, *90*, 13–29.

Pavlik, G., Major, Z., Csajági, E., Jeserich, M., and Kneffel, Z. 2013. The athlete's heart. Part II: influencing factors on the athlete's heart: types of sports and age (review). *Acta physiologica Hungarica*, *100*(1), 1–27.

Pelliccia, A., Di Paolo, F. M., and Maron, B. J. 2002. The athlete's heart: remodelling, electrocardiogram and pre-participation screening. *Cardiology in review*, *10*(2), 85–90.

Pelliccia, A., Di Paolo, F. M., Quattrini, F. M., Basso, C., Culasso, F., Popoli, G., De Luca, R., Spataro, A., Biffi, A., Thiene, G., and Maron, B. J. 2008. Outcomes in athletes with marked ECG repolarization abnormalities. *The New England journal of medicine*, *358*(2), 152–161.

Pelliccia, A., Culasso, F., Di Paolo, F. M., Accettura, D., Cantore, R., Castagna, W., Ciacciarelli, A., Costini, G., Cuffari, B., Drago, E., Federici, V., Gribaudo, C. G., Iacovelli, G., Landolfi, L., Menichetti, G., Atzeni, U. O., Parisi, A., Pizzi, A. R., Rosa, M., Santelli, F., Santilio F, Vagnini, A., Casasco M. and Di Luigi, L. 2007. Prevalence of abnormal electrocardiograms in a large,

unselected population undergoing pre-participation cardiovascular screening. *European heart journal*, *28*(16), 2006–2010.

Pellicia, A. and Maron, B. J. 1995. Preparticipation cardiovascular evaluation of the competitive athlete: perspectives from the 30-year Italian experience. *American Journal Cardiology,* 75, 827-9.

Pelliccia, A., Maron, B. J., Di Paolo, F. M., Biffi, A., Quattrini, F. M., Pisicchio, C., Roselli, A., Caselli, S. and Culasso, F. 2005. Prevalence and clinical significance of left atrial remodeling in competitive athletes. *Journal of the American College of Cardiology*, 46, 690–696.

Pelliccia, A., Maron, B. J., Spataro, A., Proschan, M. A. and Spirito P. 1991. The upper limit of physiological hypertrophy in highly trained elite athletes. *New England Journal of Medicine,* 324, 295-301.

Pfister, G. 2003. The challenges of women's football in East and West Germany: a comparative study. *Soccer and Society*, 4 (2–3), 128–148.

Pfister, G., Puffer, J. C. and Maron B. J. 2000. Preparticipation cardiovascular screening for US collegiate student-athletes. *JAMA*, 283: 1597-1599

Pluim, B. M., Zwinderman, A. H., Van der Laarse, A. and Van der Wall, E. E. 2000. The Athlete's Heart: A Meta-Analysis of Cardiac Structure and Function. *Circulation*, 101, 336–344.

Qasem, M., Utomi, V., George, K., Somauroo, J., Zaidi, A., Forsythe, L., Bhattacharrya, S., Lloyd, G., Rana, B., Ring, L., Robinson, S., Senior, R., Sheikh, N., Sitali, M., Sandoval, J., Steeds, R., Stout, M., Willis, J. and Oxborough, D. 2016. A Meta-Analysis for the echocardiographic assessment of right ventricular structure and function in ARVC. *Echo Research and. Practice*, 3, 95-104.

Ramirez, A. H., Schildcrout, J. S., Blakemore, D. L., Masys, D. R., Pulley, J. M., Basford, M. A., Roden, D. M. and Denny, J. C. 2011. Modulators of normal electrocardiographic intervals identified in a large electronic medical record. *Heart Rhythm*, 271-7.

Randers, M. B., Andersen, L. J., Ørntoft, C. Ø., Bendiksen, M., Johansen, L., Horton, J and Krustrup, P. 2013. Cardiovascular health profile of elite female football players compared to untrained controls before and after short-term football training. *Journal of Sports Sciences*, 31(13), 1421-1431

Randhawa, K. 2003. Netball gets the boot as more girls bend it like Beckham. The Daily Mail 17-03-2003.

Rao, S. V., McCoy, L. A., Spertus, J. A., Krone, R. J., Singh, M., Fitzgerald, S. and Peterson, E. D. 2013. An updated bleeding model to predict the risk of post-procedure bleeding among patients undergoing percutaneous coronary intervention: a report using an expanded bleeding definition from the National Cardiovascular Data Registry CathPCI Registry. *JACC. Cardiovascular interventions*, *6*(9), 897–904.

Rawlins, J., Bhan, A and Sharma S. 2009. Left ventricular hypertrophy in athletes. *European Journal of Echocardiography*, 10(3):350-356

Rawlins, J., Carre, F., Kervio, G., Papadakis, M., Chandra, N., Edwards, C., Whyte, G. P. and Sharma, S. 2010. Ethnic differences in physiological cardiac adaptation to intense physical exercise in highly trained female athletes. *Circulation*, *121*(9), 1078–1085.

Reed, B. G. and Carr, B. R. 2018. The normal menstrual cycle and the control of ovulation. In K. R. Feingold (Eds.) et. al., *Endotext*

Redfield, M. M., Jacobsen, S. J., Borlaug, B. A., Rodeheffer, R. J. and Kass, D. A. 2005. Age and gender related ventricular-vascular stiffening: a community-based study. *Circulation,* 112(15), 2254–2262.

Reilly, T. and Brooks, G. A. 1990. Selective persistence of circadian rhythms in physiological responses to exercise. *Chronobiology International*, *7*(1), 59–67.

Reilly, T. and Gilbourne, D. 2003. Science and football: a review of applied research in the football codes. *Journal of Sports Sciences*, *21*(9), 693–705.

Reilly, T. and Thomas V. 1979. Estimated daily energy expenditures of professional association footballers. *Ergonomics,* 22(5):541-8.

Riding, N. R., Salah, O., Sharma, S., Carré, F., George, K. P., Farooq, A., Hamilton, B., Chalabi, H., Whyte, G. P., and Wilson, M. G. 2014. ECG and morphologic adaptations in Arabic athletes: are the European Society of Cardiology's recommendations for the interpretation of the 12-lead ECG appropriate for this ethnicity? *British Journal of Sports Medicine*, *48*(15), 1138–1143.

Roden, D. M. 2008. Clinical practice. Long-QT syndrome. *New England Journal Medicine*, 10; 358(2):169-76.

Rosano, G. M., Leonardo, F., Sarrel, P. M., Beale, C. M., De Luca, F., and Collins, P. 1996. Cyclical variation in paroxysmal supraventricular tachycardia in women. *Lancet.* 23;347(9004):786-8.

Rowland, T. and Roti, M. 2010. Influence of sex on the "Athlete's Heart" in trained cyclists. *Journal Science Medicine Sport,* 13(5):475-8.

Rudski, L., Lai, W., Afilalo, J., Hua, L., Handschumacher, M., Chandrasekaran, K., Solomon, S., Louie, E. and Schiller, N. 2010. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography: endorsed by the European Association of Echocardiography. *Journal of the American Society of Echocardiography*, 23(7), 685–713.

Salerno, J. C., Schmied, C. M., Sharma, S., Stein, R., Vetter, V. L. and Wilson, M. G. 2013. Electrocardiographic interpretation in athletes: the 'Seattle criteria'. *British Journal Sports Medicine*, 47, 122-4

Scharf, M., Brem, M. H., Wilhelm, M., Schoepf, U. J., Uder, M., and Lell, M. M. 2010. Cardiac magnetic resonance assessment of left and right ventricular morphologic and functional adaptations in professional soccer players. *American heart journal*, *159*(5), 911–918.

Schwartz P. J. 1997. The long QT syndrome. *Current problems in cardiology*, *22*(6), 297–351.

Schwartz, P. J., Periti, M. and Malliani, A. 1975. The long QT syndrome. *American heart journal*, 89, 378-390.

Sharma, S. 2003. Physiological Society Symposium – The athlete's heart athlete's heart – effect of age, sex, ethnicity and sporting experimental physiology: regular physical training is associated with several physiological and biochemical adaptations which enable. *Experimental Physiology,* 88, 665–669.

Sharma, S., Drezner, J. A., Baggish, A., Papadakis, M., Wilson, M. G., Prutkin, J. M., La Gerche, A., Ackerman, M. J., Borjesson, M., Salerno, J. C., Asif, I. M., Owens, D. S., Chung, E. H., Emery, M. S., Froelicher, V. F., Heidbuchel, H., Adamuz, C., Asplund, C. A., Cohen, G., Harmon, K. G., Marek, J. C., Molossi, 229 S., Niebauer, J., Pelto, H. F., Perez, M. V., Riding, N. R., Saarel, T., Schmied, C. M., Shipon, D. M., Stein, R., Vetter, V. L., Pelliccia, A. and Corrado, D. 2017. International recommendations for electrocardiographic interpretation in athletes. *Journal of the American College of Cardiology*, 69, 1057–1075.

Sharma, S., Maron, B. J., Whyte, G., Firoozi, S., Elliott, P. M., and McKenna, W. J. 2002. Physiologic limits of left ventricular hypertrophy in elite junior athletes' relevance to differential diagnosis of athlete's heart and hypertrophic cardiomyopathy. *Journal of the American College of Cardiology*, 40, 1431- 1436.

Sharma, S., Whyte, G., Elliott, P., Padula, M., Kaushal, R., Mahon, N. and McKenna, W. J. 1999. Electrocardiographic changes in 1000 highly trained junior elite athletes. *British Journal of Sports Medicine,* 33,319–324.

Sharma, S., Whyte, G., and McKenna, W. J. 1997. Sudden death from cardiovascular disease in young athletes: fact or fiction? *British journal of sports medicine*, *31*(4), 269–276.

Sheppard, M. 2012. Aetiology of sudden cardiac death in sport: a histopathologist's perspective. *British Journal of Sports Medicine*, 46, i15–i21

Shurlock B. 2009. 'Sudden Cardiac Death' makes the headline, but 'Football for Health' is the message. *European heart journal*, *30*(8), 877–879.

Silberstein, S. D., and Merriam, G. R. 2000. Physiology of the menstrual cycle. *Cephalalgia: an International Journal of Headache*, *20*(3), 148–154.

Smith, M. and Wrynn, A. 2013. Women in the 2012 Olympic and Paralympic games: an analysis of participation and leadership opportunities. Ann Arbor: SHARP Center for Women and Girls.

Somauroo, J., Pyatt, J. R., Jackson, M., Perry, R. A., and Ramsdale, D. R. 2001. An echocardiographic assessment of cardiac morphology and common ECG findings in teenage professional soccer players: reference ranges for use in screening. *Heart*, 85(6):649-54.

Somouroo, J., Robert, M. C., Simon S M., Stephen W. and De Silva, R. 2012. Lessons learnt from a tragic loss. *Br J Cardiol*, 19:161

Spataro, A., Pelliccia, A., Caselli, G., Amici, E. and Venerando, A. 1985. Echocardiographic standards in top-class athletes. *Journal Sports Cardiology*, 2, 17–27

Speroff, L. and Fritz, M. 2005. The clinical gynaecologic endocrinology and infertility. 7th Edition, Lippincott Williams and Wilkins, Philadelphia.

Steinvil, A., Chundadze, T., Zeltser, D., Rogowski, O., Halkin, A., Galily, Y., Perluk, H. and Viskin, S. 2011. Mandatory electrocardiographic screening of athletes to reduce their risk for sudden

death proven fact or wishful thinking? *Journal of the American College of Cardiology*, *57*(11), 1291–1296.

Stricker S. A. 2006. Structural reorganizations of the endoplasmic reticulum during egg maturation and fertilization. *Seminars in cell and developmental biology*, *17*(2), 303–313.

Stølen, T., Chamari, K., Castagna, C. and Wisløff U. 2005. Physiology of soccer: an update. *Sports Med,* 35(6):501-36.

Surawicz, B., Childers, R., Deal, B. J. and Gettes, L. S. 2009. AHA/ACCF/HRS Recommendations for the standardization and interpretation of the ECG: Part III: intraventricular conduction disturbances. *Journal American Collection Cardiology,* vol. 53 (pg. 976-981)

Su, H. W., Yi, Y. C., Wei, T. Y., Chang, T. C., and Cheng, C. M. 2017. Detection of ovulation, a review of currently available methods. *Bioengineering and Translational Medicine*, *2*(3), 238–246.

St John Sutton, M. G., Lie, J. T., Anderson, K. R., O'Brien, P. C. and Frye, R. L. 1980. Histopathological specificity of hypertrophic obstructive cardiomyopathy. Myocardial fibre disarray and myocardial fibrosis. *British Heart Journal*, *44*(4), 433–443.

Tamer, K., Günay,. M, Tiryaki, G., Cicioolu, I. and Erol, E. 1997. Physiological characteristics of Turkish Female Soccer players. Science and Footbal III. London: E and FN Sport, 37-39.

Thiene, G., Corrado, D. and Basso, C. 2007. Arrhythmogenic right ventricular cardiomyopathy/dysplasia. *Orphanet Journal of Rare Diseases*, *2*, 45.

Thünenkötter, T., Schmied, C., Dvorak, J. and Kindermann, W. 2010. Benefits and limitations of cardiovascular pre-competition screening in international football. *Clinical research in cardiology: official journal of the German Cardiac Society*, *99*(1), 29–35.

Timmermans, C., Smeets, J. L., Rodriguez, L. M., Vrouchos, G., van den Dool, A. and Wellens, H. J. 1995. Aborted sudden death in the Wolff-Parkinson-White syndrome. *The American Journal of Cardiology*, *76*(7), 492–494.

Todd, M., Scott, D. and Chisnall, P. 2002. Science and Football IV. En Spinks W, Reilly T, Murphy A. Science and Football IV. Proceedings of the 4th World Congress of Science and Football. London: *Routledge*, p. 374-381.

Tumilty, D. and Darby, S. 1992. Physiological characteristics of female soccer players. *Journal Sports Science*. 10, 144.

Tungsanga, K., and Sriboonlue, P. 1993. Sudden unexplained death syndrome in northeast Thailand. *International journal of epidemiology*, *22*(1), 81–87.

Utomi, V., Oxborough, D., Ashley, E., Lord, R., Fletcher, S., Stembridge, M., Shave, R., Hoffman, M. D., Whyte, G., Somauroo, J., Sharma, S. and George, K. P. 2014. Predominance of normal left ventricular geometry in the male 'athlete's heart'. *Heart (British Cardiac Society*), 100, 1264–1271.

Utomi, V., Oxborough, D., Whyte, G., Somauroo, J., Sharma, S., Shave, R., Atkinson, G. and George, K. P. 2013. Systematic review and meta-analysis of training mode, imaging modality and body size influences on the morphology and function of the male athlete's heart. *Heart,* 99(23), 1727–33.

Varnava, A. M., Elliott, P. M., Mahon, N., Davies, M. J., and McKenna, W. J. 2001. Relation between myocyte disarray and outcome in hypertrophic cardiomyopathy. *The American Journal of Cardiology*, *88*(3), 275–279.

Vakrou, S. and Abraham, M. R. 2014. Hypertrophic cardiomyopathy: a heart in need of an energy bar? *Frontiers in physiology*, *5*,

Vollman, R. F. 1977. The menstrual cycle. Major Problem Obstetric Gynecol. 1-193.

Wagner, G. S., and Strauss, D. G. 1994. Marriot's Practical Electrocardiography. Baltimore: Williams and Wilkins; 13th edition.

Wang, Q., Curran, M. E., Splawski, I., Burn, T. C., Millholland, J. M., VanRaay, T. J., Shen, J., Timothy, K. W., Vincent, G. M., de Jager, T., Schwartz, P. J., Toubin, J. A., Moss, A. J., Atkinson, D. L., Landes, G. M., Connors, T. D., and Keating, M. T. 1996. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nature genetics*, *12*(1), 17–23.

Wang, Q., Shen, J., Splawski, I., Atkinson, D., Li, Z., Robinson, J. L., Moss, A. J., Towbin, J. A., and Keating, M. T. 1995. SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell*, *80*(5), 805–811.

Wasfy, M., DeLuca, J., Wang, F., Berkstresser, B., Ackerman, K., Eisman, A., Lewis, G., Hutter, A., Weiner, R. and Baggish, A. 2015. ECG findings in competitive rowers: normative data and the prevalence of abnormalities using contemporary screening recommendations. *British Journal of Sports Medicine, 49(3), 200–206.*

Wasfy, M. M., Hutter, A. M., and Weiner, R. B. 2016. Sudden cardiac death in athletes. *Methodist DeBakey Cardiovascular Journal*, *12*(2), 76–80.

Weiner, R. B. and Baggish, A. L. 2012. Exercise-induced cardiac remodelling. *Program Cardiovascular,* 54(5):380-6.

Whyte, G. P., George, K., Sharma, S., Firoozi, S., Stephens, N., Senior, R. and McKenna, W. J. 2004. The upper limit of physiological cardiac hypertrophy in elite male and female athletes: the British experience. *European Journal of Applied Physiology*, 92, 592–597.

Wigle, E. D., Rakowski, H., Kimball, B. P. and Williams, W. G. 1995. Hypertrophic cardiomyopathy. Clinical spectrum and treatment. *Circulation*, *92*(7), 1680–1692.

Wigle, E. D., Sasson, Z., Henderson, M. A., Ruddy, T. D., Fulop, J., Rakowski, H. and Williams, W. G. 1985. Hypertrophic cardiomyopathy. The importance of the site and the extent of hypertrophy. A review. *Progress in cardiovascular diseases*, *28*(1), 1–83.

Willianson, D. 1991. Belles of the ball: early history of women's football. Devon, R and D associates.

Williams, J. and Woodhouse, J. 1991. 'Can Play, Will Play? Women and Football in Britain', in Williams, John and Wagg, Stephen (eds.) British football and social change: getting into Europe (Leicester: Leicester University Press), pp. 85-108.

Wilson, M. G., Basavarajaiah, S., Whyte, G. P., Cox, S., Loosemore, M. and Sharma, S. 2008. Efficacy of personal symptom and family history questionnaires when screening for inherited

cardiac pathologies: the role of electrocardiography. *British journal of sports medicine*, *42*(3), 207–211.

World Health Organisation. 2007 (feb) Cardiovascular diseases.

Http: //www. Who. Int/mediacentre/factsheets/fs317/enlindex. Html

Zaidi, A., Ghani, S., Sharma, R., Oxborough, D., Panoulas, V. F., Sheikh, N., Gati, S., Papadakis, M. and Sharma, S. 2013. Physiological right ventricular adaptation in elite athletes of African and Afro-Caribbean origin. *Circulation*, 127, 1783– 92.

Zipes, D. P., Ackerman, M. J., Estes, N. A., 3rd, Grant, A. O., Myerburg, R. J. and Van Hare, G. 2005. Task Force 7: arrhythmias. *Journal of the American College of Cardiology*, *45*(8), 1354–1363.

Zorzi, A., Calore, C., Vio, R., Pelliccia, A. and Corrado, D. 2018. Accuracy of the ECG for differential diagnosis between hypertrophic cardiomyopathy and athlete's heart: comparison between the European Society of Cardiology (2010) and International (2017) criteria. *British journal of sports medicine*, *52*(10), 667–673.

Appendix 1- LIVERPOOL JOHN MOORES UNIVERSITY PARENT CONSENT FORM

LIVERPOOL JOHN MOORES UNIVERSITY

PARENT CONSENT FORM

The Athletic Heart Phenotype in Elite Female Footballers

Researchers: Aleah Mohammad School of Sport and Exercise Science, Faculty of Science.

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered ☐
2. I understand that my child participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐
3. I understand that any personal information collected during the study will be anonymised and remain confidential. ☐
4. I agree to let my child take part in the above study. ☐

Name of Parent	Date	Signature
Name of Researcher	Date	Signature
Name of Person taking consent *(if different from researcher)*	Date	Signature

Aleah Mohammad
Tom Reilly Building Liverpool John Moores
Liverpool John Moores University
Liverpool, UK

Email: A.B.Mohammad@ljmu.ac.uk

Appendix 2- Athlete Consent form

Professor John Somauroo
MB BS BMedSci(Hons) FRCP(Lond) FRCP(Edin) FFSEM
Consultant Cardiologist and Physician
Honorary Professor in Cardiovascular, Sports and Exercise Medicine

Grosvenor Nuffield Hospital
Wrexham Road
Chester
CH4 7QP
Tel: 01244 657946

Consent Form for Cardiac Screening

It is extremely important that you read and understood the information below.

Test Procedure:

The aim of the screening is to look closely at the structure and function of your heart using an ECG and Echocardiogram. You may also be asked to perform an exercise test.

An **Electrocardiogram (or ECG)** is a simple, non-invasive and painless test that examines the electrical activity within your heart. Small stickers are placed on your chest. Flexible leads from the ECG machine are then attached to these stickers. The electrical rhythm of your heart is recorded and printed out on paper.

You may also be asked to perform an **Exercise Test**. This is similar to an exercise machine at the gym. We keep a close eye on your ECG and blood pressure during the test. There is a small risk of causing a change in heart rhythm during the exercise test, but this is one of the reasons that we perform this test as it may uncover a problem with your heart.

An **Echocardiogram (ECHO)** is an ultrasound scan of the heart that measures cardiac dimensions and the flow of the blood in and out of the heart. Just like a sonogram of a pregnant woman, the scan is painless, non-invasive and takes no more than 20 min. All medical personnel who are linked to Prof Somauroo are verified and approved by him. All results are treated in the strictest of confidence.

Results:

It should be noted that the results will appear abnormal in a small percentage of cases and follow up tests may be required to look more closely at your heart.

STATEMENT: **I have read and understood the information above. I understand that in the rare event an abnormality is confirmed, this may affect some types of mortgage and health/life insurance applications and that it may also affect some careers.**

Questions concerning the testing procedure have been answered to my satisfaction. I also understand that I am free to withdraw consent and discontinue participating in any procedures without giving a reason.

I have also been informed that the information derived from these tests is confidential and will not be disclosed to anyone other than my doctor or others who are involved within my care. However, I do agree that the information from these tests will be held on a database at Liverpool John Moores University and by Prof John Somauroo and can be used anonymously for research purposes.

(SIGNATURE).....DATE .....

NAME OF ATHLETE
(PRINTED).....AGE.....

CONTACT TEL. NO. .....

PARENT'S SIGNATURE (required if aged under 16) .....DATE .....

Prof John Somauroo 16.8.12 (Based on documents from CRY)