The Impact of Prolonged Strenuous Exercise on Right Ventricular Structure and Function: Insights from Novel Echocardiography and Electrocardiography

Rachel Nia Lord

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Liverpool John Moores University

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Abstract

The body of evidence investigating post-exercise changes in left (LV) and right ventricular (RV) structure and function, commonly referred to as exercise induced cardiac fatigue (EICF), demonstrates a reduction in both systolic and diastolic function. RV dilatation and reduced LV filling are also evident. The mechanism responsible for this phenomenon is not fully understood, however a recent theory suggests that an elevated afterload on the RV and subsequent interaction between the ventricles may be implicated.

Based on this, the aims of this thesis were: 1) to assess the RV structural and functional response to a 100 mile ultra-marathon applying novel techniques to determine the transient or persistent nature of RV post-exercise changes, 2) to provide a holistic assessment of all cardiac chambers, including the simultaneous derivation of structure and function for both the LV and RV in the same cardiac cycle following a 100 mile ultra-marathon, 3) to establish the acute response to a 100 mile ultra-marathon in both the 12-lead ECG and right-sided ECG and 4) to assess RV structure and function during a 6 hour upright cycling exercise bout, including the simultaneous estimation of PAP.

The key finding from the Chapter 3 was a 10% reduction (P = 0.007) in RV strain and early diastolic strain rate immediately post-race that remained depressed following 6 hours of recovery. The application of area-deformation loops in Chapter 4 highlighted a leftward shift of the LV loop and rightwards shift of the RV loop immediately post-race with

concomitant reduction in both LV and RV strain (-29 to -26% and -23 to -19% respectively, $P = 0.01$). The LV loop reflected a change in cardiac mechanics immediately post-race, supported by the increase in the systolic-diastolic strain gradient ($P < 0.05$). Following 6 hours of recovery, the strain gradient returned to baseline values but both RV and LV loops were still displaced from baseline values. There was a 22% elevation in the ST segment in leads V2R and V3R following the 100 mile ultra-marathon in Chapter 5. A heterogeneous response was observed with respect to T wave changes with 50% of right-sided ECGs demonstrating a significant change pre to post-race.

The laboratory based study in Chapter 6 established the most appropriate technique with respect to reliability, feasibility and appropriate absolute values to quantify RV function during exercise. MST and TVI provide disparate RV strain values at 50 (25 and 30%), 70 (20 and 35%) and 90% (15 and 32%) maximum heart rate. CoV for global RV strain during exercise ranged from 7 to 11% for TVI and 14 to 73% for MST. In Chapter 7, the RV in-exercise response was assessed throughout prolonged cycling exercise with concomitant estimation of RV afterload. In contrast to the field-based studies, an initial increase in RV strain (-26 to -28%) was maintained as exercise progressed with no elevation in RV afterload or subsequent structural adaptation.

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Preface

A number of the chapters in this thesis have resulted in jointly authored peer reviewed publications or are currently under review and these are presented below.

Chapter 2:

Lord R, Oxborough D, Somauroo J, George K. (2014). Echocardiographic Evidence of the Cardiac Stress of Competing in Ultra-Endurance Exercise*. Deutsche Zeitschrift für Sportmedezin 65: 93 - 101.*

Chapter 3:

Lord R, George K, Somauroo J, Stembridge M, Jain N, Hoffman MD, Shave R, Haddad F, Ashley E, Jones H, Oxborough D. (2015). The right ventricle following ultra-endurance exercise: insights from novel echocardiography and 12-lead electrocardiography. *European Journal of Applied Physiology 115: 71-80*.

Chapter 4:

Lord R, George K, Somauroo J, Stembridge M, Jain N, Hoffman MD, Shave R, Haddad F, Ashley E, Jones H, Heemels A, Oxborough D. Alterations in Cardiac Mechanics following Ultra-endurance Exercise: Insights from Left and Right Ventricular Area-Deformation (ε) Loops. *The Journal of Physiology, In review.*

Chapter 5:

Lord R, George K, Somauroo J, Jain N, Reese K, Hoffman MD, Shave R, Haddad F, Ashley E, Jones H, Heemels A, Oxborough D. Exploratory Insights from the Right-sided Electrocardiogram following Prolonged Endurance Exercise**.** *Scandinavian Journal of Medicine and Science in Sports, In review.*

Chapter 6:

Lord R, George K, Jones H, Somauroo J, 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.*

Abbreviations

2D	two-dimensional
3D	three-dimensional
A	peak late diastolic trans-mitral blood flow velocity
A’	peak late diastolic myocardial tissue velocity
AP4CH	apical four chamber view
ARVC	arrhythmogenic right ventricular cardiomyopathy
ASE	American Society of Echocardiography
BOO	atrial booster volume
BP	blood pressure
cMRI	cardiac magnetic resonance imaging
CON	atrial conduit volume
CoV	coefficient of variation
E	peak early diastolic trans-mitral blood flow velocity
E’	peak early diastolic myocardial tissue velocity
ECG	electrocardiography
EDA	end diastolic area
EDV	end diastolic volume
EF	ejection fraction
EI	eccentricity index
EICF	exercise-induced cardiac fatigue
ESV	end systolic volume
FAC	fractional area change
FPS	frames per second

FS	fractional shortening
HR	heart rate
HRmax	maximum heart rate
IVCT	isovolumic contraction time
IVRT	isovolumic relaxation time
LA	left atrium
LoA	limits of agreement
LV	left ventricle
MST	myocardial speckle tracking
PA AccT	pulmonary artery acceleration time
PAP	pulmonary artery pressure
PHT	pulmonary hypertension
PLAX	parasternal long axis
PSAX	parasternal short axis
QTc	corrected QT interval
RA	right atrium
RAD	right axis deviation
RES	atrial reservoir volume
ROI	region of interest
RV	right ventricle
RVAd	right ventricular diastolic area
RVAs	right ventricular systolic area
RVD1	right ventricular inflow minor axis – basal level
RVD2	right ventricular inflow minor axis – mid level
RVD3	right ventricular major axis

RVH	right ventricular hypertrophy
RVOT	right ventricular outflow tract
RVOT1	right ventricular outflow tract parasternal short axis at aortic valve level
RVOT2	right ventricular outflow tract parasternal short axis at infundibulum
RVOTplax	right ventricular outflow tract parasternal long axis at the aortic valve level
RVSp	right ventricular systolic pressure
S’	peak systolic myocardial tissue velocity
SR	strain rate
SRA	peak late diastolic strain rate
SRE	peak early diastolic strain rate
SRS	peak systolic strain rate
SV	stroke volume
TAPSE	tricuspid annular plane systolic excursion
TDI	tissue Doppler imaging
TrE/A	trans-tricuspid early to late blood flow velocity ratio
TsPeak	time to peak strain
T_{SRA}Peak	time to peak late diastolic strain rate
T_{SRE}Peak	time to peak early diastolic strain rate
T_{SRS}Peak	time to peak systolic strain rate
TVI	tissue Velocity imaging
VOL ED	atrial volume at end diastole
VOL ES	atrial volume at end systole

VOL pre A	atrial volume prior to P wave
VTI	velocity time integral
ε	strain

Chapter 1 - **General Introduction**

It is well established that regular moderate exercise results in a range of beneficial adaptations to the cardiovascular system (Lee et al., 1993, Green et al., 2006) that promote reduced morbidity and mortality. Prolonged strenuous exercise, typically defined as lasting greater than 4 hours in duration (Whyte, 2006), has been shown to have a deleterious effect on cardiac function which has previously been termed “exercise induced cardiac fatigue” (EICF). The first evidence of EICF associated with prolonged exercise was demonstrated in 1964 by Saltin and Stenberg who reported a significant decrease in left ventricular (LV) stroke volume (SV) during a bout of cycle exercise in the presence of unaltered haemodynamic loading. Since this landmark paper numerous studies have investigated EICF following endurance exercise, focusing initally on LV systolic and diastolic function with more recent attention given to the right ventricle (RV). Initial studies focussing on the right ventricle (RV) have clinical relevance, in that specific case studies reported pulmonary oedema in two athletes on completion of a 161 km endurance trail run (Davila-Roman et al., 1997).

Recent developments in echocardiography have allowed a more in depth analysis of the RV which is challenging to assess given its complex shape and location compared to the LV (Ho and Nihoyannopoulos, 2006). A mechanistic theory to explain EICF has been proposed implicating a reduction in RV SV and subsequent LV dysfunction in recovery from prolonged endurance exercise (Oxborough et al., 2011). The development

of novel techniques affords the opportunity to derive additional RV structural and functional parameters and this has yet to be fully explored. It would also be pertinent to establish the interaction between the RV and LV in this setting in an attempt to add further mechanistic insight. The application of the standard 12-lead and specific right-sided chest leads may expose a link between electrical activity and structural and functional responses to a bout of ultra-endurance running.

The new mechanistic theory linking purporting a serial impact of a reduction in RV function, as a result of elevated wall stress, on LV filling in recovery (La Gerche et al., 2011) may go some way to explain post-exercise functional and structural cardiac responses. The temporal relationship between the ventricles and timing of any changes in cardiac mechanics during exercise is yet to be established and is an important step in fully understanding the complex, multifactorial mechanisms of EICF. It is pertinent to firstly establish the reproducibility and feasibility of new echocardiographic techniques, specifically strain imaging, during exercise. This will inform the interpretation of data when these novel echocardiographic techniques are applied in an attempt to quantify the temporal cardiac mechanics of the RV during a prolonged exercise bout with simultaneous estimation of pulmonary artery pressures (PAP). The serial/parallel RV impact on the LV is dependent on an elevation in PAP. The quantification of exercising PAP could expose the timing of structural and functional RV responses in relation to changes in PAP and is an important development in our knowledge of EICF.

It is clear that additional work is required to fully elucidate the impact of acute prolonged, strenuous exercise on cardiac structure and function. This concept is the primary driver for the development of this thesis, where field-base studies, mechanistic studies and laboratory studies are linked to develop our knowledge in this field.

Chapter 2 **- Literature Review**

2.1 Introduction

Prolonged strenuous exercise, typically defined as lasting greater than 4 hours in duration (Whyte, 2006), has been shown to have a deleterious effect on cardiac function which has previously been termed “exercise induced cardiac fatigue” (EICF). The first evidence of EICF associated with prolonged exercise was demonstrated in 1964 by Saltin and Stenberg who reported a significant decrease in left ventricular (LV) stroke volume (SV) during a bout of cycle exercise in the presence of unaltered haemodynamic loading. Since this landmark paper numerous studies have investigated EICF following endurance exercise, focusing initally on LV systolic and diastolic function with more recent attention given to the right ventricle (RV). Although the existence of a post-prolonged exercise reduction in cardiac function is now well established, the specific impact and magnitude of changes are variable across studies. This diversity across individual studies may be a consequence of the different modes of exercise, exercise duration and/or intensity as well the heterogeneous demographics associated with participant groups. In addition, the assessment of cardiac function in this setting has evolved in line with developments in non-invasive imaging (e.g. echocardiography). The structure and focus of this review is aimed at providing an overview of the extant literature in this field initially concentrating on standard echocardiographic imaging of global ventricular systolic and diastolic function. This is then followed by a shift of focus to those studies that

have integrated advanced quantitative echcardiographic tools, notably ε imaging, and how these have impacted upon our understanding of EICF and will likely drive future research ideas.

2.2 Exercise-Induced Cardiac Fatigue: A Historical Perspective

Early studies of EICF evaluated global LV systolic and diastolic function pre- and post-exercise using M-mode, two dimensional (2D) and Doppler echocardiography (see Table 2.1). Indices of systolic function included fractional shortening (FS), fractional area change (FAC) and ejection fraction (EF), whereas global diastolic function was often represented by early (E) and late (A) diastolic flow velocities and their ratio E/A. A decrease in FS, FAC and EF have been demonstrated over a range of exercise modes and durations, however findings are not always consistent (see Table 2.1). For example, Douglas et al. (1990) demonstrated a decline in LV systolic function following an Ironman triathlon, whereas La Gerche et al. (2004) observed no functional changes after the same exercise exposure. This type of disparity is likely a consequence of; heterogeneous research designs, different workloads, lack of attention to fluid loss and loading changes, varied training status of the athletes, small sample sizes as well as technical imaging developments over time.

In an attempt to overcome some of these limitations, a meta-analysis was undertaken by Middleton et al. (2006a). A sample of 294 trained and untrained participants completing endurance exercise ranging from 1 – 24

hours were included in the analysis. The overall effect was a small but significant 2% decrease in LV EF within 30 min of completion of a bout of endurance exercise. A sub-analysis on untrained subjects taking part in exercise of > 3 hours duration and trained athletes competing in exercise > 10 hours provided evidence for a profound decline in LV EF of 5.5 and 4 %, respectively. Training status appears to mediate the severity of decline in EF with untrained subjects being affected at lower exercise durations. In trained athletes a significant decrease in EF was only seen following ultra-duration exercise suggesting a a causal link to exercise volume. In view of this further work is needed to determine the exact role and importance of training status on EICF. Moreover, there is limited work looking at the impact of exercise duration/mode in a study design using repeated measures assessments. Whilst this provides some support for the phenomenon of EICF it is important to note that changes in EF were strongly associated with estimates of preload. This suggests that these changes are not due to intrinsic myocardial fatigue or dysfucntion.

Numerous early studies assessing LV diastolic function have reported a decline in E velocity and a compensatory rise in A velocity and therefore a reduction in the E/A ratio independent of exercise mode and duration (see Table 2.1). This was further highlighted by the meta-analysis conducted by Middleton et al. (2006a) which also reported no association of diastolic function to post-exercise changes in heart rate or preload (LV end diastolic volume). Despite this Hart et al. (2007) demonstrated that a post-marathon reduction in diastolic function, as determined by pulsed-wave

Doppler indices, partially returned to a baseline level following a post-race postural manoevre (the Trendelenburg position which augments preload). These data suggest that blood volume shifts may, in part, explain the post-exercise reduction in function observed in previous studies.

Table 2.1 – Studies utilising standard 2D and Doppler indices in the assessment of left ventricular function

Author	Year	Exercise	n	Systolic Indices			Diastolic Indices		
				↓	-	↑	↓	-	↑
Crawford	1979	Exhaustion <20 min	27	FS					
Niemela	1984	24 hr run	13	FS					
Perrault	1986	Marathon	13		EF, FS				
Douglas	1987	Ironman Triathlon	21	FS					
Seals	1988	Exhaustion (160-180mins)	12	FS					
Carrio	1990	6 hr race	10			EF			
Douglas	1990	Ironman Triathlon	41	EF	FAC			E/A	
Manier	1991	Marathon	11		FS			E/A	
Vanoverschelde	1991	20 km run	23	EF					
Ketelhut	1994	Exhaustion (1 hour)	10	EF		EF			
Palatini	1994	Exhaustion (61min semi supine)	16			EF			
Eysmann	1996	Exercise to exhaustion (95 mins)	10	EF					
Davila-Roman	1997	100 mile race	14		EF				
Douglas	1998	Ironman Triathlon	15	EF					
Lucia	1999	Marathon	17 male and 5 female VO2max: 55.7		EF		E/A		
Rifai	1999	Ironman Triathlon	11 male, 12 female	EF, WM					
Whyte	2000	Full and Half-Ironman	14	EF (full)	EF (half)		E/A		
Haykowsky	2001	Half-Ironman Triathlon	9	EF					
Shave	2002	Mountain marathon (2 days)	11	EF, FS			E/A		
Shave	2002	30 min running	8 VO2max: 67.4		SBP/ESV		E/A		
McGavock	2003	Olympic Triathlon	13 females			FAC			
George	2004	Marathon	35 finishing time 157-341 min				E/A		
Shave	2004	100 mile	8 VO2max: 65.6 +/- 7.0		EF		E/A		
Shave	2004	50 mile	8		EF			E/A	
Stickland	2004	Exercise to exhaustion (2.5 – 3.5 hrs)	11			EF			
La Gerche	2004	Ironman Triathlon	15	WM (1 sub)	EF			E/A	

Shave	2004	Half-Ironman Triathlon	9	LV EF			E/A		
George	2005	Marathon	29				E/A, PV		
Whyte	2005	Marathon	43 male 9 female		EF		E/A		
Dawson	2005	4 hour	16				E/A		
Welsh	2005	Half-ironman Triathlon	9	SBP/ESV					
Middleton	2006	Marathon	14				E/A, FPV		
Neilan	2006	Marathon	20				E/A		
Oxborough	2006	Marathon	35				E/A		
Scharhag	2006	1 hr / 2hr run	27 VO2max: 60±5		FAC				
Hassan	2006	Ironman Triathlon	39		EF		E/A		
Neilan	2006	2000m Rowing	17		EF		E/A, FPV		
Middleton	2007	Repeated bouts (4 days)	10	EF			E/A, FPV		
Alshaher	2007	135 mile road race	14 males, 10 females				E/A	FPV	
Hart	2007	Marathon	13 male, 1 female				E/A		
La Gerche	2008	Ironman Triathlon	20 male, 7 female		EF				
Poh	2008	2000m speed skating	39 (elite and control)						E/A
Oxborough	2010	Marathon	17		EF		E/A		
Banks	2010	150 minutes	18 maximal aerobic power = 55.1	EF			E/A		
Oxborough	2011	100 mile run	16	EF, SV			E/A		E dec
La Gerche	2011	Endurance race 3-11 hours	40	ESV	EF		E/A		
Nie	2011	2 x 45 minutes	12	EF			E/A		
Williams	2011	Cycling (RAAM)	4		EF			E/A	
Banks	2011	150 minutes	36 (young and old)				E/A		
Oosthuyse	2012	Multi stage cycling 4 days	11	EF	SBP, SV		E, E/A	E dec	
Nottin	2012	2 hour exercise	20				E, E/A		
Passaglia	2013	24 hour run	24		EF		E/A		

EF – Ejection Fraction, E/A – Early to Late Diastolic Ratio, E decel – E deceleration Time, SBP – Systolic Blood Pressure, FPV – Flow Propagation Velocity, FS – Fractional Shortening, WM – Wall Motion Abnormalities, FAC – Fractional Area Change, SV – Stroke Volume, ESV – End Systolic Volume, PV – Pulmonary Vein,

The introduction of tissue Doppler imaging (TDI) in the assessment of EICF attempted to overcome some of the load-dependent limitations of standard 2D and Doppler techniques as well as providing local or regional functional assessment. Studies applying TDI to assess LV systolic function have reported no change in peak systolic myocardial velocity (S') after exercise of differing modes and durations (see Table 2.2). Although these studies suggest that LV systolic function is either unchanged, or even improved, following exercise, it is important to note that the exercise duration was often limited to shorter endurance exercise exposures (e.g. marathon races). TDI can assess diastolic parameters of wall motion across the cardiac cycle, notably peak early diastolic (E') and late diastolic (A') myocardial velocities (see Table 2.2). George at al. (2006) and Neilan et al. (2006a) assessed E' in six LV wall segments post-marathon, with a consistent regional decline noted. Interestingly, in Hart et al. (2007) the post-exercise depression in E' was not modified by the Trendelenburg postural manoeuvre, suggesting a relatively load-independent (intrinsic) LV functional change. The application of TDI indices provide further support for a reduction in both systolic and diastolic LV function following prolonged endurance exercise as demonstrated using conventional 2D and Doppler echocardiography.

Table 2.2 - Studies utilising tissue Doppler and Myocardial Speckle tracking indices in the assessment of the left ventricle

Author	Year	Exercise	n	Systolic Indices			Diastolic Indices		
				↓	-	↑	↓	-	↑
George	2005	Marathon	29				E'/A'	E/E'	
Whyte	2005	Marathon	43 male 9 female				E'/A'		
Neilan	2006	Marathon	20	Sep ε, SRS			Sep E', E', SRE		
Oxborough	2006	Marathon	35		S'		E'/A'		
Scharhag	2006	1 hr / 2hr run	27 VO2max: 60		S'			E'/A'	
Neilan	2006	2000m Rowing	17			S', Torsion	E'		
George	2006	Marathon	30		S'				
Hart	2007	Marathon	13 male, 1 female				E'/A'		
La Gerche	2008	Ironman Triathlon	20 male, 7 female	long ε					
Poh	2008	2000m speed skating	39 (elite and control).		S'				
Dawson	2008	Marathon	15		long, rad,circ ε,SRS		rad, circ, long SRE, SRE/A		
Scott	2009	100 mile trial race	25 (finishing time 25.5 +/- 3.2 h	rad, circ, long ε & SRS			rad, circ, long SRE		
George	2009	60 mile race	19	rad, circ ε SRS	long ε and SRS		SRE		
Nottin	2009	Ironman Triathlon	23	rad, circ, long ε, SRS, torsion			rad, circ, long SRE, Untwist		
Oxborough	2010	Marathon	17	rad, circ, long ε			E', SRE, untwist, SRE/A circ long rad		Torsion, rotation
Chan-Dewar	2010	Marathon	14	sub epi rad ε, sub endo circ ε					
Banks	2010	150 minutes	18 VO2 = 55.1	ε			E'/A', SRE/A		
Oxborough	2011	100 mile run	16	long, rad, circ ε, SRS circ	SRS long, rad		E', torsion, SRE circ		
La Gerche	2011	Endurance race 3-11 hours	40		ε, SR				
Williams	2011	Cycling (RAAM)	4		ε, SRS				SRA
Banks	2011	150 minutes	36 (young and old)			ε, SRS	SRE/A		
Oosthuyse	2012	Multi stage cycling 4 days	11				E' sep E'/A'		
Nottin	2012	2 hour exercise	20		ε, SR		E'	SRE, SRA	

S' – Systolic Myocardial Velocity, E' – Early Diastolic Myocardial Velocity, A' – Late Diastolic Myocardial Velocity, SRS – Systolic Strain Rate, SRE – Early Diastolic Strain Rate, SRA Late Diastolic Strain Rate , Sep – Septal, long – Longitudinal, rad – Radial, circ – Circumferential, sub epi – Sub Epicardial, sub endo – Sub Endocardial

Tissue Dopper (like Doppler flow imaging) has a number of limitations with absolute velocity values being potentially influenced by translation, tethering and the angle of insonation (Marwick, 2006) and most work to date has only assessed LV longitudinal function (Cho et al., 2006). Progress in echocardiographic techniques and the advent of myocardial strain (ε) imaging, providing an objective measurement of absolute myocardial shortening has overcome these issues. This facilitates the assessment of LV Eulerian ε and strain rate (SR) in multiple planes of motion, providing a "richer" and more physiologically complete assessment of cardiac function. Although tissue Doppler (TVI) derived ε and SR still suffers from a dependency on the angle of insonation, it is less affected by translation and tethering and hence has been applied to the assessment of the LV, by Neilan et al. (2006b), following a marathon. A reduction was observed in septal ε, systolic (SRS), early (SRE) and late diastolic SR (SRA) which all returned to baseline 24 hours post-exercise completion. La Gerche et al. (2008) reported a decrease in LV longituinal ε only following an ironman triathlon.

Although TVI derived ε and SR have provided further evidence for intrinsic changes in contraction and relaxation following prolonged endurance exercise, most ε and SR data acquired after prolonged exercise has been obtained from speckle tracking technology (MST). This technique allows the assessment of regional and global angle-independent Lagrangian ε and SR data in multiple planes (Table 2.2). George et al. (2009) demonstrated changes in LV ε and SR in all planes with a predominance

seen in radial and circumferential motion. This was further supported by a reduction in LV longitudinal and radial ε and SR following a marathon (Oxborough et al., 2010b) and 100 mile ultramarathon (Oxborough et al., 2011, La Gerche et al., 2012b). George et al. (2009) specifically noted a case of reduced ε in only septal wall segments after a 90 km run. This localised impact on cardiac function suggested an intrinsic, rather than load-related, mechanism as well as raising the question of ventricular interaction and more specifically the impact from the right ventricle (RV). MST techniques also allow the assessment of ventricular diastolic function by providing SR in early (SRE) and late (SRA) diastole and their ratio (SRE/SRA). Empirical data pertaining to these indices are summarised in Table 2.2 and these largely support a global change in diastolic function after prolonged exercise.

Strain imaging also allows the assessment of LV rotation and consequently LV twist and untwist. LV untwisting is fundamental in the development of an intra-ventricular pressure gradient that drives early diastolic filling (Notomi et al., 2008) and thus can provide further detail in relation to diastolic EICF. A reduction in twist and untwist has been reported following a marathon (Oxborough et al., 2010b), ironman triathlon (Nottin et al., 2009) and 100 mile race (Oxborough et al., 2011). These novel indices add to the body of evidence using conventional, Doppler and tissue Doppler echocardiography and provide further support for a global reduction in LV systolic and diastolic function in recovery from a range of prolonged endurance exercise modes and durations.

Chan-Dewar et al (2010a) reported an electromechanical dissociation in 19 subjects in the LV and RV following an 89km running race, indicating that there may be an impact on the mechanical activation within the ventricles post-exercise. In addition, Sahlén et al. (2010) reported an increase in time to peak S' between basal and mid-level in the LV indicative of myocardial dysynchrony in runners completing a 30 km cross country race. Interestingly, this ventricular dysynchrony was only evident in the participants who had not completed a race of this magnitude previously. A delay in mechanical activation adds further support to an intrinsic reduction in myocardial function in both the LV and RV.

Data interrogating the electrical response in the LV following a bout of prolonged endurance exercise is limited. Stewart et al. (2014) and Sahlén et al. (2009) have applied the standard 12-lead electrocardiogram (ECG) following prolonged endurance exercise bouts of a 2 hour cycle and 30 km cross country race respectively. Both studies reported a prolonged corrected QT (QTc) alluding to an alteration in the electrical conduction of the ventricles. It is pertinent to build on these studies applying 12-lead ECG in the EICF setting to fully understand the electrical responses of the LV and to determine their significance as well as any relationship with changes in echocardiographic indices.

In summary, there is a large body of historical evidence indicating that a reduction in both systolic and diastolic function, assessed using conventional and more novel techniques, is evident in the LV during

recovery from prolonged endurance exercise. Continual developments in echocardiography allow the application of new techniques to further expand this database whilst also affording their application to assess the RV. A renewed focus on the RV response to prolonged endurance exercise has been initiated by these technological advances and a possible mechanistic link between LV and RV dysfunction.

2.3 The Right Ventricle: Challenges and Implications

The RV is an integral component of the cardiovascular system and is responsible for pumping blood into the pulmonary circulation to prompt gas exchange in the alveoli/lungs. During acute exercise, oxygen demand in the working muscles increases and thus the RV is challenged to match supply to the increased demand. The RV is inherently difficult to assess given its location, complex geometry comprising of outflow and inflow chambers and marked hypertrabeculation (Ho and Nihoyannopoulos, 2006). Multiple echocardiographic windows must be utilised to obtain a range of RV structural and functional indices in an attempt to establish RV inflow and outflow dimensions, pulmonary blood flow, RV filling and systolic and diastolic function (Rudski et al., 2010). Ultra endurance exercise challenges the RV to provide an increased oxygen supply to the pulmonary system for a prolonged period of time. Adaptation of the RV during acute exercise and chronic training programmes for ultra-endurance sports is vital to underpin enhanced exercise and performance capacities. Working in a double circulatory system, the right and left sides of the heart are linked and must be able to generate the same stroke

volume to maintain a physiologically functional heart. To fully understand the post-exercise response of the LV and RV, a holistic approach to cardiac assessment should be applied, acquiring data for all 4 cardiac chambers using a range of conventional and novel echocardiographic and electrocardiographic techniques. Assessment of the atria provides additional data pertaining to preload and therefore has the potential to provide a more in depth understanding of any interaction between the ventricles.

A direct, and potentially negative, impact of prolonged exercise on RV function has been suggested for some time. Two case studies of pulmonary oedema, suggestive of right sided heart failure, were noted in the 1970's after the completion of a 90-km foot-race (McKechnie et al., 1979). La Gerche et al. (2008), Neilan et al. (2006a), Carrio et al. (1990) and Davila-Roman et al. (1997) built on the findings from these initial case reports by applying conventional echocardiography to pre and post-exercise assessments. Collectively, these studies indicated impaired RV systolic function supported by a decrease in RV FAC after a triathlon, marathon, 6 hour race and ultra-marathon, respectively (see Table 2.3). In contrast, Douglas et al. (1990) reported no change in RV EF after an Ironman triathlon. The disparity between these studies may be as a result of differing sample sizes and the training status of the participants chosen for the studies. Using standard Doppler blood flow indices to assess diastolic filling of the RV, a reduction in the ratio of tricuspid early and late diastolic blood flow velocities (TrE/A) was reported by Douglas et al.

(1990) following an ironman triathlon. Oxborough et al. (2006) supported these findings reporting a decreased TrE/A ratio following a marathon (see Table 2.3). These data are suggestive of a reduced filling in the RV following prolonged exercise that mirror those observed in the LV, perhaps linked to the loading conditions imposed.

As echocardiographic techniques have developed, the advent of tissue Doppler imaging, purported to be less load dependent than conventional Doppler indices (Hart et al., 2007), was applied to the assessment of RV function in the EICF setting. Oxborough et al. (2006) reported no change in RV S' but a reduction in RV E'/A' ratio suggesting a reduction in diastolic filling but preserved systolic function following a marathon, supported by a reduction in TrE/A. Neilan et al. (2006b) reported a decrease in RV S' and E', indicating decreased systolic and diastolic function after exercise of the same duration. La Gerche et al (2008) and Banks et al. (2010) also demonstrated a reduction in systolic function post ultra-endurance triathlon and 150 minute run respectively and reported a decrease in RV S' (see Table 2.3). A recent meta-analysis by Elliot and La Gerche (2014) added further support to the notion of reduced RV function following intense endurance exercise with measureable reductions in both systolic and diastolic indices in 293 participants over a range of exercise stimuli.

Table 2.3 - Studies utilising conventional, Doppler, tissue Doppler and Myocardial Speckle tracking indices in the assessment of EICF in the RV

Author	Year	Exercise	n	Systolic Indices			Diastolic Indices		
				↓	-	↑	↓	-	↑
Carrio	1990	6 hour run	10	EF					
Douglas	1990	Ironman Triathlon	41		EF		E/A		
Davila-Roman	1997	161 km race	14	FAC					
Neilan	2006	Marathon	20	FAC, S', ε, SRS			E'		
Neilan	2006	Marathon	60		FAC		E', A'		
Oxborough	2006	Marathon	35 (18-50 yr)		FAC, S'		E/A, E'/A', E'		A'
La Gerche	2008	Ironman Triathlon	20 male, 7 female	FAC, S', ε					
Poh	2008	2000m speed skating	39 (young and old)			FAC, SRS			
Banks	2010	150 minutes	18 VO2 = 55.1	S', ε					
Oxborough	2010	Marathon	17	FAC					
Oxborough	2011	100 mile run	16	FAC, ε					
La Gerche	2011	Endurance race 3-11 hours	40	FAC, ε, SRS					

EF – Ejection Fraction, E/A – Early to Late Diastolic Ratio, FAC – Fractional Area Change, S' – Systolic Myocardial Velocity, E' – Early Diastolic Myocardial Velocity, A' – Late Diastolic Myocardial Velocity, SRS – Systolic Strain Rate.

2.4 The Right Ventricle: Application of Novel Indices

A reduction in TVI derived RV ε was reported by La Gerche et al. (2008) following an ultra-endurance triathlon. Banks et al. (2010) applied TVI strain imaging to the RV following a 150 minute running exercise sessions at a moderate and high intensity, reporting a reduced RV ε following both of the exercise intensities. Again these data confirm the consistent nature of changes in RV diastolic function and provide further evidence for an impact on RV systolic function complementing those observed within the LV (Banks et al., 2010, Oxborough et al., 2011, Oxborough et al., 2010b). MST has been applied to the RV in the EICF setting during recovery from a 100 mile trail running race and ironman triathlon in two recent studies (La Gerche et al., 2012b, Oxborough et al., 2011). These studies supported the use of MST as well as conventional and tissue Doppler indices of RV, LA and LV structure and function to provide a holistic approach assessing all cardiac chambers. Both of these studies reported a decrease in RV ε (-27 to -24%) in recovery from the respective exercise bouts. A dilatation was evident in the RV inflow of 3 mm and the outflow by 3 mm (Oxborough et al., 2011).

In an attempt to understand the holistic cardiac response to a bout of prolonged endurance exercise, Oxborough et al. (2010b) assessed the interaction between LA, LV and RV structure and function in recovery from a marathon by applying standard 2D echocardiography and MST. In support of an interaction between the RV and LV after a marathon, a

decrease in LA end-systolic volume (ESV) (6 ml reduction) and LV end-diastolic volume (EDV) (7-10 ml reduction) was evident, indicating reduced LV. Significant correlations were reported between LV transmitral E/A ratio and LA end systolic and reservoir volumes as well as between circumferential strain rate ratio SRE/SRA and LA end systolic and reservoir volumes respectively. This is indicative of either an intrinsic reduction in LV relaxation and its consequent impact on LA filling and emptying or reduced LA filling impacting on LV relaxation. Of particular interest was the negative correlation between RVFAC and LV longitudinal diastolic strain rate ratio SRE/SRA. This link between changes in RV and LV function provides strong evidence to suggest a ventricular interaction in recovery from prologed streuous exercise and this has a possible mechanistic implication.

A new interaction theory between LV and RV function has emerged based on work from Oxborough et al. (2010b, 2011) and La Gerche et al. (2011). They implicated a relatively elevated pulmonary artery pressure (PAP) and therefore a disproportionately higher stroke work load in the RV than the LV (La Gerche et al., 2011). Initially, the RV is able to cope by increasing contractility and wall stress to overcome this afterload. The thin walled RV myocardium is not able to sustain this for a prolonged time period (La Gerche et al., 2012b, Oxborough et al., 2011). A reduction in contractility ultimately reduces the volume of blood that the RV is able to eject and therefore LA preload is impaired (Oxborough et al., 2010b), subsequently impacting on LV filling. Landmark studies by Oxborough et al. (2011) and

La Gerche et al. (2012b) reported RV dilatation and reduced RV ε with a concomitant reduction in LV EDV following prolonged exercise. RV SV is reduced in these two studies and therefore it seemed that this serial impact of the RV on LV filling was evident following prolonged endurance exercise.

There may also be a parallel component to the RV LV interaction. In patients with a chronically elevated RV afterload such as pulmonary hypertension and embolism (Olson et al., 2010), RV EDV is increased and RV ε is reduced. The RV dilatation in these patients is marked and is causing a volume overload on the interventricular septum. This has been known to cause septal displacement, specifically in diastole (Ryan et al., 1985). If this is the case, the displaced, flattened interventricular septum is affecting the structural integrity of the LV and will impact on LV mechanics. This could consequently reduce the suction effect and pressure gradient caused by early LV relaxation and reduce LV filling. Evidence of septal displacement is reported by both La Gerche et al. (2012b) and Oxborough et al. (2011) following prolonged endurance exercise, highlighting the likelihood both a serial and parallel impact of the RV response to a relative elevation in pulmonary afterload.

2.5 Temporal Assessment of Structure and Function

Both structure and function are dependent on the loading conditions imposed and linking structure and function within the same chamber in the same cardiac cycle may generate some understanding of the pressure-

volume relationship within the cavity and aid the interpretation of post-exercise responses. Exploratory work by Gibson and Brown (1976) aiming to simultaneously link cardiac structure and function was initially undertaken in the late 1970s. The starting point for Gibson and colleagues was the demonstration of excellent agreement between the timing of the upstroke on LV displacement curves as determined using apexcardiography and the upstroke in LV pressure as measured using a simultaneous invasive LV pressure catheter. Having effectively validated displacement as a surrogate for pressure, they utilised M-mode echocardiography to define structure with simultaneous apexcardiography assessing displacement allowing for the generation of dimension-displacement loops as a non-invasive surrogate of pressure-volume loops. They then applied their methodology in a range of pathological conditions with varying loading conditions such as aortic stenosis, ischaemic heart disease and mitral regurgitation as well as healthy controls and were able to validate their technique (Venco et al., 1977). Myocardial ε is strongly correlated to LV and RV displacement as determined by apexcardiography, M-mode echocardiography and tissue Doppler derived 'tissue tracking' (Sutherland et al., 2004). Myocardial ε has fewer limitations, such as tethering, translation and rotation, which are inherent to displacement from M-mode and Tissue tracking (Korinek et al., 2005). Improvements in 2D imaging allow for more accurate assessment of ventricular area and there may be some value in taking forward the concepts established by Gibson and colleagues and applying the novel techniques now available such as MST and conventional assessment of

ventricular area. The combination of these indices may provide a more accurate non-invasive surrogate for a pressure-volume loop with the additional value in providing an assessment of the RV. The application of this novel technique may have value in proving or disproving the theories proposed by Oxborough and La Gerche by exposing any interaction between the ventricles. This technique was therefore employed to assess the LV and RV, and any interaction, prior to, on completion of and 6 hours into recovery from a 100 mile ultra-marathon in Chapter 4.

2.6 The role of the 12-lead Electrocardiogram

An important addition to the EICF literature would be the application of the 12-lead ECG in this setting. Although some data is available focused on the LV response to acute endurance exercise, the RV ECG is yet to be addressed. In the clinical setting, the 12-lead ECG is used in the diagnosis of pulmonary embolism with the incidence of partial right bundle branch block (RBBB), T-wave inversion and J point elevation in lead V1 reported (Stein et al., 2013) alongside a reduction in RV ε (Kucher et al., 2003). There may be a link between the reduction in RV function discussed in this literature review and an electrical response in the 12-lead ECG and it is pertinent to investigate this further. Moreover, right-sided precordial leads have been added to the standard 12-lead ECG in the diagnosis of myocardial infarction with right coronary artery involvement (Zehender et al., 1993), pulmonary hypertension and pulmonary embolism (Akula et al., 2003, Chia et al., 1997). These leads have been shown to be more sensitive and specific than the standard 12-lead ECG alone in detecting

ST segment and T wave changes indicative of these pathological conditions in the RV. The proposed mechanistic interaction link between the LV and RV following prolonged endurance exercise is dependent on an elevated afterload, similar to pulmonary hypertension. It is sensible to consider that this exercise-induced elevation in PAP may manifest in the right-sided ECG, as seen in pathology, and the application of this technique in the EICF setting is therefore important. This provided the focus for Chapter 5 where the right-sided ECG in ultra-endurance athletes is obtained prior to and on completion of a 100 mile ultra-marathon.

2.7 Insights from In-Exercise Assessment

There are a limited number of studies assessing cardiac function during exercise (see Table 2.4). Studies have applied conventional 2D, Doppler and tissue Doppler indices and reported no change in LV EF during a 150 minute cycle and a reduction in LV EF during running exercise of the same duration (Goodman et al., 2001, Banks et al., 2010) (see Table 2.4). A reduction in LV SV has been reported during a 120 minute cycle and 150 minute run (Banks et al., 2010, Goodman et al., 2008). Conversely, an increase in RV and LV S' has been demonstrated during submaximal cycling exercise (Tan et al., 2009, Goebel et al., 2007) (see Table 2.4). With regards to diastolic function, an increase in LV E, A, E' and A' has been demonstrated during submaximal cycling and 150 minute running exercise bout resulting in a reduction in the E/A and E'/A' ratios (Goebel et al., 2007, Tan et al., 2009, Giannaki et al., 2008, Banks et al., 2010) (see Table 2.4). Collectively, these studies reflect some similarity to the pre and

post-exercise EICF literature in the LV with a reduction in systolic and diastolic function evident, whilst highlighting the lack of data available for the RV.

In contrast, studies applying novel ε imaging techniques to interrogate the in-exercise response of LV function demonstrate an increase in LV longitudinal, radial and circumferential ε and SRS during progressive or submaximal cycling exercise of a short duration (Pierre-Justin et al., 2005, Hanekom et al., 2007, Goodman et al., 2008, Tan et al., 2009, Doucende et al., 2010, Soullier et al., 2012) (see Table 2.4). Doucende et al. (2010) also reported an increased SRE during the progressive submaximal cycle session in their study. Few studies have applied novel techniques in the assessment of RV function during exercise. Goebel et al. (2007), Tan et al. (2009) and La Gerche et al. (2012a) applied ε imaging to the RV during submaximal cycling, stress echo and progressive maximal cycling respectively and reported an increase in RV ε (see Table 2.4). These studies do not demonstrate any link to the reduction in LV and RV ε and SR seen in recovery from more prolonged exercise in EICF, however the in-exercise response to the prolonged exercise bouts in EICF research is not known. Unlike many of the in-exercise studies, Banks et al. (2010) report contradictory data highlighting a reduction in LV and RV ε, SRE and SRA following 150 minutes of running. The study design employed does not truly assess in-exercise function as participants were transferred to a standard supine echocardiographic position without any continuous

exercise stimulus. This may, in part, explain the disparity observed as heart rates will decline and loading conditions are altered.

Applying novel techniques to the temporal assessment of the LV and RV to prolonged endurance exercise may help to reveal any interaction between the ventricles and provide further mechanistic insight, whilst adding to the understanding of the timing and mechanisms responsible for EICF. Chapters 6 in this thesis aimed to determine the feasibility and reliability of different techniques to assess RV ε during exercise. Based on the recommendations from Chapter 6, an in-exercise assessment of RV function throughout a 6 hour bout of cycling exercise was undertaken in Chapter 7 using the most reliable and accurate technique to derive RV ε.

Table 2.4 - Studies assessing the in-exercise functional responses of the LV and RV

Author	Year	Exercise completed	n	Systolic Indices			Diastolic Indices		
				↓	-	↑	↓	-	↑
Goodman	2001	150 min cycle	15		LV EF, ESV		LV EDV		
Pierre-Justin	2005	Progressive cycle	18			LV ε, SRS			
Goebel	2007	Cycle at 2 W/kg body weight	45			RV S', ε, LV S', ε			
Hanekom	2007	Stress echo	150			LV ε, SRS			
Giannaki	2008	Progressive supine cycle	16				LV E/A, E'/A'		LV E, A, E', A'
Goodman	2008	120 min cycle	12, VO2 = 51.7	LV SV		LV EF, long ε, SRS			
Esch	2009	Submaximal cycle	6			LV torsion			LV untwist
Tan	2009	Submaximal cycle	27			LV SV, S', long and rad ε			LV E', E, untwist rate
Teske	2009	Stress echo	34			RV ε			
Banks	2010	150 minute run	18 VO2 = 55.1	LV EF, SV, LV ε, RV ε					
Doucende	2010	Progressive submaximal cycle	20			LV SV, long + circ ε and SRS, twist, twist rate			LV long + circ SRE, untwist rate
Stohr	2010	Knee extension	10			LV twist			
La Gerche	2012	Progressive maximal cycle	40 athletes, 15			RV ε, SRS			
Soullier	2012	Progressive cycle to exhaustion	20			LV long, circ + rad ε, twist			LV untwist

LV – Left Ventricle, RV – Right Ventricle, EF – Ejection Fraction, E - Early Trans-mitral filling velocity, FAC – Fractional Area Change, SV – Stroke Volume, ESV – End Systolic Volume, EDV – End Diastolic Volume, S' – Systolic Myocardial Velocity, E' – Early Diastolic Myocardial Velocity, A' – Late Diastolic Myocardial Velocity, SRS – Systolic Strain Rate,

SRE – Early Diastolic Strain Rate, SRA - Late Diastolic Strain Rate , long – Longitudinal, rad – Radial, circ – Circumferential.

2.8 Development of echocardiographic techniques

The current body of research related to EICF has been developed over the past 30 years and has utilised a variety of ultrasound techniques to illuminate any changes in cardiac function. The techniques applied to the research have developed in tandem with the evolution of echocardiography as a modality to provide quantitative indices of LV, RV, LA and RA function. Following development of the B-mode and M-mode techniques based on the acoustic mismatch, the next step utilised a number of beams to generate B-mode signals across a sector. Depth and brightness were established for each beam, allowing the first 2D moving echocardiographic images (Bom et al., 1973, Griffith and Henry, 1974, see Figure 2.1). Henry and colleagues subsequently proposed the first systematic approach to standard 2D and M-mode echocardiography for assessment of the LV (Henry et al., 1980). Over time this has developed and the new guidelines for LV chamber quantification have been published this year (Lang et al., 2015). 2D imaging also provides opportunity to evaluate right heart structure and function (Foale et al., 1986), however this is challenging due to the lack of uniformity of the RV. Echocardiographic assessment of RV structure and function has long been considered to be problematic (Ho and Nihoyannopoulos, 2006) and compounded by excess trabeculation, and a separate infundibulum (Levine et al., 1984). Foale et al. (1986) suggested an approach to overcome these problems by assessing both inflow and outflow

dimensions from a range of acoustic windows. This formed the basis for the current guidelines for RV chamber quantification (Rudski et al., 2010).

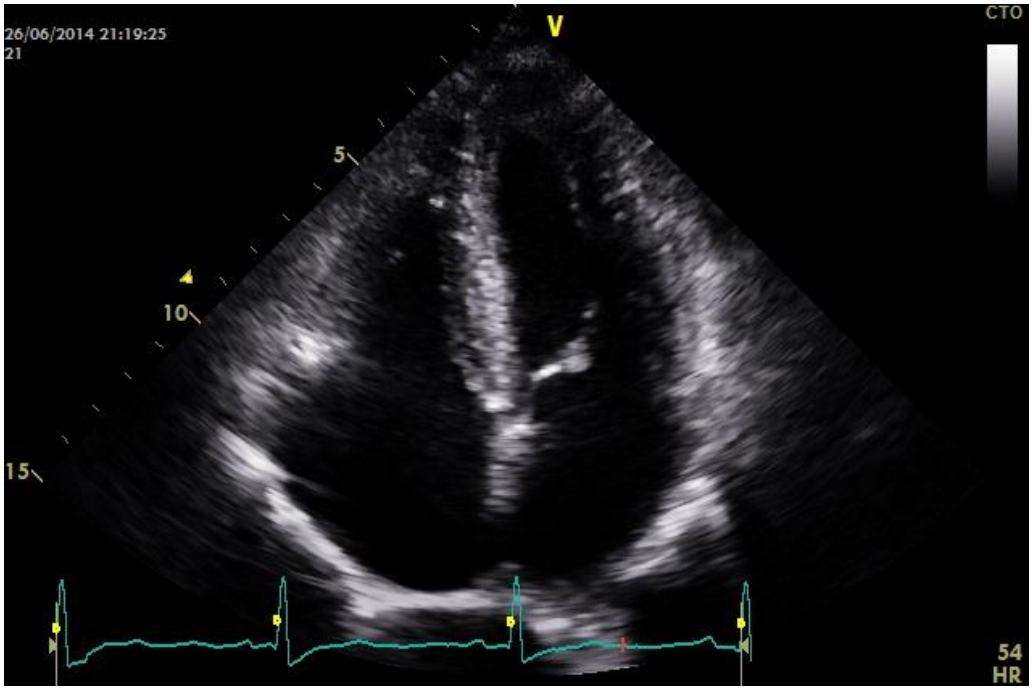


Figure 2.1 – Exemplar 2D apical 4 chamber image

The practicalities and principles for utilising Doppler to assess blood flow were established by Baker (1970). The trans-mitral Doppler signal has clear clinical significance and was incorporated into the standard echocardiographic examination. This provided the measurements of early (E) and late diastolic (A) filling, allowing the calculation of the E/A ratio. (Rakowski et al., 1996, see Figure 2.2). It is important to remember that the trans-mitral Doppler measures LV filling and not just LV diastolic function (Nagueh et al., 2009).

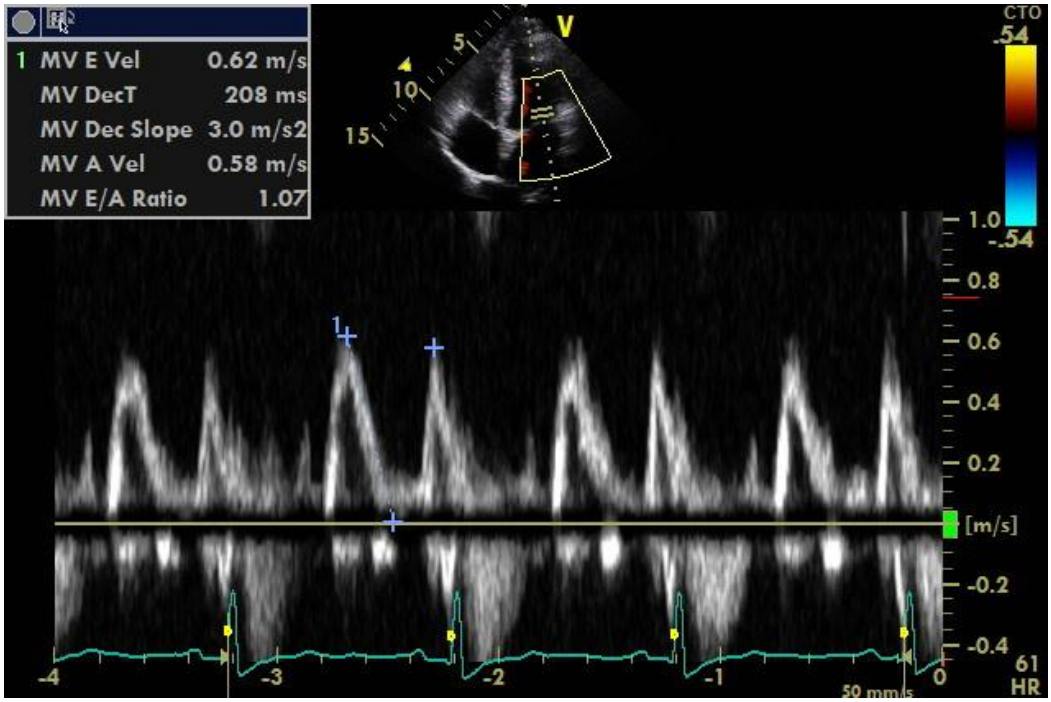


Figure 2.2 – Exemplar transmitral Doppler flow profile

The use of standard PW Doppler to interrogate myocardial wall velocity, as opposed to blood flow, was first introduced by Isaaz et al., (1989). The technique was quickly adopted into the echocardiographic assessment due to the ease of aligning the myocardium with the ultrasound beam and it's ability to assess regional function. TDI provides systolic (S'), early diastolic (E') and late diastolic (A') myocardial velocities for different myocardial wall segments (see Figure 2.3) and has excellent temporal and spatial resolution whilst being less dependent on 2D image quality.

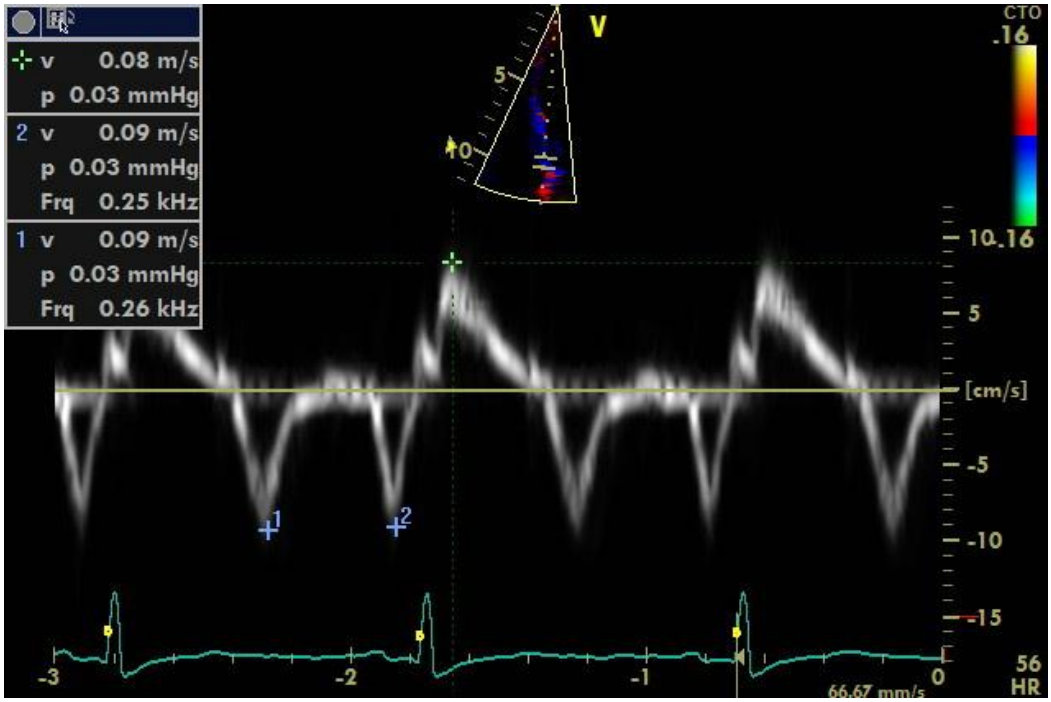


Figure 2.3 – Exemplar assessment of septal myocardial tissue velocities using tissue Doppler.

Sutherland (1995) developed the standard PW TDI technique by applying a colour Doppler method. This allowed the assessment of myocardial deformation (strain) (ε). Myocardial ε describes the change in length (as a percentage) of the myocardium rather than movement provided by velocity data (Sutherland et al., 2004, see Figure 2.4). Strain rate (SR) is the rate of deformation and is expressed in terms of length per second (l/s) (Yip et al., 2003). TDI derived ε and SR have been validated *in vitro* using phantoms (Heimdal et al., 1998, Belohlavek et al., 2001) and *in vivo* using ultrasonic crystals (Urheim et al., 2000). Unlike velocity data, ε is not affected by translation and tethering, as a result of not being relative to the transducer position (Sutherland et al., 2004). Strain imaging has similar inherent limitations to TDI. TVI ε is derived from velocities and as such it

provides a measure of 'Eulerian' ε (Pavlopoulos and Nihoyannopoulos, 2008), there is no true measurement of baseline original length (Lagrangian ε).

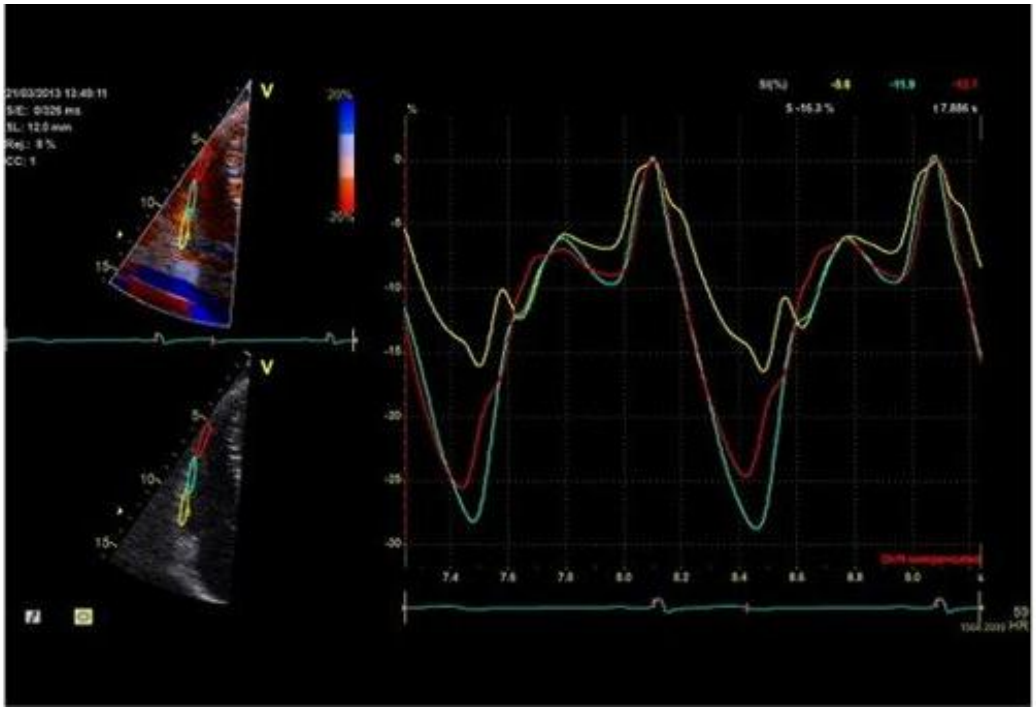


Figure 2.4 – Tissue Doppler assessment of RV myocardial strain

Myocardial speckle tracking (MST) is a technique where kernels or speckles of the myocardium are identified in 2D greyscale images and tracked through the cardiac cycle to provide strain data in systole and diastole. MST provided an echocardiographic technique that overcame many of the limitations of TDI ε imaging, including angle independency. It also provides the ability to accurately assess LV longitudinal, radial, rotation and circumferential deformation, allowing a better understanding of the complex temporal cardiac mechanics throughout the cardiac cycle. MST derived strain is a measure of lagrangian ε using the original length as the starting value (Geyer et al., 2010). MST is capable of evaluating the

subtle exercise-induced changes in LV and RV function (Mor-Avi et al., 2011). MST also allows the assessment of ventricular diastolic function by providing strain rate data in multiple planes in early (SRE) and late (SRA) diastole and their ratio (SRE/SRA) and this has been applied in the EICF setting to evaluate LV, RV and even LA function (see Figure 2.5).

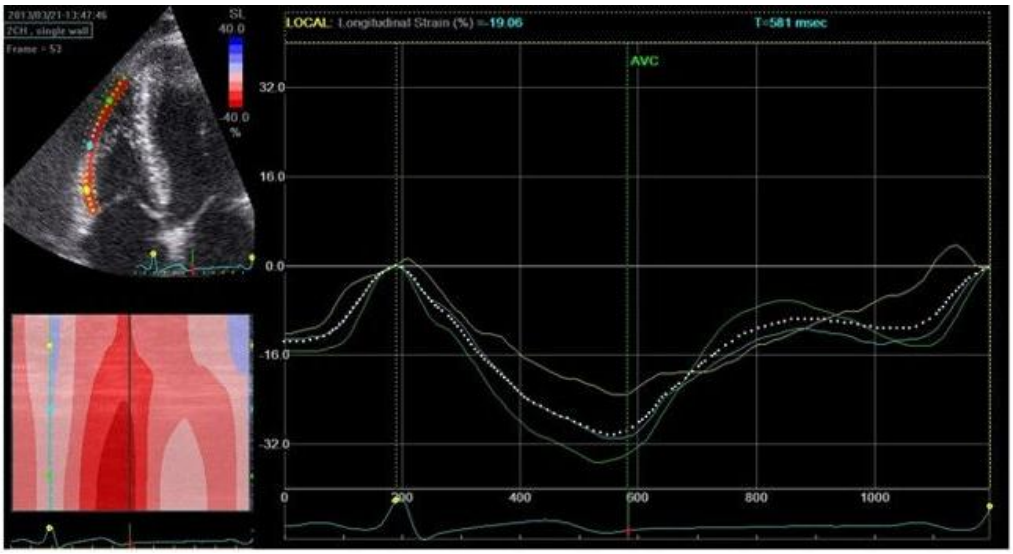


Figure 2.5 – Myocardial speckle tracking assessment of RV strain

2.9 Conclusion

This review has defined the concept of EICF and detailed the historical literature with respect to initially LV and more recently RV structural and functional response to prolonged endurance exercise. The available data generally suggests a global decrease in both systolic and diastolic RV function alongside RV dilatation. The development of echocardiographic techniques has afforded a more in depth assessment of cardiac function with the advent of MST providing a more accurate measure of intrinsic myocardial function. With reference to the RV, there is a suggestion that

there may be a more persistent nature in RV functional responses to prolonged endurance exercise. The issues highlighted by this literature review have generated the structure and focus of the studies contained within this thesis.

It is pertinent to establish whether a more persistent change is evident in RV structure and function, specifically applying the novel technique of MST following a large exercise volume. This builds the rationale for the assessment of recovery measures following a 100 mile ultra-marathon in Chapter 3; a field based study at the 2013 Western States 100 mile endurance run. Conventional echocardiographic indices were combined with novel MST ε imaging prior to, immediately on completion of and 6 hours into recovery from the race. There is a deficit of literature pertaining to the temporal relationship between structure and function and the interaction between the ventricles. Clearly the application of a technique that allows simultaneous structure and function to be derived would provide further mechanistic insight and expose any impact of the RV on the LV. The application of novel echocardiographic techniques to link area and ε, may serve as a surrogate for the pressure-volume relationship. This develops the rationale and the integration of imaging methods for a holistic assessment of all four cardiac chambers to better understand the temporal cardiac mechanics following a 100 mile ultra-marathon in field-based Chapter 4. Studies have suggested that there may be an electrical activation response to prolonged endurance exercise. Chapter 5, therefore includes the application of the standard 12-lead ECG to

determine the acute response of the electrical conduction system to the 100 mile ultra-marathon. Based on the clinical application of right-sided ECG leads being utilised due to their enhanced sensitivity and specificity to right heart changes especially in the presence of an elevated afterload, this study was a field based study focusing solely on the right-sided precordial leads at the 2014 Western States 100 mile endurance run. Specific attention was paid to the development of R' waves, ST segment and T wave changes following the race.

There is a scarcity of literature focusing on the in-exercise assessment of the RV during prolonged endurance exercise. Although the reliability of MST is known at rest, it is pertinent to establish the reliability of the technique during exercise at varying intensities and in the upright position to allow the application of this technique to the functional assessment of the RV. Different techniques have been suggested to provide differing values when utilised to derive RV ε. Chapter 6 was therefore a laboratory study that assessed the repeated measures feasibility, validity, comparability and reliability of TVI and MST derived RV ε and SR both at rest and during progressive cycling exercise in an upright position. The current data applying MST to the in-exercise assessment of the RV suggest that, in response to a short bout of exercise, the RV responds in a physiological manner with a proportional increase in contractility with increasing exercise intensity. Still lacking however is the assessment of the RV during a prolonged exercise bout, and this provides the rationale for Chapter 7, a laboratory study assessing RV structure and function of

participants during a 6 hour upright cycling exercise bout. It has been demonstrated that PAP can be elevated up to 60 mmHg during short, progressive exercise, and considering the implication of an elevated PAP in the mechanisms responsible for post-exercise changes in RV structure and function, Chapter 7 also included the estimation of PAP in the upright cycling session. The simultaneous assessment of RV structure and function alongside PAP may help to expose the temporal relationship between these variables during prolonged endurance exercise and develop the mechanistic understanding of RV exercise-induced responses.

2.10 Aims and Hypotheses

1. To assess RV structure and function following a 100 mile ultra-marathon applying novel techniques to determine the immediate and 6 hour post-race responses.
2. To provide a holistic assessment of all cardiac chambers, including the simultaneous derivation of structure and function for both the LV and RV in the same cardiac cycle following a 100 mile ultra-marathon.
3. To establish the acute P wave, ST segment and T wave changes in response to a 100 mile ultra-marathon in both the 12-lead ECG and right-sided ECG.
4. To determine the comparability and reliability of MST and TVI techniques to derive RV strain during progressive cycling exercise.

5. To assess RV structure and function during a 6 hour upright cycling exercise bout, including the simultaneous estimation of PAP.

   H_0: There will be no change in any variable assessing RV structure or function following a 100 mile ultra-marathon

   H_0: There will be no change in any variable assessing RV or LV structure and function and therefore there will be no interaction between the ventricles following a 100 mile ultra-marathon.

   H_0: There will be no change in P wave amplitude, ST segment or T wave amplitude in either the 12-lead or right sided ECG following a 100 mile ultra-marathon.

   H_0: There will be no difference between RV strain values or the reproducibility of these values derived using TVI and MST during progressive exercise.

   H_0: There will be no change in any variable assessing RV structure or function during a 6 hour cycle.

Chapter 3 - The Right Ventricle following Ultra-endurance Exercise: Insights from Novel Echocardiography and 12-lead Electrocardiography

This Chapter is published in the European Journal of Applied Physiology and can be accessed at the following address:

http://www.ncbi.nlm.nih.gov/pubmed/25204280

Chapter 4 - Alterations in Cardiac Mechanics following Ultra-endurance Exercise: Insights from Left and Right Ventricular Area-Strain Loops

4.1 Introduction

As described in Chapter 3, prolonged endurance exercise has a negative impact on both the RV and LV (Middleton et al., 2006a). A number of theories describing the possible mechanisms responsible for these findings have been proposed including beta-adrenergic receptor desensitization, oxidative stress and impaired calcium metabolism (Dawson et al., 2005), however these have yet to be substantiated. The theory suggested to explain exercise-induced changes in the RV in Chapter 3 may also be implicated in any interaction between the ventricles.

Echocardiographic techniques such as ε imaging have allowed a more comprehensive assessment of LV and RV function and these have recently been employed in the post-prolonged exercise setting in Chapter 3 adding further support to previous studies (George et al., 2009, Oxborough et al., 2011, Oxborough et al., 2010b, La Gerche et al., 2012b). Collectively, the data in Chapter 3 in addition to the body of published evidence highlight a reduction in peak LV and RV ε alongside alterations in chamber dimensions, but the impact of ultra-endurance exercise on temporal cardiac mechanics remains largely unknown. In this

setting, the interaction of RV and LV structure and function has received limited attention and a comprehensive evaluation of simultaneous structure and ε throughout the cardiac cycle has not been attempted. The combination of echocardiographic modalities may help to reveal mechanical changes in cardiac function whilst offering a more comprehensive understanding of exercise-related structural and functional adaptation. The concept of assessing area-ε relationships (loops) within the ventricles is novel and provides the potential for determining the contribution of longitudinal ε to area change in both ventricles. The underpinning theory for the construction of the area-ε loop was developed in 1976 by Gibson and Brown. Their exploratory work demonstrated excellent agreement between the timing of the upstroke on LV displacement curves as determined using apexcardiography and the upstroke in LV pressure as measured using a simultaneous invasive LV pressure catheter. Having validated displacement as a surrogate for pressure, they utilised M-mode echocardiography to define structure with simultaneous apexcardiography allowing for a non-invasive surrogate of pressure-volume loops in a range of disease states (Venco et al., 1977). Myocardial ε is strongly correlated to LV and RV displacement as determined by apexcardiography, M-mode echocardiography and tissue Doppler derived ‘tissue tracking’ (Sutherland et al., 2004). Myocardial ε has fewer limitations, such as tethering, translation and rotation, which are inherent to displacement from M-mode and Tissue tracking (Korinek et al., 2005). Improvements in 2D imaging allow for accurate assessment of ventricular area and therefore an area-ε loop takes forward the concepts

established by Gibson and colleagues. In view of the improvements in quantitative analysis over the preceding decades it is likely that area-ε loops are a more accurate non-invasive surrogate for a pressure-volume loop with the additional value in providing an assessment of the RV.

In view of this, the current study utilises a novel approach by assessing echocardiographic derived temporal area-ε loops in conjunction with conventional 2D and Doppler indices, in order to establish the relative impact of prolonged strenuous exercise (100 mile endurance run) on RV and LV structure and function. Furthermore, the study aims to establish whether any changes in cardiac mechanics persist 6 hours into recovery from the exercise bout. These broad aims allow the generation of the following specific hypotheses: 1) The RV area-ε loop will be displaced to the right as a result of RV dilatation and hence RV SV will be reduced. This will have a serial impact on the LV area-ε loop, which will consequently be displaced to the left indicative of under-filling. 2) After 6 hours of recovery, the RV area-ε loop will return to baseline values and therefore LV filling will be restored and the area-ε loop will return to baseline.

4.2 Methods

4.2.1 Sample Population and Protocols

The same sample as described in Chapter 3 is utilised for the data in this Chapter with additional methods detailed below.

4.2.1 Echocardiographic Assessments

All echocardiographic images were acquired using a commercially available ultrasound system (Vivid Q, GE Medical, Horten, Norway) with a 1.5-4 MHz phased array transducer. Images were obtained by a single experienced sonographer with the participant in the left lateral decubitus position. Images were recorded to DVD in raw DICOM format and data were analysed offline by a single experienced sonographer using commercially available software (EchoPac version 7, GE Medical, Horten, Norway). A minimum of three cardiac cycles were averaged for all peak indices.

4.2.2 Conventional 2D, Doppler and Tissue Doppler Echocardiography

The RV was assessed in accordance with ASE guidelines (Rudski et al., 2010a) as described in Chapter 3.

In addition, a comprehensive assessment of LV structure and function was undertaken in accordance with ASE guidelines (Lang et al., 2005). LV EDV and ESV were estimated using Simpsons biplane methodology allowing the calculation of SV and EF. LV diastolic function was assessed using trans-mitral Doppler providing peak E, A and their ratio E/A. Pulsed wave tissue TDI assessment of the lateral and septal annulus provided peak myocardial velocities S', E' and A' and the average of both walls reported. E/E' was calculated as a non-invasive surrogate of left atrial (LA)

pressure. RVSp was derived from the tricuspid regurgitant jet using continuous wave Doppler. PAP was calculated as (PAP (mmHg) = RVS_p + 5mmHg). RV and LV end-systolic wall stress was calculated using the formula ES-σ = Pr/2h as previously described (La Gerche et al., 2011).

A full assessment of LA and RA structure and volumetric function was assessed using a Simpson biplane method as previously described (Oxborough et al., 2010b). LA and RA volumes at end systole (VOL ES), end diastole (VOL ED) and pre A (VOL pre A) were calculated allowing the derivation of reservoir (RES), LA conduit (CON) and booster (BOO) volumes.

4.2.3 2D Myocardial Speckle Tracking

A focused apical four chamber orientation was acquired for assessment of the LV whilst a modified image with lateral transducer movement was acquired for assessment of the RV. For the assessment of LV circumferential function, rotation and torsion, images of the LV were acquired from a parasternal short axis view at the base, mid and apex. For all images the system was optimised as previously described (Oxborough et al., 2011). Offline analysis allowed the assessment of peak global longitudinal RV ε (calculated as an average of 3 myocardial segments from base to apex of the RV lateral wall, peak global longitudinal LV ε and peak global LV circumferential ε as an average of 6 myocardial segments at basal mid and apical levels. Peak basal and apical rotation and rotation rates in systole and early and late diastole were obtained to allow the

calculation of peak twist and twist rate as the net difference between basal and apical rotation and rotation rate respectively.

4.2.4 Area-Strain Loops

In order to standardise for variable HR, temporal data was obtained throughout the entire cardiac cycle using cubic spline interpolation in Microsoft Excel (2010) to provide 300 data points for both systole and diastole as previously described (Burns et al., 2010a). The splined data of longitudinal RV and LV ε were used to derive time points for the simultaneous area and ε calculations. Both systole and diastole were divided into 10% increments, essentially providing 20 time points and subsequent ε values across the full cardiac cycle. The original image and cardiac cycle that was used to derive the ε values was then re-analysed for RV/LV area in 2D at each corresponding time point, hence providing a simultaneous RV/LV ε and RV/LV area (see Figure 4.1). This was undertaken for each individual participant and the mean area-ε at percentage increments were calculated across the cohort. Data was plotted as area against ε (area-ε loop) for the whole cohort for RV and LV longitudinal motion using commercially available software (GraphPad Prism).

Polynomial regression analysis of the order $y=mx^2+mx+c$ was performed on each individual participants area-ε loops for systole and diastole independently at pre, post and post 6 hours. Using the polynomial equation ε values in systole and diastole were calculated for 10%

increments of the chambers end diastolic area (EDA) within the range 40-90% for the LV and 60-90% for the RV to reflect physiological functional area change in each ventricle. The difference between the same percentage of EDA in systole and diastole was calculated and termed *systolic-diastolic ε gradient*.

Reliability data for the RV and LV area-ε loops in 20 control subjects was similar across EDA ranges (40 to 80%) with coefficient of variation values ranging from 7-21% for simultaneous ε, area and systolic-diastolic gradient. Comprehensive reliability data for each 10% change in EDA is provided in Tables 4.1-4.3).

Gibson et al. (1976) employed apex cardiograms and plotted displacement against LV dimension changes which they were able to validate against invasive pressure-dimension assessments. They then assessed their displacement-dimension loops and invasive pressure-dimension loops in different populations with pathological conditions with varying loading conditions such as aortic stenosis, ischaemic heart disease and mitral regurgitation as well as healthy controls and were able to validate their technique. It is argued that the current deployment of area- ε loops is a sensible and linked development of this “old” technique bringing it into the 21st century with current echocardiographic tools.

Table 4.1 - Coefficients of variance (CoV) and intra-class coefficients (ICC) for left ventricular and right ventricular strain and systolic-diastolic strain gradients derived from polynomial regression at percentage end diastolic areas

Area %	Systolic Strain CoV (%) (95% CI)	Systolic Strain ICC (95% CI)	Diastolic Strain CoV (%) (95% CI)	Diastolic Strain ICC (95% CI)	Gradient ICC (95% CI)
RIGHT VENTRICLE					
80	20.7 (15.8-30.3)	0.699 (0.240-0.881)	17.4 (13.3-25.3)	0.805 (0.508-0.923)	0.760 (0.393-0.905)
70	12.9 (9.8-18.7)	0.833 (0.578-0.934)	12.3 (12.2-17.6)	0.866 (0.662-0.947)	0.722 (0.298-0.890)
60	9.4 (7.2-13.6)	0.901 (0.749-0.961)	9.0 (6.9-13.1)	0.910 (0.774-0.965)	0.531 (-0.186-0.814)
50	8.3 (6.3-12.0)	0.919 (0.795-0.968)	8.3 (6.3-12.0)	0.916 (0.789-0.967)	0.372 (-0.587-0.751)
40	9.0 (6.8-13.0)	0.909 (0.770-0.964)	9.6 (7.4-14.0)	0.890 (0.723-0.957)	0.661 (0.143-0.866)
LEFT VENTRICLE					
80	19.2 (14.7-28.0)	0.637 (0.083-0.856)	10.6 (8.1-15.4)	0.914 (0.783-0.966)	0.207 (-1.003-0.686)
70	13.4 (10.2-19.4)	0.729 (0.316-0.893)	9.1 (7.0-13.2)	0.903 (0.755-0.962)	0.265 (-0.856-0.709)
60	9.7 (7.4-14.0)	0.788 (0.464-0.916)	7.9 (6.0-11.5)	0.893 (0.730-0.958)	0.372 (-0.586-0.752)
50	7.4 (5.6-10.7)	0.828 (0.565-0.932)	7.2 (5.5-10.5)	0.874 (0.682-0.950)	0.485 (-0.301-0.796)
40	6.8 (5.2-9.8)	0.834 (0.582-0.934)	7.2 (5.5-10.4)	0.837 (0.588-0.935)	0.198 (-1.026-0.683)

Table 4.2 – Coefficients of variance (CoV) and intra-class coefficients (ICC) for right ventricular strain and area

Percentage of cardiac cycle	Area ICC (95% CI)	Strain ICC (95% CI)	Area CoV (%) (95% CI)	Strain CoV (%) (95% CI)
80	0.986 (0.965-0.995)	0.957 (0.892-0.983)	4.1 (3.1-6.0)	8.0 (6.1-11.5)
75	0.986 (0.965-0.995)	0.953 (0.880-0.981)	4.0 (3.1-5.8)	7.2 (5.5-10.3)
70	0.981 (0.952-0.993)	0.954 (0.883-0.982)	5.3 (4.1-7.7)	5.4 (4.1-7.8)
65	0.990 (0.975-0.996)	0.953 (0.881-0.981)	4.2 (3.2-6.1)	4.8 (3.6-6.9)
60	0.985 (0.961-0.994)	0.953 (0.881-0.981)	5.3 (4.0-7.7)	4.5 (3.5-6.6)
55	0.972 (0.930-0.989)	0.950 (0.874-0.980)	5.6 (4.3-8.1)	4.4 (3.4-6.4)
50	0.979 (0.946-0.992)	0.940 (0.849-0.976)	5.3 (4.1-7.7)	4.0 (3.1-5.8)
45	0.974 (0.934-0.990)	0.921 (0.801-0.969)	6.7 (5.1-9.7)	6.7 (5.1-9.7)
40	0.984 (0.959-0.994)	0.970 (0.924-0.988)	5.7 (4.4-8.2)	9.7 (7.4-14.0)

Table 4.3 - Coefficients of variance (CoV) and intra-class coefficients (ICC) for left ventricular strain and area

Percentage of cardiac cycle	Area ICC (95% CI)	Strain ICC (95% CI)	Area CoV (95% CI)	Strain CoV (95% CI)
80	0.987 (0.968-0.995)	0.930 (0.823-0.972)	3.3 (2.5-4.8)	11.9 (9.1-17.3)
75	0.980 (0.950-0.992)	0.919 (0.794-0.968)	4.4 (3.4-6.4)	8.7 (6.6-12.6)
70	0.967 (0.916-0.987)	0.912 (0.777-0.965)	5.7 (4.4-8.3)	7.3 (5.6-10.5)
65	0.957 (0.891-0.983)	0.901 (0.751-0.961)	6.6 (5.0-9.5)	6.5 (5.0-9.5)
60	0.955 (0.888-0.982)	0.883 (0.705-0.954)	5.8 (4.4-8.4)	6.4 (4.9-9.2)
55	0.960 (0.899-0.984)	0.881 (0.699-0.953)	6.1 (4.7-8.8)	6.1 (4.7-8.9)
50	0.930 (0.823-0.972)	0.885 (0.709-0.954)	7.5 (5.7-10.8)	5.9 (4.5-8.5)
45	0.928 (0.794-0.968)	0.910 (0.771-0.964)	8.0 (6.1-11.6)	7.2 (5.5-10.5)
40	0.985 (0.985-0.962)	0.963 (0.907-0.985)	5.4 (4.1-7.7)	9.0 (6.9-13.1)

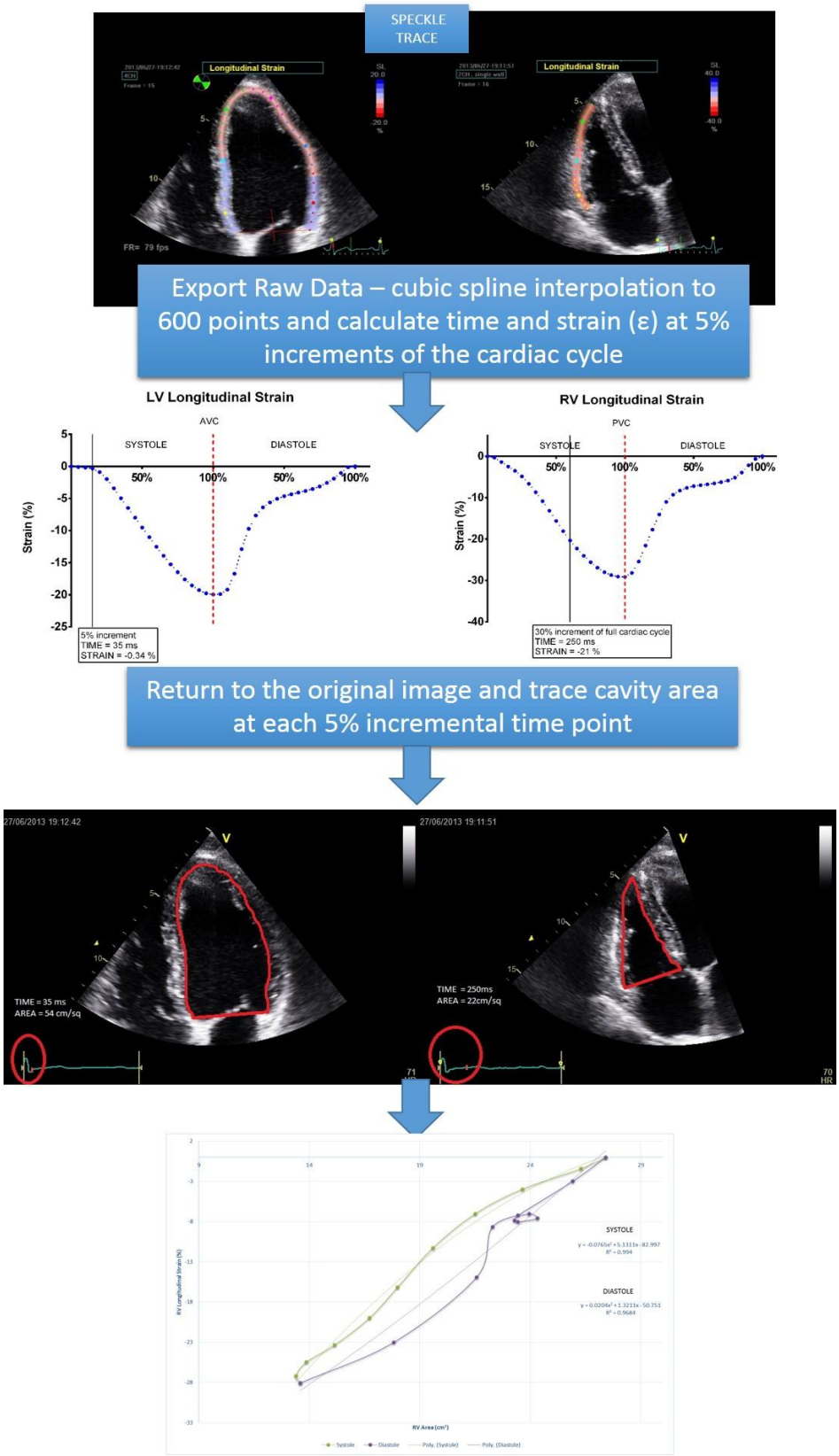


Figure 4.1 - Systemic methodology for generation of area-strain loops

4.2.5 Statistical Analysis

All echocardiographic data were analysed for normality of distribution using a Shapiro-Wilk test. Due to the reduced sample size from post-race to post 6 hour data collection, pre-race versus post-race data were compared using Student's Paired T-tests and recovery data reported for descriptive purposes. All statistical tests were performed using commercially available software (IBM SPSS version 21) and statistical significance was set at $P < 0.05$.

4.3 Results

4.3.1 Demographics

Systolic and diastolic BP were significantly reduced post-race. Heart rate and body mass were not different at pre and post-race assessments (see Table 4.4).

Table 4.4 - Participant demographics pre and post-race and after 6h of recovery

Parameter	Pre	Post	Recovery
Body mass (kg)	70.1 ± 8.8	68.8 ± 7.8*	66.1 ± 7.9
Systolic BP (mmHg)	134 ± 11	114 ± 12*	117 ± 12
Diastolic BP (mmHg)	84 ± 10	76 ± 8*	77 ± 8
Heart rate (bpm)	63 ± 10	70 ± 10	71 ± 12

* indicates statistical significance pre to post race ($P<0.05$). Data presented as mean ± SD

4.3.2 Conventional 2D, Doppler and Tissue Doppler Echocardiography

There was a post-race increase of 13% in RVOTplax, 13% in RVOT1, 12% in RVD1 and 16% in RVD2 (*P* = 0.004, 0.002, 0.003 and 0.001, respectively) whilst there was a 10% decrease in RV FAC and 18% reduction in RV S' and E' (*P* = 0.02, 0.005 and 0.049, respectively; Table 4.5). RV SV was maintained with no significant reduction observed post-race. RV wall stress was elevated compared to baseline immediately post-race and in recovery and PAP was reduced post-race compared to pre-race measures albeit not significantly. There was an 11% decrease in LV EDV and 13% increase in LV ESV (*P* = 0.005 and 0.009 respectively) resulting in a 23% and 12% reduction in LV SV and LV EF (*P* < 0.001). LV S' was reduced by 11% post-race (*P* = 0.04). There was an 18 % decrease in trans-mitral E (*P* = 0.001) and a subsequent 19% decrease in the E/A velocity ratio (*P* = 0.003). LV E' and A' were reduced by 16 and 10% (*P* < 0.001 and 0.007 respectively) post-race and hence E/E' was unchanged pre to post-race. LV EI in systole was elevated pre to post-race (*P* = 0.04). LV wall stress was reduced post-race and in recovery compared to pre-race values, albeit not significantly. LA VOL ES, pre A, ED and RES volumes were not different post-race (*P* > 0.05) whereas both CON and BOO volumes were reduced by 36 and 19% (*P* = 0.02 and 0.03 respectively). There was no change in RA VOL ES, pre A, ED, RES and BOO volumes pre to post-race (*P* > 0.05).

Table 4.5 - Left and right ventricular and atrial structural and functional data pre-race, post-race and after 6 hours of recovery

Parameter	Pre	Post	Recovery
RIGHT VENTRICLE			
$RVOT_{plax}$ (mm)	30 ± 4	33 ± 3*	33 ± 4
$RVOT_1$ (mm)	32 ± 4	36 ± 4*	35 ± 5
$RVOT_2$ (mm)	25 ± 2	28 ± 2	27 ± 3
RVD_1 (mm)	43 ± 4	48 ± 5*	47 ± 6
RVD_2 (mm)	32 ± 3	37 ± 3*	36 ± 3
RVD_3 (mm)	84 ± 6	83 ± 7	82 ± 6
RVFAC (%)	54.1 ± 5.8	48.8 ± 4.7*	50.3 ± 8.2
TAPSE (mm)	24 ± 4	23 ± 4	26 ± 3
RV S' (cm/s)	17 ± 3	14 ± 3*	16 ± 1
RV E' (cm/s)	17 ± 2	14 ± 4*	14 ± 3
RV A' (cm/s)	13 ± 5	12 ± 3	13 ± 3
RV SV (ml)	92 ± 25	89 ± 25	102 ± 35
PAP (mmHg)	25 ± 4	22 ± 8	23 ± 2
RV Wall Stress (kdynes/cm^2)	3.97 ± 1.93	4.39 ± 1.30	2.94 ± 2.24
LEFT VENTRICLE			
LV EDV (ml)	123 ± 15	109 ± 16*	112 ± 17
LV ESV (ml)	41 ± 5	47 ± 9*	39 ± 8
LV SV (ml)	82 ± 11	63 ± 11*	73 ± 11
LV EF (%)	66 ± 3	58 ± 6*	65 ± 3
MV E (m/s)	0.84 ± 0.17	0.69 ± 0.18*	0.74 ± 0.17
MV A (m/s)	0.50 ± 0.09	0.51 ± 0.11	0.53 ± 0.08
MV E/A	1.70 ± 0.38	1.37 ± 0.37*	1.38 ± 0.26
LV S' (cm/s)	13 ± 2	12 ± 1*	13 ± 2
LV E' (cm/s)	16 ± 2	13 ± 3*	15 ± 2
LV A' (cm/s)	10 ± 1	9 ± 2*	9 ± 2
E/E'	5.29 ± 1.01	5.20 ± 1.05	5.11 ± 1.26
EI Diastole	1.16 ± 0.11	1.22 ± 0.10	1.14 ± 0.08
EI Systole	1.09 ± 0.07	1.15 ± 0.12*	1.14 ± 0.08
LV Wall Stress (kdynes/cm^2)	16.82 ± 2.34	14.42 ± 2.40	12.83 ± 1.66
LEFT ATRIUM			
LA VOL ES (ml)	55 ± 8	57 ± 11	60 ± 12
LA VOL pre A (ml)	33 ± 5	34 ± 8	37 ± 10
LA VOL ED (ml)	17 ± 3	21 ± 5	22 ± 6
LA RES (ml)	38 ± 6	36 ± 6	38 ± 8
LA CON (ml)	44 ± 13	26 ± 8*	35 ± 5

LA BOO (ml)	16 ± 74	13 ± 4*	15 ± 6
RIGHT ATRIUM			
RA VOL ES (ml)	62 ± 23	62 ± 14	58 ± 22
RA VOL pre A (ml)	42 ± 14	46 ± 13	40 ± 13
RA VOL ED (ml)	28 ± 9	29 ± 11	27 ± 8
RA RES (ml)	34 ± 15	33 ± 9	31 ± 15
RA BOO (ml)	14 ± 7	17 ± 8	14 ± 7

*****indicates statistical significance pre to post race ($P<0.05$). Data presented as mean ± SD

4.3.3 Myocardial Strain Imaging

Peak RV longitudinal ε was reduced by 10% pre to post-race (*P* = 0.007). LV longitudinal ε was reduced by 9% post-race (*P* = 0.01). LV basal, mid and apical circumferential ε were all reduced (19, 14 and 15%, *P* = 0.001, 0.008 and 0.01 respectively) pre to post-race as were basal and apical rotation, twist and systolic and diastolic twist rates (39, 46 and 46%, *P* = 0.007, 0.002, <0.001, 0.004 and <0.001 respectively, see Table 4.6).

Table 4.6 - Left and right ventricular strain data pre-race, post-race and after 6 hours of recovery

Parameter	Pre	Post	Recovery
RV longitudinal ε (%)	-28.6 ± 3.8	-25.8 ± 2.8*	-27.4 ± 4.1
LV longitudinal ε (%)	-18.3 ± 1.5	-16.6 ± 2.7*	-18.5 ± 2.4
LV basal circumferential ε (%)	-22.7 ± 2.0	-18.5 ± 3.7*	-21.2 ± 2.4
LV mid circumferential ε (%)	-20.4 ± 3.3	-17.6 ± 3.6*	-21.1 ± 2.7
LV apical circumferential ε (%)	-39.1 ± 8.1	-33.2 ± 6.6*	-35.9 ± 7.3
Basal rotation (°)	-8.7 ± 3.5	-5.3 ± 3.1*	-5.2 ± 3.2
Apical rotation (°)	16.5 ± 6.0	9.0 ± 5.0*	11.7 ± 3.2
Twist (°)	24.8 ± 6.6	13.5 ± 6.3*	16.5 ± 3.7
Systolic twist rate (°/s)	121.7 ± 25.9	90.1 ± 25.8*	120.3 ± 16.3
Early diastolic twist rate (°/s)	-150.6 ± 26.1	-83.8 ± 33.6*	-149.3 ± 38.5
Late diastolic twist rate (°/s)	-79.0 ± 20.9	-78.5 ± 41.3	-81.9 ± 33.1

* indicates statistical significance pre to post race (P<0.05). Data presented as mean ± SD

4.3.4 Area-Strain Loops

The RV area-ε loop demonstrated a rightward shift immediately post-race with increased RV area and reduced peak RV ε dictating that RV ε was elevated for any given area. That aside the polynomial regression equations were similar compared to baseline and the systolic-diastolic ε gradient was unchanged reflected by the similar shape of the loop (see Table 4.7, complete ε values are provided in Table 4.8). The RV area-ε loop at 6 hr recovery was almost identical to the post-race loop (see Figure 4.2).

A leftward shift was observed in the LV area-ε loop post-race, secondary to reduced LV area and reduced peak ε. Hence for any given area, absolute ε values were lower. There was a change in LV longitudinal systolic-diastolic ε gradient post-race at 80, 70 and 40% EDA (see Table 4.7, complete ε values are provided in Table 4.9). This is also corroborated by the change in shape of the LV post-race area-ε loop. In recovery, the systolic-diastolic ε gradient returned close to baseline values, however, the LV loop remained shifted to the left (see Figure 4.2).

Table 4.7 - Systolic-diastolic strain gradients for right and left ventricles pre-race, post-race and after 6 hours of recovery

% EDA	Pre Race	Post Race	Recovery
RIGHT VENTRICLE			
90	-4.2	-4.2	-4.4
80	-5.8	-6.3	-6.4
70	-5.7	-6.2	-6.8
60	-4.1	-4.0	-5.4
LEFT VENTRICLE			
90	-1.0	-1.8	-0.7
80	-0.9	-2.8*	-0.9
70	-0.7	-2.4*	-0.9
60	-0.3	-0.5	-0.6
50	0.2	2.8	-0.2
40	0.8	7.5*	0.5

* indicates statistical significance pre to post race ($P<0.05$). Data analysed using paired t-tests.

Table 4.8 - Systolic and diastolic strain and systolic-diastolic strain gradient data for the right ventricle pre-race, post-race and in recovery derived using polynomial regression equations from area-strain loops and for percentage end diastolic areas.

	Pre Race	**Post Race**	**Recovery**
Systolic ε at 90% EDA (%)	-2.3	-2.2	-2.4
Systolic ε at 80% EDA (%)	-6.3	-6.2	-6.4
Systolic ε at 70% EDA (%)	-11.5	-11.5	-11.5
Systolic ε at 60% EDA (%)	-18.0	-18.2	-17.8
Diastolic ε at 90% EDA (%)	-6.6	-6.4	-6.8
Diastolic ε at 80% EDA (%)	-12.1	-12.4	-12.8
Diastolic ε at 70% EDA (%)	-17.3	-17.7	-18.3
Diastolic ε at 60% EDA (%)	-22.1	-22.1	-23.3
Sys-Dia Gradient at 90% EDA (%)	-4.2	-4.2	-4.4
Sys-Dia Gradient at 80% EDV (%)	-5.8	-6.3	-6.4
Sys-Dia Gradient at 70% EDA (%)	-5.7	-6.2	-6.8
Sys-Dia Gradient at 60% EDA (%)	-4.1	-4.0	-5.4

Table 4.9 - Systolic and diastolic strain and systolic-diastolic strain gradient data for the left ventricle pre-race, post-race and in recovery derived using polynomial regression equations from area-strain loops and for percentage end diastolic areas.

	Pre Race	**Post Race**	**Recovery**
Systolic ε at 90% EDA (%)	-3.5	-3.0	-4.5
Systolic ε at 80% EDA (%)	-7.6	-6.9	-8.9
Systolic ε at 70% EDA (%)	-12.0	-11.3	-13.1
Systolic ε at 60% EDV (%)	-16.8	-16.2	-17.2
Systolic ε at 50% EDA (%)	-21.8	-21.7	-21.2
Systolic ε at 40% EDA (%)	-27.1	-27.7	-25.1
Diastolic ε at 90% EDA (%)	-4.4	-4.9	-5.1
Diastolic ε at 80% EDA (%)	-8.5	-9.7	-9.7
Diastolic ε at 70% EDA (%)	-12.7	13.7	-14.0
Diastolic ε at 60% EDA (%)	-17.1	-16.7	-17.9
Diastolic ε at 50% EDA (%)	-21.6	-18.9	-21.4
Diastolic ε at 40% EDA (%)	-26.2	-20.2	-24.6
Sys-Dia Gradient at 90% EDA (%)	-1.0	-1.8	-0.7
Sys-Dia Gradient at 80% EDA (%)	-0.9	-2.8	-0.9
Sys-Dia Gradient at 70% EDA (%)	-0.7	-2.4	-0.9
Sys-Dia Gradient at 60% EDA (%)	-0.3	-0.5	-0.6
Sys-Dia Gradient at 50% EDA (%)	0.2	2.8	-0.2
Sys-Dia Gradient at 40% EDA (%)	0.8	7.5	0.5

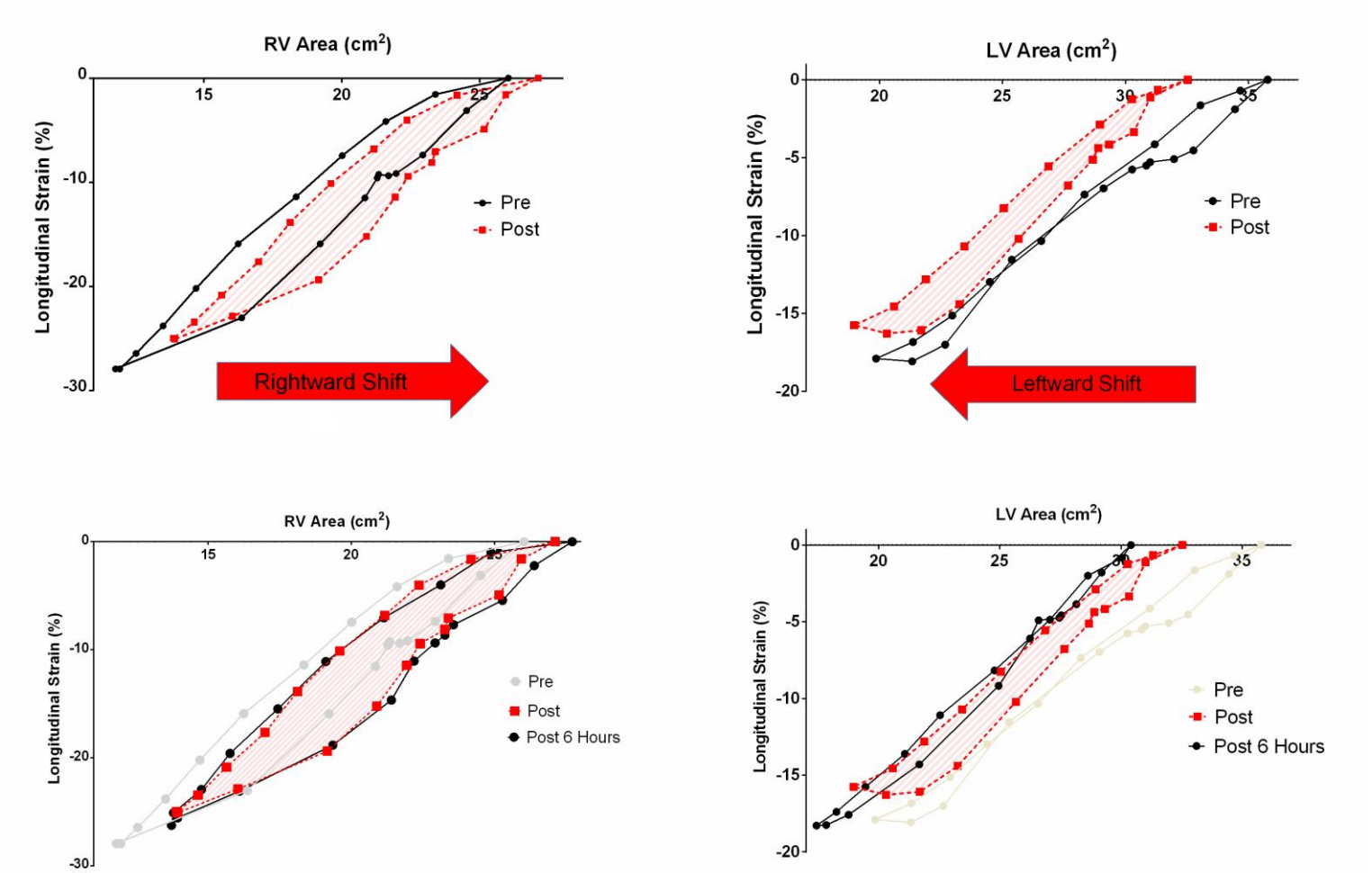


Figure 4.2 - Right and left ventricular area-strain loops pre to post-race and post-race to recovery.

4.4 Discussion

This is the first study to determine simultaneous area and ε relationships in the RV and LV in response to prolonged strenuous exercise. We observed that, 1) prolonged strenuous exercise resulted in RV dilatation and a reduction in contractility reflected by the rightward shift in the area-ε loop, although RV SV was maintained, and 2) post-exercise the LV is under filled as demonstrated by the leftward shift in the area-ε loop. The lack of change in the RV loop in the presence of a return towards baseline of the LV systolic-diastolic gradient at 6 hours recovery indicates an intrinsic reduction in relaxation that does not appear to be primarily driven by changes in RV structure and function.

4.4.1 Impact of Prolonged Strenuous Exercise

Previous studies on the LV and RV following prolonged strenuous exercise using conventional 2D and Doppler indices have reported a decrease in both LV and RV systolic and diastolic function (Oxborough et al., 2011, La Gerche et al., 2012b, George et al., 2009). The data in the current study builds on Chapter 3 and supports the findings from this study and previous research with a depression in LV and RV systolic and diastolic function evident post-exercise. LV and RV structural indices in the current study are also in support of exercise-induced adaptation previously reported with a reduction in LV and increase in RV size previously documented (Oxborough et al., 2011, Oxborough et al., 2010b, La Gerche et al., 2012b) and reflected by the findings in this study.

The data from area-ε loops describes detailed changes in cardiac mechanics following prolonged endurance exercise whilst illuminating potential mechanisms. The area-ε loops identify a post-exercise increase in RV size without any change in longitudinal contribution to area change as identified by higher ε values at any given area, relative to a higher initial starting area. In view of an unchanged area-ε relationship and no change in the longitudinal systolic-diastolic ε gradient it is likely that the reduced peak contractility observed post-exercise is a consequence of the larger volume only. Our findings of a maintained RV SV and no change in LA end systolic volume or E/E' suggest a lack of intrinsic dysfunction of the RV myocardium. The LV area-ε loop data demonstrates post-exercise under-filling of the LV with a concomitant reduction in peak longitudinal ε. Although systolic ε is lower at any given area post-exercise, it is clear the area-ε relationship in systole is similar to baseline and is therefore likely to be a consequence of reduced filling. LV wall stress and blood pressure are both reduced post-race and therefore LV afterload is reduced. In the presence of a reduced LV afterload, myocardial ε should increase due to a relative reduction in myocardial workload (Burns et al., 2010b). In contrast, we observed a reduction in post-race ε providing further support for an intrinsic reduction in function. That aside there is a significant change in the longitudinal contribution in diastole post-race as demonstrated by an increased LV loop systolic-diastolic ε gradient. These changes in diastolic mechanics are in the presence of a reduced LA conduit volume and therefore may, in part, be responsible for the under-filling observed post-

exercise. This is further evidenced by a maintenance of LA preload / volume and RV SV.

As suggested in Chapter 3, RV dilatation and dysfunction has been suggested to be secondary to a sustained exposure to a relatively elevated wall stress (La Gerche et al., 2011) and therefore the dysfunction observed in the post-exercise setting is likely to be a 'fatigue' of the myocardium resulting in a reduced stroke volume (Oxborough et al., 2011) with a serial negative impact on LV filling (Oxborough et al., 2010b). Data from the current study significantly develops our knowledge of the post-prolonged exercise structure / function relationship of the RV but with only partial support of previous theories and no evidence indicating a serial impact of the RV on LV filling. Post-exercise wall stress in the current study is elevated, however the PAP is reduced in recovery and therefore the increase in wall stress is likely as a result of the RV dilatation seen in recovery from prolonged strenuous exercise. The mechanistic theories postulated for LV dysfunction following prolonged endurance exercise are plentiful and include oxidative stress (Vitiello et al., 2011), myocardial damage (Koller, 2001), beta-receptor desensitization (Banks et al., 2010) as well as the impact from an enlarged, dysfunctional RV (La Gerche et al., 2011). The recovery loops provide further insight into the mechanisms underpinning LV dysfunction. Whilst the RV area-ε relationship remains similar to immediately post-exercise, the systolic-diastolic ε gradient of the LV loop returns to baseline level and provides strong evidence that the changes in longitudinal contribution to area change in diastole are intrinsic

in nature and not secondary to a serial impact from the RV. That aside, the LV is still under-filled and therefore we must also speculate that there is an additional mechanism at play leading us to consider the multifactorial nature of LV post-exercise dysfunction.

A major contributing factor in LV filling is the ability of the ventricle to untwist, generating a sharp decline in LV pressure during early diastole (Burns et al., 2010). LV untwist is ultimately driven by its preceding twist as potential energy is stored within the compressed titin molecule during ventricular systole (Weiner et al., 2010) but also by the maintenance of LV structural integrity. It is apparent that any disruption to twist mechanics will impact on overall LV filling. Our data demonstrates a reduced twist and untwist immediately post-exercise primarily as a consequence of reduced basal and apical rotation which persists 6 hours into recovery. It is unlikely that twist mechanics contribute solely to the intrinsic reduction in longitudinal diastolic dysfunction immediately post-exercise, however it is possible that they contribute to LV under-filling immediately post-race and throughout the recovery period.

An alternative mechanism for the reduction in LV twist is a parallel RV impact on LV function. This has been observed in the presence of increased RV volume/pressure and results in septal displacement in both systole and diastole (Ryan et al., 1985). The displaced septum in diastole impacts on LV untwist reducing the ability of the LV to fill to capacity and may well influence diastolic filling (Puwanant et al., 2010). Septal

displacement has been observed in a few post-exercise studies (Oxborough et al., 2011, La Gerche et al., 2012b) as well as a recent case-report (Oxborough et al., 2014). The current data highlights an increased EI immediately post-exercise and theoretically, it could be argued that a 'parallel RV impact' has some influence on LV filling in the current study independent of any intrinsic reduction in LV relaxation.

4.4.2 Limitations and Future Research

A 3D technique would overcome potential geometric limitations of the current 2D imaging, however the current frame-rates for real-time acquisition of 3D volume and ε are low and provide limited scope for detecting small changes in function. The data in this Chapter and Chapter 3 add to the evidence base in the EICF setting and demonstrate a clear impact on both the RV and LV in recovery from a 100 mile ultramarathon. There is some evidence to suggest that an elevation in PAP is responsible for a reduction in RV function and RV dilatation, but limited support for any interaction between the RV and LV as a result of a reduced RV SV. That said, there is a possible parallel impact of the RV on LV filling worthy of further investigation. These studies highlight the multifaceted nature of the mechanisms responsible for EICF and it would be pertinent to build on the echocardiographic data and initial ECG data by assessing the electrical response to prolonged endurance exercise, specifically in the right-sided leads, and this provides the focus for Chapter 5. RV and LV area-ε loops were only assessed in the longitudinal plane and therefore construction of

circumferential area-ε loops may provide additional insight. The assessment of ventricular function and area-ε loops during exercise may reveal the timing of RV dilatation and determine whether LV intrinsic relaxation occurs prior to recovery. It is pertinent to establish the reliability and feasibility of novel ε techniques in-exercise to establish the boundaries of inherent measurement error. There are two techniques available to assess myocardial strain and it is important to compare the absolute values derived from these different methodologies as well as comparing their accuracy. The focus for Chapter 6 is therefore to determine the reliability, feasibility and comparability of MST and TVI derived RV strain during upright cycling exercise. Taking this forward, Chapter 7 will interrogate the in-exercise response of the RV during 6 hours of cycling exercise using the most appropriate technique based on the data from Chapter 6. PAP will be assessed at the same time point as RV function in an attempt to prove or disprove the elevated afterload theory proposed by Oxborough and La Gerche. Cardiac biomarkers were not measured during this study, however the inclusion of brain natriuretic peptide and/or cardiac troponins may aid the understanding of post-exercise changes in cardiac structure and function. Previous studies have linked post-exercise cardiac biomarker release to LV and more specifically RV dysfunction and investigating this relationship further may expose a mechanistic link.

4.4.3 Conclusion

There is evidence of a persistent post-exercise shift in the RV area-ε loop indicating RV dilatation with reduced contractility that is likely a consequence of RV structural adaptation rather than any intrinsic dysfunction. The LV area-ε loop is shifted left immediately post-exercise and the LV is under filled, likely as a result of intrinsically reduced longitudinal relaxation and impaired LV twist/untwist. The former mechanism is transient and returns to normal following 6 hours of recovery whilst LV twist/untwist remains depressed which could, in part, explain a persistent LV under-filling. Importantly from a mechanistic insight, at 6 hr post-exercise there appears to be no obligatory serial impact of reduced RV function on LV mechanics. It may be that mechanical changes with prolonged exercise in the LV and RV are independent.

Chapter 5 - Exploratory Insights from the Right-sided Electrocardiogram following Prolonged Endurance Exercise

5.1 Introduction

As evidenced in Chapters 3 and 4, recovery from prolonged strenuous exercise appears to have a profound effect on the structure and function of the heart. This includes a change in the electromechanical association in the ventricles suggesting a potential impact on electrical activation (Chan-Dewar et al., 2010a) that was also associated with post-exercise cardiac dysfunction.

The theory discussed in Chapters 3 and 4 suggesting a relative elevation in PAP and consequent disproportionate exercise stroke work in the RV may be implicated in post-exercise RV dysfunction. In Chapter 3 we assessed 12-lead ECG changes following a 100 mile ultramarathon and demonstrated an increase in the summated R wave in V1 and S wave in V5, J point elevation in V1, partial RBBB, T wave inversion in lead V1 and early repolarisation pre to post-race pointing to changes in the right-sided electrical conduction system. Nevertheless, the use of right-sided ECG leads, focusing on the right heart, have not been employed in this setting. Given the imposition of a disproportional workload on the RV during exercise and the changes reported in the standard 12-lead ECG, it is

pertinent to further investigate the electrical activity in the right side of the heart following prolonged exercise. Right-sided chest leads have been previously utilised in addition to the standard 12-lead ECG to determine RV involvement in myocardial infarctions associated with the right coronary artery (Zehender et al., 1993) and in the diagnosis of PHT and pulmonary embolism (Akula et al., 2003, Chia et al., 1997). In these clinical situations, the right-sided leads demonstrate ST segment changes that are not evident on a standard 12-lead ECG and have an increased sensitivity and specificity in diagnosing conditions affecting the RV.

Consequently, the aim of the current study was to determine whether the right-sided ECG demonstrates changes indicative of right-sided adaptation following prolonged endurance exercise. This broad aim allows for the generation of the following hypothesis: the right-sided 12-lead ECG will indicate ST segment elevation and T wave changes indicative of right-sided pressure/volume overload.

5.2 Methods

5.2.1 Sample Population

Thirty athletes (20 male, 10 female) at the 2014 Western States Endurance Run (28th June, Squaw Valley to Auburn, CA) (body mass 68 ± 12 kg, height 173 ± 11 cm, age 45 ± 10 years) were recruited to take part in the study. Participants self-reported that they had no family history or

diagnosis of cardiovascular disease or co-morbidities for cardiovascular disease, were not taking any prescribed medication and had no musculoskeletal injuries. The current training status for the cohort was 6 ± 1 days per week, 57 ± 15 miles per week, 14 ± 5 training hours per week and 35 ± 26 previously completed ultramarathons. Participants completed the race in 26.29 ± 2.95 hours. Written informed consent was obtained and Ethics approval was granted by the University Ethics Committee.

5.2.2 Study Design

The 30 participants were assessed pre-race (24 - 48 hours prior to the race) and immediately post-race (within 30 minutes of race completion). Height, body mass, resting blood pressure and right-sided 12-lead ECGs were recorded at baseline and immediately post-race. Participants were requested to avoid vigorous training and alcohol for the 24 hours prior to and caffeine for the 6 hours prior to the pre-race assessments. Participants were permitted to consume food and fluid *ad libitum* during the race and ambient temperature ranged from 0 to 31.7 °C.

5.2.3 Standard 12-lead Electrocardiogram and Posterior Leads

All subjects underwent a standard 12-lead ECG and a repeat 12-lead ECG with V4-6 relocated to posterior chest leads V7-9 prior to and on completion of the race. These ECGs were analysed for any change pre to post-race indicative of coronary artery involvement.

5.2.4 Right-sided 12-lead Electrocardiogram

Limb leads were placed in the same position as the standard 12-lead ECG, the precordial leads were mirrored from the standard 12-lead ECG onto the right side of the chest and positioned following the recommended guidelines for the standard 12-lead ECG (Kossman et al., 1967) giving leads V1R-V6R. Each of the 6 precordial leads V1R-V6R was analysed for J point elevation (>1mm), ST segment elevation (>1mm), T wave amplitude, R wave amplitude, R' wave amplitude and S wave amplitude. The ST segment was calculated using the J point and J point plus 80ms and integrating the difference between the two points and termed ST segment integral.

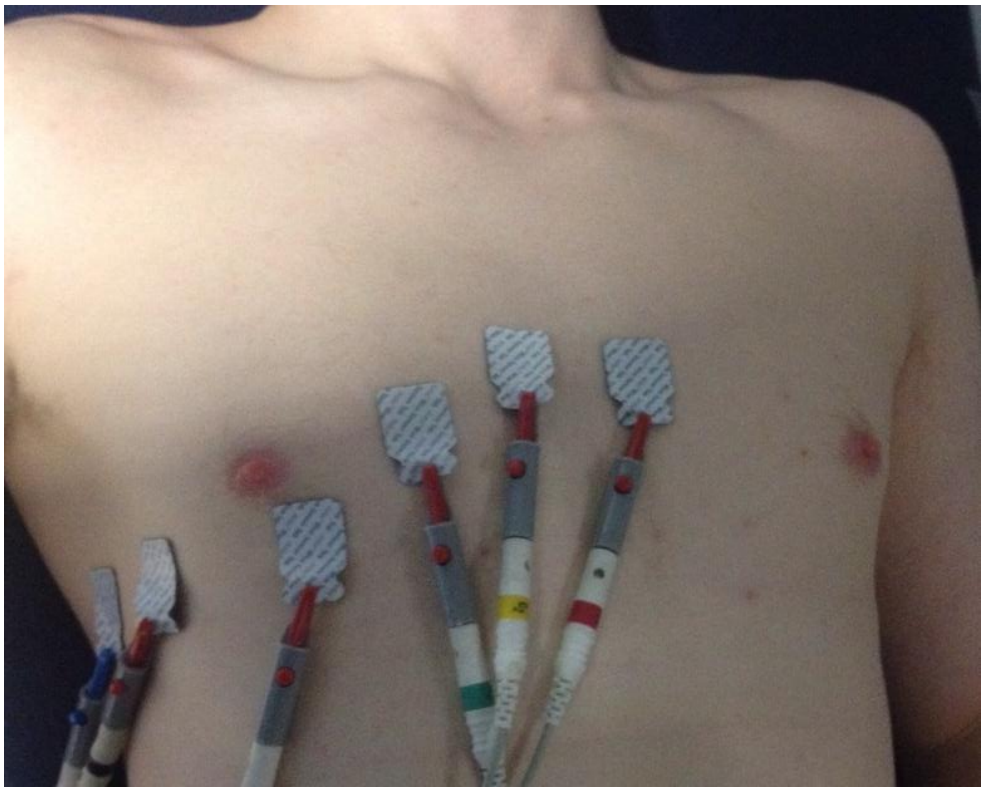


Figure 5.1 - Electrode placements for the precordial leads for the right-sided 12-lead ECG

5.2.5 Statistics

All variables were analysed for normality of distribution using a Shapiro-Wilk test. Pre to post-race peak values for each variable were compared using Student's Paired T-tests using commercially available software (IBM SPSS v21) and statistical significance was set as $P < 0.05$.

5.3 Results

5.3.1 Demographics

There was a significant increase in heart rate (58 ± 7 to 78 ± 10 bpm) and reduction in systolic (128 ±11 to 120 ±14 mmHg) and diastolic (84 ± 8 to 78 ± 10 mmHg) BP pre to post race ($P = 0.004$). There was no change in body mass pre to post race ($P = 0.08$).

5.3.2 Standard and posterior 12-lead ECGs

There was no evidence of pre to post-race myocardial ischemia as documented on standard and posterior ECGs.

5.3.3 Right-sided 12-lead ECG

There was a 12% ($P = 0.04$) reduction in S wave amplitude in lead V1R from pre to post-race (see Table 5.1). There was a 23 ($P = 0.01$) and 38% ($P = 0.03$) increase in J point amplitude in leads V1R and V2R respectively from pre to post-race (see Table 5.1). A converse response

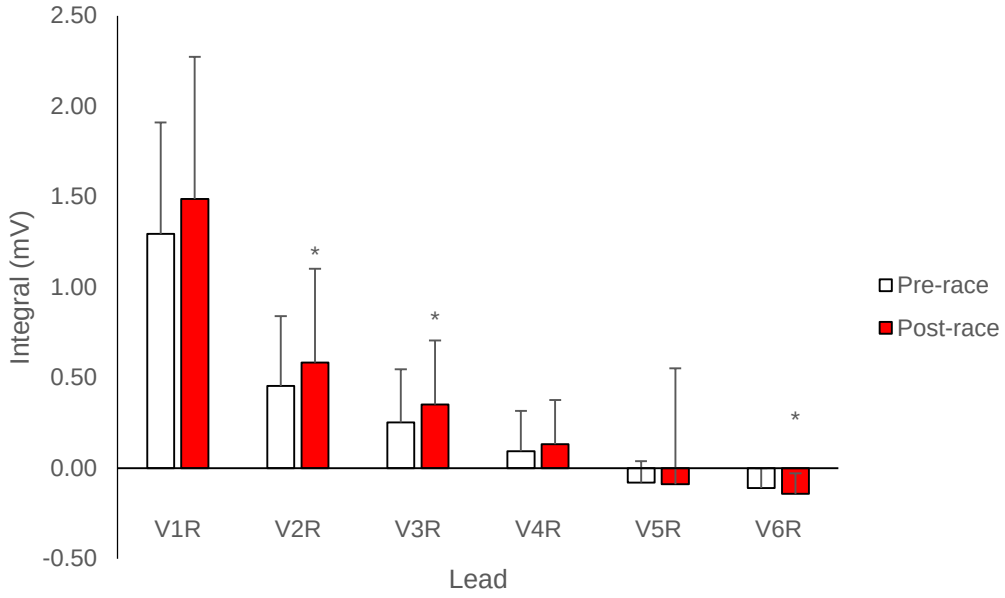
was evident in lead V6R with an 80% (*P* = 0.03) depression in J point amplitude from pre to post-race (see Table 5.1). There was a 22% (*P* = 0.05) increase in ST segment integral in V2R and V3R (see Table 5.1 and Figure 5.2) consistent with ST elevation and a 27% (*P* = 0.03) decrease in ST segment integral in lead V6R from pre to post-race (see Figure 5.2). No significant ST segment elevation was evident in leads V4R-V6R. All ST segment changes were below criteria for clinical diagnosis of myocardial ischemia/infarction. T wave inversion was evident in leads V2R-V6R in 50, 57, 73, 90 and 83% of athletes respectively.

Despite there being significant cohort changes in right-sided ECG parameters, close inspection of individual ECG traces pre- and post-exercise demonstrated heterogeneous changes in specific individuals. This is notable in two case studies where the degree of response is marked (see Figures 5.3 and 5.4). These two case reports demonstrate P pulmonale, ST segment elevation in the right-sided precordial leads V1R-V6R with marked T wave changes alongside the development of R’ waves in lead V1R and V2R.

Table 5.1- Right-sided 12 lead electrocardiogram variables

Variable	Pre	Post
LEAD V1R		
R wave amplitude (mV)	5.91 ± 2.98	5.92 ± 2.78
R' wave amplitude (mV)	0.36 ± 0.91	0.64 ± 1.74
S wave amplitude (mV)	-13.82 ± 6.50	-12.13 ± 6.39*
J point amplitude (mV)	0.92 ± 0.57	1.13 ± 0.62*
T wave amplitude (mV)	5.12 ± 3.12	4.99 ± 3.27
LEAD V2R		
R wave amplitude (mV)	2.86 ±2.08	2.77 ±1.83
R' wave amplitude (mV)	0.75 ± 2.23	0.72 ± 1.95
S wave amplitude (mV)	-9.44 ± 4.62	-9.07 ± 4.46
J point amplitude (mV)	0.32 ±0.35	0.44 ± 0.40*
T wave amplitude (mV)	-0.05 ± 1.69	0.17 ± 1.96
LEAD V3R		
R wave amplitude (mV)	1.92 ± 1.27	2.10 ± 1.36
R' wave amplitude (mV)	0.77 ± 1.94	0.71 ± 1.92
S wave amplitude (mV)	-6.81 ± 3.94	-6.69 ± 3.77
J point amplitude (mV)	0.15 ± 0.30	0.24 ± 0.30
T wave amplitude (mV)	-0.61 ±1.31	-0.19 ± 1.58
LEAD V4R		
R wave amplitude (mV)	1.44 ± 0.88	1.43 ± 0.94
R' wave amplitude (mV)	0.69 ± 1.37	0.79 ± 1.92
S wave amplitude (mV)	-4.59 ± 3.41	-4.72 ± 2.89
J point amplitude (mV)	0.04 ± 0.24	0.10 ± 0.22
T wave amplitude (mV)	-0.95 ± 0.93	-0.78 ± 1.09
LEAD V5R		
R wave amplitude (mV)	0.83 ± 0.60	0.81 ± 0.64
R' wave amplitude (mV)	0.70 ± 0.92	0.67 ± 0.95
S wave amplitude (mV)	-2.96 ± 2.19	-2.71 ± 1.78
J point amplitude (mV)	-0.03 ± 0.15	-0.05 ± 0.15
T wave amplitude (mV)	-1.30 ± 0.48	-1.22 ± 0.57
LEAD V6R		
R wave amplitude (mV)	0.88 ± 0.71	0.76 ± 0.68
R' wave amplitude (mV)	0.41 ± 0.69	0.41 ± 0.73
S wave amplitude (mV)	-1.90 ± 1.94	-1.62 ± 1.72
J point amplitude (mV)	-0.05 ± 0.12	-0.09 ± 0.11*
T wave amplitude (mV)	-1.18 ± 0.46	-1.14 ± 0.53

* indicates statistical significance pre to post race ($P<0.05$). Data presented as mean ± SD



* indicates significant difference pre to post-race

Figure 5.2 - ST integral pre-race and post-race for leads V1R-V6R

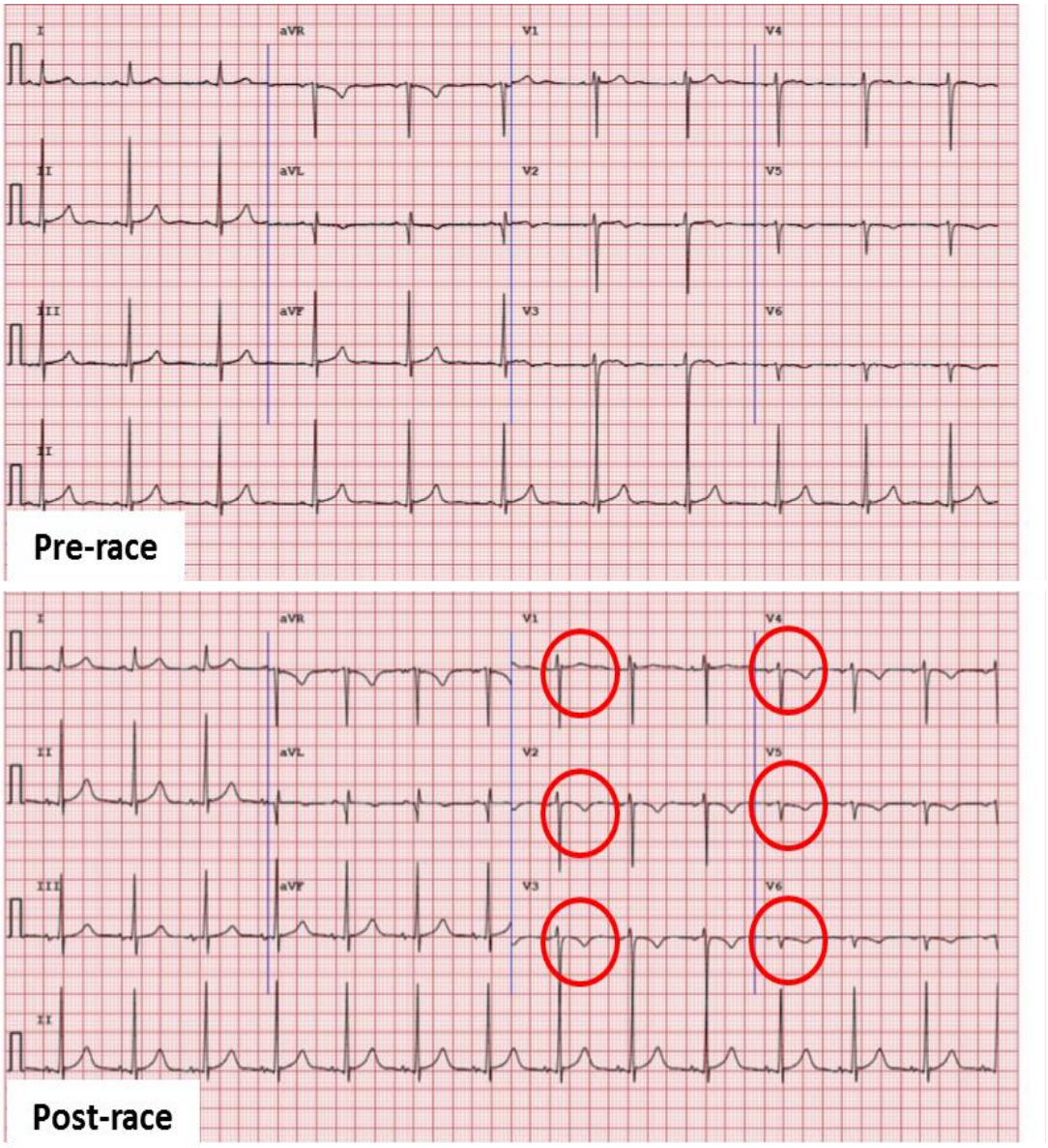


Figure 5.3 - Case Study 1 demonstrating T wave changes from pre-race to post-race in leads V1R-V6R indicated using red circles. Note precordial leads V1-V6 on the ECG represent right-sided precordial leads V1R-V6R.

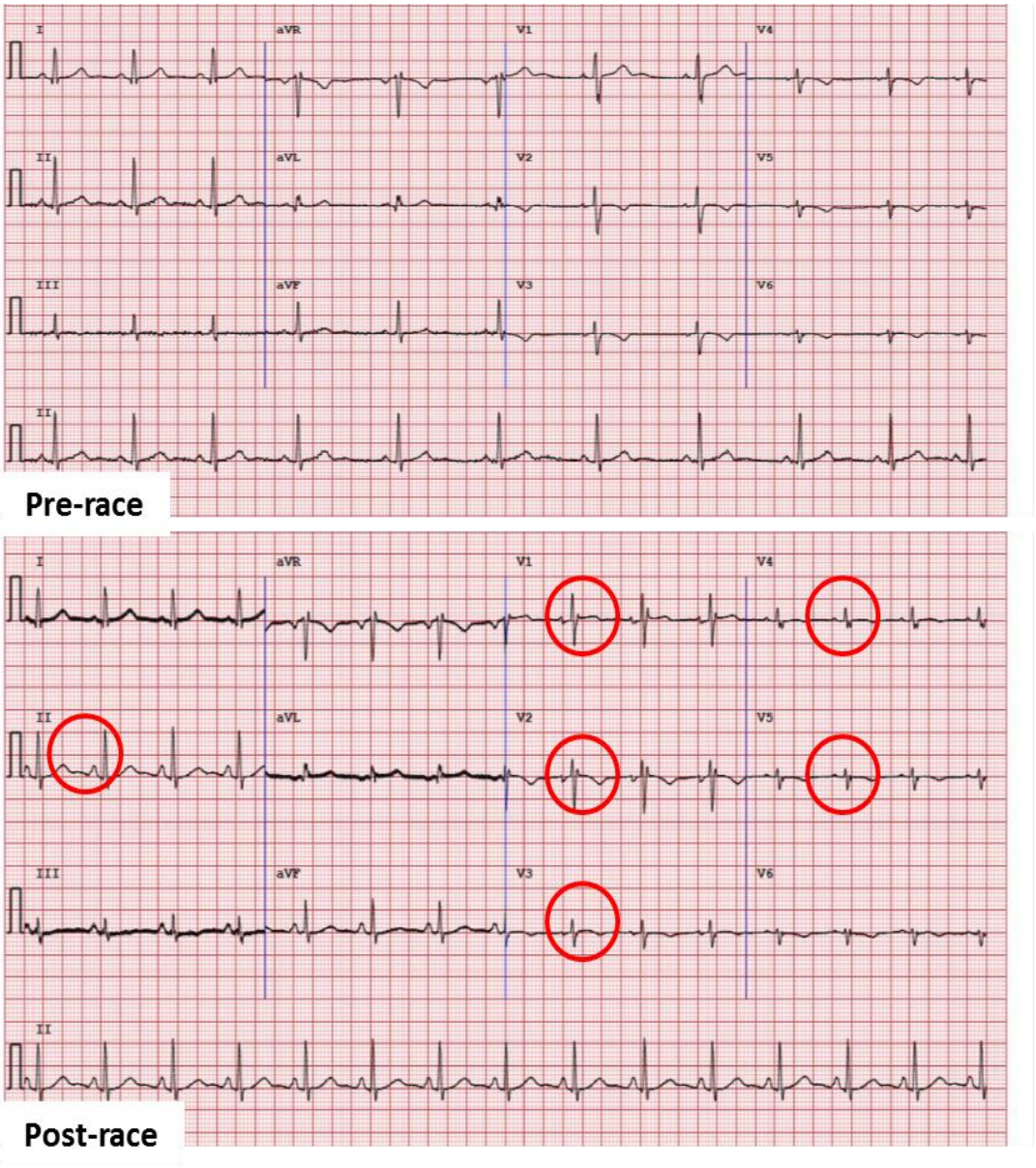


Figure 5.4 - Case Study 2 demonstrating post-race P pulmonale in lead II, development of R' waves in the anterior leads V1R and V2R and ST segment changes in leads V1R-V5R indicated by red circles. Note precordial leads V1-V6 on the ECG represent right-sided precordial leads V1R-V6R

5.4 Discussion

The key findings from this study are evidence of ST segment elevation in leads V1R and V2R, ST depression in lead V6R and T wave changes in leads V1R-V5R following the race with no concomitant changes in the standard or posterior 12-lead ECGs. Importantly, the data in the current study demonstrated a heterogeneous response with some athletes demonstrating clear ST segment and T wave changes in the right-sided leads and generation of R' waves in the anterior precordial leads as demonstrated in the case reports. This study applied right-sided precordial leads in the assessment of electrical changes following a bout of prolonged endurance exercise. Although a clear response across the whole sample is not evident, some data are indicative of a heterogeneous response to the 100 mile ultra-marathon with some individuals demonstrating a response similar to that seen in the presence of an elevated RV afterload (Akula et al., 2003, Chia et al., 1997). This varied response to an exercise stimulus and the consequent RV afterload has been reported previously using both standard 12-lead ECG (Chapter 3) and echocardiography (George et al., 2009, Oxborough et al., 2011).

The elevation in J point and ST segment in leads V1R-V3R and converse depression in lead V6R were demonstrated across the cohort following a 100 mile ultra-marathon and may indicate repolarisation changes. T wave changes were also evident in numerous athletes in leads V2R-V4R following the ultra-marathon giving further support to a possible change in

repolarisation. These changes likely indicate an exercise-induced right-sided adaptation in electrical conduction and structure following prolonged endurance exercise. The mechanism behind these changes and the clinical implications for the athlete are at present unknown but these post-exercise electrical adaptations are likely to represent physiological modifications in response to the disproportionate workload placed on the RV during exercise (La Gerche et al., 2011) and could be linked to the echocardiographic data indicating a reduced function and structural adaptation in the RV and LV in Chapters 3 and 4 in this thesis following prolonged strenuous endurance exercise.

There was a significant increase in P wave amplitude in lead II from pre to post race in the current study and although the value does not reach the criteria for P pulmonale (Stein et al., 1975, Stein et al., 1991), this may be indicative of RA enlargement or restrictive filling in the presence of an acute pressure/volume overload on the RA (Stein et al., 1991). Interestingly, P pulmonale has been reported in cases where an acute afterload is placed on the right side of the heart and the increase in P wave amplitude in the current study may therefore indicate a manifestation of the transient elevation in PAP previously reported following ultra-endurance exercise (La Gerche et al., 2011, Kovacs et al., 2009). In this case, the increase in P wave amplitude could be indicating both right atrial enlargement and/or a pressure overload on the right atrium.

RA and LA enlargement has been reported using transthoracic echocardiography (Oxborough et al., 2010b) and studies have also observed RV dilatation (La Gerche et al., 2012b, Oxborough et al., 2011) and a reduction in LV EDV (La Gerche et al., 2012b) in recovery from ultra-endurance exercise. Cardiac function has also been assessed using echocardiography and a global decrease in both systolic and diastolic RV (Neilan et al., 2006b, Poh et al., 2008) and LV (George et al., 2009, Banks et al., 2011) function in recovery from prolonged endurance exercise reported in conjunction with the acute structural adaptations. The data from Chapters 3 and 4 adds support to the body of evidence demonstrating reduced function, RV dilatation and a reduction in LV filling and the right-sided ECG changes evident in the current study may represent an electrical adaptation linked to these structural and functional changes. This data provides further insight into the acute electrical adaptations in response to a bout of ultra-endurance exercise. The mechanistic implications for this data with respect to post-exercise cardiac dysfunction are important to consider and they may provide further support for a disproportionate exercise load on the RV. Acute ECG changes can also be caused by disturbances to blood electrolytes (Diercks et al., 2004), especially after a prolonged endurance exercise bout (Stewart et al., 2014, Overgaard et al., 2002). This is an important consideration and warrants further investigations to allow firm conclusions to be drawn on post-exercise electrical responses.

In Case Study 1 (Figure 5.3), there is new T wave inversion evident in leads V2R-V6R post-race compared to pre-race. Case Study 2 (Figure 5.4) demonstrates an increase in P wave amplitude pre to post race in lead II, normalisation of the ST segment and T wave inversion in leads V3R-V5R and development of R' waves in V1R and V2R. Two previous studies analysing right-sided ECG in the presence of an elevated afterload have reported T wave inversion in leads V4R-V6R (Chia et al., 1997) and conversely reported ST segment elevation with flattened T waves in leads V3R-V6R (Chia et al., 1997, Akula et al., 2003). The two cases in the current study demonstrate similar changes to those presented in conditions of RV afterload but to a lesser degree. These two case studies indicate a varied response to a similar stimulus and further support the notion of heterogeneity in response to an elevated afterload. This is likely dependent on the heterogeneous demographic of the athlete.

Studying acute ECG changes may help to understand the mechanisms involved in both acute and chronic electrical remodelling seen in endurance athletes and also inform echocardiographic assessments following ultra-endurance exercise. Chronic exercise training in the athlete results in structural and functional cardiac adaptation as determined by diagnostic imaging such as transthoracic echocardiography (Oxborough et al., 2012b, Utomi et al., 2014) and the standard 12-lead ECG (Corrado et al., 2009). These changes are well documented and inform the criteria for interpretation of the 12-lead ECG in athletes (Corrado et al., 2010b, Drezner et al., 2013) but the adaptation process and mechanisms are not

fully understood. The acute changes in the current study of ST segment elevation and the development of R' waves could be linked to chronic adaptation in athletes such as early repolarisation and incomplete right bundle branch block. A longitudinal investigation over a period of training may expose some mechanistic links between acute exercise responses and chronic electrical remodelling.

5.4.1 Clinical Perspective

Whilst the right-sided ECG changes demonstrated in the current cohort are likely due to a physiological response to a prolonged exercise bout as opposed to pathology, the clinical consequence of these changes and the translation from acute response to chronic remodelling is unknown. A period of endurance exercise training has been proven to result in electrical remodelling of the standard 12-lead ECG in athletes with clear implications evident for pre-participation screening. The data in this study suggest that a mechanistic link could be evident between the acute changes evident in the R' wave development and ST segment changes and long-term remodelling of incomplete RBBB and early repolarisation and is worthy of further investigation.

5.4.2 Limitations and Future Research

This exploratory study has provided the first data for right-sided precordial leads in the assessment of athletes following prolonged endurance

exercise, there are however some limitations. There is a lack of normative data for the right-sided ECG in endurance athletes and therefore the electrical adaptation to endurance training is not known for the right-sided precordial leads. That said, the pre-race data can be considered as a small sample of the typical right-sided ECG in endurance athletes. Data were only acquired at two time points, before the race and immediately following the race and therefore the transience of the observed changes is not known and would be a useful addition in future studies to determine the recovery period. Echocardiographic data at the same time point as the right-sided ECG would provide a full structural and functional assessment of the right heart allowing structural changes to be correlated with electrical changes to determine any relationship between these variables. This was not feasible in the current study due to post-race logistics. The three field based studies in Chapters 3-5 have developed our understanding of EICF providing further evidence for changes in RV and LV structure and function in recovery from prolonged endurance exercise. The original application of the right-sided ECG revealed post-exercise electrical responses indicative of an increased RV afterload, in support of a reduced RV ε and dilatation evident in Chapters 3 and 4. Chapter 4 provides insightful data for cardiac mechanics and questions the serial impact of the RV on the LV, however the temporal response of the ventricles during exercise remains unclear. The laboratory based studies in Chapters 6 and 7 aim to address this using in-exercise echocardiography and simultaneous assessment of PAP in a controlled environment.

5.4.3 Conclusion

Completion of a 100 mile endurance run results in electrical adaptation in the right-sided precordial ECG leads affecting P wave, ST segment and T wave amplitude post-race. Furthermore, in a number of athletes there were pronounced changes indicative of acute exercise induced afterload and right heart electrical and/or structural adaptation.

Chapter 6 - Reliability and Feasibility of Echocardiographic Derived Right Ventricular Strain: Impact of Technique, Body Position and Exercise Intensity

This Chapter is published in Echo Research and Practise and is available at the following address:

http://www.echorespract.com/content/1/1/31.full?sid=b1d2cecc-9369-4dc8-97a6-2b6710fa91f6

Chapter 7 - Right Ventricular Structure and Function during Prolonged Endurance Exercise

7.1 Introduction

Chapters 3 and 4 in this thesis demonstrate a clear reduction in RV function in recovery from prolonged running exercise. To our knowledge, the response of the RV in recovery from prolonged cycling exercise has not been investigated.

The vast majority of studies investigating cardiac fatigue have focused on comparisons between pre-race and recovery measures, with a void in the literature focusing on the in-exercise response of the RV. Over a short progressive exercise bout, RV function has been successfully assessed using echocardiography (La Gerche et al., 2012a) with results indicating a physiological linear response between increasing exercise intensity and increasing contractility. A previous study assessed RV ε in athletes 15 minutes and 150 minutes into a running exercise bout (Banks et al., 2010). Participants in this study were transferred from the treadmill to echo bed and stopped exercising for the duration of the assessment and therefore this study has clear limitations. Heart rate drops significantly on exercise cessation and the echocardiograms were obtained with subjects in a supine as opposed to upright position therefore impacting on load and not giving a true reflection of in-exercise RV function. The authors also applied speckle-tracking to derive RV ε, a technique where inferior reliability and feasibility is evident compared to TVI derived ε above 50% maximum heart rates

(Chapter 6). A decrease in RV ε was reported following the 150 minute running session, however this is likely due to a parasympathetic response to the removal of the exercise stimulus and therefore the temporal response of the RV to a prolonged cycling bout remains unknown.

Linked to the theories proposed by La Gerche et al and Oxborough et al discussed in Chapters 3-5, La Gerche et al. (2011) quantified disproportionate RV exercise stroke work in a semi-supine progressive cycling exercise fitness test and reported significantly elevated PAP up to 61 mmHg during exercise. The use of cardiac magnetic resonance imaging (cMRI) to derive RV end systolic volume and echocardiography to estimate PAP does not afford simultaneous assessment of the components that RV wall stress is calculated from and although the authors attempt to correct for the two techniques involved in the measurement, there is a large potential for error. The concurrent assessment of RV structure and function alongside PAP using echocardiography may aid the understanding of temporal exercise induced changes in a prolonged upright exercise bout. The recommendation based on data from Chapter 6 that only TVI provides valid data above 50% of the maximum heart rate limits the assessment of RV ε in this Chapter to this technique and therefore area-ε loops cannot be generated in this study.

The aim of this study is therefore to build on previous research and assess RV structure and function and PAP at 2 hour intervals during a 6 hour cycling exercise bout. The key hypothesis based on this aim is that an elevation in

PAP will consequently result in a reduction in RV function and structural remodelling.

7.2 Methods

7.2.1 Sample Population

Based on an *a priori* power calculation, eight well trained male ultra-endurance athletes (Data presented as mean ± SD, Body mass 77.8 ± 11 kg, height 179 ± 6 cm, BP 136/88 mmHg, age 40 ± 7 years, VO2 max 51.9 ± 10 $ml.kg.min^{-1}$) were recruited and volunteered to participate in this study. Participants self-reported: no known cardiovascular disease, no prescribed medications and no comorbidities or family history of cardiovascular disease. Written informed consent was obtained and ethics approval granted by the University Ethics Committee.

7.2.2 Protocols

Participants underwent a maximal oxygen uptake test (Oxycon, Care Fusion, Hoechberg, Germany and SRM bike, Jülich, Germany) at 30 watt increments every 3 minutes to determine their maximum heart rate on a separate day to the 6 hour cycling session. Participants were requested to avoid vigorous training, alcohol and caffeine for a minimum of 24 hours prior to the assessment. On the day of the cycling session, systolic and diastolic blood pressure was assessed prior to and immediately after exercise using standard auscultation (Dinamap pro, GE Healthcare, Horten, Norway).

Participants were assessed prior to and at 2, 4 and 6 hour intervals during the exercise bout using echocardiography and all images were acquired using a commercially available ultrasound system (Vivid Q, GE Medical, Horten, Norway) with a 1.5-4 MHz phased array transducer. Images were obtained by a single experienced sonographer with the participant on their road cycling bike fixed to a turbo trainer device cycling at 75% maximum heart rate. Images were recorded to DVD in raw DICOM format and data were analysed offline by a single experience sonographer using commercially available software (EchoPac version 7, GE Medical, Horten, Norway). A minimum of three cardiac cycles were averaged for all peak indices.

7.2.3 Conventional 2D, Doppler and Tissue Doppler Echocardiography

The RV was assessed in accordance with ASE guidelines (Rudski et al., 2010a) providing structural indices at the outflow tract ($RVOT_{plax}$, $RVOT_1$ and $RVOT_2$) and at the inflow (RVD_1, RVD_2, RVD_3). RVAd and RVAs were measured and FAC calculated. A pulsed wave TDI sample positioned at the tricuspid annulus allowed the assessment of RV S', E' and A' myocardial velocities.

RVSp was derived from the tricuspid regurgitant jet using continuous wave Doppler. The regurgitant signal was improved for resting and exercise measurements in a supine position using agitated saline administered via a three way stop cock cannula inserted into the antecubital vein as previously described (Badesch et al., 2009). This technique has been shown to improve

the accuracy of both resting and exercising assessments of PAP (Lopes et al., 2008) and agitated saline was therefore administered to our participants immediately prior to echocardiographic assessment at rest and at 2, 4 and 6 hour intervals into the cycling session. PAP was calculated as (PAP (mmHg) = RVS_p + 5mmHg) as described in Chapter 3 and 4.

7.2.4 Tissue Velocity Strain Imaging

Based on a previous study by our group assessing feasibility, comparability and reliability of MST and TVI to derive RV longitudinal ε at 70% maximum heart rate, we have utilized TVI derived ε for the current study given the superior reliability and feasibility of this technique and the limited temporal resolution of speckle tracking (Chapter 6). An AP4CH view was modified laterally to ensure the longitudinal movement of the RV lateral wall was aligned with the ultrasound beam. A colour tissue velocity ROI was superimposed and a narrow sector was utilised to obtain frame rates in excess of 200 FPS and gain, filter, pulse repetition frequency and depth were adjusted to optimise colour saturation and eliminate aliasing. All TVI acquisitions were analysed offline using the same software package and a large sample volume of 24 mm x 4 mm was used to maximise capture of the basal, mid or apical segment. The sample was anchored to the myocardium and adjusted throughout the cardiac cycle to ensure consistency of its anatomical position. Longitudinal ε was obtained for each segment and a global value was calculated as an average of all 3 segments.

7.2.5 Statistics

Echocardiographic data were analysed for normality of distribution using a Shapiro–Wilk test. Peak data at 2, 4 and 6 hours into exercise were compared using a one-way ANOVA. All statistical tests were performed using commercially available software (IBM SPSS version 22) and statistical significance was set as $P < 0.05$.

7.3 Results

7.3.1 Demographics

Body mass (77.8 ± 11 and 78 ± 11.3 kg), systolic (136 ± 13 and 123 ± 10 mmHg) and diastolic BP (78 ± 8 and 70 ± 5 mmHg) were unchanged pre to post exercise ($P > 0.05$). HR was significantly higher during exercise at 2, 4 and 6 hour compared to baseline ($P = 0.04$, 0.04 and 0.03 respectively, see Table 7.1).

7.3.2 Exercise Responses

There was a significant increase ($P = 0.003$) in TAPSE from baseline to 4 hours and RV S' and RV A' were also significantly elevated ($P = 0.001$, 0.015 and 0.006 and >0.001 respectively) from baseline to 2, 4 and 6 hours into exercise. There was no significant difference in RV structural parameters RVOTplax, RVOT1, RVOT2, RVD1, RVD2, RVD3, RVAd and RVAs across assessment points. There was no significant difference in RVFAC from baseline to during exercise. There was no significant difference in RV global

longitudinal ε, PAP or RV wall stress from baseline to in-exercise measures, see Table 7.1.

Table 7.1- Right and Left Ventricular Structural and Functional Indices

Variable	Baseline	2 hour	4 hour	6 hour
Heart rate (bpm)	96 ± 23	131 ± 12 ɣ	134 ± 16 *	146 ± 27 Θ
$RVOT_{plax}$ (mm)	30 ± 3	31 ± 2	32 ± 3	33 ± 3
RVOT1 (mm)	32 ± 4	33 ± 6	34 ± 4	33 ± 5
RVOT2 (mm)	22 ± 3	22 ± 3	24 ± 2	23 ± 2
RVD1 (mm)	46 ± 5	47 ± 3	49 ± 5	47 ± 5
RVD2 (mm)	28 ± 5	28 ± 4	29 ± 3	30 ± 4
RVD3 (mm)	85 ± 10	89 ± 6	90 ± 4	89 ± 4
RVDa (cm^2)	26.4 ± 4.7	27.0 ± 2.7	28.5 ± 3.8	27.9 ± 3.1
RVSa (cm^2)	12.9 ± 1.7	13.3 ± 2.5	13.2 ± 1.6	13.8 ± 1.8
RVFAC (%)	50.6 ± 6.0	50.9 ± 5.4	53.5 ± 6.2	50.5 ± 6.2
TAPSE (mm)	28 ± 3	32 ± 2	35 ± 4 *	32 ± 3
RV S' (cm/s)	17 ± 3	34 ± 6 ɣ	31 ± 7 *	32 ± 5 Θ
RV E' (cm/s)	17 ± 41	20 ± 4	20 ± 6	20 ± 9
RV A' (cm/s)	13 ± 3	29 ± 4 ɣ	34 ± 3 *	34 ± 2 Θ
RV global ε (%)	-26.4 ± 2.7	-27.8 ± 4.5	-26.4 ± 4.3	-24.2 ± 2.7
PASP (mmHg)	17 ± 10	20 ± 9	21 ± 9	26 ± 9
RV Wall Stress (kdynes/cm^2)	6.2 ± 2.4	5.8 ± 3.4	5.5 ± 2.2	7.1 ± 3.0

Statistical significance indicated as ɣ = baseline to 2 hours, * = baseline to 4 hours, Θ = baseline to 6 hour

7.4 Discussion

This study focused on the in-exercise response to a six hour cycling bout and demonstrates no change in RV structure and function throughout exercise in contrast to the data provided by Chapters 3 and 4. RV afterload does not elevate during exercise as indicated by similar PAP and RV wall stress during exercise. This disparity between Chapters 3 and 4 where RV changes secondary to an elevated RV afterload are evident and the current study where the RV is unchanged throughout exercise are intriguing.

The current study demonstrates an initial response in RV functional parameters to the exercise bout which is in fitting with previous studies assessing the response to short duration progressive exercise. In these studies and in the current cohort, RV contractility and relaxation increased from baseline to during exercise. This is the normal physiological response to exercise brought about by sympathetic stimulation and blood pressure regulation and indicates that exercise reserve in the current cohort is in line with previous studies in the athletic population (Stoylen et al., 2005, La Gerche et al., 2012a).

Given the large exercise volume imposed on the participants in the current study, we hypothesised that PAP would elevate and drive an exercise induced cardiac fatigue response in the RV, as demonstrated in Chapters 3 and 4. In contrast, the current data demonstrates maintained RV

function and no RV structural remodelling, in fact the RV appears to maintain contractile function at the same level as the initial exercise induced response in short duration exercise. Whilst surprising given that RV dysfunction has been reported following exercise durations much lower than 6 hours (Poh et al., 2008, Neilan et al., 2006b, Oxborough et al., 2006), the PAP and RV wall stress in the current study is not significantly higher during exercise suggesting that RV afterload is not elevated in the current cohort. The elevated afterload mechanism suggested to cause RV dysfunction is dependent on increased PAP during exercise and this is not present in the current study, likely explaining the maintained function and structural integrity of the RV.

Few studies have assessed RV structure, function or PAP during exercise in an upright position and the effect of gravity and loading on these parameters remains to be determined. This could have implications for future research in this setting. Indeed, the previous studies estimating PAP during exercise have done so with participants in a supine or semi-supine position and the effect of modifying body position when obtaining the tricuspid regurgitant signal during exercise is unknown and could explain the differing values reported in the current study compared to previous work. Furthermore, studies using echocardiography to investigate RV exercise induced cardiac fatigue have assessed participants prior to and on completion of exercise in a supine position and not considered the effect of body position on cardiac structure and function. Most exercise is undertaken in an upright position and to truly

understand the effects of exercise on cardiac structure and function, it is pertinent for assessments to be undertaken in the exercising body position to ensure that loading conditions are identical for measurements taken before, during and in recovery from exercise. Assessment of PAP is more accurate using agitated saline and further work using this technique is required to establish the response of the pulmonary circulation across a range of exercise modes, intensities and durations to determine the relative afterload placed on the RV during exercise and the consequent acute structural and functional adaptations.

Another important consideration is the exercise mode chosen for the study, cycling is the most practical for in-exercise echocardiography but has significantly lower energy demands than running, rowing and triathlon (Millet et al., 2009) and this may explain the maintenance in RV structure and function when compared to the 100 mile ultramarathon exercise stimulus in Studies 3-5. Furthermore, the RV response in recovery from a bout of prolonged cycling exercise has not been documented and it is possible that cycling does not result in EICF in the RV.

7.4.1 Limitations and Future Research

The small sample sized included in the current study likely influences statistical power and may, in part, explain the lack of statistical change evident. This study used TVI derived ε imaging given that Chapter 6 in this thesis suggests that speckle tracking is not a reliable technique above 50% maximum heart rates. The use of a semi-supine cycle ergometer

may improve image quality and afford the use of speckle tracking to assess athletes during exercise and allow the assessment of RV and LV function using a superior technique and in multiple planes. This would allow the construction of area-ε loops which provide a more in depth understanding of temporal cardiac mechanics. It is pertinent to apply this technique during exercise to fully elucidate the timing of exercise-induced changes in structure and function as well as determining any interaction between the ventricles. The use of 3D echo would improve structural assessment, however the frame rates are not sufficient to allow valid assessment of function especially during exercise at higher heart rates.

7.4.2 Conclusion

A 6 hour cycling exercise bout at 75% maximum heart rate results in an initial increase in RV contractility and relaxation. This increased function is maintained throughout exercise and there is no evidence of RV structural adaptation. PAP is not elevated above normal resting values during the exercise bout and suggest that the RV is not placed under a higher afterload during prolonged cycling exercise.

Chapter 8 - Synthesis

8.1 Brief Summary of Findings

Chapters 3, 4 and 5 were field based studies at the 2013 and 2014 Western States Endurance Run, a 100 mile trail running race. Chapter 3 provided evidence for exercise induced changes in RV structure and function following the race that were still evident after 6 hours of recovery suggesting that RV acute adaptation is more persistent than expected. In addition, this study provided evidence of an acute post-exercise response in the 12-lead ECG with findings indicative of right-sided electrical adaptation to the 100 mile race.

Chapter 4 aimed to build on the echocardiographic data acquired in Chapter 3 and applied the novel technique of constructing area-ε loops to both the RV and LV in an attempt to create a non-invasive surrogate for pressure-volume loops. This technique also allowed a more in depth analysis of the interaction between the RV and LV at rest, immediately post-exercise and after 6 hours of recovery. Whilst RV dilatation was evident post-exercise and in recovery, at the same time RV ε was reduced. Despite this, the RV actually maintained output. With respect to the LV, there was a change in mechanics immediately post-exercise alongside reduced filling of the LV. Importantly, this mechanical change had reverted to baseline values after 6 hours of recovery. With no change in the RV area-ε loop from post-exercise to recovery, this indicates that the change in the LV area-ε loop is likely due to an intrinsic reduction in LV

relaxation. The lack of change in RV structure and function from post-exercise to recovery suggests that LV changes are independent of RV function and the mechanism responsible for these changes is not a serial impact of the RV on the LV.

Chapter 3 also led to a more in depth investigation of right-sided ECG adaptation to prolonged endurance exercise in Chapter 5 using right-sided chest leads for the 12-lead ECG prior to and on completion of the 100 mile ultramarathon. The key findings from this study were an elevation in ST segment and T wave changes indicative of an elevated afterload on the right heart.

These field based studies provided more in depth analysis of the RV and LV response in recovery from prolonged endurance exercise, however there was still a void in the literature with respect to in-exercise response. These studies require a laboratory environment to control environmental conditions and allow exercise to be undertaken on equipment that allows in-exercise echocardiographic assessment. Before applying novel indices such as ε imaging to obtain RV function in exercise, it was important to establish the feasibility, reliability and comparability of the available techniques to derive ε indices. This was the key focus in Chapter 6 where both TVI and MST derived ε were obtained at rest in a supine and seated position and during progressive upright cycling exercise from 50-90% maximum heart rate. The key findings from this study were that only TVI ε

provided acceptable reliability and valid data at heart rates above 70% maximum heart rate.

Based on this, the in-exercise ε measurements obtained in Chapter 7, alongside conventional 2D and Doppler measures of RV structure and function, utilized TVI as participants were cycling at 75% maximum heart rate. This study indicated that RV function increased in response to exercise and that this increased function is maintained across 6 hours of exercise with no structural adaptation or functional decrement as exercise progressed. PAP was also assessed during exercise in an attempt to understand RV loading conditions and were unchanged throughout exercise.

Overall, the results from this thesis have developed our understanding of acute changes in LV and RV structure and function during recovery from prolonged endurance exercise with respect to both ECG and echocardiographic data whilst also providing some exploratory in-exercise data for the RV. The mechanistic processes responsible for exercise related responses in echocardiographic and electrocardiographic indices remain unclear. There is a suggestion of an independence between the RV and LV, in contrast to the theory based on a serial impact of the RV on the LV and it is clear that exercise-induced changes are complex to understand and likely multifactorial in nature.

8.2 Overarching Issues

8.2.1 Exercise-induced cardiac fatigue: Possible mechanisms

This thesis aimed to develop our understanding of exercise-induced RV changes and to add further insight into the mechanisms responsible for EICF both in recovery from and during prolonged endurance exercise. With respect to possible mechanisms, a newer theory of a serial impact of the RV on the LV as a consequence of disproportionate elevation in RV stroke work has been suggested based on research by La Gerche et al. (2011) and Oxborough et al. (2010b, 2011). They reported that PAP during prolonged exercise are elevated in athletes up to 65 mmHg (La Gerche et al., 2011, Kovacs et al., 2009) and are therefore above those diagnosed clinically in pulmonary hypertension. This elevation in pulmonary artery pressure is due to a relative inability of the pulmonary circulation to vasodilate subsequently elevating pulmonary vascular resistance and thus pulmonary artery pressure is increased. This results in a disproportionate increase in afterload on the RV compared to the LV which affects the pressure-volume relationship within the RV cavity and elevates RV wall stress. La Gerche et al. (2011) quantified wall stress in their study and compared values to wall stress in the LV and identified a disproportionate stroke work in the RV as a result of a comparatively greater wall stress. There is therefore a greater demand on the RV myocardium to generate a greater force, however the thin walled RV may be unable to cope with an increased afterload in the same manner as the LV. RV contractility is therefore compromised either through a fatigue process or a direct physiological response to the afterload (Braunwald,

1997). A reduction in RV function decreases the RV ejection volume (SV) and therefore increases the residual volume in the RV at end systole (ESV) and as a result of this combined with increased venous return, the RV EDV increases and RV dilates.

The serial impact of reduced SV from the RV causes a reduction in LA and subsequently LV preload and would ultimately reduce LV filling and LV EDV will decrease and consequently LV SV will be reduced. According to Frank-Starlings Law, this reduced LV preload could result in a decrease in LV contractility as this is dependent on the volume of the cavity at end-diastole. Evidence of a dilated, dysfunctional RV was reported in two key studies, one by La Gerche et al. (2012b) and the other by Oxborough et al. (2011) following prolonged endurance exercise. In these studies, the reduced RV function was deemed to have had a serial impact on LV filling and ultimately reduced LV preload as a result of a decreased RV SV secondary to an elevated RV afterload.

The data from Chapters 3 and 4 in this thesis build on the body of evidence focusing on RV EICF with only partial support of a serial impact of the RV on the LV. Whilst RV dilatation is evident following the 100 mile ultra-marathon and there is reduced filling in the LV, the application of the area-ε loops give further insight into post-exercise mechanics in both ventricles. Although the RV is dilated and ε is reduced, RV SV is maintained following the exercise bout and there is no change in the

systolic-diastolic ε gradient relationship indicating that the longitudinal contribution to area change is similar to baseline values. Conversely, the LV EDA is reduced with a reduction in LV ε following the race, more importantly there is a change in the systolic-diastolic ε gradient suggesting that the longitudinal contribution to area change is altered in the post-exercise setting. The key element of Chapters 3 and 4 is the assessment of the RV and LV 6 hours into recovery from exercise. The data provided from the area-ε loops at this time point is intriguing and calls into question a serial impact of the RV on the LV as recovery progresses. Whilst there is no change in the RV loop, the LV loop demonstrated a return to baseline of the systolic-diastolic ε gradient. With no change evident in the RV loop at this time point, this would suggest that any change in the LV loop is not likely to be as a result of a serial RV impact and more probably a case of intrinsic myocardial contraction/relaxation or a parallel impact of the RV on the LV. That said, the response and interaction between the ventricles during exercise remains unknown given that the data in Chapter 7 did not induce an elevated RV afterload likely due to the lower exercise volume in this study compared to the 100 mile running race.

8.2.2 The Stroke Volume Paradox

In order to maintain normal cardiac structure and function, the SV across the pulmonary valve should equal the SV across the aortic valve (Klabundle, 2011). When there is a disparity between what goes in and what leaves, as in cases of valve disease or congenital abnormalities, cardiac enlargement and dysfunction entails (Friedberg and Mertens,

2012, Lancellotti et al., 2010). In Chapters 3 and 4 the RV and LV SV were measured by different techniques and therefore it is understandable that the values were not identical. That aside the direction and magnitude of change immediately following prolonged strenuous exercise was markedly different between both chambers; RV SV was maintained but LV SV reduced. In this setting it would be expected for the excess volume to be taken up by the atria or the pulmonary circulation. Our data demonstrates no change in LA volume in the immediate post-exercise setting suggesting that it is unlikely that the heart has been exposed to this SV disparity for a prolonged duration i.e. during the race. Consequently, it is unlikely that LV relaxation abnormalities exist during the exercise bout (unless RV SV equally reduces) and this would suggest that the mechanism for systolic-diastolic ε gradient changes is more likely to be beta-receptor desensitization rather than any active ischemic or oxidative stress. Interestingly LA VOL ES has started to elevate 6 hours post-exercise and may be indicative of the exposure to the disparate SV following race completion. Whilst it is difficult to fully explain this finding, it appears that the LA may act as a 'buffer' for reduced LV SV in the post-exercise setting and equally, it could be argued, may be the stimulus for LA adaptation seen in endurance athletes (Oxborough et al., 2012b). These thoughts are speculative but provide some 'food for thought' when considering future work in this area.

Furthermore, the right-sided ECG data obtained in Chapter 5 is indicative of an elevated RV afterload following the 100 mile ultra-marathon and

there may be a “mechanistic” link between these electrical responses and the structural and functional changes in the ventricles following prolonged endurance exercise that warrants further investigation to fully determine the complex, multifactorial nature of the mechanisms involved. Interestingly, the right-sided ECG changes seen in this thesis are a milder manifestation of those reported in cases of pulmonary embolism (Akula et al., 2003, Chia et al., 1997). Although more likely to be indicative of a physiological response to an elevated afterload, therefore providing additional support for an elevated pulmonary vascular resistance and subsequent RV afterload from ECG indices alongside echo data, the clinical significance of these ECG changes is yet to be determined. A small number of case studies have reported pulmonary embolism in athletes following prolonged running exercise (McKechnie et al., 1979) and these reports stimulated the early research interest into right-sided post-exercise cardiac responses. These issues still appear to have an application more than 50 years later, highlighting the multifaceted nature of EICF and difficulty in determining true mechanistic understanding.

It is important to consider that the echocardiographic estimation of RV SV applied in this thesis has a certain degree of measurement error associated and is not considered to be the gold standard technique to derive values. The technique is however recommended in the guidelines for the assessment of the RV using echocardiography (Rudski et al., 2010a). Although cMRI is the gold standard to assess RV structure due to the complex location, geometry, and excess trabeculation of the ventricle

(Ho and Nihoyannopoulos, 2006), there are significant limitations in its use in RV functional assessment. A number of cardiac cycles are required to provide structural and functional indices for both the RV and the LV and frame rates are very low. Therefore cMRI is not a viable technique to construct area-ε loops from. The application of these loops adds further mechanistic insight specifically with respect to cardiac mechanics and further application of this technique is likely to develop knowledge and understanding of cardiac fatigue. This also supports the use of echocardiography rather than cMRI in post-exercise assessment even when other limitations are considered. That said, the subtle responses evident in exercise-induced cardiac fatigue are challenging to reveal using all echocardiographic variables and it is important to consider measurement error as well as intrinsic changes to myocardial function when interpreting pre to post race changes.

There is a clear disparity in the reliability of speckle tracking and TVI derived ε at rest over multiple image acquisitions in Chapter 6 which builds on previous research and supports the use of speckle tracking with resting subjects. The benefit of calculating the measurement error for speckle tracking derived ε is that this data can be applied in the interpretation of data for the studies in this thesis to determine that pre to post-exercise changes are larger in magnitude than inherent variation within the technique itself.

Clearly this thesis calls into question the idea of a serial impact of the RV on the LV and seems to suggest that recovery of function in the two ventricles may be independent. Indeed, if a reduced RV SV is not responsible for a reduction in LA preload and subsequent effect on the LA LV pressure gradient for filling, another mechanism must be at play for the LV to be under filled. The RV and LV are structurally diverse but interlinked by the nature of the RV being attached to and wrapped around the LV. The application of area-ε loops in the assessment of resting RV and LV mechanics provides some novel information that we feel deserves some commentary. We termed the difference between ε in systole and diastole at any given area “systolic – diastolic ε gradient” and assessed this at rest prior to any exercise. This systolic / diastolic relationship in the LV is such that longitudinal ε is similar in systole and diastole throughout the range of LV size suggesting that there is a similar contribution of longitudinal function to systole and diastole in the healthy human LV. The relationship in the RV at rest is very different with a much lower contribution of longitudinal function to area change in diastole i.e. increased longitudinal systolic – diastolic ε gradient. It is clear therefore that diastolic mechanics are very different between the two chambers in a resting state. It has been proposed that RV filling in a healthy heart is primarily driven by kinetic energy generated from gravity similar to that observed in a hydraulic ram (Sengupta and Narula, 2013). Conversely, LV filling is primarily driven by the generation of a suction pump and is essentially a vortex impeller (Sengupta and Narula, 2013). It is therefore likely that the longitudinal fibre relaxation is partially responsible for the

sharp decline in LV pressure and vital in maintaining the vortices and LV filling. This type of relaxation is not required in the resting state of the RV and, therefore, we must speculate that there is a shift in diastolic mechanics to enable the more passive filling, possibly to a circumferential predominance. This phenomenon is intriguing and raises the potential for the application of this type of imaging in a range of conditions. It would be pertinent to establish the impact of posture, exercise, disease, load and respiration on the longitudinal contribution to RV and LV filling in order to provide further insight into ventricular physiology and its acute and chronic adaptation.

The mechanics of the LV are dependent on maintaining the structural integrity of the cavity to allow contraction and relaxation of the double helical myocardium in multiple planes. If this structural integrity is compromised then this can impact on the sharp pressure decline in early diastole and consequently impact on LV filling (Weiner et al., 2010, Burns et al., 2009). In patients with chronic RV afterload such as pulmonary hypertension and embolism (Olson et al., 2010), RV EDV is increased and RV ε is reduced. This RV volume overload can displace and flatten the interventricular septum affecting the structural integrity of the LV especially at the basal level. In turn, this impacts the mechanics of the LV, reducing torsion and circumferential ε whilst longitudinal ε is also reduced (Puwanant et al., 2010). The authors speculate that changes in LV geometry and flattening of the septum impact on circumferential and torsional mechanics. Untwist is a sentinel event in LV relaxation to

maintain the pressure gradient between the LA and the LV (Burns et al., 2010a, Notomi et al., 2008). If untwist is reduced due to a change in the structural integrity of the LV as a result of a septal displacement this impact on LV circumferential and radial mechanics could consequently reduce the suction effect and pressure gradient caused by early LV relaxation and therefore impact filling. In Chapters 3 and 4, post-exercise RV dilatation is evident similar to that seen in pulmonary hypertension patients and as a result of an elevation in PAP. It is possible that the RV dilatation is causing a volume overload causing septal displacement specifically in diastole (Ryan et al., 1985) and this is further supported by evidence of an increase in the LVEI in diastole and also a prolongation of the IVRT. In these studies, RV enlargement may therefore have a parallel impact on LV untwist in early diastole (Nottin et al., 2011), especially the septal wall segments (George et al., 2009), and ultimately reduce filling. This indicates that there is in fact a possible interaction between the RV and LV but not via reduced output. Although we did not apply area-ε loops to LV circumferential motion, data from Chapter 4 indicate that LV circumferential ε, rotation and torsion are all reduced immediately post-exercise at the basal level. The challenge remains to explain whether these changes are as a result of a parallel impact of the RV on the LV or an intrinsic reduction in LV relaxation and this is yet to be determined.

If the LV changes seen post-race in Chapter 4 are intrinsic in nature, they are possibly as a result of a down regulation of beta receptors. This has been eloquently demonstrated by Banks et al (2010) following a 150

minute run. Their participants underwent dobutamine administration before and immediately following the exercise bout and a reduction in response was evident post-exercise. The cause of this down regulation is not currently known, but may be a protective mechanism to a sustained exposure to a high heart rate and circulating catecholamines (Eysmann et al., 1996). In patients with prolonged atrial fibrillation where an ablation is performed, the LV of these patients demonstrates a phenomenon known as myocardial stunning with pseudo-restrictive filling where little atrial component to filling is evident, suggestive of atrial dysfunction even when reverted back to sinus rhythm. A similar situation is demonstrated with tachycardic cardiomyopathy where, after ablation to correct the tachycardia, the LV does not function normally and filling is still restricted, again with the patients back in sinus rhythm. Beta down-regulation has been implicated in these cases as a protective mechanism to the overload caused by an arrhythmia and the same principle may apply with ultra-endurance exercise given that total exercise duration can be in excess of 24 hours and heart rate will be elevated for a large percentage of this time period. Further studies are required to fully understand both the mechanism behind down-regulation and whether this is the case in intrinsic exercise-induced responses to prolonged strenuous exercise.

8.2.3 Transient versus Persistent Phenomenon

Exercise-induced cardiac fatigue in the LV is a transient phenomenon with post-exercise changes reverting to baseline given a sufficient recovery

period as reported by Neilan et al. (2006a) and Oxborough et al. (2010b). This does not seem to be the case with the RV where more persistent changes have been reported following a marathon by Neilan et al. (2006b). This has been linked to the relatively elevated workload of the RV compared to the LV. Data from Chapters 3 and 4 in this thesis add support to a more persistent nature of exercise-induced RV changes given that when assessed after 6 hours of recovery from the 100 mile trail race, alternations in RV structure and function had not returned to baseline values whereas the LV began to recover. The consequence of a more persistent adaptation in RV structure and function is not fully understood and importantly has implications for both recovery periods between races and the timing of pre-participation screening. If an athlete were assessed in the days following a prolonged bout of endurance exercise, there may be some residual modifications in RV structure and function that could result in a false positive result in a screening. It is also pertinent to consider the effect of further exercise before complete recovery of the RV from previous bouts and the time course of this recovery process for the RV should be established over a range of exercise modes and durations to further inform both athletes and practitioners.

8.2.4 Heterogeneous Responses

Studies on both the LV and RV have reported contradictory findings from the same exercise duration (Middleton et al., 2006a, Oxborough et al.,

2010a) which makes the interpretation of post-exercise changes in cardiac structure and function challenging. This disparity has been suggested to be as a result of study design, reliability of techniques used, measurement error and statistical power of studies using different sample sizes. Another potential issue is the heterogeneity of individual responses. Two specific studies have analysed individual as opposed to cohort responses to prolonged endurance running, one specifically focusing on the LV (George et al., 2009) and the other on the RV (Oxborough et al., 2011) and reported a heterogeneous impact of the exercise bout on their participants. This varied response may cause a masking effect with respect to the subtle changes evident following prolonged endurance exercise and explain why a significant response is not evident following similar exercise across all sample populations. In this thesis, a heterogeneous response is evident in participants in Chapters 3, 4 and 5 in both ECG and echo data over the same exercise duration and may explain why only a small number of variable are statistically significant especially in the ECG data.

Explanations for heterogeneous responses to the same exercise bout have been linked to training status of the athlete and exercise finishing time/exercise intensity. Oxborough et al. (2011) correlated training status and race finishing time to changes in RV structural and functional indices and reported a strong inverse relationship between training years and finishing time with the severity of RV changes. It appears that those athletes who have trained for a number of years may have induced a

protective effect on the RV response to prolonged endurance exercise, perhaps as a result of chronic remodeling of the RV to a greater volume with lower ε at rest (Oxborough et al., 2012b) but a superior exercise reserve (La Gerche et al., 2012a). It is also possible that exercise intensity during the exercise bout could have a mediating effect on the degree of acute adaptation in the RV. It could be argued that the disparity between results in Chapters 3 and 4 where post-exercise RV changes are evident and Chapter 7 where no RV response is present is as a consequence of a reduced exercise intensity in Chapter 7. The diverse field based and lab based exercise modes in this thesis may have exacerbated a disparity in exercise intensity and had a consequent impact on the degree of RV response.

8.2.5 Impact of body position

Cardiac function is dependent on the loading conditions imposed. The challenge when investigating the impact of prolonged endurance exercise is to quantify the loading conditions on the cardiac chambers. The field based studies in this thesis assessed participants immediately prior to and on completion of exercise. Whilst there may be remodelling and functional changes evident at post-exercise assessment, any changes in load that are responsible for these adaptations during exercise will not be present once exercise is completed.

Preload is defined as the end diastolic pressure that creates the largest dimension of the right or left ventricle. It can be affected by sympathetic stimulation, respiratory and skeletal muscle pumps, blood pressure and rate of venous return and many of these mediating factors are altered during exercise resulting in an increased preload. This is turn through Frank Starling's law will have a consequent impact on contractility. Once the exercise stimulus is removed, the increase in preload returns to baseline levels and can make the analysis of any post-exercise changes in cardiac structure and function complex and authors often extrapolate post-exercise findings to the conditions they would anticipate during exercise. To further complicate the understanding of changes in cardiac function following prolonged endurance exercise, athletes are often dehydrated which can reduce preload over the duration of exercise.

Afterload is the wall stress developed in the myocardium during ejection in either the right or left ventricle. According to Laplace's law, the tension in the myocardial fibres (the end systolic wall stress) is equal to the pressure in the ventricle multiplied by the volume within the ventricle divided by the wall thickness. The pressure in the ventricle at the point of ejection must be greater than the pressure it is ejecting against and therefore afterload is a consequence of either aortic or PAP for the left and right ventricle respectively. An elevation in afterload will subsequently increase end systolic volume and therefore decrease SV. Chapter 7 aimed to quantify an exercise induced increase in pulmonary artery pressure and therefore

identify an elevation in RV afterload, however values in this study were not above normal limits.

Echocardiographic assessments undertaken in accordance with American Society of Echocardiography guidelines (ASE) (Lang et al., 2015) have the participant in the left lateral decubitus (supine) position to provide the best image quality and remove as much lung artefact as possible. Whilst optimal image acquisition is important for valid and reliable measurement, images obtained in alternate positions may be useful in clinical and research situations where loading, filling and blood flow are assessed. Given the importance of quantifying load discussed above, of particular interest is the upright position as exercise is usually performed in this position and to truly understand the physiological demand on the RV, participants should be assessed in the correct orientation.

Chapter 6 assessed the reliability, feasibility and comparability of RV ε derived using TVI and MST and results suggest that only TVI provides acceptable data for use above 50% maximum heart rate, although TVI is less reliable at rest than MST. Chapter 7 therefore applied TVI to derive RV ε values, but also utilized standard 2D and Doppler indices to derive a range of structural and functional RV variables. It is pertinent to consider the effect of body position and exercise on all of these parameters to establish the reliability, validity and feasibility of in-exercise echocardiography and to determine the effect of changing body position

on loading and filling of the cardiac chambers. Altering body position from supine to upright drives a number of physiological responses including a drop in blood pressure, increased sympathetic drive and heart rate. This makes comparing the results from Chapters 3 and 4 to Chapters 6 and 7 complex given the different body position used between the studies and could explain the lack of change in RV structure and function in Chapter 7 alongside other mediating factors such as exercise volume and mode.

The vast majority of literature assessing cardiac responses in recovery from prolonged endurance exercise typically assesses participants in the ASE recommended supine left lateral decubitus position where loading conditions may be different to those imposed during the exercise bout. Altering the body position of these assessments to an upright one could well have an impact on the key findings in the studies and is an important consideration for future research. Without knowing the normative resting and exercising values for both supine and upright measurements, one must compare results from opposing body positions with caution as any differences may be due to measurement error or changes in load as well as any dependent variable under investigation. That said, the assessment of cardiac structure and function in an upright position is more challenging than supine echocardiography and may not provide valid and reliable data. The absolute values derived using MST in Chapter 6 above 50% maximum heart rate are indicative of pathology, which is extremely unlikely given the current cohort and is likely due to the inability of the tracking algorithm to detect sufficient change in the myocardium speckle

pattern as well as increased noise at high frame rates. This will have the effect of under sampling (a temporal resolution of approximately 11 ms) with the potential of missing the true peak ε values when changes in RV function are small in amplitude and fast acting. The higher frame rate for TVI (>200 FPS) appears to derive an absolute value in fitting with the known physiological response to exercise of increased RV ε. At a higher exercise intensity, image quality deteriorates due to upper body movement and increased respiratory activity. MST is highly dependent on image quality and out-of-plane motion, more so than TVI derived ε and may, in part, explain the findings from this study. MST is a superior technique to TVI as it is more load independent and affords the assessment of ε in multiple planes as well as the construction of area-ε loops. As evidenced in Chapter 4, these have the potential to provide a surrogate for a pressure-volume relationship within the ventricle as well as providing a more in depth understanding of the mechanical changes evident following prolonged endurance exercise. The application of these loops in-exercise is the next important step in understanding the complex multifactorial nature of exercise-induced cardiac responses, to enable this to occur, RV function must be maintained at the exercising heart rate and participants should continue to exercise throughout the echo assessment. Transferring subjects from the exercise equipment to a semi-supine cycle ergometer could perhaps provide a suitable compromise for echocardiography and overcome the limitations of upright exercise scanning to afford images of a sufficient quality to construct area-ε loops.

8.3 Implications

8.3.1 Clinical/Research Perspective

A recent editorial by La Gerche (2015) and research paper by Saberniak et al. (2014) have promoted a clinical interest in the link between "dose response" of exercise and arrhythmogenic right ventricular cardiomyopathy (ARVC) phenotype expression. The first study hypothesizing that exercise could modify gene expression of the ARVC phenotype exercised a sample of mice with a gene mutation resulting in earlier RV dysfunction and arrhythmias than the sedentary control mice (Kirchhof et al., 2006). This has since been translated to the human population initially by comparing ARVC patients to endurance athletes where a larger and more dysfunctional RV was reported in the endurance athletes (Sen-Chowdhry et al., 2007). James et al. (2013) developed this further by conducting an exercise related study in 87 patients with a known desmosomal gene mutation. ARVC patients were divided into athletic and non-athletic groups using a cut off of 50 hours of exercise per year and athletic ARVC patients developed the phenotype at an earlier age and were more likely to meet Task Force criteria, develop arrhythmias and heart failure. The most recent study (Saberniak et al., 2014) defined athletic status as ≥4 h per week of strenuous exercise for at least 6 years and identified 37 athletes in their cohort of 110 ARVC patients who either met Task Force criteria (Marcus et al., 2010) or were a relative of a gene-confirmed ARVC patient. They supported the findings of James et al. (2013) with a greater expression of the phenotype with increased incidence of symptoms. Interestingly, multimodality imaging revealed a

greater impairment in both RV and LV function in the athletes compared to sedentary ARVC patients. Furthermore, a dose-response relationship was evident between exercise intensity and duration and the degree of RV and LV dysfunction, suggesting that exercise volume may modify the expression of the ARVC phenotype. Of concern for the clinician is the fact that the exercise volume assessed by Saberniak et al. (2014) is significantly lower than that routinely undertaken by professional endurance athletes in their training programmes. The editorial by La Gerche (2015) suggested that this volume of exercise could promote an ARVC phenotype in athletes with a mild genetic predisposition. Indeed, the authors go one step further and hypothesise that extreme exercise may act as a substrate for the development of ARVC in its own right. This is based on research by Heidbüchel et al (2003) focusing on RV dysfunction and arrhythmias in professional cyclists with palpitations where almost two-thirds of these athletes met diagnostic criteria for ARVC with no evidence of the familial disease and only 12.8% of the cohort with desmosomal mutations (La Gerche et al., 2010). The pathophysiological mechanism for the development of exercise-induced ARVC has been linked to the elevated RV wall stress during exercise, transient RV dysfunction following an acute prolonged exercise bout and greater chronic remodelling of the RV when compared to the LV, all of which La Gerche suggests result in a pro-arrhythmogenic adaptation of the RV myocardium with disrupted desmosomal integrity due to the haemodynamic stresses of prolonged endurance exercise termed the “Heidbuchel syndrome”.

Although this theory is still controversial, the “marathon rat” studies by Mont and colleagues add some support to this theory. Benito et al., 2011 implemented a 16 week endurance exercise programme in 10 rats compared to 9 sedentary rats, assessing them with echocardiography, electrophysiology studies and post mortem analysis of RV fibrosis. Their findings demonstrated eccentric hypertrophy, diastolic dysfunction, increased ability to induce ventricular arrhythmias and increased levels of RV myocardial fibrosis in the endurance exercise rats. Although undertaken in rats and not humans, these findings highlight the importance of undertaking similar training studies in athletes and normal healthy controls quantifying exercise volume and the subsequent chronic remodelling of the RV to determine if exercise-induced ARVC develops and if so, at what training volume. At present, there is a growing body of evidence that environmental factors could be as important as genetic predisposition/family history respect to the development of ARVC and is worthy of further investigation. The more persistent nature of RV change indicated by data in this thesis adds to the concern for RV structural and functional integrity especially in athletes completing a large training volume and not undertaking sufficient rest to recovery fully. La Gerche (2015) also suggested that repeated exposure to ultra endurance exercise may ‘weaken the interstitial matrix of the RV’ providing a substrate for arrhythmias to develop. Additional evidence of myocardial fibrosis in the LV of athletes who suffer sudden cardiac death and increased incidence of atrial fibrillation (Sharma et al., 2010, Wilson et al., 2011) provide some

concern with regard to the long-term impact of endurance exercise in the veteran population. This also poses the question of environmental risk factors in the development of other cardiomyopathies such as hypertrophic cardiomyopathy (HCM). It is already established that exercise acts as a trigger for arrhythmias in athletes with HCM and there may be a dose-response relationship with exercise and phenotypical expression of the disease. With respect to cardiac screening, the timing of exercise prior to these assessments is important and it is vital to obtain full training details for athletes and ensure that the maximum possible rest period is given in order for a true resting assessment to be made and avoid any misdiagnosis of an exercise induced physiological response as pathological or “grey zone” athletic adaptation.

8.3.2 Athlete’s Perspective

This thesis provides some interesting findings from an athlete’s perspective. The most important of these is that, although acute RV responses in structure and function are demonstrated following prolonged running, there is no evidence of a decline in exercise performance, in fact the RV seems to adapt to maintain sufficient output under the loading conditions imposed and the intensity demanded. It is important to build on the in-exercise data derived during cycling exercise in Chapter 7 suggestive of a maintained function and structure in response to 6 hours of cycling, especially in different exercise modes, intensities and durations alongside quantification of PAP in an attempt to better understand the

multifactorial mechanisms responsible for exercise-related RV changes. Athletes should be aware of the persistent nature of these RV responses and consider adequate rest periods between training sessions, races and any pre-participation screening. It is important for athletes to self-monitor any symptoms induced by exercise especially with regard to pulmonary oedema.

8.4 Future Research

Whilst this thesis has answered some of the questions posed, it has also created a number that need addressing. Future research could focus on a number of directions in an attempt to further develop our understanding of exercise-induced changes in cardiac structure and function.

The in-exercise data collected in Chapter 7 is the first novel exploratory insight into RV structure, function and afterload throughout a 6 hour exercise session with no significant change evident. To build on the understanding of in-exercise cardiac responses and to determine the effect of exercise mode, duration and intensity and also training status on these responses, it is vital to design a number of studies that aim to assess a range of sample populations with specific exclusion and inclusion criteria. These studies should encompass pre-exercise, in-exercise and recovery measurement time points to develop understanding of the temporal response of cardiac chambers and loading conditions both during and in recovery from prolonged endurance exercise and determine

whether any response during exercise is persistent during the recovery period. It would be advantageous to include the assessment of regional RV and LV myocardial function, given that a base to apex gradient has been suggested to exist in the RV of trained athletes at rest (Teske et al., 2009b) and it would be beneficial to build on the understanding of segmental function during exercise (La Gerche et al., 2012a). Chapters 3 and 5 highlight the importance of assessing the electrical activity in the RV and it would be pertinent to develop this research to include in-exercise data. This would also afford the opportunity to relate ECG and echocardiographic data for the same participant at the same time point and may help to expose a mechanistic link between electrical and structural/functional changes. An important consideration and limitation of this thesis is that blood samples were not obtained in any participants. With respect to cardiac biomarkers, a correlation has been reported between brain naturetic peptide and delta changes in RV structure and function (La Gerche et al., 2008) and there may be a mechanistic link with the elevated wall stress theory worthy of further investigation. ECG changes have been correlated with changes in blood electrolytes and rheology (Diercks et al., 2004, Stewart et al., 2014) and it is important to establish whether this is the case in post-exercise changes or if they are a response to an elevated afterload and future studies should aim to establish this.

The next essential step is to apply the area-ε loop technique to both the RV and LV during exercise whilst simultaneously assessing the loading

conditions placed on each ventricle. Chapter 7 is the first study to assess PAP during exercise using agitated saline and suggest that the major limitation of much of the previous work focused on the RV, the inability to quantify PAP, may be overcome using this technique. It is vital that the most accurate assessment of PAP in undertaken in order to relate any alteration to a structural, functional or electrical acute adaptation. This will develop the understanding of the time course of changes in RV and LV structure and function and the mechanisms responsible including any interaction between the ventricles and will be a significant contribution to the exercise-induced cardiac fatigue field.

The long-term cumulative effect of multiple repeated bouts of prolonged endurance exercise on electrical activity, RV structure and function needs to be determined, especially with more persistent RV changes evident in Chapters 3 and 4 and a possible link between the acute right-sided ECG changes in Chapter 5 and athletic remodeling evident in the standard 12-lead ECG of endurance athletes. Studies utilizing longitudinal assessments over a period of training may help to explain the progression of chronic RV remodeling and reveal athletes at risk of developing myocardial fibrosis or cardiac arrhythmias and help to determine if environmental factors can cause expression of a cardiomyopathy phenotype without a genetic mutation.

8.5 Overall Conclusions

Using new techniques in the assessment of EICF, this thesis has significantly built on our understanding of this phenomenon. It is clear that a more persistent response to a bout of prolonged endurance exercise is evident in the RV and the clinical consequence of this remains unclear. The application of area-ε loops has allowed a more in depth understanding of the temporal changes in cardiac mechanics in the LV and RV and suggest that there may not be a serial impact of the RV on the LV in recovery from prolonged endurance exercise. EICF is a complex, multifactorial issue and the mechanisms responsible for exercise-induced changes in cardiac structure, function and electrical activation are yet to be fully understood. The application of the 12-lead and right sided ECG has revealed an acute response of the electrical conduction system to prolonged endurance exercise that may indicate an elevated RV afterload. The assessment of RV ε in-exercise at heart rates above 50% maximum are confined to TVI derived values and a 6 hour bout of cycling exercise does not appear to evoke elevated PAP in-exercise or reciprocal RV responses. To conclude, it is pertinent to reflect on the hypotheses of this thesis and determine whether the studies discussed allow the acceptance or rejection of these:

H_0: There will be no change in any variable assessing RV structure or function following a 100 mile ultra-marathon

REJECT

H_0: There will be no change in any variable assessing RV or LV structure and function and therefore there will be no interaction between the ventricles following a 100 mile ultra-marathon.

REJECT

H_0: There will be no change in P wave amplitude, ST segment or T wave amplitude in either the 12-lead or right sided ECG following a 100 mile ultra-marathon.

REJECT

H_0: There will be no difference between RV strain values or the reproducibility of these values derived using TVI and MST during progressive exercise.

REJECT

H_0: There will be no change in any variable assessing RV structure or function during a 6 hour cycle.

PARTIALLY ACCEPT

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Appendices

Appendix 1 – Ethics Approval

Proportionate Review – Full Ethical Approval: Application for Ethical Approval No.: 12/SPS/039 - The impact of training status as well as acute exercise intensity and duration on RV structure and function

Dr Sue Spiers and Dr Adam Mackridge have considered the application on behalf of Liverpool John Moores University Research Ethics Committee (REC). I am pleased to inform you that ethical approval has been granted and the study can now commence.

Approval is given on the understanding that:

- any adverse reactions/events which take place during the course of the project are reported to the Committee immediately;
- any unforeseen ethical issues arising during the course of the project will be reported to the Committee immediately;
- the LJMU logo is used for all documentation relating to participant recruitment and participation eg poster, information sheets, consent forms, questionnaires. The LJMU logo can be accessed at http://www.ljmu.ac.uk/corporatecommunications/60486.htm

Where any substantive amendments are proposed to the protocol or study procedures further ethical approval must be sought.

Applicants should note that where relevant appropriate gatekeeper / management permission must be obtained prior to the study commencing at the study site concerned.

For details on how to report adverse events or request ethical approval of major amendments please refer to the information provided at http://www.ljmu.ac.uk/RGSO/RGSO_Docs/EC8Adverse.pdf

Please note that ethical approval is given for a period of five years from the date granted and therefore the expiry date for this project will be 19th September 2017. An application for extension of approval must be submitted if the project continues after this date.

Yours sincerely

PP:

Proportionate Review – Full Ethical Approval: Application for Ethical Approval No.: 13/SPS/011 12-Lead Electrocardiography and Echocardiography in the Assessment of Veteran and Elite Athletes completing the Western States 100 mile Endurance race: The Impact of Prolonged Strenuous Exercise on Cardiac Structure and Function.

Dr Sue Spiers, Dr Dave Harriss and Dr Helen Poole have considered the application on behalf of Liverpool John Moores University Research Ethics Committee (REC). I am pleased to inform you that ethical approval has been granted and the study can now commence.

Approval is given on the understanding that:

- any adverse reactions/events which take place during the course of the project are reported to the Committee immediately;
- any unforeseen ethical issues arising during the course of the project will be reported to the Committee immediately;
- the LJMU logo is used for all documentation relating to participant recruitment and participation eg poster, information sheets, consent forms, questionnaires. The LJMU logo can be accessed at http://www.ljmu.ac.uk/corporatecommunications/60486.htm

Where any substantive amendments are proposed to the protocol or study procedures further ethical approval must be sought.

Applicants should note that where relevant appropriate gatekeeper / management permission must be obtained prior to the study commencing at the study site concerned.

For details on how to report adverse events or request ethical approval of major amendments please refer to the information provided at http://www.ljmu.ac.uk/RGSO/RGSO_Docs/EC8Adverse.pdf

Please note that ethical approval is given for a period of five years from the date granted and therefore the expiry date for this project will be **11th March 2018**. An application for extension of approval must be submitted if the project continues after this date.

Yours sincerely

PP:

Appendix 2 – Participant Information Sheet

LIVERPOOL JOHN MOORES UNIVERSITY
PARTICIPANT INFORMATION SHEET

Title of Project: *The RV functional response to an acute bout of prolonged endurance exercise.*

Name of Researcher (Student): Rachel Lord
Name of Research Supervisor: Dr David Oxborough
Department: Research Institute for Sport and Exercise Sciences
Contact details: r.lord@2009.ljmu.ac.uk

You are being invited to take part in a research study. Before you decide to take part, it is important that you understand why the research is being done and what it involves. This sheet gives you information about what you will need to do and what will be tested. If you would like any further information or have any questions then please feel free to contact me. Please take time to decide if you would like to participate in the study.

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

The aim of this study is investigate the right ventricular effects of a prolonged bout of endurance exercise to try to better understand what happens in the right side of the heart and how this affects training and competition performance.

2. **Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you volunteer, you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason and this will not affect your rights or access to studies from the university in the future.

3. **What will happen to me if I take part?**

The study will take place in the laboratory in Tom Reilly building within the Research Institute for Sport and Exercise Sciences at Liverpool John Moores University.

You will be required to attend the laboratory for a two visits. Visit 1 will be a VO2max and lactate threshold test. Following 15 minutes lying rest, you will undergo a 12 lead ECG where electrodes are attached to each wrist and ankle in addition to 6 chest electrodes. Leads are attached to the electrodes and pick up the electrical signals of the heart. This allows us to check for possible underlying silent cardiovascular disease. All electro and echocardiograms will be performed or interpreted by an experienced clinical physiologist with expertise in the assessment of cardiac disease. In the very unlikely event that a cardiac abnormality is detected then the following pathway will be initiated. The significance of the abnormality will be established and the participant will follow one of two routes:

1) In the case of a minor non-life threatening abnormality the participant will be informed of the possible implications and potential diagnosis. They will be advised to make an appointment with their General Practitioner (GP) and the Clinical Physiologist (Dr David Oxborough) will write to the GP detailing the electrocardiographic and echocardiographic findings, suggesting they refer the participant to a local Cardiologist (if deemed appropriate). Until a firm diagnosis is made within the hospital setting it would be considered inappropriate to provide patient information leaflets at this early stage, however reassurance and the ability for the participant to directly contact the Clinical Physiologist will be available.

2) In the very unlikely setting where a more urgent referral is deemed appropriate the Clinical Physiologist will discuss directly with a Consultant Cardiologist (Prof. John Somauroo) and the appropriate action will be undertaken i.e. referral to secondary or tertiary care.

Resting brachial artery systolic and diastolic blood pressure will be taken from the left arm using a standard blood pressure cuff and machine. Body mass will be recorded using Seca scales. We will then run a VO2 max test sat on a bicycle where the intensity is increased every 2 minutes until you cannot continue and oxygen levels monitored to give us an idea of how fit you are. The lactate threshold test is much shorter but follows a similar pattern with exercise intensity increasing every minute and a finger prick blood sample being taken to analyse lactate levels. When these levels increase the test is stopped.

On Visit 2, we will scan your heart using an ultrasound machine; you will be required to lie on your left side then a small receiver will be placed on the skin surface with a small amount of gel to improve the image being recorded, the device is then moved to different positions on the chest to obtain the required data. Clips are saved to be analysed later. To improve some of the blood flow measures, we will inject a small amount of saline flushed with air (agitated saline) into a catheter placed in your lower arm. The catheter will be left in your arm for the duration of testing and the agitated saline will be given before each heart scan.

You will then undertake a 6 hour simulated ultra-endurance race on a bike, pacing will be up to you. At 2, 4, 5 and 6 hours during the exercise, the testing protocol described above will be carried out. The testing protocol will then also be carried out 30 minutes after finishing exercise.

Initial Requirements
All subjects will complete a PAR-Q questionnaire to clear them for exercise. You will need to complete a food and drink diary for the 24 hours prior to testing. Please avoid any form of exercise for the 24 hours before testing. You will also need to abstain from alcohol and caffeine for the 12 hours prior to testing.

4. Are there any risks, discomforts, benefits involved?
There is a very small risk of infection due to the finger prick blood sample and of a blood clot developing where we insert the catheter. This will be done by Professor Somauroo who has never had any problems with the procedure during his career. In addition to receiving free screening for CV disease, participants will be exposed to cutting edge research tools that will help you to understand the effects of prolonged endurance exercise on the cardiovascular system. This could help you to adapt your training to maximise performance.

5. Will my taking part in the study be kept confidential?

Data collected in this investigation will be fully anonymised using codes with no way of linking data to you. Data collected may be reported at national or international conferences and/or in journal publications but your identity will be protected by the use of a pseudonym. All data will be stored in a password protected computer file that only myself and the project supervisor will have access to. On completion of the study this data will be destroyed by electronic deletion and any hard copies shredded. You will have access to your personal results at the end of the study should you wish to obtain these.

Contact Details of Researcher
r.lord@2009.ljmu.ac.uk

Contact Details of Supervisor
d.l.oxborough@ljmu.ac.uk

Appendix 3 – Consent Form

LIVERPOOL JOHN MOORES UNIVERSITY
CONSENT FORM

The RV functional response to an acute bout of prolonged endurance exercise.

Researcher: Rachel Lord (School of Sport and Exercise Sciences)

Please tick in the boxes to give your consent and sign and date the form at the bottom.

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 satisfactorily ☐

2. I understand that my 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 the collection, storage and analysis of venous blood samples as described in the information sheet. ☐

5. I agree to the administration of agitated saline by qualified personnel as described in the information sheet. ☐

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

Name of Participant Date
Signature

Name of Researcher Date
Signature

Appendix 4 – Health and Training Questionnaire

Sport and Exercise Sciences Pre-testing health and activity questionnaire

Personal Information

1. Name:_____
2. DOB:_____Age:_____
3. Height:_____Weight: _____
4. Address _____

   _____

   _____
5. Telephone number:

   Home: _____
   Mobile:_____
6. Email:_____
7. What is your ethnic group (please tick box)

Caucasian	Hispanic	Black	Asian	Chinese	Other
☐	☐	☐	☐	☐	☐

Personal Medical History Assessment (circle answer)

8. Has your doctor ever said that you have had a heart condition? Yes
   No

   If yes, please give details, including dates

   _____

9. Have you ever had a real, or suspected, heart attack? Yes
   No

If yes, when did it occur
_____

10. Have you ever experienced rapid heart beating or palpitations? Yes
No

If so, please give details, including what you were doing at the time
_____
_____
_____
_____

11. Have you ever had angina or a sharp heavy pain in your chest as the result of physical activity? Yes
No

If so, please circle level of activity: low moderate strenuous

12. Do you lose your balance because of dizziness? Yes
No

13. Do you ever lose consciousness? Yes
No

14. Have you ever had a resting or exercise ECG taken? Yes
No

If yes, was the ECG normal? Yes
No

15. Have you ever been severely breathless as a result of low/moderate level exercise? Yes
No

16. Do you suffer from high or low blood pressure? Yes
No

If yes, which one? Low High

17. Are you currently taking prescribed medication to control your blood pressure? Yes
No

If yes, give name and dosage
_____

18. Have you ever been told your blood cholesterol is too high? Yes
No

If yes, please state your cholesterol level (if known)
_____

19. Are you currently taking prescribed medication to control your cholesterol ? Yes
No

If yes, state name and dosage
_____

20. Do you suffer from any kidney problems now or in the past? Yes
No

If yes please specify condition and medication
_____

_____
_

21. Do you suffer from diabetes? Yes
No
If yes, how is it controlled **(please tick)**
a) Dietary means ☐ b) Insulin injection ☐
c) Oral medication ☐ c) Uncontrolled ☐

22. Are you currently taking or being described **ANY** medication from your Doctor?
If yes, please specify the condition, medication and dosage
_____
_____
____

Physical Activity Assessment

23. What year did you start training? _____
_

24. What is your typical training mileage per week?_____
_

25. How many hours a week do you train?_____

26. How many marathons and ultra-marathons have you completed?____
_____
_____

27. When was your last training session, and what did it involve?_____
_____
_

28. When was your last >2hr training session? _____
_

28. Please detail any further information you would like to tell us regarding your health or training status _____
_____
_____
_____
_____
_____
_____
_____

Participant signature: _____

Thank you for completing this questionnaire

Once complete please return to:-

Rachel Lord
PhD Student
Research Institute of Sport and Exercise Science
Liverpool John Moores University
Liverpool, UK

Email: r.lord@2009.ljmu.ac.uk