The Impact of Training Quantity on Structure-Function Relationships of the Cyclist's Heart

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Abstract

Structural and functional adaptations of the left ventricle (LV) in response to chronic exercise training termed the “athlete’s heart” (AH) are central to a road cyclist’s (RC) performance capacity. As a result, RC athletes complete very high training hours, which generate a stimulus for cardiac remodelling. In some cases, the profound adaptation observed in these athletes can mimic pathological processes, presenting a risk of false-positive identification of cardiomyopathy at pre-participation screening or during follow-up. Furthermore, emerging data suggest acute transient post-strenuous exercise reductions in LV systolic and diastolic function termed “exercise induced cardiac fatigue” (EICF) may be extended to short-term periods such as overload training where very high training hours and limited recovery exist (i.e. training camps and/or stage races). The magnitude and possible mechanism(s) responsible for persistent EICF in overload training, and implications for pre-participation screening/follow-up of RC are not fully understood.

Based on this, the aims of this thesis were: 1) establish the impact of moderate and very high chronic training hours on structural, functional and mechanical remodelling of the road cyclist’s LV, 2) determine how the LV responds to variations in training hours across a competitive road cycling season, 3) assess the impact of short-term overload endurance training on LV structure-function-mechanical relationships of the road cyclist’s LV, and 4) evaluate the relationship between LV function and road cycling performance following short-term overload endurance training.

Study 1 (Chapter 4) highlighted that LV eccentric hypertrophy is commonly presented by elite cyclists (EC) (35%), but not sub-elite cyclists (SEC) (3%). Increases in LV mass between non-athletes (NA) and SEC (133 ± 24 vs 163 ± 26 g, $P<0.001$) were

predominantly driven by chamber expansion, whereas increased chamber concentricity between SEC and EC (5.85 ± 0.98 vs 7.11 ± 1.08 g/ml$^{2/3}$, P<0.001) drove further LV mass development (133 ± 24 vs 210 ± 31 g, P<0.001). Marked structural remodelling in EC was also associated with a high prevalence of reduced (<52%) LV ejection fraction (LV EF) (12 %) and mildly reduced diastolic function.

Study 2 (Chapter 5) established a progressive increase in LV mass between off-season and end-season in parallel with an accumulation of training hours in RC (143 ± 17 vs 162 ± 31, P<0.05), which was eccentric in nature. Although RC presented mildly decreased early diastolic function during the most rapid increase in training hours, both resting and in-exercise mechanics remained unchanged across all timepoints.

In study 3 (chapter 6), 3-weeks of overload (OL) training elicited acute fatigue in RC, which was associated with dilatation of the LV (50.8 ± 2.9 vs 51.8 ± 3.2 mm, P<0.05), a decreased ability to augment LV EF (67 ± 5 vs 63 ± 3, P=0.056), and an increased atrial contribution to diastolic filling in-exercise (9 ± 3 vs 12 ± 2 cm/s, P<0.05). Decreased LV twist (17.7 ± 4.5 vs 15.3 ± 3.3, P<0.05) and global longitudinal strain (GL ε) (-20.2 ± 1.0 vs 19.2 ± 1.3, P= 0.063) are indicative of intrinsic contractile dysfunction and suggest similar mechanisms are involved in both acute and persistent EICF.

The application of conventional and novel echocardiographic techniques have provided further understanding of normal physiological adaptation of the LV in response to short-, medium- and long-term high training hours in RC. These insights may lead to improvements in pre-participation screening and influence the training practices of this athlete group.

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Preface

Two of the chapters included in this thesis have resulted in jointly authored peer-reviewed publications. These are presented below.

Chapter 2:

Brown, B., Somauroo, J., Green, DJ., Wilson, M., Drezner, J., George, K. and Oxborough D. (2017). The complex phenotype of the athlete's heart: Implications for preparticipation screening. *Exercise and Sport Science Reviews*. *45(2):96-104.*

Chapter 4:

Brown, B., Millar, L., Somauroo, J., George, K., Sharma, S., La Gerche, A., Forsythe, L. and Oxborough, D. (2020). Left ventricular remodelling in elite and sub-elite road cyclists. *Scandinavian Journal of Medicine & Science in Sports*. *DOI: 10.1111/sms.13656*

Abbreviations

2D	Two-Dimensional
A	Peak Late Mitral Diastolic Velocity
A’	Peak Late Diastolic Myocardial Velocity
AH	Athletes’ Heart
ARVC	Arrhythmogenic Right Ventricular Cardiomyopathy
ASE	American Society of Echocardiography
AVC	Aortic Valve Closure
BSA	Body Surface Area
BP	Blood Pressure
CoV	Coefficient of Variation
DCM	Dilated Cardiomyopathy
E	Peak Early Mitral Diastolic Velocity
E’	Peak Early Diastolic Myocardial Velocity
EC	Elite Cyclist
ECG	Electrocardiogram
EF	Ejection Fraction
EICF	Exercise Induced Cardiac Fatigue
ESC	European Society of Cardiology
GC ε	Global Circumferential Strain
GL ε	Global Longitudinal Strain
HCM	Hypertrophic Cardiomyopathy
HD	High Dynamic
HR	Heart Rate
HR_{max}	Maximum Heart Rate

HS	High Static
IVC	Inferior Vena Cava
LA	Left Atrium
LV	Left Ventricle
LV EDV	Left Ventricular End Diastolic Volume
LV ESV	Left Ventricular End Systolic Volume
LVH	Left Ventricular Hypertrophy
LVIDd	Left Ventricular Internal Diastolic Dimension
LVISd	Left Ventricular Internal Systolic Dimension
MWT	Mean Wall Thickness
NA	Non-Athlete
RC	Road Cyclist
RV	Right Ventricle
RWT	Relative Wall Thickness
S’	Peak Systolic Myocardial Velocity
SCD	Sudden Cardiac Death
SEC	Sub-Elite Cyclist
SR	Strain Rate
SRA	Late Diastolic Strain Rate
SRE	Early Diastolic Strain Rate
SRS	Systolic Strain Rate
STE	Speckle Tracking Echocardiography
SV	Stroke Volume
TDI	Tissue Doppler Imaging
ε	Strain

Chapter 1 **General Introduction**

1.1 Introduction

Road cycling (RC) is an endurance sport, whereby performance is largely determined by an athlete's maximal aerobic capacity ($VO_{2\ max}$), economy, and ability to sustain a high percentage of $VO_{2\ max}$ for long periods of time (Faria *et al*., 2005). The role of cardiac adaptation in developing appropriate physiological capacities is therefore inherent and can be described by a modified Fick equation ($VO_{2\ max}$ = stroke volume maximum [SV_{max}] x heart rate maximum [HR_{max}] x (maximum arterio-venous oxygen difference [a-vO_2 $diff_{max}$]) (Levine *et al*., 2008).

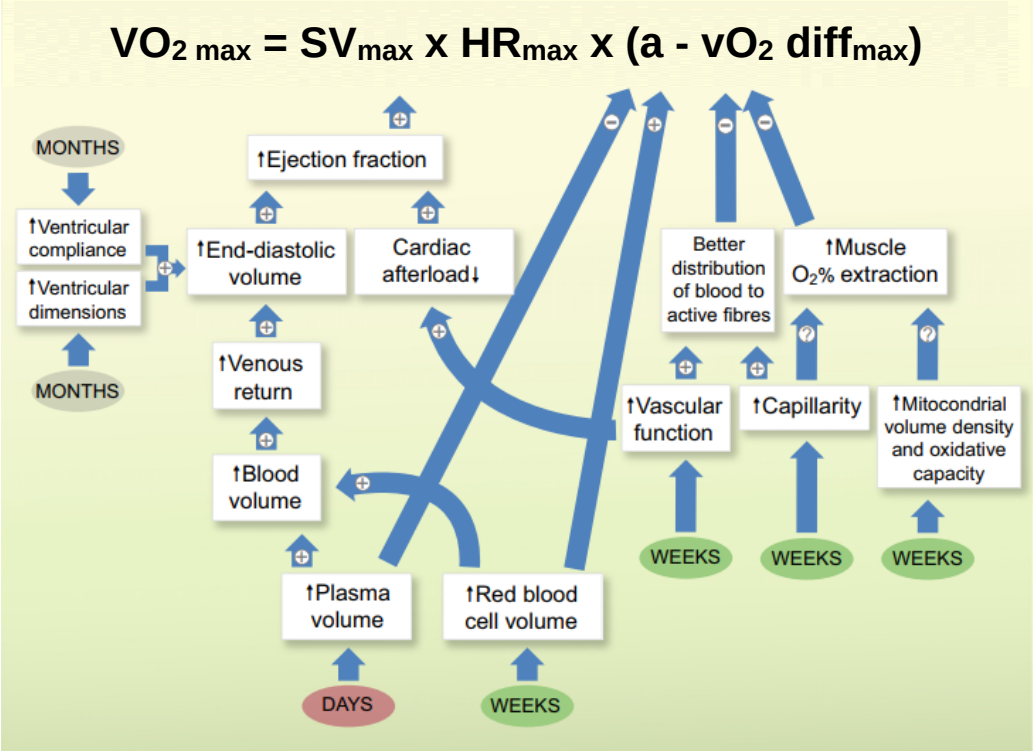

Figure 1. Trainable parameters of the modified Fick Equation, adapted from Lundby, Montero and Joyner (2017)

As HR_{max} is not a trainable parameter, athletes utilise training regimes which seek to optimise SV_{max} and a-vO_2 $diff_{max}$ (Levine *et al*., 2008) (see figure 1). Red blood cell volume, intra-muscular capillarity and mitochondrial density/function all enhance delivery and utilisation of oxygen to the working musculature, and increase/decrease

(to a meaningful extent) within 2-6 weeks of (de-)training (Mujika *et al*., 2019; Jensen *et al*., 2004; Granata *et al*., 2018). However, factors affecting oxygen utilisation depend on the central parameter of SV. Aerobic training is known to elicit increases in chamber volume (and thus SV), however, the time frame of meaningful change in an athletic context, remains relatively unknown. Furthermore, only recently have advances in echocardiographic technology and technique facilitated comprehensive assessment of chamber mechanics and compliance (essential to SV generation) (Marwick *et al*., 2006).

As physiological adaptations favourable to cycling performance are primarily driven by quantity of training, it is unsurprising that elite RC complete very high training hours (Metcalfe *et al*., 2017). However, training hours are not distributed equally across a training season, as load and recovery demands of multi-stage races and training camps generate considerable peaks and troughs (Metcalfe *et al*., 2017). Significant challenges to recovery often result in states of transient over-reaching or acute-fatigue, whereby performance is decreased or fails to super-compensate in response to a training stimulus (Aubry *et al*., 2015).

The phenotype of the elite RC is known to present challenges in the clinical differentiation between physiology and pathology, due to the extent of remodelling (Abergel *et al*., 2004). Furthermore, the impact of an acute bout of strenuous exercise acts as an additional confounding factor to differential diagnosis, resulting in a transient bi-ventricular dysfunction similar in appearance to arrythmogenic right ventricular cardiomyopathy (ARVC) and dilated cardiomyopathy (DCM) (Lord *et al*., 2018; Oxborough *et al.,* 2010). However, the "normal response" to repeated bouts of

strenuous exercise with insufficient recovery (i.e. multi-stage races or training camps) remains unknown. Characterisation of cardiac structure, function and mechanics in this context is required to aid distinction between physiological and pathological processes.

Speckle tracking echocardiography (STE) represents a novel echocardiographic technique, which has improved understanding of normal mechanical function of the AH phenotype. In the case of RC, STE has the potential to clarify structure-function relationships of the LV and generate new knowledge regarding training induced remodelling. Additionally, in-exercise assessment will provide insight into development of the functional reserve that characterises the endurance AH phenotype, and how this responds to short- and long-term training quantity alterations. Specifically, this information is likely to be useful for clinicians, whereby pre-participation screening of elite RC is mandated by the Union Cycliste Internationale (UCI), whereas the specific time point of screening within a competitive season is not standardised.

1.2 Overarching Aims

The overarching aim of this thesis is to provide a comprehensive assessment of the RC's LV in response to different short-, medium- and chronic- periods of high training hours, using conventional echocardiography and STE at rest and in-exercise.

Elite RC are known to present with profound LV remodelling, in part due to the high dynamic, high static nature of the sport, and in part due to the extreme quantity of training undertaken by these athletes (Abergel *et al*., 2004; Metcalfe *et al*., 2017).

Although physiological cut-off values for structural remodelling of the LV to exclude pathology in this group are defined (Whyte *et al*., 2004; Makan *et al*., 2005), quantification of "normal" physiological function is less clear. Previous assessment of the elite RC heart was carried out in the late 1990's, and (by the authors' admission), were likely impacted by widespread use of illicit performance enhancing drugs (Abergel et al., 2004). It is now known that that blood doping, exogenous erythropoietin (EPO) and anabolic steroid abuse were particularly prevalent during this period.

Blood doping and EPO administration increase red blood cell content, resulting in enhanced oxygen carrying capacity (and associated $VO_{2\ max}$ improvement). These practices also have significant effects on total blood volume (increasing and reducing, respectively), and are therefore highly likely to impact pre-load dependant measures of LV function previously highlighted as characteristics of the elite RC heart (Kumar et al., 2004; Lord et al., 2018a). Administration of exogenous anabolic steroids increase an athlete's capacity to recover between repeated bouts of strenuous endurance exercise (such as a multi-stage race), where excessive energy expenditure may result in suppression of HPA-axis function (Lucía et al., 2001). Abuse of exogenous anabolic steroids is also known to drive myocyte hypertrophy, resulting in concentric-type remodelling of the LV and the potential for mildly depressed early diastolic function (due to decreased myocardial elastance) (Angell et al., 2012).

Although blood doping, exogenous EPO and anabolic steroid abuse are likely still present in RC, introduction of the "Athlete Biological Passport" in 2008 has (at the very least) decreased the manipulation of bloods to near-physiological levels (Pottgiesser et

al., 2011). Advances in liquid chromatography and mass spectrometry methodologies have also enabled identification of exogenous anabolic steroids, previously undetectable when the elite RC heart was characterised (Botrè, 2008).

This thesis therefore sought to comprehensively assess the LV in elite RC, who carry out very high chronic training hours (Metcalfe *et al*., 2017). Comparison to sub-elite RC who complete moderate chronic training hours, and non-athletes would provide reference for the magnitude of adaptation, and insight into the adaptive process.

Medium-term, seasonal variations in training hours have been shown to impact echocardiographic parameters of the LV in soccer and swimming (D'Ascenzi *et al*., 2015; Csajagi *et al*., 2015), but remain unknown in RC. The highly variable and seasonal nature of training hours in competitive road cyclists appears to increase the potential for medium-term alterations in LV structure, function and mechanics. Clarification of how these (de-)training responses manifest are likely to provide value in a pre-participation screening setting.

The negative impacts of acute strenuous bouts of exercise on LV function and mechanics are well established (Lord *et al*., 2018). The nature and magnitude of these alterations present a significant challenge to differential diagnosis of the AH phenotype. Although recent work has indicated short-term overload training may result in similar dysfunction (Le Meur *et al*., 2014), echocardiographic evidence is required to confirm

this. STE assessment of LV mechanics may also provide additional insight into mechanisms of fatigue-induced LV dysfunction.

These overarching aims generate several specific aims for this thesis:

1. To establish the impact of moderate and very high chronic training hours on structural, functional and mechanical remodelling of the road cyclist's LV.
2. To determine how the LV responds to variations in training hours across a competitive road cycling season.
3. To assess the impact of short-term overload endurance training on LV structure-function-mechanical relationships of the road cyclist's LV.
4. To evaluate the relationship between LV function and road cycling performance following short-term overload endurance training.

Chapter 2 **Literature Review**

2.1 Introduction

It is well established that the athlete's heart (AH) undergoes physiological adaptation in response to chronic exercise training, however much of the early literature focused on the left ventricle (LV) with little acknowledgement of right ventricular (RV), atrial, or functional adaptation. Interest in "whole" cardiac adaptation has significantly increased in the last 10 years, and original theories of load induced, dichotomous adaptations have been questioned (Spence et al., 2013). The impact of other factors on cardiac phenotype have also been raised, including sporting discipline, training load, ethnicity, body size, sex and age, on the magnitude and nature of adaptation (see figure 2) (Basavarajaiah et al., 2008; Weiner et al., 2010; Utomi et al., 2013; Kaku et al., 2014; Riding et al., 2014).

The reasons behind this growth of interest appear to have been driven by developments in imaging technology and high-profile cardiac events which continue to occur in the seemingly healthiest of the population. Although contradictory schools of thought exist regarding the efficacy of pre-participation screening (Harmon et al., 2014), there is a growing demand for sports physicians to undertake screening in order to exclude inherited cardiac diseases, which account for the majority of sudden cardiac death (SCD) cases in individuals under 35 years of age (Chandra et al., 2013). A comprehensive understanding of cardiac structure and function in a heterogeneous athletic population is therefore fundamental to facilitate identification of normal and abnormal features on the athlete's electrocardiogram (ECG) and echocardiogram. This review aims to provide a balanced perspective on current understanding of the

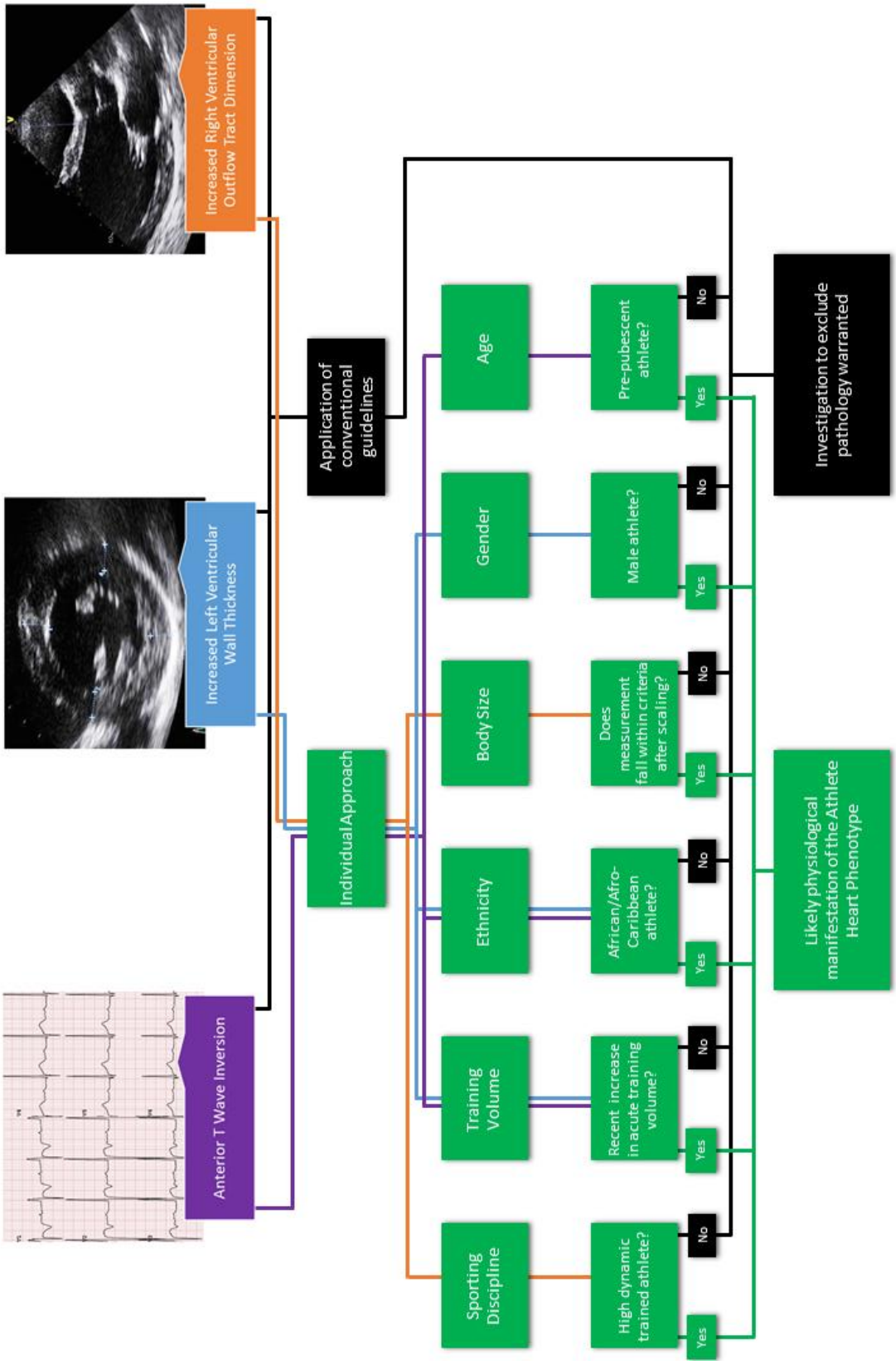

Figure 2. The multi-factorial nature of the athlete's heart phenotype

athlete's heart, with a focus on the impact of sporting discipline, training load, ethnicity, body size, sex and age. It was hypothesised that an individualised approach to diagnostic testing is required to differentiate between physiological and pathological cardiac adaptation in athletic

2.2 The Impact of Training Modality on Athletic Cardiac Development

Although it is known that exercise training elicits physiological adaptation of the heart, understanding of how specific training stimuli are related to this adaptation is less clear. The seminal work of Morganroth et al. (1975) suggested endurance and strength-based training elicit eccentric hypertrophy (primarily through volume overload) and concentric remodelling (primarily through pressure overload) of the LV, respectively. More recent work has challenged this (Spence et al., 2011; Utomi et al., 2013), highlighting the need for a more comprehensive study of the AH phenotype, and greater clarity in the classification of sporting disciplines.

The 12-Lead Electrocardiogram

Knowledge of training-induced ECG changes is predominantly based upon large cohort studies, where little consideration has been given to sport specific cardiovascular demands. In view of this, Brosnan et al. (2014) analysed the resting ECG of endurance athletes and non-endurance athletes using the 2010 European Society of Cardiology (ESC) guidelines (See table 1), which specify group 1 (common training related) and group 2 (abnormal non-training related) criteria. A higher prevalence of both group 1 and group 2 changes were observed in endurance athletes compared to non-endurance athletes. The false positive rate of almost 30%, suggests the specificity of these criteria

are relatively poor. In view of this, refined criteria have been developed which better-reflect normal "training-related" changes in the ECG (Brosnan et al., 2014; Sheikh et al., 2014). Brosnan et al. (2014) reported a significant decrease in the false-positive rate through application of the Seattle criteria.

Table 1. Classification of ECG characteristics based on the 2010 ESC recommendations

ESC Classification of ECG Abnormalities in athletes	
Group 1 (training-related)	**Group 2 (training-unrelated)**
Sinus Bradycardia	T-wave inversions
First degree AV Block	ST-segment depression
Incomplete RBBB	Pathological Q-waves
Early Repolarisation	Left Atrial Enlargement
Isolated QRS voltage criteria for LVH	Left axis deviation / left anterior hemiblock
	Right axis deviation / left posterior hemiblock
	Right Ventricular Hypertrophy
	Ventricular pre-excitation
	Complete LBBB or RBBB
	Long QT or short QT interval
	Brugada-like early repolarisation

The Left Ventricle

Many studies have attempted to quantify the magnitude of cardiac adaptation and to understand any relationship to the type of exercise stimulus. Recent work (Utomi et al., 2013; Utomi et al., 2014) has challenged the traditional theory of dichotomous adaptations induced by endurance and resistance training. Whilst LV cavity dilatation was observed in endurance athletes, neither concentric remodelling nor concentric hypertrophy were discerned in resistance trained athletes (Spence et al., 2011; Utomi et

al., 2013). A more recent study has provided further support for this, describing a predominance of normal LV geometry in both endurance and resistance trained athletes (Utomi et al., 2014). It is unclear whether the differences between findings in these studies can be accounted for by improvements in imaging quality, or changes in athletes' training methods over the last 40 years. The inclusion of more aerobic conditioning in resistance trained athletes' programmes, and more strength training in endurance athletes' programmes may provide some explanation for the similar LV geometry between groups.

Longitudinal studies have demonstrated endurance training is associated with progressive increases in LV cavity dimension and wall thickness, in a close relationship with development of fat-free mass (FFM) (Baggish et al., 2008; Spence et al., 2011; D'Ascenzi et al., 2012). Spence et al. (2011) described no structural changes to the LV, whereas Baggish et al. (2008) observed concentric hypertrophy following 3-6 months of resistance training. These disparate findings may be explained by differences in study specific training loads, training status of the participants, or imaging methodologies (Spence et al., 2013). Further longitudinal studies are required to assess the impact of training load on structural adaptation in both endurance and resistance training settings.

Previous research has highlighted decreased resting systolic function in endurance athletes, with up to 12% of this group presenting with an "abnormally" low ejection fraction (EF) (Abergel et al., 2004). This decrease in EF appears to be a consequence of an increased LV end-diastolic volume (EDV) (see table 2), resulting in the need for a lower contraction force to generate the necessary stroke volume. Previous large-scale studies have observed no differences in regional or global peak longitudinal strain (ε)

between endurance and resistance trained athletes (Utomi et al., 2014). Furthermore, no differences in peak longitudinal, circumferential, and rotational ε values have been reported between endurance athletes and sedentary controls (Utomi et al., 2014). However, superior ability to augment systolic function during exercise has previously been demonstrated in athletes (La Gerche et al., 2012a). Therefore, in-exercise assessment of LV systolic function provides a useful screening tool in athletes who present with decreased contractility, regardless of sporting discipline.

Assessment of diastolic function is complex, as conventional Doppler parameters are not directly related to overall volume, and are dependent upon atrial and LV pressures. It is therefore unsurprising that many studies observe no differences in peak E velocity between endurance trained, resistance trained, and sedentary individuals (Utomi et al., 2013). That aside, the atrial component, and E/A ratio are often significantly different in endurance athletes, indicative of enhanced early diastolic filling. This is supported by the increased early myocardial mitral tissue velocities (E') displayed by endurance athletes (George et al., 2010). It is thought untwisting of the LV plays an important role in lowering LV pressure, and enhancing early diastolic filling during exercise (Weiner et al., 2010). The reduction in peak LA longitudinal ε observed in high-dynamic athletes provides complimentary evidence for enhanced early diastolic filling, and a decreased atrial component (D'Ascenzi et al., 2012).

Neither endurance nor resistance training appear to elicit changes in global diastolic function during training periods up to 6 months (Baggish et al., 2008; Spence et al., 2011).

The Right Ventricle

As approximately 4-23% of all sudden cardiac death cases in athletes are due to arrythmogenic RV cardiomyopathy (ARVC), understanding the nature and magnitude of physiological training-induced RV remodelling is vital (Chandra et al., 2013). Increased RV cavity, inflow and outflow dimensions are observed in endurance athletes, compared to resistance athletes, who have similar chamber dimensions to sedentary individuals (D'Andrea et al., 2013). In addition to this, a large proportion of endurance athletes exceed "normal" values for RV inflow and outflow dimensions (57% and 40% respectively) (Oxborough et al., 2012). The prevalence of abnormal RV/LV ratios within this population (66%) also provide evidence for disproportionate loading on the RV during endurance exercise (Oxborough et al., 2012).

The work of Spence et al. (2011) provides further support for this phenomenon, as increased RV cavity dimensions were observed in participants who completed 6 months of endurance training, but not in those who completed resistance training. A study of longer duration (12 months) has also demonstrated a progressive increase in RV:LV ratio in response to high dynamic training, providing further support for disproportionate loading on the RV acting as a stimulus for more marked remodelling compared to the LV (Arbab-Zadeh et al., 2014).

RV enlargement and an increased prevalence of T-wave inversion in endurance athletes, presents a challenge in the differential diagnosis from ARVC. It is therefore important to note that global function is maintained when assessed by tricuspid plane systolic excursion (TAPSE), RV fractional area change (RV FAC), RV myocardial

tissue velocities, and peak RV global longitudinal ε (Oxborough et al., 2012; D'Andrea et al., 2013). Decreased myocardial function at the base of the lateral RV wall has also been reported in some endurance athletes (La Gerche et al., 2012a). This appears to occur in combination with concomitant RV dilatation, generating ambiguity in the pre-participation screening setting (La Gerche et al., 2012a). Importantly, ε values are only impacted at a regional level and do not fall as low as those seen in the ARVC patients (La Gerche et al., 2012a). Furthermore, a normal physiological response to exercise is maintained, highlighting the potential diagnostic role of exercise echocardiography in ambiguous cases (La Gerche et al., 2012a).

The Atria

Left atrial dilatation is commonly observed in patients with hypertrophic cardiomyopathy (HCM) (McClean et al., 2015), highlighting the importance of defining normal, training-induced adaptation. Bi-atrial dilatation and increased functional volume is observed in athletes from high-dynamic sporting disciplines (McClean et al., 2015). In contrast, there are no structural differences between sedentary individuals and athletes from low-dynamic sporting disciplines (McClean et al., 2015). Although cross-sectional studies suggest there are no differences in atrial longitudinal ε between high-dynamic, low-dynamic or sedentary groups (McClean et al., 2015), an 8 month assessment of athletes completing high dynamic training has demonstrated a progressive decrease in LA ε (D'Ascenzi et al., 2015). It is suggested that, in the presence of normal LV/RV diastolic function, atrial dilatation represents a normal physiological manifestation of the AH.

2.3 The Impact of Training Load on Athletic Cardiac Development

Although the role of training modality in development of the AH phenotype is relatively well established, understanding of this relationship is predominantly based on cross-sectional analysis of subjects with a high/low chronic training load (i.e. training quantity x intensity). Longitudinal data relating cardiac adaptation to training load are limited, due to the logistical challenge of data collection and methodological challenge of "load" calculation (Sanders et al., 2017). Despite this, emerging research has highlighted the importance of acute (2-38 hour) and medium-term (1-3 month) alterations in training load on structural and functional characteristics of AH (Weiner et al., 2010; Lord et al., 2018b).

The landmark work of Weiner et al. (2015) has provided insight into the non-linear process of physiological structural adaptation in response to increased training hours, and the mechanical alterations that facilitate normal cardiac function during this period. Considerable attention has also been afforded to "exercise induced cardiac fatigue" (EICF), whereby the training load generated by an acute exercise bout is sufficient to alter mechanics and decrease cardiac function through sub-clinical levels of cardiomyocyte damage, beta-adrenergic desensitisation and/or altered ventricular interaction (Lord et al., 2018b).

Furthermore, recent work has developed a more holistic understanding of how central factors (i.e. hypothalamic-pituatry-adreno axis) may negatively influence cardiac function (and thus performance) during medium-term periods of elevated training load (Le Meur et al., 2014).

12-Lead Electrocardiogram

Although relatively rare in the athletic population (6.6%), the prevalence of training unrelated ECG changes are associated with high chronic training load (Dores et al., 2016). It is noteworthy however, that high training intensity (defined as having at least one high dynamic or static component) acts an independent predictor of ECG abnormality, whereas training hours do not (Dores et al., 2016). Left atrial enlargement, left axis deviation, and T-wave inversion form the most common abnormal ECG patterns observed within this population (Dores et al., 2016). A large cohort study (*n*= 52,755) of cross-country skiers provides further support for a causal link between high dynamic training and electrocardiographic abnormalities, as authors were able to develop a dose-response curve for correlates of physical fitness and cumulative risk of AF (Andersen et al., 2013). Athletes who completed a 90km cross-country skiing race within 60% of the winner's time had 1.3-fold greater risk of AF compared to those who took more than twice the winner's time (Andersen et al., 2013).

Medium-term increases in training hours appear to be associated with increased prevalence of training-related ECG changes. Most notably, early repolarisation pattern, whereby incidence has been shown to increase from 40 % to 63 % in response to 3 months of increased training hours (of a high dynamic, high static nature) (Noseworthy et al., 2011). Transient lateral T-wave inversion has also been reported in response to rapidly increased quantity of training (320% habitual training hours per week) in the case-study of a professional boxer (Oxborough et al., 2014a). It should be noted however, that T-waves had normalised after 2 weeks of training, and remained normal

(despite maintenance of training hours) for the following 10 weeks (Oxborough et al., 2014a).

Acute bouts of strenuous exercise are associated with increased presentation of training-related and training-unrelated ECG changes (Lord et al., 2015; Kaleta et al., 2018). Significant increases in the prevalence of left and right atrial enlargement have been observed in recreational athletes (38 % and 43 % respectively) upon completion of a marathon (Kaleta et al., 2018). By comparison, the increased demands of an ultra-marathon appear to result in more (and more severe) ECG changes (Lord et al., 2015). Most notably, Long QTc, which has been observed in 6 % of subjects upon completion of a 100-mile ultra-marathon (Lord et al., 2015).

The Left Ventricle

Contrary to previous assumptions (based on cross-sectional assessments), structural remodelling of the LV in response to elevated high dynamic training hours appears to be phasic in nature, rather than concurrent (D'Ascenzi et al., 2015; Weiner et al., 2015). Chamber expansion takes place in a medium time-span (< 3 months), whereas wall thickness development forms a longer-term process (9 months – 3 years) (D'Ascenzi et al., 2015; Weiner et al., 2015). It is proposed the timespans of these adaptations can be accounted for by the differing rates of exercise-induced plasma volume expansion and adaptive myocyte hypertrophy (Weiner et al., 2015).

During the initial adaptive phase, athletes can be expected to present with increased pre-load sensitive functional/mechanical parameters, including E, E/A ratio, apical

rotation and LV Twist (D'Ascenzi et al., 2015; Weiner et al., 2015). It is suggested that these "supranormal" measures of diastolic function are maintained during ventricular hypertrophy, observed during the secondary (chronic) adaptive phase (Weiner et al., 2015).

EICF, in contrast, is characterised by a significant decrease in systolic and diastolic function (Lord et al., 2018b). Although these decreases are partially mediated by post-exercise alterations in loading conditions, the failure of pre-load augmentation to normalise ventricular contractile/relaxation is indicative of intrinsic dysfunction (Hart et al., 2007). STE assessment provides additional evidence for intrinsic dysfunction, as global longitudinal ε and systolic SR are also reduced following strenuous exercise (Lord et al., 2018b).

Many studies have explored the association between EICF and blood biomarkers of cardiomyocyte damage (Shave et al., 2008). Cardiac troponin T (cTnT) and I (cTnI) are regulatory proteins unique to the myocardium, which control the calcium mediated actin and myosin interaction (Sharma, Jackson and Makan, 2004). Increased serum concentrations of these troponins (such as in myocardial infarction) are therefore indicative of damage intrinsic to the myocardium. Significant correlations between LV function and cardiac troponins have been observed following extended exposure of the myocardial wall to increased volume/pressure load (Rifai et al., 1999; Neilan et al., 2006), suggesting cardiomyocyte damage may be responsible for decreased chamber contractility/compliance. It is noteworthy, however, that there is little evidence supporting a temporal relationship between EICF and blood biomarker expression (Shave et al., 2010). It is therefore likely that localised cardiomyocyte damage does not

contribute to EICF in the LV, and represents a benign response to acute strenuous exercise. In contrast, an exercise-duration mediated blunting of the inotropic response provides support for β-adrenoreceptor desensitisation as a causative mechanism of EICF (Eysmann et al., 1996; Douglas, O'toole and Katz, 1998; Welsh et al., 2005). It is unclear, however, how β-adrenoreceptor desensitisation directly impacts diastolic function, if at all (Lord et al., 2018b).

Structural, functional and mechanical aspects of EICF subside within 48 hours of exercise cessation (Lord et al., 2018b). Despite this clear timeframe, the impact of subsequent bouts of strenuous exercise completed with <48 hour recovery (such as endurance stage races, or training camps) on EICF is unclear. Recent work utilising impedance cardiography found a decreased cardiac output at maximal exercise intensities in triathletes following completion of 3-weeks structured overload training (Le Meur et al., 2014). Although a significant reduction in exercise-adrenaline response was proposed as the driver of decreased cardiac output in this study, further work utilising echocardiography is required to determine whether a medium-term increase in training-load results in EICF-like dysfunction.

The Right Ventricle

Intense high-dynamic exercise presents a significant haemodynamic challenge to the RV, as the pulmonary vascular bed is only capable of reducing its resistance by 30-50%, compared to >75% in the systemic vasculature (Heidbüchel and La Gerche, 2012). The resulting increase in wall stress during exercise is far greater in the RV (170%) compared to the LV (25%) (Heidbüchel and La Gerche, 2012).

As a result, athletes with chronically high training load (of a high dynamic nature) present with proportionally greater RV structural remodelling compared to that of the LV (Oxborough et al., 2012). Reflecting the extent of this adaptation, athletes with chronically high training load can also be expected to present with lower resting RV free-wall ε and RV EF compared to non-athletes (La Gerche et al., 2012a). These functional and mechanical characteristics represent an increased reserve volume, as exercise echocardiography reveals superior augmentation (La Gerche et al., 2012a).

To the authors' knowledge, the only human study assessing medium-term RV adaptation in response to differing training loads is that of D'Ascenzi et al. (2016). Completion of 4 months elevated moderate-high dynamic training load was found to increase RV cavity dimension and chamber area (D'Ascenzi et al., 2016). End-diastolic RV/LV diameter ratio also increased during this period, highlighting more pronounced remodelling of the RV (compared to LV) in response to elevated training load (D'Ascenzi et al., 2016). Upon completion of a further 4 months moderate-high dynamic training, End-diastolic RV/LV ratio normalised (despite maintenance of RV cavity dimension and area), suggesting medium-term LV remodelling may be similar in magnitude, but require a longer time span (D'Ascenzi et al., 2016). Despite significant structural remodelling, D'Ascenzi et al. (2016) observed no changes in functional or mechanical parameters throughout the study period.

Acute strenuous bouts of high dynamic exercise lasting 3-11 hours have also been shown to disproportionately affect the RV, resulting in significant chamber dilatation, decreased RV EF and RV free wall ε which normalise within 6 h – 11 days (La Gerche

et al., 2012b). In contrast to the LV, reductions in RV function (EF) are significantly correlated with biomarkers of cardiomyocyte damage (La Gerche et al., 2012b). The considerable wall stress placed upon the relatively thin myocardium of the RV is proposed to result in cardiomyocyte damage, which in-turn forms the primary causative factor for EICF in this ventricle (Heidbüchel and La Gerche, 2012). Furthermore, diminished systolic function of the RV has clear "downstream" implications for LV diastolic function, clearly demonstrating serial ventricular interaction plays a key role in EICF (Lord et al., 2018b).

The damage and dysfunction generated by acute strenuous exercise has raised concerns regarding the potential for longer term pathological adaptation of the RV, particularly in the case of repeated bouts of strenuous exercise with insufficient recovery (i.e. endurance stage races and/or training camps) (Heidbüchel and La Gerche, 2012). Although "exercise-induced ARVC" have been observed (exclusively) in athletes completing high dynamic, high static exercise, no studies (to the authors' knowledge) have manipulated/training high training load in-line with RV assessment.

The Atria

Little is known regarding the impact of training hours on atrial adaptation. Although cross-sectional assessments of the atria have demonstrated an association between chronically high training loads (of a high dynamic, high static nature) and development of AF, this process has not been captured within a longitudinal study design (Andersen et al., 2013).

Completion of a strenuous bout of high dynamic exercise appears to have little impact on RA structure, as no change in RA area are observed upon completion of an ultra-marathon (Lord et al., 2018b). Dilatation of the RV appears to elicit a protective effect on the RA in this situation, preventing the RA (and venous system) from relative increases in afterload mediated by downstream elevation in PA pressure (McClean et al., 2015).

In contrast to the RA, LA structure and function are significantly altered upon completion of strenuous exercise (Oxborough et al., 2010a). Intrinsic impairment of LV relaxation/compliance and reduced LA reservoir volume (mediated by reduced upstream RV systolic function) observed in cases of EICF are associated with a shift towards late diastolic filling of the LV (Lord et al., 2018b). In conjunction with decreased early diastolic filling, athletes with EICF can be expected to present with decreased atrial SRe, and peak ε (Oxborough et al., 2010a).

2.4 The Impact of Ethnicity on Athletic Cardiac Development

The 12-Lead Electrocardiogram

Knowledge of the athlete's ECG is primarily based on studies in Caucasian athletes (Brosnan et al., 2014). Recent studies have attempted to document the impact of some ethnicities. In a large study of elite athletes, Sheikh et al. (2014), reported African/Afro-Caribbean athletes were more likely to present an abnormal ECG compared to their Caucasian counterparts (11.5% vs 5.3%). An earlier study found that T-wave inversion was present in 23% of African/Afro-Caribbean athletes (primarily in contiguous

anterior leads), compared to only 3.7% of Caucasian athletes (Papadakis et al., 2012). This T-wave inversion expression is in stark contrast to African/Afro-Caribbean HCM patients, who generally exhibit T wave inversions in the lateral leads (76.9%). T wave inversion extending into lateral leads therefore warrants investigation for the exclusion of pathology in all cases, irrespective of ethnicity (Papadakis et al., 2012). African/Afro-Caribbean athletes also exhibit a higher prevalence of ST-segment elevation. Furthermore, T-wave inversion and convex profile ST-segment elevation were commonly found together in contiguous anterior leads and are likely to represent a physiological, training-induced characteristic of the African/Afro-Caribbean AH (see table 3). In addition, a higher prevalence of early repolarisation (ERP), RV hypertrophy, LA enlargement and RA enlargement were evident in African/Afro-Caribbean athletes.

The prevalence of training-related ECG changes appears to be lower in Arabic/Middle Eastern athletes compared to their Caucasian counterparts, whilst non-training related changes were similar between groups (Riding et al., 2014). Based on this, it is recommended that current guidelines are relevant and appropriate in the pre-participation screening of these athletes (Riding et al., 2014). It should also be noted however, that a small number of Arabic/Middle Eastern athletes can be expected to present T-wave inversion in contiguous leads, in combination with a convex profile ST-segment elevation (as previously highlighted in African/Afro-Caribbean athletes).

There is a lack of ECG data pertaining to South/East Asian athletes, although it is possible to draw some information from a large cohort study (n= 18,497) characterising ECG findings in young Singaporean army recruits (Ng et al., 2012). Seven percent of subjects exhibited an ECG abnormality. For those who received further assessment, the

most common abnormality was increased R/S voltage, followed by right and left axis deviation, right bundle branch block and pathological Q wave expression. Accordingly, East/South Asian athletes are likely to present a similar prevalence of ECG changes to Caucasian and Arabic/Middle Eastern athletes.

The Left Ventricle

Similar to understanding of ECG adaptation, at present, knowledge of the athlete's LV is predominantly based on studies of Caucasian athletes (Whyte et al., 2004). These data have been used to discern "normal" limits for LV wall thickness in males and females. In a comparison between highly trained African/Afro-Caribbean and Caucasian male athletes, a higher proportion of African/Afro-Caribbean athletes presented LV wall thickness values >12 mm (Basavarajaiah et al., 2008). In addition, profound LV hypertrophy (≥15 mm) was demonstrated in a small number (3%) of African/Afro-Caribbean athletes, but not in any Caucasian athletes. Similarly, a higher prevalence of LV hypertrophy has been observed in female African/Afro-Caribbean athletes compared to Caucasian counterparts (Papadakis et al., 2012). Interestingly, no differences have been observed in the LV wall thicknesses of African/Afro-Caribbean and Caucasian sedentary individuals, suggesting African/Afro-Caribbean individuals exhibit a more pronounced training response, rather than a pre-disposition to greater wall thicknesses (Basavarajaiah et al., 2008).

Athletic training is associated with increased LV trabeculation, which may mimic LV non-compaction cardiomyopathy (LVNC). Increased trabeculation is more prevalent in African/Afro-Caribbean athletes, compared with Caucasian counterparts (Gati et al.,

2013). Gati et al. (2013) also found more African/Afro-Caribbean athletes met two criteria for LVNC, than Caucasian athletes. Of these athletes, a higher proportion of African/Afro-Caribbean individuals also presented deep T-wave inversion and reduced LV EF (3.4% vs 0.5%) (Gati et al., 2013). It should be noted however, that T wave inversion is generally expressed in anteroseptal leads in athletes fulfilling LVNC criteria, in stark contrast to LVNC patients where a greater prevalence of inferolateral T wave inversion is observed (Gati et al., 2013). Although increased trabeculation appears to be a physiological process in both African/Afro-Caribbean and Caucasian ethnic groups, differentiation between physiological adaptation and LVNC appears to be more challenging in the African/Afro-Caribbean athletic population, with more athletes falling into the diagnostic "grey zone" (Gati et al., 2013).

Structural remodelling in Arabic/Middle Eastern athletes appears similar in nature to that of Caucasian athletes. Although the magnitude of adaptation appears to be smaller in Arabic/Middle Eastern athletes, differences in LV mass can be negated via scaling to BSA, suggesting this finding may simply express differences in body-size (Riding et al., 2014). Furthermore, a similar prevalence of LV hypertrophy was presented by Arabic/Middle Eastern, African/Afro-Caribbean and Caucasian athletes (Riding et al., 2014). Global measures of LV function appear to be consistent across Arabic/Middle Eastern, African/Afro-Caribbean and Caucasian athletes, with all groups presenting normal systolic function and diastolic filling.

Although data regarding LV adaptation in South/East Asian athletes is sparse, both male and female Chinese athletes appear to display a similar magnitude and prevalence of LV cavity dilation and hypertrophy to Caucasian counterparts (Ma et al., 2007).

Structural morphology has also been studied in a group of Japanese ultramarathon runners. Extreme LV dilatation (LVIDd ≥70 mm) was reported in 11.3% of their cohort, combined with LV wall thickness values up to 19 mm (Nagashima et al., 2003). Due to the lack of data available for comparison, it is impossible to confirm whether these findings reflect an ethnically mediated physiological adaptation to ultra-endurance training, a unique training load, pathologic expression, or simply weak measurement methodology, although the latter is most likely as these findings have not been reproduced in similar studies.

Global systolic and diastolic measures of function can be expected to fall within normal ranges for African/Afro-Caribbean, Arabic/Middle-Eastern, South/East Asian and Caucasian athletes (Ma et al., 2007; Basavarajaiah et al., 2008; Riding et al., 2014). Currently, there are no data pertaining to ethnically mediated adaptation in LV mechanics. Further study is this area is warranted.

The Right Ventricle

To the best of the author's knowledge, there is only one study which has assessed RV structure in African/Afro-Caribbean athletes, highlighting similar RV structural adaptation to Caucasian athletes (Zaidi et al., 2013). Importantly, in the context of pre-participation screening, the combination of ECG and structural criteria for ARVC is more commonly met by African/Afro-Caribbean athletes compared to Caucasian athletes, creating a greater diagnostic challenge in this group. The lack of data for other ethnicities highlights the need for further work to establish the impact of ethnicity on RV structure and function.

The Atria

While there is no data pertaining to RA adaptation in African/Afro-Caribbean athletes, larger LA dimensions have been discerned in this group of athletes compared to Caucasian athletes (Papadakis et al., 2012). The clinical/physiological consequences of this finding remain unclear. There are no data pertaining to other ethnic groups and therefore until further work is undertaken to establish ethnic variance, existing normal ranges should be applied to all athletes.

2.5 The Impact of Body Size on Athletic Cardiac Development

Although athletic training is known to increase cardiac dimensions, inter-population comparison is challenging due to anthropometric differences. Indexing of cardiac dimensions aims to provide body-size and/or body-composition independent values, providing a better platform for comparison (George et al., 2001). The many methods of indexing come with their own merits and flaws which may impact on interpretation. Simple ratio-metric scaling is the most common approach to scaling, whereby a cardiac measurement is indexed to a body size parameter (i.e. y/x). This method has been criticised as relationships between cardiac dimensions and body-size are rarely linear (George et al., 2001). In contrast, allometric scaling methods produce a curvi-linear "line of best fit", and come close to generating body-size independent values (George et al., 2001). In order to scale allometrically, the size parameter should be indexed to the scaling factor raised to the power of the defined exponent ($y = ax^b$). Once determined, the resultant scaled index will not correlate with the original body size

factor. The value of indexing is also reliant upon the body size variable selected (body mass, body surface area, height or fat free mass (FFM)).

The Left Ventricle

Height, body mass, and body surface area (BSA) represent the most common indexing parameters due to their ease of access. A number of large scale studies have sought to generate an appropriate *b* exponent to facilitate between-study comparison of LV mass (George et al., 2001). Using height as an example, *b* exponent values generated by this work range from $^{1.97\text{-}3}$, reflecting differences in cohort age, sex, and physical fitness (George et al., 2001). A similar range of *b* exponents have been described for indexing to body mass, highlighting the challenge of producing a "one size fits all" value. More recently, the efficacy of FFM as an indexing variable has become clear (Whalley et al., 2004; Spence et al., 2011; D'Ascenzi et al., 2015). In order to determine FFM (fat mass subtracted from total body mass), firstly an individual's body composition must be measured. This measurement may be carried out using skinfold callipers, dual energy x-ray absorptiometry (DEXA), or magnetic resistance imaging (MRI). DEXA, which uses two different x-ray intensities to differentiate between lean and fat body mass, is commonly used in the literature because of its greater accuracy compared to skinfold calliper measurements, and relative inexpensiveness compared to MRI.

Whalley et al. (2004) found ratiometric scaling of LV mass and LVIDd to FFM provided a stronger correlation than BSA or height$^{2.7}$. This method appears to overcome many of the limitations of extreme body anthropometry observed in athletes, as LV mass and FFM develop concurrently (D'Ascenzi et al., 2015). It should be noted

however, that even in athletes displaying extreme anthropometry, physiological adaptation of the LV appears to be proportional to body size and remain within "normal" limits (Riding et al., 2012). Scaling of LV structures to FFM therefore appears to be optimal, although use of BSA with a population specific *b* exponent will also generate acceptable, body-size independent values (see table 4).

The Right Ventricle

Data pertaining to scaling of RV structural parameters is limited, likely representing the difficulty of imaging the RV, and its challenging geometry (George et al., 2001). Although a linear relationship between RVIDd and BSA has previously been described, this may have been fortuitous, as George et al. (2001) found no significant linear relationships between RVIDd measurements and body mass, height or BSA. Body size independent measurements of RVOT, RVI and RV length are feasible however, using allometric scaling with population specific *b* exponents (Oxborough et al., 2012). Use of these indexing methods may provide greater sensitivity in the identification of ARVC, when compared to conventional guidelines, which are commonly exceeded by athletes. Furthermore, Oxborough et al. (2012) observed body-size independence in functional assessment of the RV (using absolute ε values).

The Atria

Relatively little research has been carried out regarding scaling of the LA, and no data pertaining to indexing of the RA are available. Like the LV, the LA appears to confirm to conventional geometrical similarity (George et al., 2001). George et al. (2001) observed a significant linear relationship between height and LAD using ratiometric scaling, suggesting this simple approach may be appropriate for body-size independent

measurement. More recent work has questioned this, and found indexing of LAD to BSA with a population specific *b* exponent provided more valid, body-size independent values (Giraldeau et al., 2015). The efficacy of scaling LA volume to FFM has also been described, with a cohort specific *b* exponent of $^{0.7}$ being optimal for both male and female collegiate-level athletes (Giraldeau et al., 2015).

The Aorta

Although this literature review is focused on athletic development of cardiac chambers, it is also important to acknowledge the impact of body size on adaptation of the aorta in the athlete's heart. Particularly as a small number of SCD cases in athletes can be attributed to aortic dissection. Although correlations between aortic root dimension (at the sinus of Valsalva) and both BSA and height have previously been identified (Riding et al., 2012; Oxborough et al., 2014b; Boraita et al., 2016), investigation to exclude Marfan syndrome is warranted in males presenting dimensions >40 mm (or >34 mm in females), irrespective of extreme anthropometry (Riding et al., 2012; Engel, Schwartz and Homma, 2016).

2.6 The Impact of Sex on Athletic Cardiac Development

The relative scarcity of data defining "normal" athletic adaptation of all four cardiac chambers in female athletes presents a challenge to clinicians. Inter-sex differences in body-size, body composition and hormonal profile therefore present challenges in pre-participation screening (Rowland and Roti, 2010). While early research has consistently demonstrated smaller cardiac dimensions in female athletes (compared to males), recent

work has sought to eliminate inter-sex differences in body-size and composition to isolate the influence of athletic training on the female AH (Giraldeau et al., 2015).

The 12-Lead Electrocardiogram

In a comparison between elite female and male rowers, a similar prevalence of abnormal ECG findings were observed between sexes (3% and 4% respectively), but with profound differences in the prevalence of specific training-related changes (Wasfy et al., 2015a). A higher prevalence of early repolarisation pattern (ERP) was reported for male athletes compared to their female counterparts (76% vs 23%) (Wasfy et al., 2015a). Interestingly the increased overall prevalence of ERP in male athletes appears to be driven by a higher prevalence of anterior lead ERP, as the prevalence of lateral and/or inferior lead ERP was similar between males and females. Females are also far less likely to display isolated QRS voltage criteria for LV hypertrophy compared to their male counterparts (8% vs 51%) (Wasfy et al., 2015a).

The Left Ventricle

Male athletes consistently display larger LV cavity dimensions and wall thicknesses compared to their female counterparts (Giraldeau et al., 2015). LV hypertrophy in female athletes (wall thickness >11mm) is extremely rare, compared to male athletes where a minority of athletes (2.5-5% prevalence in males) can be expected to present thicknesses >12mm (Whyte et al., 2004). Whether these differences can be accounted for by body-size, or whether a sex-specific difference in physiological remodelling exists is a contentious issue (Giraldeau et al., 2015). Rowland and Roti (2010) reported

larger LV dimensions in male athletes compared to their female counterparts despite indexing for BSA, $BSA^{0.5}$, and FFM^{-1} (derived from skin-fold measurements). These findings may represent weak scaling methodology as opposed to physiological differences however, as scaling of LV structural parameters to FFM (derived using dual energy x-ray absorptiometry) eliminates any meaningful inter-sex differences (see table 5) (Giraldeau et al., 2015). Remodelling of the LV in females appears to follow a similar pattern, time-scale and relative magnitude to that observed in male athletes (Giraldeau et al., 2015).

Global systolic function is similar between male and female athletes, with differences in absolute LV SV being eliminated by scaling to FFM (Giraldeau et al., 2015). Although Giraldeau et al. (2015) reported a slightly higher LV EF and global longitudinal ε in females compared to males (66% vs 63% and -22% vs -20.6% respectively), this did not translate into meaningful differences in LV SV index, or systolic longitudinal SR. Furthermore, Giraldeau et al. (2015) observed a higher early diastolic longitudinal SR in female athletes compared to their male counterparts (1.81 %/s vs 1.56 %/s). Further examination in a large heterogeneous cohort is required to confirm these findings.

One potential confounding factor in the pre-participation screening of female athletes is the menstrual cycle, and possible influence of contraceptive methods. Fluctuations in oestrogen throughout the menstrual cycle are known to impact both central and peripheral cardiovascular factors, including blood volume and total peripheral resistance, yet disagreement exists as to whether these factors impact LV function (Green et al., 2016). (Fuenmayor, Ramírez and Fuenmayor, 2000) observed a

significant difference in mitral valve E/A ratio between follicular and luteal phases, whereas George et al. (2000) reported no meaningful differences in functional parameters between these time-points. Further examination into the impact of the menstrual cycle on LV function, including STE indices, will provide important insight for pre-participation screening.

The Right Ventricle

Much like the LV, larger RV structural dimensions are observed in male athletes compared to their female counterparts (Giraldeau et al., 2015). Giraldeau et al. (2015) observed that inter-sex differences in chamber dimensions can be eliminated by indexing to FFM, suggesting differences in structural parameters can be accounted for by body-size (Giraldeau et al., 2015).

Giraldeau et al. (2015) observed a higher early diastolic longitudinal SR in the RV free wall in females compared to males, suggestive of slightly enhanced diastolic function at rest in female athletes. No other inter-sex differences were observed in STE derived functional indices. To the best of the author's knowledge, no research has been carried out to examine the impact of the menstrual cycle on RV function. This may represent an excellent research opportunity, as any effect of the menstrual cycle on LV function is likely to be magnified in the RV, due to disproportionate haemodynamic loading.

The Atria

Training-induced bi-atrial dilatation is observed in male and female athletes, with smaller absolute dimensions being displayed by female athletes (Baggish et al., 2008; Giraldeau et al., 2015). The relative magnitude of physiological adaptation in LA dimensions appears to be similar between male and female athletes (Giraldeau et al., 2015). There are insufficient data to comment on the inter-sex difference in RA adaptation. Like ventricular structures, inter-sex differences in LA and RA volume are eliminated when indexed to FFM, indicating a close relationship to body size (Giraldeau et al., 2015).

2.7 The Impact of Age on Athletic Cardiac Development

In a large study (*n*= 1210) of SCD in the US general athletic population, Harmon et al. (2014) found the mean age of cases to be only 17 years. This finding is supported by Italian, Israeli, Danish and Swedish groups, who described similar prevalence of SCD in young athletes (Harmon et al., 2014). The efficacy of cardiovascular screening in school-age athletes has previously been demonstrated by a reduction in sudden death rates from 1:28,000 to 1:250,000 following implementation of a screening programme (Harmon et al., 2014), yet there is no consensus on how age should impact classification of normal/abnormal findings in athletic individuals. The growth of participation in competitive sport at masters and veteran levels has also increased the need for understanding of the impact of ageing upon cardiac adaptation.

The 12-Lead Electrocardiogram

Youth athletes (aged 10-15 years) can be expected to present fewer abnormal (10% vs 40%) and mildly abnormal (3-8% vs 19-36%) ECG traces compared to their senior counterparts (Koch et al., 2014). This is likely a result of fewer cumulative training hours and may also be influenced by the higher levels of body fat and lower levels of sex hormones observed in this group (Koch et al., 2014). Within the abnormal patterns presented by youths, there is a strikingly high prevalence of anterior T-wave inversion, raising concerns over ARVC (Attisani et al., 2011). However, T-wave inversion appears to be a feature of immaturity rather than pathology in this group, and a progressive decline in precordial T-wave prevalence is observed during adolescence (32.2% in 6-8 year olds compared to 3.3% in 16-18 year olds) (Attisani et al., 2011) (see table 6). Development of refined criteria, which factor in the chronological age and anthropomorphic characteristics of young athletes may be appropriate to minimise the rate of false positive ARVC diagnoses (Attisani et al., 2011). The prevalence of LV hypertrophy (using isolated Sokolow criteria) is considerably lower in junior male athletes (15%) compared to senior male athletes (51%) (Bessem, De Bruijn and Nieuwland, 2015). The lack of data pertaining to training and non-training related ECG changes in veteran athletes, warrants further exploration.

The Left Ventricle

Development of an increased LV cavity size is generally observed in non-athletic males and females between birth and 30 years of age, followed by a progressive decline as age increases (Kaku et al., 2014). LV cavity enlargement and wall thickness are

increased in junior athletes compared to age matched non-athletic controls, but are less pronounced compared to their senior counterparts due to a lack of physical maturity and fewer cumulative training hours (Sharma et al., 2002; Makan et al., 2005; Sheikh et al., 2013). Despite this, an LV cavity dimension >60 mm in the presence of diminished systolic or diastolic function represents an appropriate indicator for the investigation of dilated cardiomyopathy (DCM) in both adolescent and senior athletes (Makan et al., 2005). Similarly, conventional guidelines which warrant investigation to exclude HCM (LV wall thickness >12 mm in males or >11 mm in females) are also applicable to the athletic adolescent population (Sharma et al., 2002).

It is clear that a relationship between lifelong exercise "dose" and LV cavity size exists. Carrick-Ranson et al. (2014) observed significantly higher LV EDV index values in master athletes, compared with those of age-matched sedentary individuals and casual exercisers. Furthermore, some structural adaptation initiated by athletic training may remain present more than ten years after cessation of participation (Carrick-Ranson et al., 2014). It should be noted however, that sporting discipline appears to be a factor in the longevity of structural adaptation, as preservation of the AH phenotype has been discerned in retired wrestlers, but not in retired marathon runners (in an age-matched cohort).

Ageing does not appear to be associated with changes in global measures of systolic function in healthy non-athletic individuals (Carrick-Ranson et al., 2014). Furthermore, no differences in EF are observed between sedentary young individuals, junior athletes, sedentary older individuals, and master athletes (Sharma et al., 2002; Makan et al., 2005). A progressive decrease in peak longitudinal ε is observed throughout the lifespan

of healthy, non-athletic individuals (Kaku et al., 2014). Whilst some evidence suggests exercise may have a protective effect on this decrease in LV longitudinal deformation (Kaku et al., 2014), further clarification is needed. Although there does not appear to be an age-related change in peak rotational, circumferential or radial ε, a shift in the base-apex deformation gradient has been identified within these planes (Kaku et al., 2014). It therefore appears that systolic function is maintained with progressing age through increased action of the apical region of the LV, which compensates for decreased deformation at basal level (Kaku et al., 2014).

Global diastolic function is comparable between junior athletes and age matched non-athletic individuals (Sharma et al., 2002; Makan et al., 2005). Following maturation, a gradual decrease in the trans-mitral E/A ratio is observed, as a greater reliance is placed upon the atrial component of LV filling (Kaku et al., 2014). Although master athletes and sedentary age-matched individuals display similar contractile function, master endurance athletes display significantly greater ventricular compliance and decreased wall stress, resulting in LV pressure-volume relationships similar to those of young healthy individuals (Arbab-Zadeh et al., 2004). Future research should seek to clarify the impact of age on temporal diastolic deformation characteristics, in rotational, circumferential, and radial planes.

The Right Ventricle

As in the LV, development of RV cavity size is observed throughout adolescence in young athletes (George et al., 2001), likely reflecting an accumulation of training hours and physical maturation. At the other end of the spectrum, decreasing RV chamber area

is observed in non-athletic, ageing individuals (Henein et al., 2014). Whether life-long training has a protective effect on these decreases is unknown, and represents an opportunity for future research.

To the authors' knowledge, there are no data available pertaining to global function of the adolescent athlete's heart. Systolic function in ageing individuals is characterised by decreases in RV S', and RV peak ε (Chia et al., 2015). In addition, RV systolic reserve appears to decrease with advancing age, resulting in a reduced ability to augment RV deformation (Chia et al., 2015). Diastolic function of the RV diminishes with advancing age in sedentary individuals. A decrease in trans-tricuspid E/A ratio is observed, along with decreased ability to augment diastolic function during exercise stress (Chia et al., 2015).

The Atria

Data regarding the impact of ageing on the athlete's atrial structure and function are limited. It is clear however, that LA cavity size increases throughout adolescence in junior athletes, most likely as a function of cumulative training hours and physical maturation (George et al., 2001). Life-long endurance athletes can also be expected to present significantly larger atria compared to sedentary age matched controls (Wilhelm et al., 2012). To the best of the author's knowledge, there are no data available regarding the impact of age on the RA. Future research which develops understanding of structural and functional adaptation of the atria with advancing age will therefore be highly valuable to practitioners.

2.8 Technical Development of Pre-Participation Screening Methods

12-Lead Electrocardiogram

The first recorded application of the "ECG" was carried out in the mid-19th century to detect electrical activity in frogs (Matteuci, 1842), and subsequently, in humans (Waller, 1887). This technology developed into the 3-lead ECG (Einthoven, 1901), and with the addition of precordial (Wolferth and Wood, 1932) and unipolar leads (Goldberger, 1942), into the 12-Lead Electrocardiogram device now commonly used.

Although a standardised protocol and interpretation of the 12-lead ECG was established in 1954 (Wilson et al., 1954), soon after, it was recognised that electrophysiological adaptations associated with athletic training could lead to false-positive identification of pathology in athletes (Gott, Roselle and Crampton, 1968). Much later, the first criteria for interpretation of the 12-lead electrocardiogram in athletes were defined (Corrado et al., 2005). Large-scale studies of athletes have facilitated refinement of these criteria, resulting in greater specificity of ECG detectable pathology while maintaining sensitivity (Corrado et al., 2010; Drezner et al., 2013; Sheikh et al., 2014). As a result, the false-positive rate has fallen from 21.5 % using the 2010 ESC Guidelines, to 6.6 % using the 2014 "refined criteria" (Sheikh et al., 2014).

Conventional 2D Echocardiography

Study of the AH phenotype has developed in tandem with echocardiographic technology over the last 60 years. M-mode echocardiography, in which the reflection

of a single-beam of ultrasound from the cardiac wall is recorded, was carried out as early as 1953 (Fraser, 2001). Real-time 2D echocardiographic imaging, whereby depth and brightness were established for multiple ultrasound beams, represented the next major development (Bom et al., 1973; Griffith and Henry, 1974). This method would become the backbone of echocardiographic examination, and was used as early as 1979 to visualise LV mass in athletes (Morganroth et al., 1975). A systematic approach for assessment of the LV using M-mode and 2D echocardiography would be proposed shortly afterwards (Henry, Gardin and Ware, 1980).

Pulsed-wave Doppler emerged in tandem with 2D Echocardiography imaging (Gowda et al., 2004). Utilising the observations of (Doppler, 1842) and ultrasonic developments, Baker, Rubenstein and Lorch (1977) established principles for the use of Pulsed-wave Doppler to determine the direction and velocity of blood flow (via positive/negative Doppler shift). The utility of Pulsed-wave Doppler to assess valvular defects was quickly realised (Hatle, Angelsen and Tromsdal, 1980), and a framework for the assessment of LV diastolic filling patters was subsequently proposed (Kitabatake et al., 1982). Additionally, colour-flow Doppler became commercially available in the late 1980's (Omoto and Kasai, 1987). The added ability to visualise blood flow, providing particular value in the study of atrial and ventricular defects, as well as mitral and tricuspid valvular regurgitation.

Tissue Doppler Imaging

Isaaz et al. (1989) used pulsed-wave Doppler to target the posterior wall of the LV, as opposed to red blood cells, and in doing so introduced Tissue Doppler Imaging.

Quantification of myocardial velocities in systole and diastole provided insight into mechanical aspects of structure-function relationships within the heart for the first time. Furthermore, this technique facilitated regional assessment of the LV, allowing wall motion abnormalities common to hypertrophic cardiomyopathy (HCM) (Nagueh et al., 2001; Cardim et al., 2002; Ho et al., 2002). This is of particular relevance to the AH phenotype, where profound structural adaptation and reduced global function at rest commonly present a challenging differentiation between physiology and pathology.

Speckle Tracking Echocardiography

Speckle tracking echocardiography (STE) was introduced in 1994 (O'Donnell et al., 1994). Using images acquired from conventional 2D-echocardiography, this offline method tracks the motion of characteristic speckle patterns generated by ultrasound reflection from the myocardial wall (Mor-Avi et al., 2011). In doing so, it is possible to quantify deformation of the myocardium in relation to its original size (strain), and the rate at which this takes place (strain rate) (Mor-Avi et al., 2011). In contrast to TDI assessment, which is a highly angle-dependant method of measuring linear deformation (via Doppler shift), STE is partly angle-independent, and can be used to assess displacement in longitudinal, circumferential, radial, and rotational planes (Mor-Avi et al., 2011). Like TDI, STE facilitates regional assessment of myocardial deformation. However, unlike TDI, it is possible to segment myocardial regions and carry out simultaneous strain and strain rate measurements from a single image (Forsythe, George and Oxborough, 2018). High prognostic value in the diagnosis of HCM and DCM has been demonstrated for longitudinal strain in particular (Forsythe, George and Oxborough, 2018). As a result, application of longitudinal strain is recommended in

the most recent European guidelines, with a value of less than -15 % suggested to be indicative of pathology (Pelliccia et al., 2018).

Exercise-stress Echocardiography

Profound structural ventricular remodelling presented by athletes is often accompanied by reduced resting measures of function and mechanics, making differentiation diagnosis of the AH phenotype and cardiomyopathy challenging (George et al., 2012). Technological development of 2D echocardiography have increased temporal resolution and increased the quality of images (Forsythe, George and Oxborough, 2018). As a result, more accurate assessment of structure, function and mechanics are feasible during exercise (Forsythe, George and Oxborough, 2018). Furthermore, development of ergometers whereby exercise is completed in the left-lateral decubis position has overcome logistical challenges to in-exercise assessment.

Exercise circumferential strain, radial strain and twist have been found to provide distinction between physiological adaptation and HCM, in the case of ambiguous resting function/mechanics (Soullier et al., 2012). Soullier et al. (2012) observed significantly lower rest-exercise augmentation in circumferential (5 %), radial (4 %) and twist (1 %) for HCM patients compared to healthy controls (35 %, 18 %, and 63 % respectively). Additionally, temporal mechanical analysis revealed altered systolic-diastolic coupling in HCM patients, which was not present in control subjects (Soullier et al., 2012). Similarly, exercise augmentation of RV free wall strain rates have been presented as an effective method to differentiate endurance AH and ARVC phenotypes, which both present with reduced function at rest (La Gerche et al., 2012a).

Chapter 3 General Methodology

3.1 Ethical Approval

Ethical approval was granted for each study from the local ethics committee at Liverpool John Moores University. All participants were provided with a participant information sheet, and written informed consent was gained prior to enrolment.

3.2 Preliminary Procedures

All participants were free of known cardiovascular disease and abstained from alcohol and caffeine consumption for at least 24 hours prior to each data collection. Participants also refrained from training activities for at least 6 hours prior to each data collection. Subjects completed a health questionnaire to exclude cardiovascular symptoms, family history of sudden cardiac death (SCD) and other cardiovascular history and/or abnormalities.

3.3 Anthropometric Assessment

Body mass (Seca 217, Germany) and height (Seca Supra 719, Germany) were recorded. Body surface area (BSA) was calculated as previously described (Mosteller, 1987). Systolic and diastolic blood pressures were recorded using an automated sphygmomanometer (Dinamap 300, GE, USA) after at 5 minutes of seated rest.

3.4 12-Lead Electrocardiogram

A standard, resting 12-lead electrocardiogram (CardioExpress SL6, Spacelabs, USA) was undertaken in accordance with AHA guidelines (Mason, Hancock and Gettes, 2007). Results were reviewed against current international criteria (Drezner et al., 2017) by a sports cardiologist to exclude pathology.

3.5 Conventional 2D Echocardiography

A standard resting echocardiogram was undertaken by a single experienced sonographer, using a commercially available ultrasound system (Vivid Q, GE, Norway and Vivid E95, GE, Norway) and a 1.5-4 MHz phased array transducer. All images were acquired in accordance with the American Society of Echocardiography (ASE) guidelines (Lang et al., 2015). Images were analysed offline (Echopac v202, GE, Norway) by a single experienced researcher. A minimum of three cardiac cycles were averaged for all acquisitions.

LV linear dimensions (LVIDd and LVIDs) were measured at the level of or immediately below the mitral valve tips in a parasternal long axis orientation. LVIDd was measured at the point when the cavity was its largest (see figure 3), and LVIDs at the point when the cavity was smallest (see figure 4).

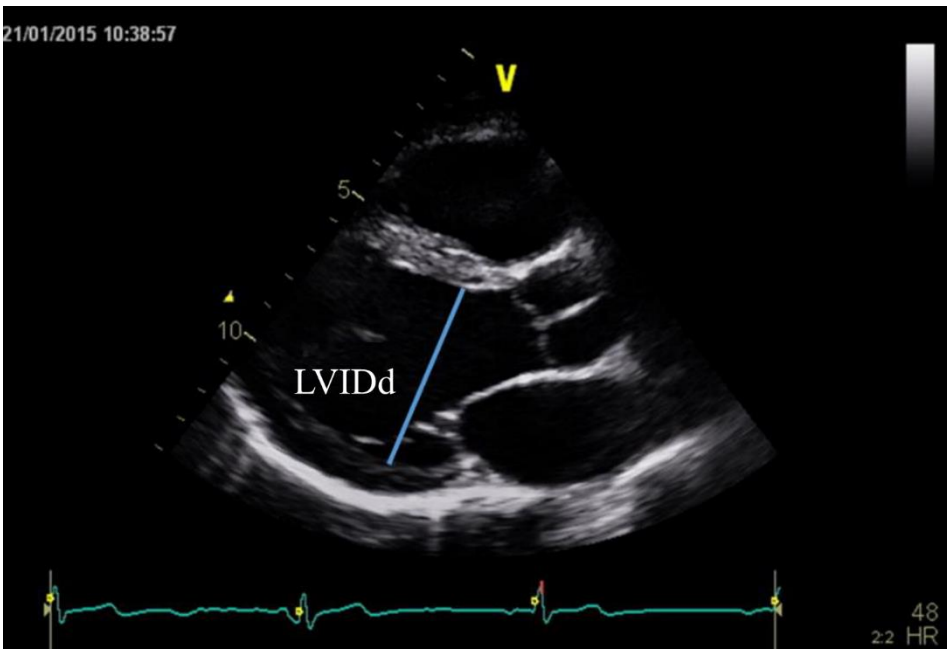

Figure 3. Parasternal long axis orientation, demonstrating LVIDd

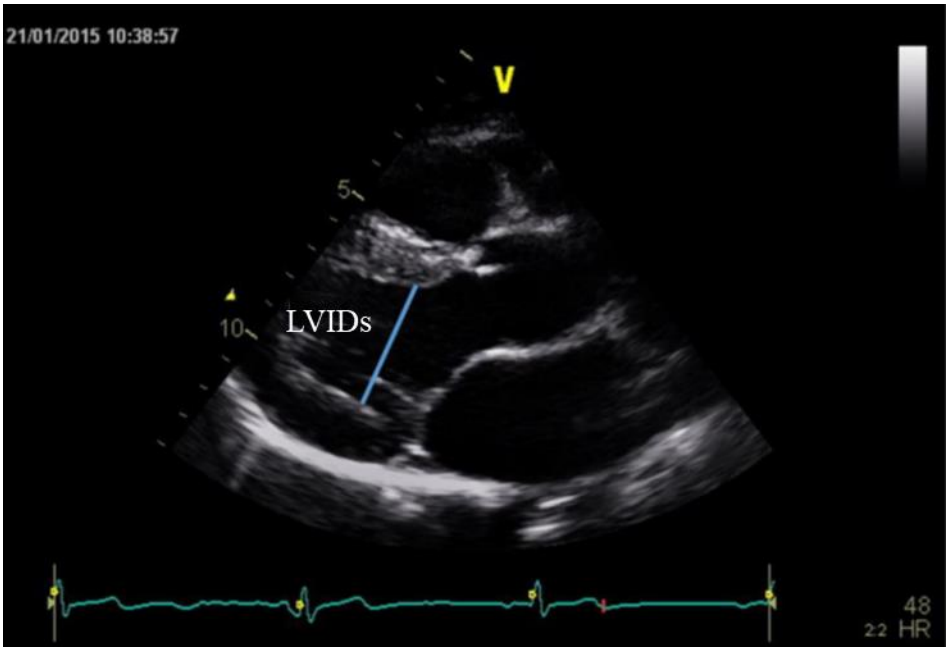

Figure 4. Parasternal long axis orientation, demonstrating LVIDd

To provide a comprehensive assessment of LV wall thickness, eight measurements were made from a parasternal short axis orientation at basal (see figure 5) and mid-levels (see figure 6) from the antero-septum, infero-septum, posterior wall and lateral wall (Wigle et al., 1985).

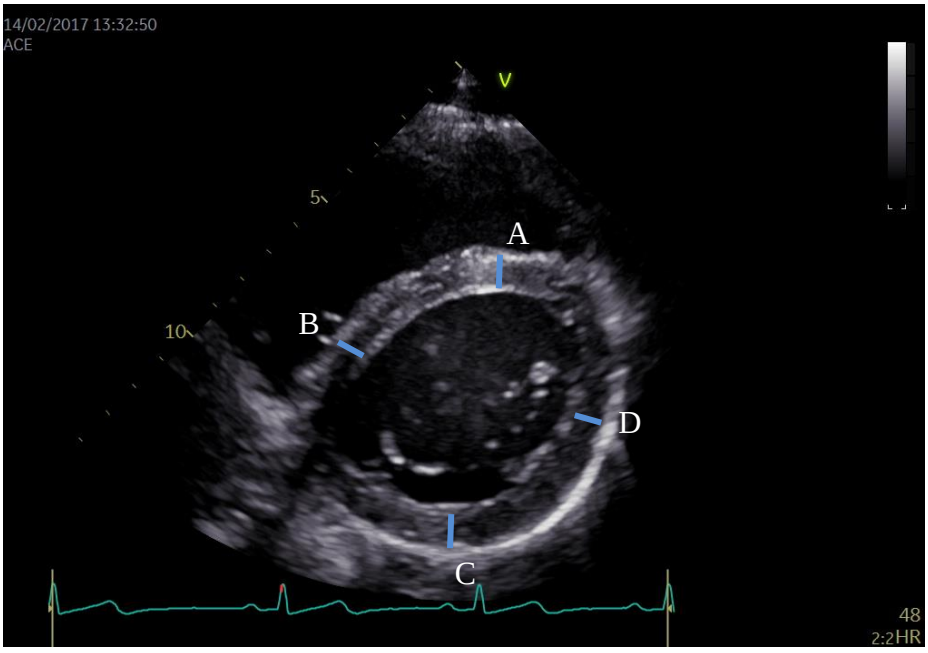

Figure 5. Short axis orientation demonstrating basal level wall thickness. (A) antero-septum, (B) infero-septum, (C) posterior wall, (D) lateral wall.

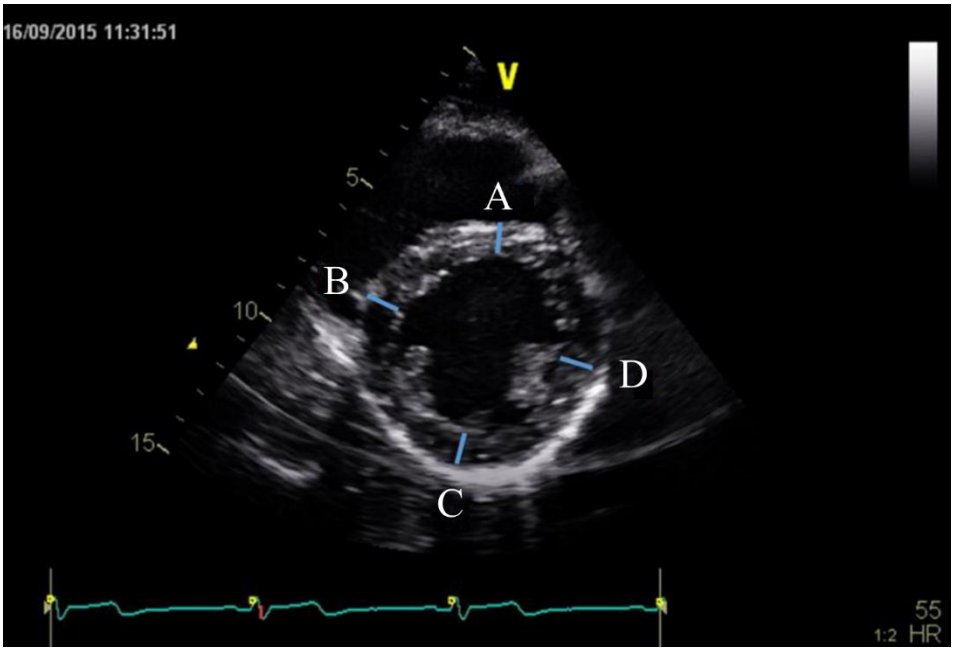

Figure 6. Short axis orientation demonstrating mid-level LV wall thickness. (A) antero-septum, (B) infero-septum, (C) posterior wall, (D) lateral wall.

Mean wall thickness (MWT) was calculated as an average of all eight segments. Conventional relative wall thickness (RWT) was calculated using the formula [(IVSWTd + PWTd)/LVd] (where IVSWTd denotes mean diastolic basal infero-septal and anterior-septal wall thicknesses and PWTd denotes diastolic basal posterior wall thickness). LV linear dimensions and wall thicknesses facilitated calculation of LV mass using the ASE corrected equation (LV Mass (g) = 0.8 + 0.6). LV end-diastolic volume (LV EDV) and LV end-systolic volume (LV ESV) were calculated using the Simpsons biplane method from measurements made in a modified apical 4-chamber orientation and apical 2-chamber orientation (see figure 7).

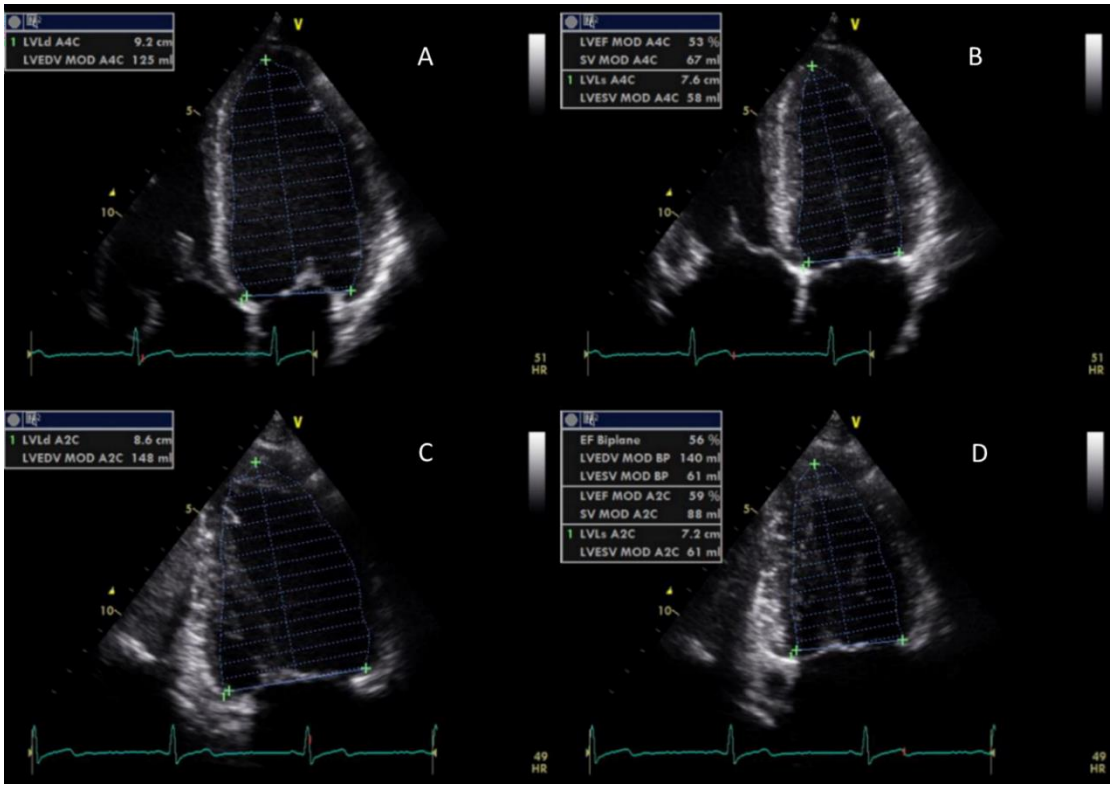

Figure 7. Biplane Calculation of LV EDV and LV ESV using (A) apical 4-chamber orientation at end-diastole, (B) 4-chamber orientation at end-systole, (C) apical 2-chamber orientation at end-diastole, and (D) apical 2-chamber at end-systole.

LV concentricity was calculated as [LV mass/LV $EDV^{2/3}$] (Trachsel et al., 2018). LV geometry was assessed using a four-tier method, whereby geometry was defined as 1) normal (LV mass <116 g/m^2, concentricity <9.1 $g/ml^{(2/3))}$), 2) concentric remodelling (LV mass <116 g/m^2, concentricity ≥9.1 $ml^{(2/3))}$), 3) concentric non-dilated LVH (LV mass ≥116 g/m^2, concentricity ≥9.1 $g/ml^{(2/3)}$ and LV EDV/BSA <76 ml/m^2), 4) concentric dilated LVH (LV mass ≥116 g/m^2, concentricity ≥9.1 $g/ml^{(2/3)}$ and LV EDV/BSA ≥76 ml/m^2), 5) eccentric non-dilated LVH (LV mass ≥116 g/m^2, concentricity <9.1 $g/ml^{(2/3)}$ and LV EDV/BSA <76 ml/m^2) and 6) eccentric dilated LVH (LV mass ≥116 g/m^2, concentricity <9.1 $g/ml^{(2/3)}$ and LV EDV/BSA ≥76 ml/m^2) as previously described by Trachsel et al. (2018) (see Figure 8).

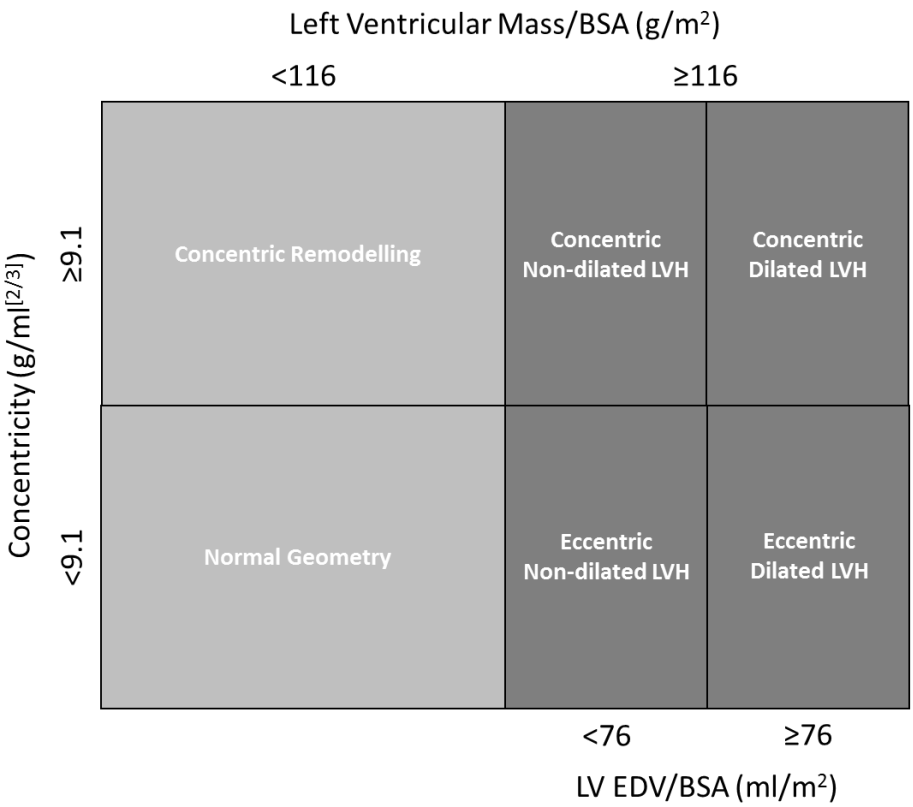

Figure 8. Four-tier classification of LV geometry

Stroke volume (SV), and EF were calculated from LVEDV and LVESV respectively. Doppler imaging was used to assess blood flow velocity across the mitral valve during early diastolic (E) and late diastolic (A) phases (see figure 9).

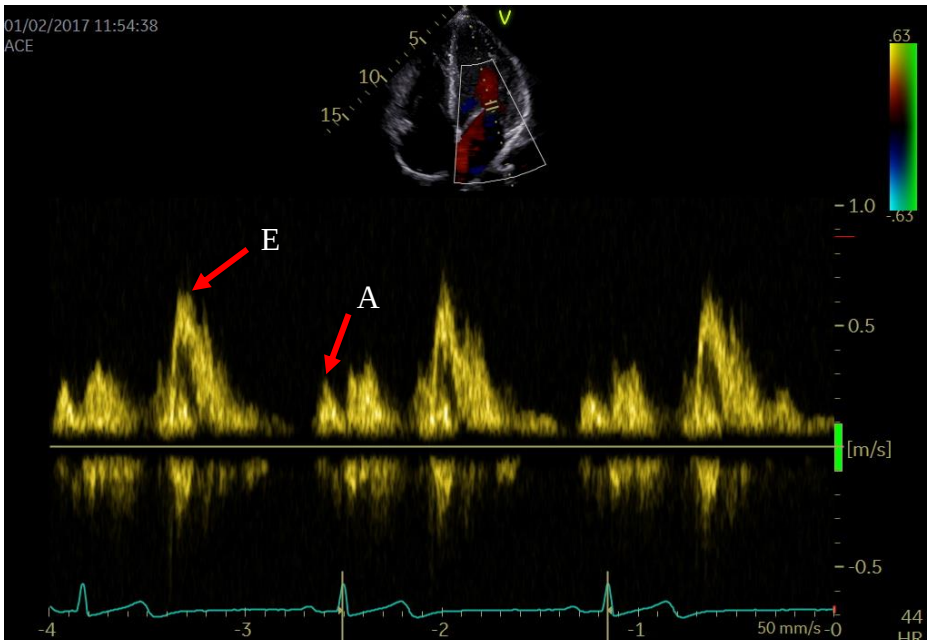

Figure 9. Doppler assessment of mitral valve blood flow, demonstrating E and A

Pulsed-wave Tissue Doppler Imaging (TDI) was used to assess peak longitudinal motion velocity of the infero-septum and lateral wall in systolic (S'), early diastolic (E') and late diastolic (A') phases (see figure 10). The pulsed-wave sample volume was placed in the basal tissue just above the annulus of the mitral valve on both the infero-septum and lateral walls.

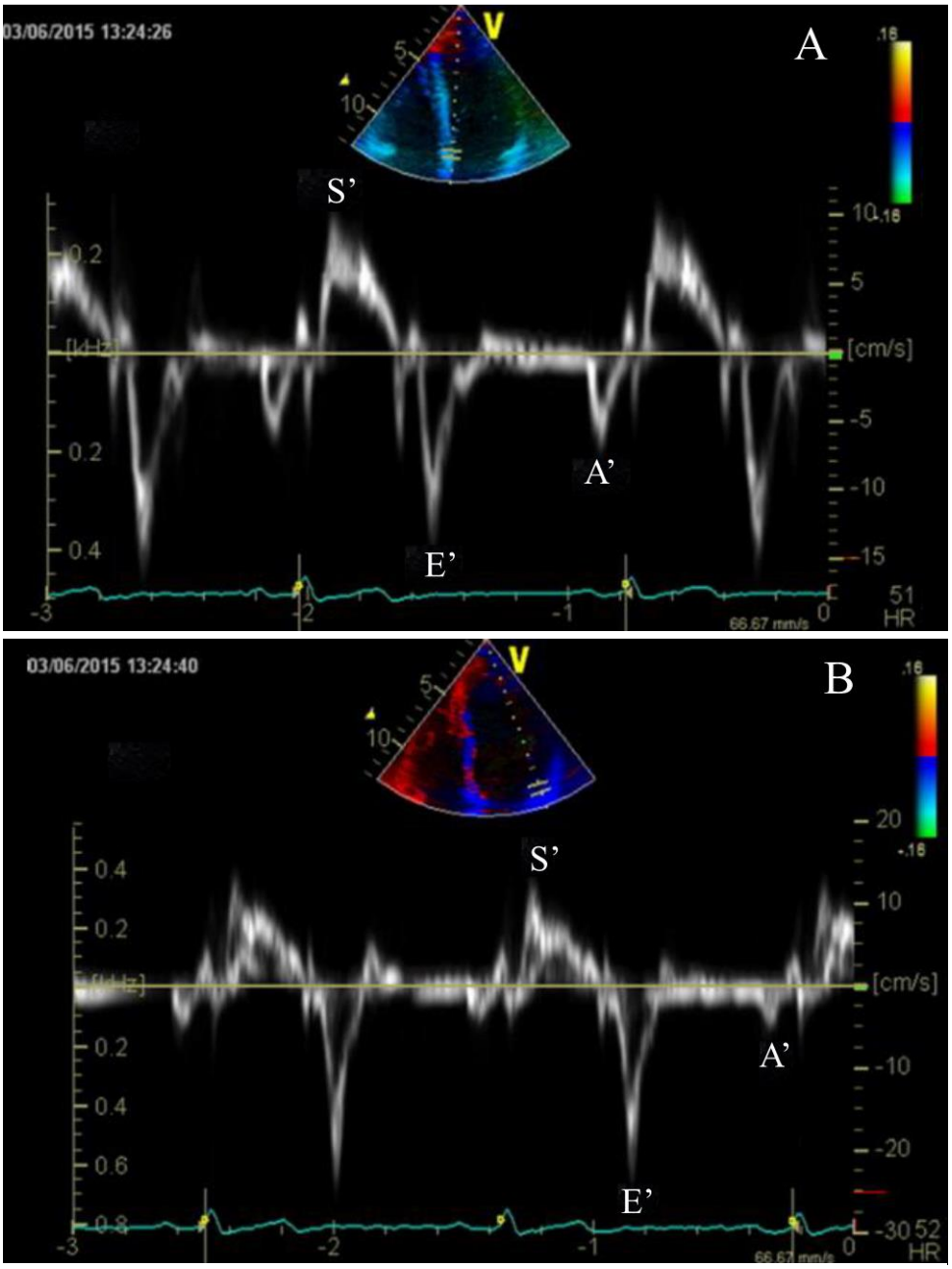

Figure 10. Pulsed-wave TDI demonstrating S', E', and A' of the (A) infero-septum and (B) lateral wall

All structural indices are presented as absolute values as well as being scaled allometrically to BSA based on the principle of geometric similarity (Batterham and George, 1998; Dewey et al., 2008). Linear dimensions were scaled to $BSA^{0.5}$, areas directly to BSA, and volumes to $BSA^{1.5}$.

3.6 Myocardial Speckle Tracking

All images were acquired at frame rates between 40 and 90 frames per second, and settings were adjusted to provide optimal endocardial delineation. During offline analysis (Echopac v202, GE, Norway), the endocardial border was manually traced, and the region of interest was adjusted to encompass the full myocardium.

Longitudinal Mechanics

GL ε and longitudinal SR were assessed using apical four-chamber, two-chamber and three-chamber orientations (see figure 11). The focal point was position at the level of the mitral valve, and aortic valve closure (AVC) time was set using the aortic continuous wave Doppler trace. In the apical four-chamber orientation, the range of interest (ROI) was traced around the myocardium from infero-septum to basal lateral wall. In the three-chamber orientation, the ROI was traced from basal posterior wall to basal antero-septum. In the two-chamber orientation, the ROI was traced from the basal inferior wall to the basal anterior wall.

Each apical orientation provided 6 segments, which facilitated global ε and SR to be calculated as an average of 18 segments. GL ε was determined as the greatest value (see figure 12), and systolic strain rate (SRS), early diastolic strain rate (SRE) and late diastolic strain rate (SRA) were identified (see figure 13).

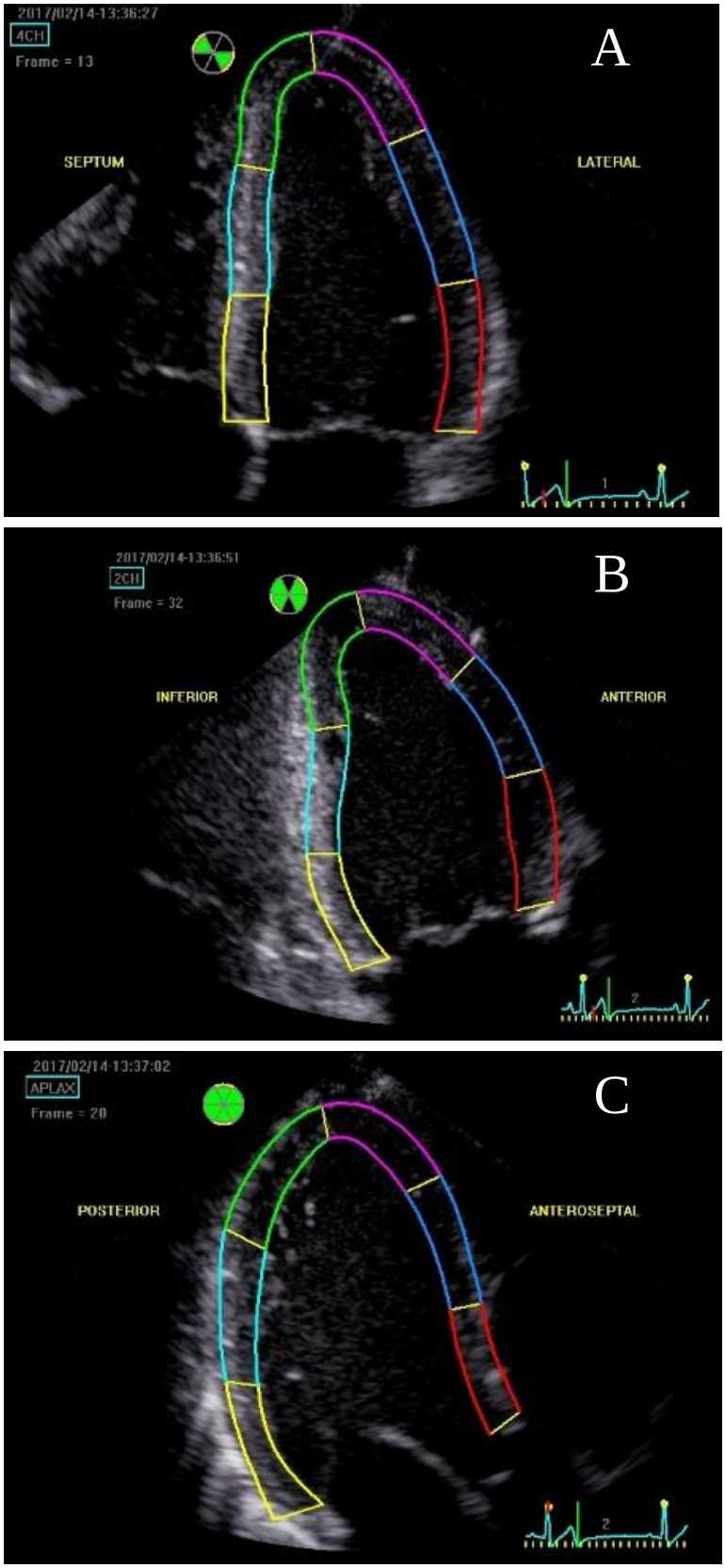

Figure 11. (A) Apical 4-chamber orientation segments, (B) Apical 2-chamber orientation segments and (C) Apical 3-chamber orientation segments

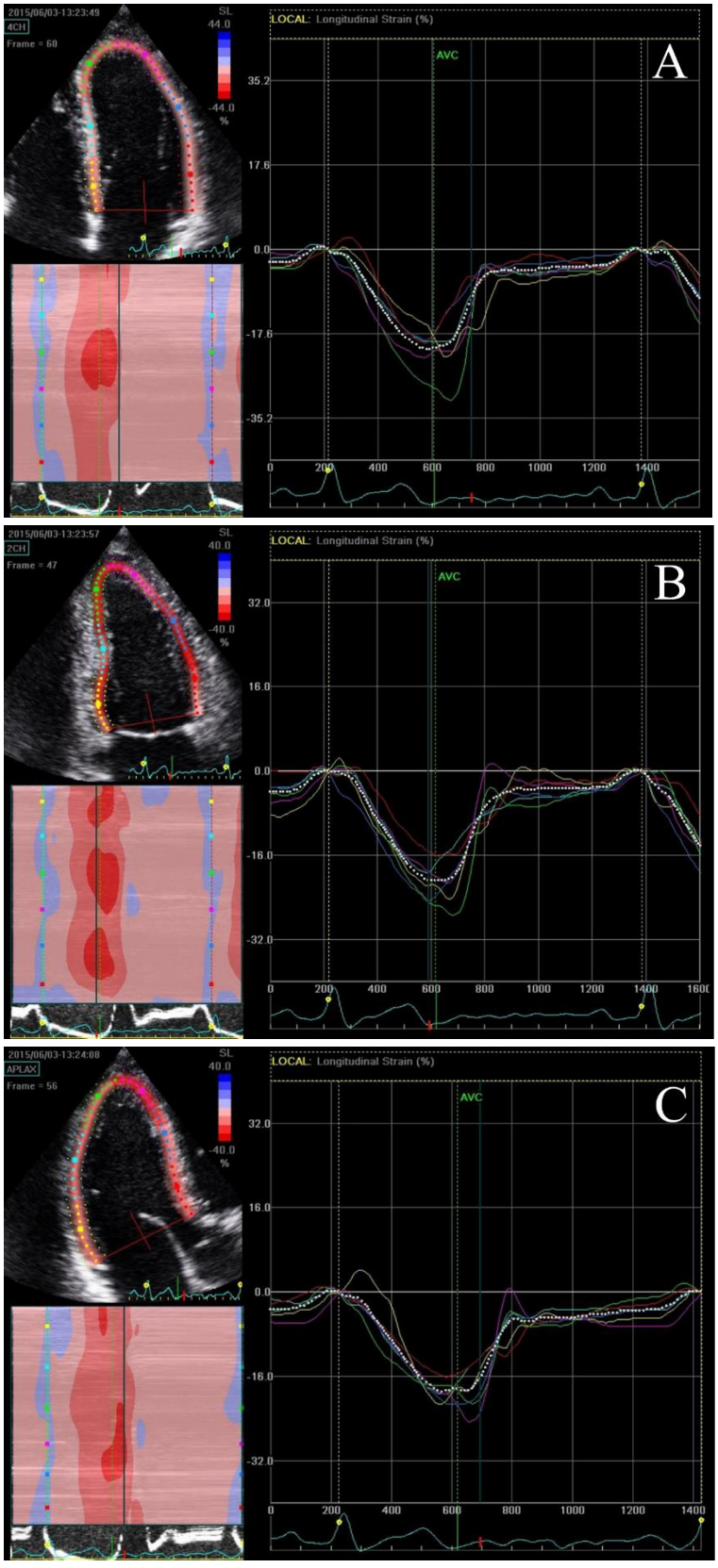

Figure 12. GL ε trace in apical (A) 4-chamber orientation, (B) 2-chamber orientation, and (C) 3-chamber orientation

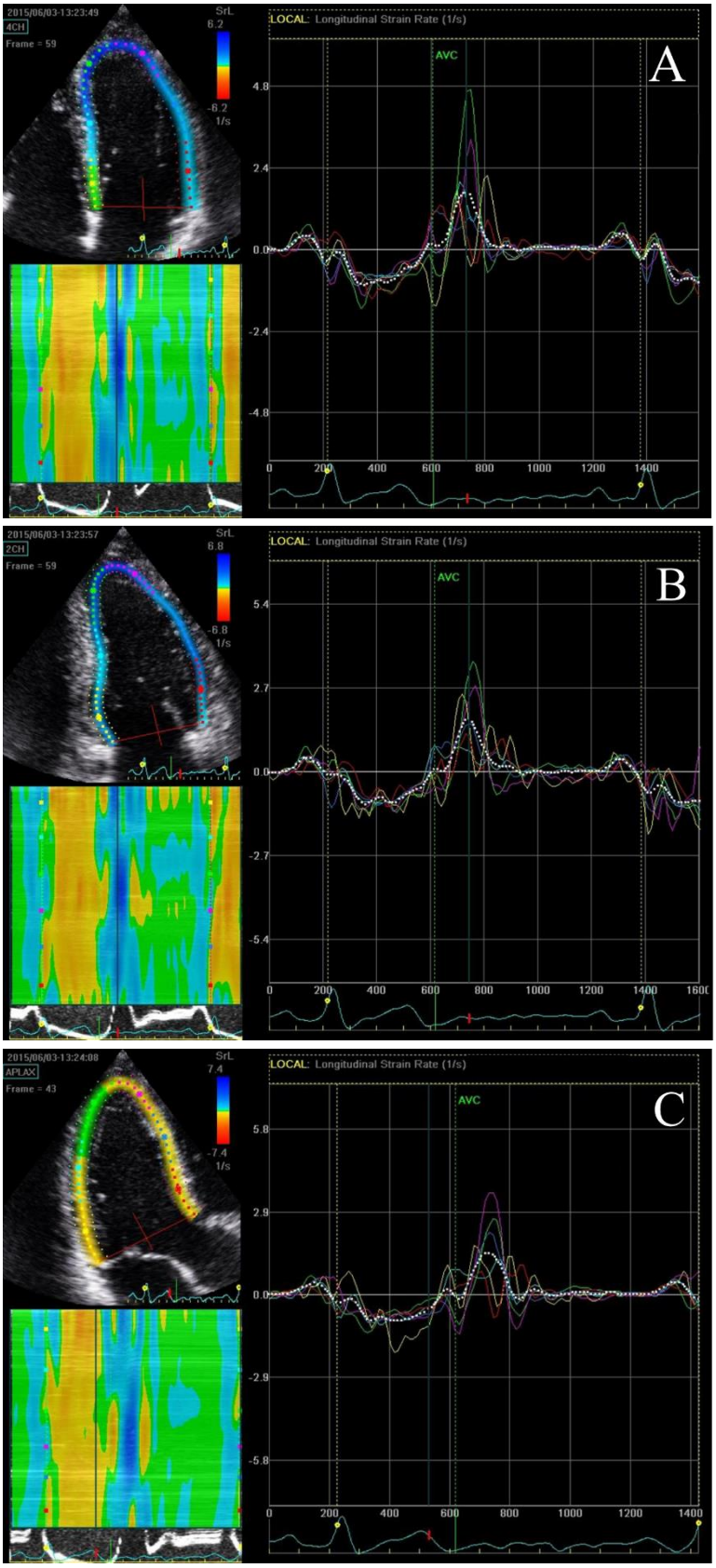

Figure 13. Longitudinal SR trace in (A) 4-chamber orientation, (B) 2-chamber orientation, and (C) 3-chamber orientation

Circumferential and Rotational Mechanics

The parasternal short-axis orientation facilitated assessment of circumferential ε at basal (mitral-valve) and mid- (papillary muscle) levels, and rotation at basal and apical (the point immediately above the point of systolic cavity obliteration) levels. In all cases, the focal point was position close to the centre of the cavity, and AVC was set using the aortic continuous wave Doppler trace. GC ε and SR values were calculated as an average of antero-septum, infero-septum, inferior, posterior, lateral, and anterior regional segments at basal and mid- cavity levels (see figures 14-16). LV twist was calculated as the net difference between apical and basal rotation (see figure 17).

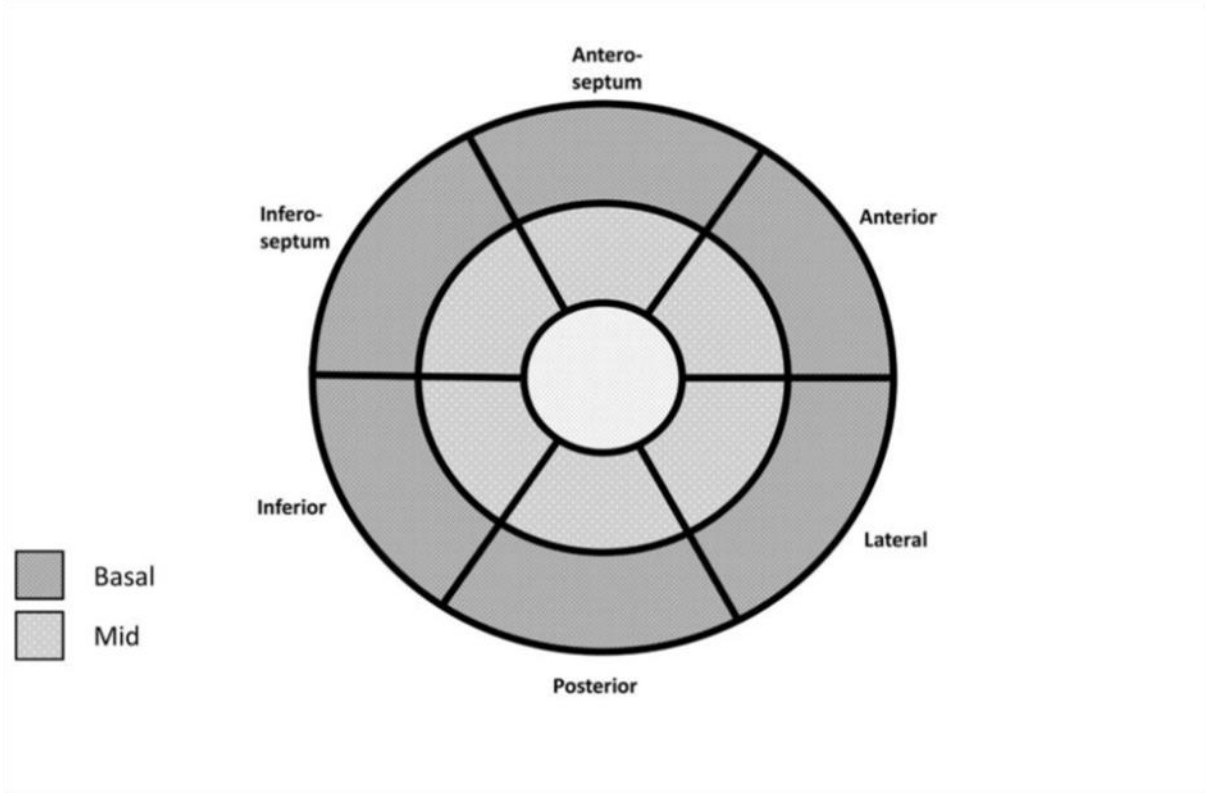

Figure 14. Basal- and mid-cavity level segments from a short axis orientation

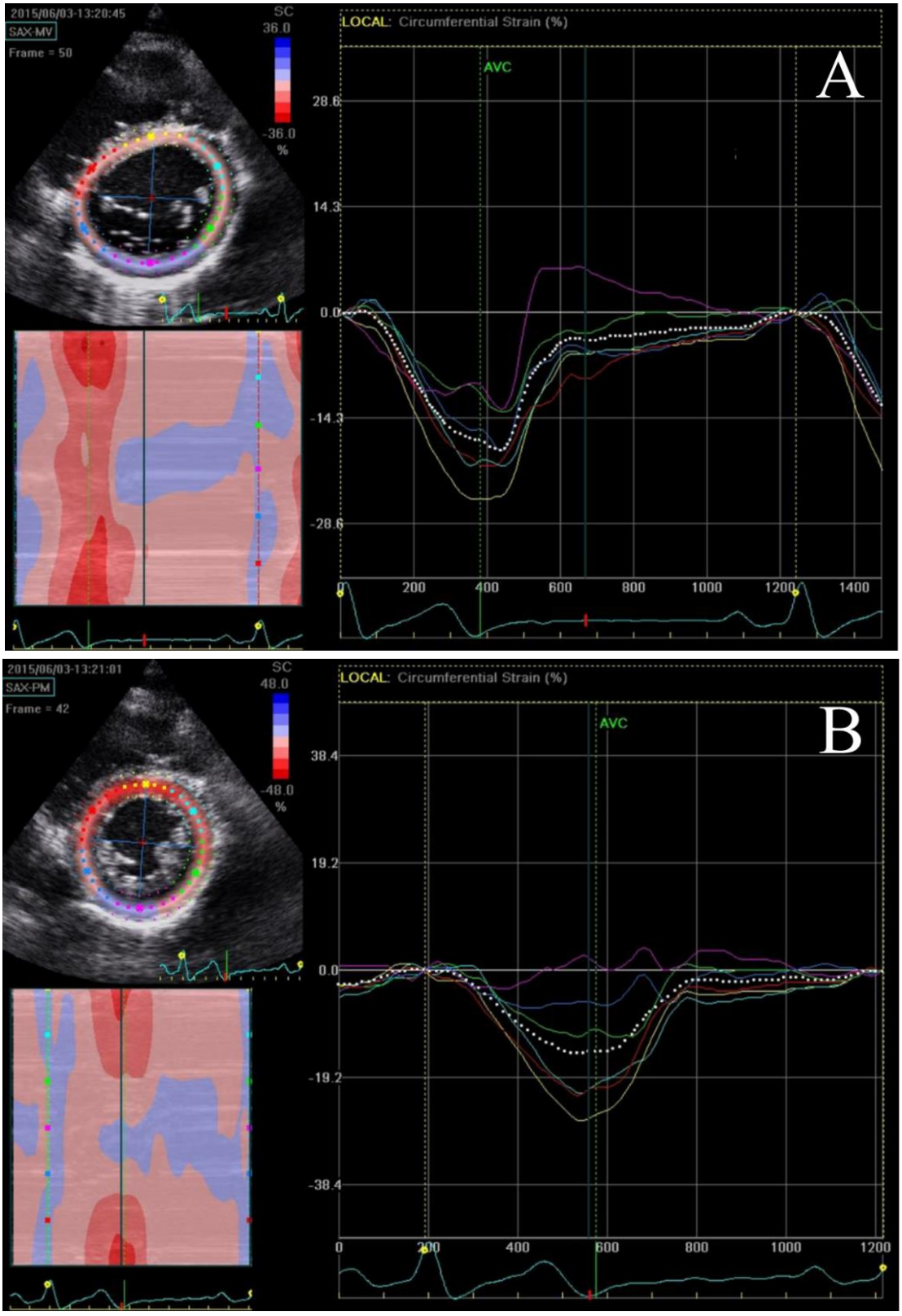

Figure 15. GC ε at (A) basal-level, and (B) mid-level

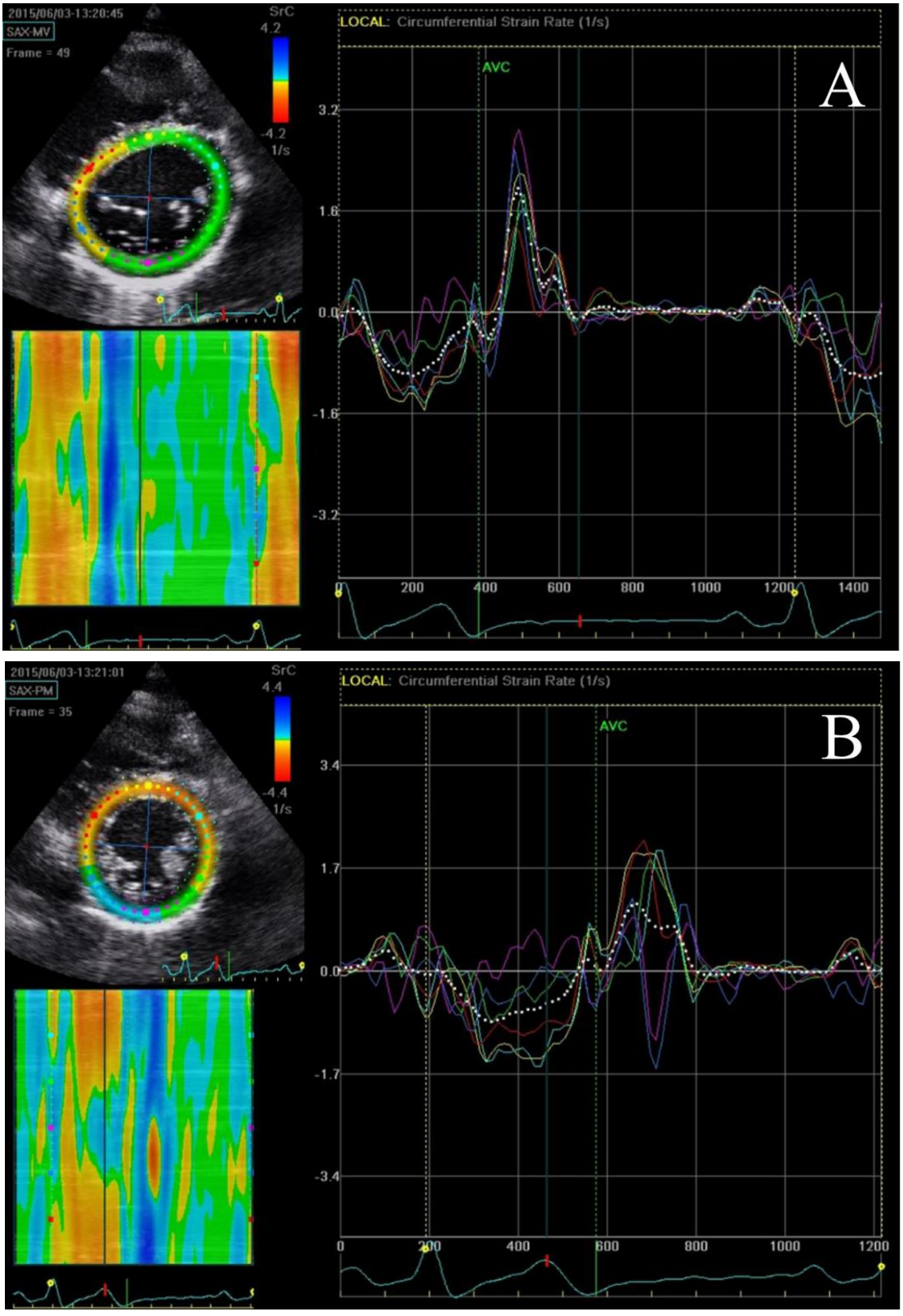

Figure 16. GC SR at (A) basal-level, and (B) mid-level

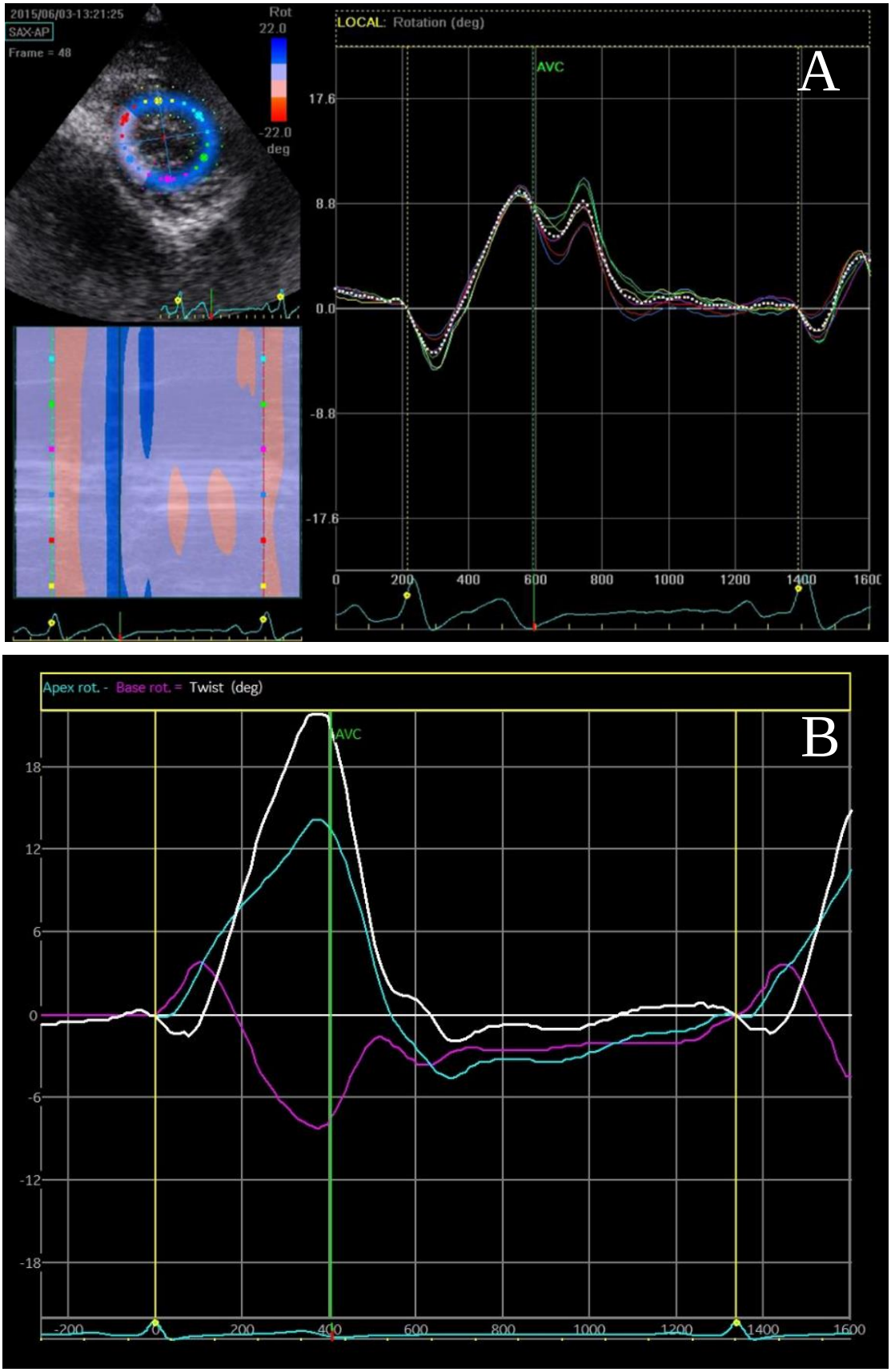

Figure 17. (A) Rotation at apical level and (B) Apical rotation, basal rotation, and net twist traces

Previous data collected in the Liverpool John Moores University laboratory has demonstrated very good agreement for peak GL ε (CoV 6%, ICC 0.807) and LV Twist (CoV 10%, ICC 0.954), and good agreement for GC ε (CoV 7%, ICC 0.781) (Oxborough, George and Birch, 2012).

3.7 Stress Echocardiography

A stress echocardiogram was undertaken using an electromagnetically braked cycle ergometer (Lode Angio, NL) in the left lateral decubis position. Exercise intensity was controlled by adjusting resistance in the hyperbolic mode to elicit 50% HR_{max} using the previously described age-predicted method (Fox 3rd and Haskell, 1968). Images were recorded in a short axis-orientation at basal-, mid- and apical-level, to determine GC ε, GC SR and LV twist as previously described (Figures 14-17). LV EF was measured from apical four-chamber and two-chamber and images, using the Simpsons Biplane method as previously described (Figure 7). Doppler imaging was used to measure blood flow velocity across the mitral valve (Figure 9), and TDI was used to determine S', E' and A' at the infero-septal and lateral walls (Figure 10). Apical four-chamber, two-chamber and three-chamber images facilitated assessment of GL ε and SR as previously described (Figures 11-13). All images were acquired in accordance with ASE guidelines (Lang et al., 2015).

Chapter 4 The Impact of Endurance Performance Level on Left Ventricular Remodelling

4.1 Introduction

Structural adaptation of the athlete's heart (AH) has been relatively well characterised, with the greatest dimensions observed in athletes who carry out high quantities of training with high dynamic and high static components, as is the case in sports such as cycling, triathlon and rowing (Levine et al., 2015). The most notable of these adaptations are proportional increases in left ventricular (LV) chamber volume and wall thickness (Abergel et al., 2004; Utomi et al., 2013; Wasfy et al., 2015b) with concomitant changes in LV mass. Exposure to extended periods of elevated preload (eliciting ventricular volume overload) and elevated wall stress appear to be the primary drivers of training-induced structural adaptation in the athlete's heart (La Gerche, Taylor and Prior, 2009; Brown et al., 2017). A training-related increase in chamber compliance and size enables the athletes to generate very high cardiac outputs that are required to sustain high dynamic exercise (Levine, 2008). Although strong correlations between LV end diastolic volumes (EDV) and aerobic capacity (La Gerche et al., 2012c) have been reported, the association between functional/mechanical adaptation and athletic performance level is not understood (Spence et al., 2011; Weiner et al., 2015).

Whilst there is some consistency in the extant literature regarding the LV structural phenotype in athletes who engage in high training hours, this has been based on absolute chamber sizes and a basic linear derivation of LV geometry (George et al., 2001). In addition, contradictory findings exist regarding the nature and magnitude of physiological adaptation in LV function (Arbab-Zadeh et al., 2004; Weiner et al., 2015). This is particularly relevant to the assessment of road cyclists, whereby application of conventional measures of function suggest 7% present with reduced ejection fraction

(EF) (Abergel et al., 2004). The application of novel indices of LV mechanics utilising myocardial speckle tracking echocardiography (STE) may be insightful by facilitating the assessment in LV longitudinal, circumferential and rotational planes of motion (Santoro et al., 2014; Utomi et al., 2014). Additionally, STE offers far greater sensitivity than conventional measures of function, with less load-dependence and angle-dependence compared to Doppler and Tissue Doppler respectively (Marwick, 2006; Forsythe, George and Oxborough, 2018).

Although positive associations between LV Mass Index (LVMi), LV End Diastolic Volume (EDV) and STE derived peak global longitudinal ε (GL ε) exist (i.e. increased LVMi results in decreased GL ε), athletes with the most pronounced structural adaptation can still be expected to present similar peak GL ε values to non-athletes (NA) (Beaumont et al., 2017; Forsythe et al., 2018). In contrast, endurance training appears to elicit no change, or mild increases in global circumferential ε (GC ε) and a reduction in LV twist (Baggish et al., 2008; Santoro et al., 2014; Utomi et al., 2014). It is unclear whether alterations in GC ε and LV twist are an acute response to training (Baggish et al., 2008), or a chronic adaptation required to maintain systolic function in the presence of marked LV structural remodelling (Santoro et al., 2014).

It has been suggested chronic high training hours are associated with development of supra-normal diastolic function, and that enhanced ventricular relaxation is an important contributor to LV filling, which in turn facilitates stroke volume generation (Caso et al., 2000; Weiner et al., 2010). That said, large cohort examinations of athletes have described similar diastolic filling (as determined by Doppler imaging) at rest between athletes and non-athletes (Pluim et al., 2000; Finocchiaro et al., 2018).

Furthermore, recent work has clearly demonstrated larger LV cavity size is associated with a lower E' velocity (Caselli et al., 2015; Finocchiaro et al., 2018).

It is noteworthy that previous investigations of the athletes' LV mechanics have focused on athlete vs non-athlete comparisons, with little consideration for differences due to athletic performance level. The only cross-sectional comparison between mechanics of elite and sub-elite athletes (to the authors' knowledge) described significant differences in systolic tissue velocities and diastolic filling (Baggish et al., 2010), highlighting the importance of characterising the mechanical phenotypes within these two distinct groups.

Consequently, this study aimed to quantify differences in LV structural remodelling between SEC and EC, and to determine the impact of sub-elite and elite level training on LV function. In view of this, it was hypothesised that: (1) greater LV structural remodelling will be observed in EC compared to SEC, and (2) conventional and mechanical measures of systolic and diastolic LV function will be lower in EC compared to SEC.

4.2 Methods

Study Population and Design

Male elite-level road cyclists (EC, *n*=69) actively competing in UCI World Tour and UCI Pro Continental level events, male sub-elite road cyclists (SEC, *n*=30) actively racing under a 1st, 2nd or 3rd category British Cycling license, and healthy, non-smoking

male non-athlete university students/staff (NA, *n*=46) engaging in fewer than 3 hours recreational activity per week were recruited into this cross-sectional study.

A very high proportion of subjects were Caucasian (97%). Of the *n*= 4 non-caucasian subjects, *n*= 2 EC athletes were Latin American, and *n*= 2 NA were mixed Caucasian/Black Caribbean. All subjects were free of known cardiovascular disease and abstained from alcohol and caffeine consumption for at least 24 hours prior to data collection. Subjects also refrained from training activities for at least 6 hours prior to data collection. Ethics approval was granted for this study by the Ethics Committee of Liverpool John Moores University and the National Research Ethics Service, Essex Research Ethics Committee in the United Kingdom.

Procedures

Subjects completed a health questionnaire to exclude cardiovascular symptoms, family history of sudden cardiac death (SCD) and other cardiovascular history and/or abnormalities. Body mass (Seca 217, Germany) and height (Seca Supra 719, Germany) were recorded. Body surface area (BSA) was calculated as previously described in chapter 3. A standard, resting 12-lead electrocardiogram was undertaken and reviewed as described in chapter 3.

All resting echocardiographic acquisition and analysis of the LV was undertaken as described in chapter 3.

Statistical Analysis

Study data were collected and managed using REDCAP electronic data capture tools hosted at Liverpool John Moores University (Harriss and Atkinson, 2013). All echocardiographic data were presented as mean ± SD (range). Statistical analyses were performed using the commercially available software package SPSS (SPSS, version 23.0 for Windows, USA). A one-way analysis of variance (ANOVA) with an alpha value set to P = 0.05 was used to examine differences between groups.

4.3 Results

Age and height was similar between EC (27±5 years and 1.80±0.06 m), SEC (25±5 years and 1.80±0.07 m), and NA (22±3 years and 1.78±0.07 m) respectively. Body mass was significantly lower in EC and SEC, compared to NA (P< 0.001 and P< 0.05 respectively) (71.0±5.9 and 73.2±8.4 vs 78.1±9.8 kg) resulting in BSA being significantly lower in EC compared to NA (P< 0.05) (1.88±0.10 and 1.96±0.14 m^2). Resting HR was also significantly lower in EC and SEC compared to NA (both P< 0.001) (51±8, 53±7 and 69±10 beats.min^{-1} respectively). No non-training related ECG changes were observed in any subjects.

Left Ventricular Structure

Conventional LV structural parameters are presented in table 2. Absolute LVd, MWT, LV mass, LV EDV and LV ESV were significantly greater in EC compared to SEC (P<0.05, P<0.001, P<0.001, P<0.05, and P<0.001 respectively) and NA (all P<0.001). Absolute parameters were also significantly greater in SEC compared to NA (P<0.05,

P<0.05, P<0.001, P<0.001 and P<0.001 respectively). LV structural indices remained significantly greater in EC compared to SEC (P<0.05, P<0.001, P<0.001, P<0.05 and P<0.001 respectively) following allometric scaling to BSA (LVD index, MWT index, LV mass index, LV EVD index, LV ESV index). All scaled parameters were also greater in SEC compared to NA (all P< 0.001).

Table 2. Left ventricular structural parameters

	Elite Cyclists	***Sub-Elite Cyclists***	***Non-Athletes***
LVd (mm)	54.8 ± 3.8*‡ [41.0 : 62.0]	52.6 ± 3.7† [44.0 : 62.0]	49.5 ± 3.7 [40.0 : 56.0]
LVD Index (mm/(m^2)$^{0.5}$)	40 ± 3.1*‡ [27.9 : 45.8]	38.1 ± 2.5‡ [34.2 : 44.4]	35.4 ± 2.8 [29.5 : 40.0]
LV EDV (ml)	162 ± 18*‡ [113 : 201]	149 ± 19‡ [107 : 182]	104 ± 21 [55 : 148]
LV EDV Index (ml/(m^2)$^{1.5}$)	63 ± 8*‡ [45 : 79]	57 ± 8‡ [39 : 71]	38 ± 8 [22 : 51]
LV ESV (ml)	70 ± 11*‡ [42 : 94]	61 ± 13‡ [33 : 89]	43 ± 9 [24 : 59]
LV ESV Index (ml/(m^2)$^{1.5}$)	27 ± 5*‡ [17 : 40]	23 ± 6‡ [13 : 34]	16 ± 3 [9 : 23]
MWT (mm)	9.6 ± 0.7**‡ [8.0 : 12.0]	8.3 ± 0.5† [7.5 : 9.5]	7.6 ± 0.6 [6.3 : 9.1]
MWT Index (mm/(m^2)$^{0.5}$)	6.9 ± 0.5**‡ [5.9 : 8.1]	6.0 ± 0.4‡ [5.5 : 6.8]	5.5 ± 0.4 [4.5 : 6.5]
RWT	0.36 ± 0.04**‡ [0.27 : 0.51]	0.33 ± 0.03 [0.26 : 0.41]	0.32 ± 0.04 [0.25 : 0.41]
LV Mass (g)	210 ± 31**‡ [141 : 313]	163 ± 26‡ [119 : 224]	133 ± 24 [81 : 187]
LV Mass Index (g/(m^2)	112 ± 17**‡ [65 : 149]	85 ± 12‡ [64 : 117]	68 ± 12 [42 : 86]
LV Concentricity (g/(ml)$^{2/3}$)	7.11 ± 1.08**‡ [4.42 : 9.82]	5.85 ± 0.98 [4.20 : 7.84]	6.02 ± 0.83 [3.91 : 7.98]

* P<0.05 vs Sub-Elite, ** P<0.001 vs Sub-Elite, † P<0.05 vs Non-Athletes, ‡ P<0.001 vs Non-Athletes

Concentricity and RWT were significantly greater in EC compared to SEC (both P< 0.001) and NA (both P< 0.001), however no differences were observed between SEC and NA. The distribution of LV geometry across all groups is presented in Figure 19. A predominance of normal LV geometry was observed across EC, SEC and NA (60.9 %, 96.7 % and 100% respectively). Eccentric dilated LV hypertrophy was more common than eccentric non-dilated LV hypertrophy in EC (33.3 % compared to 1.4 %)

and eccentric dilated LV hypertrophy was much rarer in SEC (3.3%). There were no cases of eccentric non-dilated LVH in SEC. Concentric non-dilated LV hypertrophy and concentric dilated LV hypertrophy remained rare in EC (1.4 % and 2.9 % respectively) and no cases of this geometry were observed in SEC.

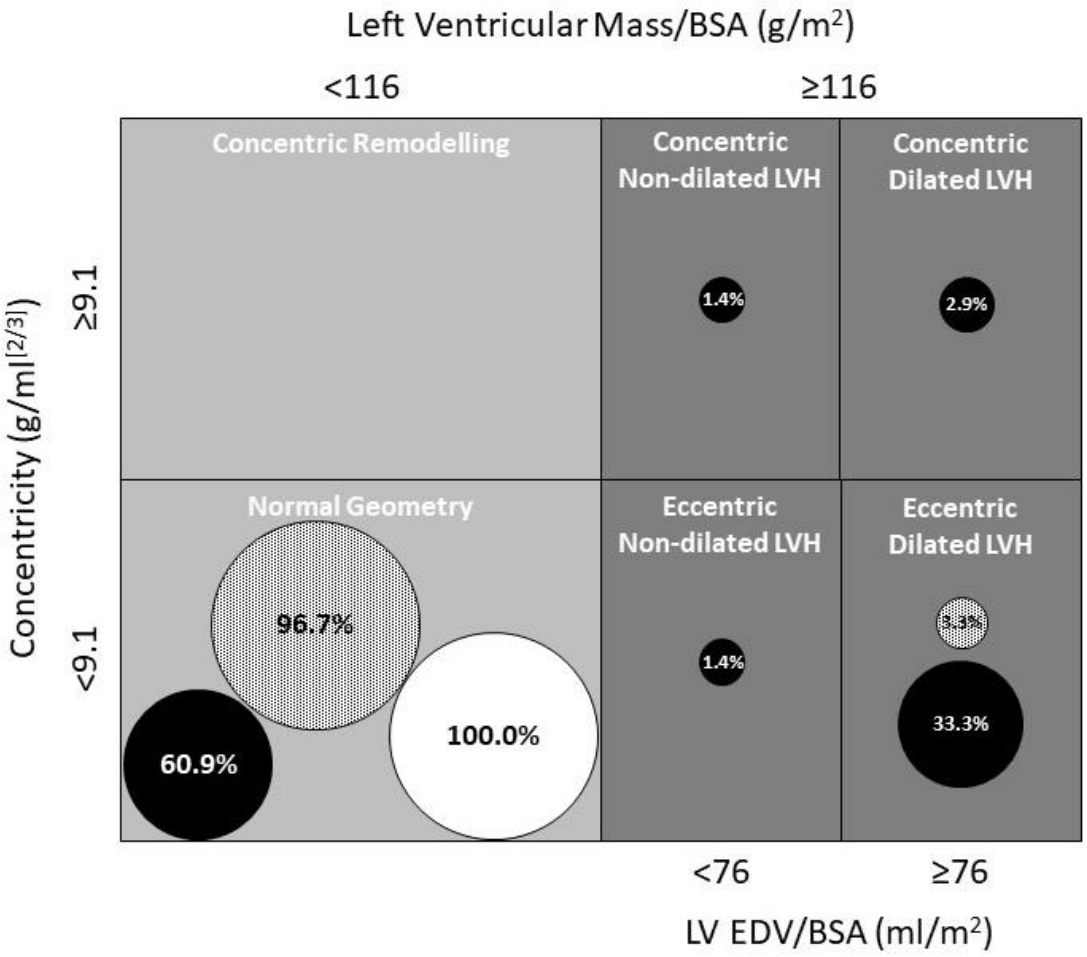

Figure 18. Four-tier LV geometry classification distribution for EC (●), SEC (▨), and NA (○)

Left Ventricular Function

Conventional indices of LV function are presented in Table 3. LV EF was lower in EC compared to NA only (P< 0.05). Reduced LV EF occurred in 11.6 % of EC and 6.8 % of SEC. Septal S' was lower in EC compared to NA only (P< 0.05).

Table 3. Left ventricular conventional resting functional parameters

	Elite Cyclists	***Sub-Elite Cyclists***	***Non-Athletes***
LV EF (%)	57 ± 5† [45 : 70]	59 ± 7 [48 : 74]	59 ± 4 [54 : 68]
E (cm/s)	0.72 ± 0.14**† [0.42 : 1.04]	0.88 ± 0.12 [0.63 : 1.14]	0.82 ± 0.15 [0.49 : 1.19]
A (cm/s)	0.37 ± 0.08*‡ [0.23 : 0.67]	0.44 ± 0.07 [0.28 : 0.61]	0.49 ± 0.10 [0.31 : 0.81]
E/A	1.98 ± 0.50† [1.17 : 3.56]	2.05 ± 0.40† [1.36 : 3.17]	1.80 ± 0.48 [0.78 : 2.91]
Septal S' (cm/s)	9 ± 1† [6 : 13]	9 ± 1 [7 : 11]	10 ± 2 [7 : 13]
Septal E' (cm/s)	12 ± 2**† [8 : 17]	15 ± 2† [11 : 20]	13 ± 3 [9 : 21]
Septal A' (cm/s)	7 ± 2† [4 : 10]	8 ± 2 [4 : 13]	8 ± 2 [5 : 12]
Lateral S' (cm/s)	12 ± 2 [8 : 18]	12 ± 3 [7 : 17]	13 ± 3 [7 : 19]
Lateral E' (cm/s)	18 ± 4*† [6 : 25]	20 ± 4 [12 : 29]	19 ± 4 [8 : 28]
Lateral A' (cm/s)	7 ± 2 [4 : 18]	7 ± 2 [5 : 12]	8 ± 2 [3 : 16]

* P<0.05 vs Sub-Elite, ** P<0.001 vs Sub-Elite, † P<0.05 vs Non-Athletes, ‡ P<0.001 vs Non-Athletes

GC and GL ε, and twist data are presented in Table 4. No differences existed between groups in peak GL ε. Peak GC ε was greater in EC and SEC compared to NA (P<0.05 and P< 0.001 respectively). No differences existed between groups in peak LV twist or basal rotation, however peak apical rotation was lower in EC compared to SEC (P< 0.05).

Transmitral E and A were both lower in EC compared to SEC (P< 0.001 and P< 0.05) and NA P< 0.05 and P< 0.001). E/A ratio was significantly higher in EC and SEC compared to NA (both P< 0.05). Septal E' and A' were lower in EC compared to NA (both P< 0.05). In addition, septal E' was lower in EC compared to SEC (P< 0.001), and greater in SEC compared to NA (P< 0.05) whilst lateral E' was lower in EC, compared to SEC (P< 0.05) and NA (P< 0.05).

Table 4. Speckle Tracking Echocardiographic parameters

	Elite Cyclists	***Sub-Elite Cyclists***	***Non-Athletes***
Global longitudinal			
Peak ε (%)	-18.3 ± 2.0 [-13.7 : -23.6]	-19.3 ± 1.7 [-16.4 : -23.3]	-18.2 ± 2.3 [-13.2 : -23.6]
Global Circumferential			
Peak ε (%)	-18.4 ± 2.4† [-14.0 : -24.1]	-19.8 ± 2.7‡ [-14.1 : -26.9]	-17.2 ± 2.6 [-12.0 : -22.3]
LV Rotation			
Peak Twist (º)	15.2 ± 5.4 [4.1 : 33.4]	17.7 ± 5.3 [9.3 : 28.0]	16.3 ± 5.3 [4.7 : 29.2]
Peak Basal Rotation (º)	-5.7 ± 2.3 [-0.8 : -11.3]	-5.0 ± 1.9 [-1.5 : -9.0]	-5.5 ± 3.0 [-0.3 : -13.5]
Peak Apical Rotation (º)	9.9 ± 5.0* [1.9 : 30.1]	13.3 ± 4.7 [3.6 : 21.4]	11.7 ± 4.1 [3.3 : 21.3]

* P<0.05 vs Sub-Elite, ** P<0.001 vs Sub-Elite, † P<0.05 vs Non-Athletes, ‡ P<0.001 vs Non-Athletes

4.4 Discussion

The main findings of this study are 1) Marked structural remodelling was observed in EC, who presented with significantly greater LV chamber volume and wall thickness compared to SEC. Over one-third of EC presented with eccentric hypertrophy, compared to just 3.3% in SEC. 2) Reduced LV EF was observed in a greater proportion of EC compared to SEC, despite similar conventional and STE measures of systolic function. Conventional measures of diastolic function were lower in EC compared to SEC.

Left Ventricular Structure

In keeping with previous findings, significantly greater LV chamber size was observed in EC and SEC compared to NA (Abergel et al., 2004; Utomi et al., 2014)., providing further support for sustained periods of elevated preload and haemodynamic volume overload acting as a primary stimulus for structural adaptation of the LV in endurance athletes. Although increased MWT was observed in EC, none of the cohort presented

thicknesses greater than 12 mm. This is in stark contrast to the work of Abergel et al. (2004), who found 8.7 % of elite cyclists presented a MWT exceeding 13 mm. It is difficult to speculate as to the reason for this disparity however the authors themselves report the potential confounding impact of performance enhancing drugs used by cyclists during the 1990's and early 2000's, many of which are known to elicit concentric LVH (Angell et al., 2014). Better endocardial border differentiation from a combination of improvement in echocardiography technology and experience in defining true endocardium from LV trabeculation may potentially have also contributed to previously erroneous measurements.

Although like Utomi et al. (2013), the majority of our EC cohort presented with normal LV geometry, a greater proportion of the cohort presented with eccentric hypertrophy (34% compared to 30%). These differences may be due to the sporting disciplines represented by the endurance trained cohort of Utomi et al. (2013), as the influence of static (% maximal voluntary contraction) demands of highly dynamic sports on adaptation of LV geometry has previously been highlighted (Wasfy et al., 2015b; Finocchiaro et al., 2017). As previously demonstrated in other sporting disciplines, concentric hypertrophy was rare in EC (4%) (Forsythe et al., 2018).

The changes observed in LV geometry highlight the contribution of LV dilatation to the increase in LV mass between NA and SEC whilst the development of a concomitant increase in wall thickness (i.e. concentricity) drives the further increase in LV mass observed in EC. This appears to be in contrast with previous studies of the endurance training process, which have either described concurrent development of LV mass and chamber volume over a period of 3-6 months (Baggish et al., 2008; Spence et al., 2011)

or increases in LV mass preceding those of chamber volume over a period of 12 months (Arbab-Zadeh et al., 2014). These findings appear to have captured a longer-term adaptation in LV geometry, very similar to that observed by Weiner et al. (2015) in their 3-year longitudinal examination of competitive rowers, albeit in a cross-sectional design with a different cohort.

Left Ventricular Function

Previous research has highlighted decreased resting systolic function in endurance cyclists, which, in addition to the profound cavity dilation presented by this population, increases the potential for a false-positive diagnosis of dilated cardiomyopathy (Abergel et al., 2004; Sharma, Merghani and Mont, 2015). The finding that 11.6 % of EC and 6.7 % of SEC present with reduced EF emphasises the challenge of differentiating physiological and pathological adaptation in this group. Claessen et al. (2018) have previously demonstrated that a low EF in this population is simply a function of increased cavity volume, which requires a lower contractile force to produce the necessary stroke volume.

Previous studies have identified GL peak ε as a potential tool to aid differentiation between physiological and pathological adaptation, as healthy athletes and non-athletes present similar GL ε values and significant decreases are observed in several pathological conditions (Beaumont et al., 2017; Pelliccia, 2019). The present findings provide further support for the clinical application of GL peak ε, as similar values were observed across all groups.

The work of MacIver (2012) identified GC peak ε as having a far greater influence on EF than that of GL peak ε at rest (67 % and 33 % respectively). It therefore seems the increased GC peak ε observed in EC and SEC, represents a compensatory mechanism which facilitates normal function at rest, despite vastly increased chamber volume.

In contrast to the recent meta-analysis of Beaumont et al. (2017), which found significantly decreased LV twist in endurance athletes, no differences between EC, SEC or NA groups were observed in the present study. However; a lower apical contribution to LV twist was observed in EC, compared to SEC. Although parallels can be drawn between this adaptation and a previous cross-sectional examination (Santoro et al., 2014), these results are in contrast to the longitudinal training-study of Weiner et al. (2010). The disparity in findings between cross-sectional assessments and acute training studies may be explained by the phasic nature of training-induced adaptations in LV twist (Weiner et al., 2015). It can therefore be proposed, that the differential acute and chronic adaptations apparent in competitive rowers (Weiner et al., 2015) could extend to sub-elite and elite cyclists, as both processes are characterised by the accumulation of training hours over time (Seiler, 2010), and phasic structural adaptation of the LV (Beaumont et al., 2017).

Although increased transmitral E/A was observed in both EC and SEC, in agreement with previous descriptions of the endurance athlete's heart (George et al., 2010), Doppler and TDI analysis shows a clear divergence in the nature of this finding between EC and SEC. SEC presented with a similar E velocity, and increased septal E' compared to NA, suggestive of enhanced chamber relaxation assisting early diastolic filling (George et al., 2010). In contrast, E velocity and E' velocity were both lower in EC

(compared to NA), which indicate lower diastolic function. The most likely explanation for these lower values may be a significantly greater reserve volume and lower resting HR in comparison to both SEC and NA, resulting in a decreased need for enhanced relaxation/suction at rest (Claessen et al., 2018).

Limitations

Due to the cross-sectional nature of this study, it is not possible to directly assess any cause-effect relationships between exercise and physiological cardiac remodelling. Although the performance levels of subjects are well defined, detailed data pertaining to the quantity and intensity of training completed by EC and SEC were not available, and as such, characterisation of training within this group was based on previous reports using athletes of a similar performance level (Metcalfe et al., 2017). Radial ε was not reported in this study, due to poor reproducibility of this parameter (CoV 19%, ICC 0.714) (Oxborough, George and Birch, 2012). It should also be noted that findings of this study are specific to males aged 20-30 years, and as such, should not be extrapolated to female, junior or veteran athletic populations. All subjects denied use of illicit performance enhancing drugs, however it is impossible to quantify this claim as no specific anti-doping controls were carried out as part of this study. As such, this should be considered a limitation of the study.

Conclusions

A significantly greater LV mass was observed in EC compared to SEC, who presented with greater LV mass compared to NA. Differences in LV mass between EC and SEC

are primarily driven by increased wall thickness (and therefore concentricity), whereas chamber dilatation differentiates SEC and NA. Increased GC ε in EC and SEC may represent a compensatory mechanism to maintain stroke volume at rest in the presence of increased chamber volume, unchanged GL ε and unchanged LV twist. Decreased E and E' velocities in EC are a novel finding, and may be indicative of a considerable functional reserve. Future research is required to elucidate this complex relationship between structural adaptation and function in elite endurance athletes.

Perspectives

In this study, a considerable difference in the magnitude of structural remodelling presented by elite and sub-elite cyclists was evident. It was also shown that marked structural adaptation is often accompanied by functional and mechanical alterations, which could appear atypical in a pre-participation screening setting. The potential application of STE for differential diagnosis in these situations should be noted, particularly in the case of localised adaptations (i.e. apical rotation). This investigation prompts further research into identification and quantification of the functional reserve observed in elite endurance athletes. In chapter 5, the variability of structure-function relationships of the LV in response to medium-term alterations in training hours will be examined. The application of stress-echocardiography will provide new insight into training induced progression/regression of functional reserve capacity.

Chapter 5 Seasonal Variation in the Cardiac Structure, Function and Mechanics of Competitive Road Cyclists

5.1 Introduction

In chapter 4, a relationship between chronic training hours and the magnitude of structural, functional and mechanical LV adaptation was established. In the context of a competitive road cycling season, RC carry out carefully planned training programmes to induce physiological adaptations in anaerobic threshold and $VO_{2\ max}$, which contribute to performance (Joyner and Coyle, 2008). The time taken to induce these adaptations, and different rates of dissipation, make variability in training hours (i.e. (periodization) a key concept for coaches to utilise (Mujika et al., 2018). Specific time points over the competitive season where physiological adaptations are optimised and fatigue is minimised (termed "peaking"), can then be targeted (Mujika et al., 2018).

Anaerobic threshold power has previously been identified as the primary contributor to performance in cycling (Støren et al., 2013). Quantity of training has been proposed to hold greater efficacy than training intensity in improving this parameter (Seiler, 2010). This likely explains the considerable variation in training hours, but not intensity completed by elite RC (Metcalfe et al., 2017).

The relationship between chronic training hours and LV structural adaptation is well defined (Brown et al., 2017), as is the intrinsic link between LV structure and aerobic capacity (La Gerche et al., 2012c). Recent work has sought to define how more acute periods (3-12 months) of increased training hours drives structural adaptations of the LV (Weiner et al., 2010; Spence et al., 2011; Arbab-Zadeh et al., 2014; Zilinski et al., 2015; Oxborough et al., 2019). Disparities exist in the literature as to whether short-term (3-6 month) increases in training hours promotes increases in cavity volume (Weiner et al., 2010; D'Ascenzi et al., 2015), cavity volume and wall thickness (Arbab-

Zadeh et al., 2014; Zilinski et al., 2015), or wall thickness only (Spence et al., 2011; Oxborough et al., 2019).

Accounting for different baseline training status' of cohorts, and proposed phasic adaptation of the LV, whereby short-term (3 months) eccentric remodelling (i.e. chamber expansion with no change in wall thickness) and subsequent long-term (3 years) eccentric hypertrophy (i.e. balanced increases in chamber volume and wall thickness) are observed in experienced high dynamic, high static (HDHS) athletes, may explain disparities in the literature (Weiner et al., 2015).

Within-season (12 months) adaptive remodelling induced by intensified training in elite soccer appears to support this, as significant increases in cavity volume, but not wall thickness are observed (D'Ascenzi et al., 2015). A within-season assessment of competitive swimmers has challenged this finding, reporting thickening of the septal and posterior LV walls in response to intensified training (Csajági et al., 2015). However, due to the adolescent cohort used in this study, it is impossible to separate the relative contributions of training hours and maturation (Makan et al., 2005). Furthermore, as these observations were made in athletes completing a low-medium static component sports, it is unclear whether these findings can be extrapolated to HDHS athletes, who generally present with more marked chronic adaptation (Brown et al., 2017).

Chronically trained endurance athletes present with similar or slightly reduced systolic function and mechanics compared to non-athletes (Brown et al., 2017). Reduced function appears to represent development of a functional reserve volume (i.e. lower

contractile force required to generate appropriate SV) in this group, as augmentation occurs under exercise stress (La Gerche et al., 2012a).

Short-term structural adaptation of the LV does not appear to impact on global systolic function, but is associated with increases in S', GL ε and LV twist, which act to preserve SV during initial stages of chamber volume expansion (Weiner et al., 2015). Increased LV twist also results in increased LV untwisting rate, which holds an important role in maintaining diastolic function in this remodelling stage (Weiner et al., 2015). Initial untwisting rate acts to increase early diastolic filling (E), through generation of a greater atrioventricular pressure gradient (Weiner and Baggish, 2011). A return of S', GL ε and LV twist to baseline (or mildly reduced) levels in response to chronically elevated training hours, is therefore indicative of functional reserve development.

To the author's knowledge, no within-season assessment of LV mechanics, which act as an intermediary for structural and functional characteristics of the developing HDHS athlete's heart, has previously been carried out. This study therefore aimed to characterise the impact of seasonal variations in road cycling training hours on LV structure, function and mechanics at rest and in-exercise. It was hypothesised that: 1) Increases in LV mass (driven by chamber expansion) would be observed in-line with training hours, 2) Global systolic and diastolic function would remain similar at all time-points, and 3) increases in LV twist would occur in concurrence with chamber expansion.

5.2 Methods

Study Population and Design

Competitive road cyclists (RC) (male *n*= 5, female *n*= 2) actively racing under a 1st, 2nd or 3rd category British Cycling licence, and healthy non-athletes (male *n*= 4, female *n*= 5) engaging in fewer than 3 hours of recreational activity per week were recruited for the purpose of this study.

All participants were free of known cardiovascular disease and abstained from alcohol and caffeine consumption for at least 24 hours prior to each data collection. Participants also refrained from training activities for at least 6 hours prior to each data collection. Ethical approval was granted for this study by the ethics committee of Liverpool John Moores University.

Procedures

Measurements were performed at the beginning of the study, after 3 months, after 7 months, and after 10 months, representing off-, early-, mid-, and end-season time-points of the British road-cycling season.

At the first visit, participants completed a health questionnaire to exclude cardiovascular symptoms, family history of sudden cardiac death (SCD) and other cardiovascular history and/or abnormalities. A standard resting electrocardiogram (ECG) was also carried out to exclude potential underlying pathologies.

In the 2 weeks prior to each laboratory visit, training hours were recorded for RC using commercially available software (TrainingPeaks, USA). During each of the four visits, body mass (Seca 217, Germany) and height (Seca Supra 719, Germany) were recorded, facilitating calculation of body surface area (BSA) as described in chapter 3. All echocardiographic acquisition and analysis of the LV was undertaken as described in chapter 3.

Lactate threshold (LT), peak oxygen consumption ($VO_{2\ peak}$), maximal aerobic power (W_{max}) and maximum heart rate (HR_{max}) were determined during an incremental cycle test performed on an electromagnetically braked cycle ergometer (Lode Excalibur, NL). The test commenced at 125 W for male participants or 75 W for female participants, and increased in 25 W increments every 3 minutes until volitional exhaustion. Blood lactate measurements were obtained in the final 30 seconds of each stage using a Lactate Plus unit (Nova Biomedical, USA). Fixed blood lactate concentrations of 2 mmol/L and 4 mmol/L were used to determine aerobic and anaerobic thresholds from a third order polynomial regression curve. Breath-by-breath measurements were obtained throughout the cycle test using an Oxycon Pro (Jaeger, USA) online gas analysis system, and $VO_{2\ peak}$ was defined by the following end-point criteria, *1) heart rate within 10 beats.min^{-1} of age-predicted maximum, 2) respiratory exchange ratio >1.1, and 3) plateau of oxygen consumption despite increased workload*. Heart rate was also recorded throughout the cycle test using a Polar H7 (Polar, Finland).

Statistical Analysis

Study data were collected and managed using REDCAP electronic data capture tools hosted at Liverpool John Moores University (Harriss and Atkinson, 2013). All

echocardiographic data were presented as mean ± SD (range). Statistical analyses were performed using the commercially available software package SPSS (SPSS, version 23.0 for Windows, USA). A paired T-test with an alpha value set to P = 0.05 was used to examine differences between groups at off-season, pre-season, mid-season and end-season time-points. Within-group comparisons were carried out using a one-way analysis of variance, with statistical significance set at P<0.05.

5.3 Results

Subject characteristics are summarised in Table 5. No significant differences existed between RC and NA for age (26.6 ± 4.1 vs 26.1 ± 2.7 years), height (1.75 ± 0.10 vs 1.70 ± 0.08 cm), weight (72.9 ± 9.5 vs 65.2 ± 6.2 kg) or resting heart rate (53 ± 6 vs 64 ± 16 bpm) at the off-season time-point. No meaningful within-group changes were observed for these parameters throughout the duration of this study.

Table 5. Subject characteristics

Variable	*Group*	*Off-Season*	*Pre-Season*	*Mid-Season*	*End-Season*
Age (y)	Cyclists	26.6 ± 4.1	-	-	-
	Non-Athletes	26.1 ± 2.7	-	-	-
Height (cm)	Cyclists	1.75 ± 0.10	-	-	-
	Non-Athletes	1.70 ± 0.08	-	-	-
Weight (kg)	Cyclists	72.9 ± 9.5	72.8 ± 9.8	70.9 ± 8.2	71.0 ± 8.2
	Non-Athletes	65.2 ± 6.2	67.3 ± 6.1	66.5 ± 5.4	66.5 ± 5.4
BSA (m^2)	Cyclists	1.88 ± 0.17	1.88 ± 0.17	1.86 ± 0.16	1.86 ± 0.16
	Non-Athletes	1.74 ± 0.10	1.78 ± 0.10	1.77 ± 0.10	1.77 ± 0.10
Resting HR (bpm)	Cyclists	53 ± 6	54 ± 6	55 ± 7	54 ± 8
	Non-Athletes	64 ± 16	63 ± 15	64 ± 11	58 ± 13

* P<0.05 vs Non-Athletes, † P<0.05 vs Off-season. ‡ P<0.05 vs Pre-season, Ø P<0.05 vs Mid-season

Performance and Physiological Variables

Performance data are summarized in Table 6. Training hours were greater at PS (08:12 ± 03:01 h), MS (09:10 ± 03:41 h) and ES (06:56 ± 02:00 h) time-points compared to OS (04:32 ± 01:29 h) for RC. A decrease in RC training hours was observed between MS and ES.

Higher power at 2 mmol/L (2.99 ± 0.58 vs 1.83 ± 0.40 W/kg), power at 4 mmol/L (3.63 ± 0.47 vs 2.49 ± 0.49 W/kg), W_{max} (5.28 ± 0.57 vs 4.03 ± 0.54 W/kg), and $VO_{2\ peak}$ (58.9 ± 6.3 vs 45.5 ± 7.7 ml/kg/min) were observed in RC compared to NA at OS. Differences in these parameters remained significant for PS, MS and ES time-points.

Power at 2 mmol/L was higher at ES compared to PS in RC (3.12 ± 0.55 vs 2.88 ± 0.56 W/kg). W_{max} was decreased at PS compared to OS (5.02 ± 0.63 vs 5.28 ± 0.57 W/kg), and at ES compared to MS (5.04 ± 0.50 vs 5.35 ± 0.38 W/kg) in RC. No significant differences in HR_{max} or La_{max} existed between groups at any point in the study. La_{max} was decreased at PS compared to OS (9.3 ± 1.8 vs 10.2 ± 2.0 mmol/L) for NA only.

Structural Parameters

Structural data are summarised in Table 7, and sex-specific data are summarised in Table 18. RC presented with greater LVd (52.9 ± 2.3 vs 48.1 ± 3.0 mm), MWT (7.6 ± 0.6 vs 6.6 ± 0.6 mm) and LV mass (143 ± 17 vs 108 ± 20 g) compared to NA at OS. Differences in these parameters remained significant at all time-points. LV EDV was higher in RC compared to NA at PS (154 ± 23 vs 123 ± 23 ml) and ES (157 ± 19 vs

123 ± 23 ml). LV concentricity was higher in RC at PS (5.38 ± 0.57 vs 3.93 g/(ml)$^{2/3}$), and remained significantly higher at MS and ES.

Table 6. Training and physiological characteristics

Variable	*Group*	*Off-Season*	*Pre-Season*	*Mid-Season*	*End-Season*
Training Hours (hh:mm)	Cyclists	04:32 ± 01:29	08:12 ± 03:01 ↑†	09:10 ± 03:41 ↑†	06:56 ± 02:00 ↑†, ↓Ø
	Non-Athletes	*<03:00*	*<03:00*	*<03:00*	*<03:00*
Power at 2 mmol/L (W/kg)	Cyclists	2.99 ± 0.58*	2.88 ± 0.56*	3.02 ± 0.37*	3.12 ± 0.55* ↑‡
	Non-Athletes	1.83 ± 0.40	1.67 ± 0.29	1.79 ± 0.57	1.75 ± 0.31
Power at 4 mmol/L (W/kg)	Cyclists	3.63 ± 0.47*	3.59 ± 0.55*	3.59 ± 0.31*	3.57 ± 0.46*
	Non-Athletes	2.49 ± 0.49	2.44 ± 0.40	2.26 ± 0.58	2.29 ± 0.43
W_{max} (W/kg)	Cyclists	5.28 ± 0.57*	5.02 ± 0.63* ↓†	5.35 ± 0.38*	5.04 ± 0.50* ↓Ø
	Non-Athletes	4.03 ± 0.54	3.75 ± 0.37	3.87 ± 0.50	3.95 ± 0.41 ↑Ø
HR_{max} (beats/min)	Cyclists	195 ± 7	192 ± 4	193 ± 7	192 ± 5
	Non-Athletes	187 ± 13	185 ± 14	186 ± 13	185 ± 13
La_{max} (mmol/L)	Cyclists	11.0 ± 1.5	9.6 ± 1.8	10.7 ± 1.8	10.8 ± 2.0
	Non-Athletes	10.2 ± 2.0	9.3 ± 1.8 ↓†	9.4 ± 2.6	9.0 ± 2.6
$VO_{2\ peak}$ (ml/kg/min)	Cyclists	58.9 ± 6.3*	55.7 ± 5.8*	57.2 ± 5.2*	58.9 ± 3.3*
	Non-Athletes	45.5 ± 7.7	43.7 ± 6.1	43.5 ± 8.1	45.5 ± 6.5

* P<0.05 vs Non-Athletes, † P<0.05 vs Off-season. ‡ P<0.05 vs Pre-season, Ø P<0.05 vs Mid-season

RC presented with a greater LV EDV at PS and ES, compared to OS (154 ± 23 and 157 ± 19 vs 144 ± 17 ml). LV concentricity was lower at MS compared to OS (3.80 ± 0.70 vs 4.54 ± 0.55 g/(ml)$^{2/3}$). RC presented with greater LV mass at MS and ES (163 ± 26 and 162 ± 31 g) compared to OS (143 ± 17 g). LVd was greater at ES compared to PS time-point in NA (48.9 ± 3.0 vs 46.1 ± 3.3 mm). LV mass was lower at MS compared to PS (93 ± 19 vs 108 ± 20 g), and greater at ES (102 ± 21 g) compared to MS in NA.

Table 7. Left ventricular structural parameters

Variable	*Group*	*Off-Season*	*Pre-Season*	*Mid-Season*	*End-Season*
LVd (mm)	Cyclists	52.9 ± 2.3*	52.1 ± 3.3*	53.7 ± 2.6*	53.1 ± 2.5*
	Non-Athletes	48.1 ± 3.0	46.1 ± 3.3	47.3 ± 4.2	48.9 ± 3.0 ↑‡
LV EDV (ml)	Cyclists	144 ± 17	154 ± 23* ↑†	148 ± 18	157 ± 19* ↑†
	Non-Athletes	129 ± 29	123 ± 23	123 ± 24	123 ± 23
MWT (mm)	Cyclists	7.6 ± 0.6*	8.1 ± 0.8*	8.4 ± 0.9*	8.0 ± 0.9*
	Non-Athletes	6.6 ± 0.6	6.8 ± 0.5	6.6 ± 0.7	6.6 ± 0.8
LV Concentricity ($g/(ml)^{2/3}$)	Cyclists	5.21 ± 0.55	5.38 ± 0.57*	6.08 ± 1.0*	5.53 ± 0.79*
	Non-Athletes	4.54 ± 0.96	3.93 ± 0.61	3.80 ± 0.70 ↓†	4.18 ± 0.87
LV Mass (g)	Cyclists	143 ± 17*	155 ± 25*	163 ± 26* ↑†	162 ± 31* ↑†
	Non-Athletes	108 ± 20	96 ± 15	93 ± 19 ↓†	102 ± 21 ↑Ø

* P<0.05 vs Non-Athletes, † P<0.05 vs Off-season. ‡ P<0.05 vs Pre-season, Ø P<0.05 vs Mid-season

Function and Mechanics

Conventional measures of resting LV function are summarised in Table 8, and sex-specific data are summarised in Table 19. No significant differences were observed between groups in conventional measures of LV function.

MV E was decreased at PS compared to OS (0.83 ± 0.07 vs 0.92 ± 0.07 cm/s). In addition, MV E:A was lower at PS and MS (1.92 ± 0.24 and 2.06 ± 0.30) compared to OS (2.28 ± 0.29) in RC. Subsequently, RC presented with greater MV E:A at ES (2.22 ± 0.39) compared to MS. NA presented with a lower MV A at ES compared to OS (0.45 ± 0.09 vs 0.49 ± 0.09 cm/s).

Resting STE measures are presented in Table 9, and sex-specific data are summarised in Table 20. No significant differences were observed between groups for resting STE

parameters. An increase in LV basal rotation was observed between PS and MS (-4.7 ± 1.3 vs -6.4 ± 2.0 º) in RC.

50 % HR_{max} STE measures are summarised in Table 10, and sex-specific data are summarised in Table 21. No significant differences were observed between groups for exercise STE parameters.

Table 8. Left ventricular conventional resting functional parameters

Variable	*Group*	*Off-Season*	*Pre-Season*	*Mid-Season*	*End-Season*
LV EF (%)	Cyclists	58 ± 6	56 ± 5	59 ± 4	58 ± 2
	Non-Athletes	58 ± 5	56 ± 5	56 ± 3	57 ± 4
MV E (cm/s)	Cyclists	0.92 ± 0.07	0.83 ± 0.07 ↓†	0.88 ± 0.12	0.91 ± 0.05
	Non-Athletes	0.95 ± 0.14	0.97 ± 0.11	0.93 ± 0.16	0.94 ± 0.14
MV A (cm/s)	Cyclists	0.41 ± 0.05	0.44 ± 0.06	0.43 ± 0.03	0.42 ± 0.08
	Non-Athletes	0.49 ± 0.09	0.51 ± 0.11	0.49 ± 0.18	0.45 ± 0.09 ↓†
MV E:A	Cyclists	2.28 ± 0.29	1.92 ± 0.24 ↓†	2.06 ± 0.30 ↓†	2.22 ± 0.39 ↑‡
	Non-Athletes	2.03 ± 0.58	1.96 ± 0.31	2.06 ± 0.55	2.15 ± 0.49
Medial S' (cm/s)	Cyclists	9 ± 1	9 ± 1	9 ± 1	9 ± 1
	Non-Athletes	9 ± 1	10 ± 1	9 ± 2	9 ± 2
Medial E' (cm/s)	Cyclists	16 ± 3	15 ± 2	16 ± 2	16 ± 2
	Non-Athletes	14 ± 2	16 ± 3	15 ± 3	15 ± 2
Medial A' (cm/s)	Cyclists	7 ± 1	7 ± 1	7 ± 1	7 ± 1
	Non-Athletes	8 ± 2	9 ± 2	8 ± 1	7 ± 1
Lateral S' (cm/s)	Cyclists	11 ± 2	11 ± 2	10 ± 1	11 ± 2
	Non-Athletes	11 ± 2	11 ± 3	11 ± 2	11 ± 3
Lateral E' (cm/s)	Cyclists	19 ± 4	20 ± 3	20 ± 4	20 ± 4
	Non-Athletes	21 ± 4	20 ± 4	20 ± 2	20 ± 3
Lateral A' (cm/s)	Cyclists	6 ± 1	7 ± 1	7 ± 1	6 ± 0
	Non-Athletes	8 ± 2	8 ± 2	8 ± 1	7 ± 2

* P<0.05 vs Non-Athletes, † P<0.05 vs Off-season. ‡ P<0.05 vs Pre-season, Ø P<0.05 vs Mid-season

Table 9. Resting Speckle Tracking Echocardiography (STE) parameters

Variable	*Group*	*Off-Season*	*Pre-Season*	*Mid-Season*	*End-Season*
Global Longitudinal Strain (%)	Cyclists	-19.0 ± 2.2	-19.3 ± 1.9	-19.1 ± 1.8	-19.1 ± 1.5
	Non-Athletes	-20.1 ± 2.1	-20.1 ± 2.1	-19.4 ± 1.7	-20.6 ± 1.9
Peak Global Circumferential Strain (%)	Cyclists	-19.6 ± 1.1	-19.0 ± 1.3	-18.8 ± 0.6	-18.9 ± 2.0
	Non-Athletes	-20.3 ± 2.5	-19.4 ± 1.5	-18.7 ± 1.8	-20.1 ± 1.3
Peak LV Twist (º)	Cyclists	16.6 ± 5.0	14.9 ± 5.3	13.9 ± 2.4	15.2 ± 6.4
	Non-Athletes	20.2 ± 4.1	15.6 ± 4.0	20.3 ± 7.7	17.4 ± 5.5
Peak Basal Rotation (º)	Cyclists	-6.5 ± 2.0	-4.7 ± 1.3	-6.4 ± 2.0 ↑‡	-5.7 ± 1.5
	Non-Athletes	-8.6 ± 3.8	-7.9 ± 2.8	-7.2 ± 2.8	-6.8 ± 2.4
Peak Apical Rotation (º)	Cyclists	11.5 ± 4.7	11.2 ± 6.2	8.5 ± 3.5	11.0 ± 6.9
	Non-Athletes	11.6 ± 3.1	9.5 ± 3.5	14.2 ± 6.4	10.7 ± 3.8

* P<0.05 vs Non-Athletes, † P<0.05 vs Off-season. ‡ P<0.05 vs Pre-season, Ø P<0.05 vs Mid-season

Table 10. 50% HR max Speckle Tracking Echocardiography (STE) parameters

Variable	*Group*	*Off-Season*	*Pre-Season*	*Mid-Season*	*End-Season*
Global Longitudinal Strain (%)	Cyclists	-21.1 ± 2.5	-20.0 ± 2.5	-20.7 ± 3.2	-20.7 ± 1.6
	Non-Athletes	-22.4 ± 2.3	-22.1 ± 1.9	-20.5 ± 1.9	-22.3 ± 2.0
Peak Global Circumferential Strain (%)	Cyclists	-20.6 ± 1.7	-21.4 ± 0.9	-21.3 ± 0.8	-21.4 ± 0.9
	Non-Athletes	-22.4 ± 2.1	-21.8 ± 2.1	-20.0 ± 3.7	-20.6 ± 2.2
Peak LV Twist (º)	Cyclists	19.4 ± 4.2	20.8 ± 4.9	17.5 ± 5.9	18.6 ± 6.8
	Non-Athletes	23.3 ± 8.6	25.0 ± 6.9	23.9 ± 6.2	27.2 ± 5.9

* P<0.05 vs Non-Athletes, † P<0.05 vs Off-season. ‡ P<0.05 vs Pre-season, Ø P<0.05 vs Mid-season

5.4 Discussion

This is the first study to determine seasonal, training quantity induced changes in LV structure, function and mechanics for RC. The main findings of this work were; 1) Development of LV mass was primarily driven by increased chamber volume, and occurred in parallel with accumulation of training hours, 2) A transient increase in the contribution of basal rotation to LV twist during the period of highest training hours, and 3) A temporary shift towards late diastolic filling following the period of greatest increase in training hours.

LV Structure

Although data assessing LV structural adaptation in relation to the variable training stimuli experienced in competitive sport (particularly in HDHS sports) are sparse (Csajági et al., 2015; D'Ascenzi et al., 2015), short-term endurance training interventions have highlighted non-parallel development of chamber dilatation and wall thickening (Weiner et al., 2010; Spence et al., 2011; Zilinski et al., 2015; Oxborough et al., 2019).

A significant increase in LV mass between OS and ES was observed, driven primarily by changes in LV EDV in the presence of unchanged MWT and concentricity. These findings are in agreement with previous descriptions of eccentric type remodelling in-line with increased high dynamic training hours (D'Ascenzi et al., 2015; Weiner et al., 2015). These data also provide additional insight into the phasic structural adaptation process proposed by Weiner et al. (2015). As the proposed hypertrophic response was

not observed within this 10-month study period, it is reasonable to suggest this chronic adaptation may occur in the period between 10-39 months (Weiner et al., 2015; Brown et al., 2017).

The only long-term (3-year) investigation of LV structure carried out in road cyclists described significant chamber dilatation, and thinning of the posterior and septal walls (Abergel et al., 2004). The authors of this investigation acknowledged the likelihood of drug abuse impacting these findings however, making comparisons to the present study inappropriate (Abergel et al., 2004).

LV Function

In agreement with previous research in high dynamic component athletes (D'Ascenzi et al., 2015), no change in LV EF was observed during the road cycling season. This is also in keeping with the findings of short-term intervention studies, suggesting increases in training quantity are not associated with changes in resting global systolic function (Weiner et al., 2010; Spence et al., 2011).

The finding that GLε and LV twist remain unchanged despite a significant increase in training hours are in stark contrast to previous literature, whereby both parameters increased in response (Weiner et al., 2010; Weiner et al., 2015). Unchanged twist may be accounted for by: 1) differences in the quantity and/or intensity of training completed by subjects, 2) different stages of maturation in cohorts, as age is an independent predictor of LV twist (Zhang et al., 2010), or 3) The higher initial LV twist observed in this cohort may have resulted in a decreased adaptive "ceiling" in this parameter.

Although LV twist remained unchanged throughout the season, an increased contribution of basal rotation to twist was observed at MS. Again, this is in contrast to the findings of Weiner et al. (2015), who described an increased apical contribution to twist with preserved basal rotation. This observation, however, aligns closely with a recent short-term intervention study, whereby untrained subjects (of a similar age to the present study) completed 6 months of endurance training (Oxborough et al., 2019). The role of age, training mode, and training intensity require further investigation to understand the nature and time-course of this adaptive response which serves as a key mechanistic link between systolic and diastolic function (Weiner and Baggish, 2011; Beaumont et al., 2017; Badano and Muraru, 2019).

Similar augmentation of GLε, GCε and LV twist between RC and NA in response to exercise stress at all time-points (despite considerable differences in LV structure) provides further evidence supporting utilisation of this method when resting echocardiographic parameters are ambiguous (La Gerche et al., 2012a).

Chronic, endurance training-induced adaptation of the LV is generally accepted to have minimal impact on conventional measures of diastolic function (Brown et al., 2017). In contrast, acute bouts of endurance exercise (>120 min) have been shown to elicit mildly altered MV E and A (Lord et al., 2018b). This alteration represents a shift towards later diastolic filling, which is not a feature of the athlete's heart phenotype. Emerging evidence suggests this transient shift in diastolic function may also be extended from acute bouts to periods of overload training (Chapter 6).

The finding of decreased MV E and increased MV A (P= 0.088) (resulting in decreased MV E:A) at the PS time-point, where training is 180% greater than OS, may represent this overload response. Particularly as performance (W_{max}) was also decreased in the corresponding physiological assessment. The return of both MV E and A to baseline levels at MS (where training hours are more stable) provides support for this. It should also be noted, that MV E, A, and MV E:A remained similar between RC and NA at all time-points.

Limitations

Measurement of RC training was limited to quantity only in this study. It is therefore impossible to quantify the contributions of training load (as a function of quantity*intensity), or intensity to the LV adaptations observed.

The use of both male and female subjects is a strength of this study. However, the timing of laboratory visits will have caused data collection to occur at different points within female subjects' menstrual cycles. Previous work has determined athletic performance and conventional measures (except resting MV E:A) are not affected by the menstrual cycle (Fuenmayor, Ramírez and Fuenmayor, 2000; Kishali et al., 2006) , though the impact on mechanical measures are unknown.

Conclusions

Seasonal variations in training quantity were associated with significant alterations in the LV structure, function and mechanics of competitive road cyclists. Accumulation of training hours during the season drove an eccentric type remodelling of the LV, whereby cavity volume increased with no change in MWT or concentricity. A rapid, significant increase in training hours between OS and PS was associated with minor, transient alterations in diastolic function, similar in nature to those observed in EICF. Peak strain and twist parameters remained unchanged throughout the study period, although an increased contribution of basal rotation to twist was observed during the period of greatest training hours.

These findings provide new insight into training-induced variability of LV structure, function and mechanics. The stability of LV systolic function and global mechanics, despite significant changes in LV structure are also highlighted. The mild, transient alterations in diastolic function observed require further investigation. Chapter 6 will seek to understand whether depressed diastolic function observed in EICF can be extended to periods of OL, and thus be considered a normal aspect of the physiological adaptation process in HDHS athletes.

Chapter 6 The Impact of Short-Term Overload Training on Cardiac Mechanics in Competitive Road Cyclists

6.1 Introduction

In chapter 5, a progressive increase in LV mass was observed throughout the competitive cycling season in parallel with accumulation of training quantity. Although functional and mechanical parameters remained similar at off-, mid- and end-season timepoints, minor transient alterations in diastolic function consistent (in nature) with EICF were observed during the period of greatest increase in training hours (pre-season).

Progressive overload of physiological systems is essential to promote adaptation and performance improvement in endurance athletes (Seiler, 2010). Strategic planning of training camps and competitions are often used to achieve this, and can cause a disproportionate imbalance between training stress and opportunity for recovery/adaptation (Aubry et al., 2015). This common practice can elicit an acute fatigue response (whereby significant mood alterations but no negative performance consequences are observed), or functional over-reaching (where significant mood alterations and negative performance consequences are observed) (Aubry et al., 2015). In both cases, a "super-compensation" effect is targeted through subsequent tapering of training load, when fatigue diminishes at a faster rate than favourable physiological adaptations (Aubry et al., 2015).

Although the performance impact of rapid overload training (OL) is well characterised, the causative physiological parameters are less clear (Le Meur et al., 2014). Much of the existing literature has focused on the roles of mood state, disordered sleep, blood markers of muscle necrosis and systemic inflammation, hormonal expression, and

autonomic nervous system balance in relation to the over-reached athlete (Kindermann and Urhausen, 2002).

Despite the intrinsic link between cardiac function and endurance performance (La Gerche et al., 2012c), the impact of OL on cardiac function remained unstudied until relatively recently. The eloquent study design employed by Le Meur et al. (2014) elicited functional over-reaching in competitive triathletes following 3 weeks of rapid overload training (129% habitual training hours). Using impedance cardiography and blood catecholamine assessment, the authors described a significant decrease in LV stroke volume (SV) and cardiac output during exercise in over-reached subjects (Le Meur et al., 2014). Decreased plasma adrenaline availability was proposed as the causative mechanism for reduced SV, through decreased beta-adrenergic stimulation of cardiomyocytes (Le Meur et al., 2014).

Although the accuracy of impedance cardiography is questionable (particularly during exercise) (Warburton et al., 1999), the similarity between these findings and resting echocardiographic assessment of athletes with acute exercise-induced cardiac fatigue (EICF) are striking. A recent meta-analysis described decreased LV SV in athletes who had completed >120 min of endurance exercise (Lord et al., 2018b).

In addition to measures of global function, speckle tracking echocardiography (STE) enables assessment of the LV at the myocardial level (Forsythe, George and Oxborough, 2018). Decreases in Longitudinal strain and LV twist, which directly impact on the LV's ability to generate SV in systole, have been observed in athletes with EICF (Nottin et al., 2009). This is in direct opposition to the short-term

physiological adaptive response previously described for athletes completing 3 months of structured (i.e. not rapid OL) high dynamic, high static (HDHS) component training (Levine et al., 2015), whereby LV twist and longitudinal strain increased (Weiner et al., 2010). These findings also contradict chronic adaptations of the athlete heart phenotype, as LV twist and longitudinal strain are expected to be similar or slightly greater than non-athletes (Beaumont et al., 2017).

Furthermore, echocardiographic assessment of the LV facilitates investigation of diastolic function and mechanics, which is not possible using impedance cardiography (Nagueh et al., 2016). Decreased early diastolic filling velocity and a compensatory increase in atrial contribution to filling represent load-dependant EICF alterations in LV function, which may precede systolic dysfunction (Hart et al., 2007).

EICF alterations in LV structure, function and mechanics appear to be transient in nature, and generally return to pre-exercise levels within 4 weeks (Neilan et al., 2006). It is unknown, however, how repeated bouts of HDHS training with limited recovery will influence athletic (mal-)adaptation of the LV.

The present study therefore aimed to characterise the impact of 3-weeks HDHS OL training, and subsequent 2-week tapered training, on LV structure, function and mechanics at rest and in-exercise. It was hypothesised that: 1) Mild non-clinical reductions in LV systolic and diastolic function would be observed in-exercise following 3-week OL, 2) In-exercise LV twist and global longitudinal strain would be reduced following 3-week OL, and 3) Any changes in global function or mechanics observed at OL would normalise to baseline levels following a 2-week taper period.

6.2 Methods

Study Population and Design

Competitive road cyclists (RC) (male *n*= 4, female *n*= 4) actively racing under a 1st, 2nd or 3rd category British Cycling licence were recruited for the purpose of this study.

All participants were free of known cardiovascular disease and abstained from alcohol and caffeine consumption for at least 24 hours prior to each data collection session. Participants also refrained from training activities for at least 6 hours prior to each data collection. Ethical approval was granted for this study by the ethics committee of Liverpool John Moores University.

Procedures

The prescribed training protocol was separated into four phases; Phase 1, 3 weeks of self-directed habitual training, from which individual target training hours for subsequent phases would be calculated. Phase 2, (PRE) 1 week at 70% of HT hours. Phase 3, 3 weeks overload (OL) at 140% habitual training hours. Phase 4, 2 week taper (TA) at 70% habitual training hours.

Prior to initiation of the training protocol, subjects visited the laboratory on three occasions; at the first visit, subjects completed a health questionnaire to exclude cardiovascular symptoms, family history of sudden cardiac death (SCD) and other cardiovascular history and/or abnormalities. Body mass (Seca 217, Germany), height

(Seca Supra 719, Germany), a standard resting electrocardiogram (ECG) to exclude potential underlying pathologies, and a $VO_{2\ max}$ test to determine maximal oxygen uptake were then completed. On the second visit, subjects undertook familiarisation of the Wingate anaerobic test (WAnT). On the third visit, participants completed familiarisation of the simulated 16.1 km time trial (16.1 km TT).

Height, weight, mood state (RESTQ-76), cardiac echocardiogram, WAnT, and 16.1 km TT were measured at subsequent Pre, OL and TA time-points. The time of day was standardised for all tests at each time-point.

Monitoring of Training

Training hours were self-reported by subjects, and monitored using commercially available software (Training Peaks, USA).

Maximal Oxygen Uptake ($VO_{2\ peak}$)

Subjects completed an incremental cycle test performed on an electromagnetically braked cycle ergometer (Lode Excalibur, NL). The test commenced at 150 W for male participants or 125 W for female participants and increased in 25 W increments every 2 minutes until volitional exhaustion. Breath-by-breath measurements were obtained throughout the test using an Oxycon Pro (Jaeger, USA) online gas analysis system, and $VO_{2\ peak}$ was defined by the following end-point criteria, *1) heart rate within 10 beats.min^{-1} of age-predicted maximum, 2) respiratory exchange ratio >1.1, and 3) plateau of oxygen consumption despite increased workload.* Heart rate was also

recorded throughout the cycle test using a Polar H7 (Polar, Finland), and used to determine each subject's HR_{max}.

Cycling Performance

WAnT and 16.1 km TT tests were completed out on the same day (separated by >7 hours) in the final week of HT, OL, and TA time-points. Testing was carried out on an air-braked cycle ergometer (Wattbike Pro, UK) using commercially available software (Wattbike Performance Computer Model B, Wattbike, UK). Subject's position on the ergometer, and laboratory environmental conditions (19-20 C, 45-50 % RH) were standardised between testing sessions.

Subjects completed a standardised warm-up consisting of 2 minutes at 2.5 W/kg, 2 minutes at 3 W/kg, 2 minutes at 3.5 W/kg, followed by 60 seconds of passive rest and 2x3 second all-out sprints separated by 20 seconds of passive rest. The warm up finished with 5 minutes at 2.5 W/kg.

Wingate Anaerobic Test (WAnT)

Upon completion of the warm-up, subjects were instructed to set themselves in a start position. A 5 second countdown was given before subjects completed the 30 second all-out effort. Air-braked resistance level was self-selected (between settings 3-5) by subjects at "Pre" and maintained between time-points. Verbal encouragement was provided by researchers throughout the 30 second all-out effort. One-second peak power output (PPO), mean power output (MPO), heart rate maximum (HR_{max}) and Blood lactate maximum (La_{max}) were recorded.

16.1 km TT

Each subject completed the standardised warm-up (excluding 2x3 second all-out sprints). Air-braked resistance level was self-selected by subjects at "Pre" and kept consistent between time-points. Researchers provided a 5 second countdown prior to the trial, during which, only time elapsed, simulated speed (km/h) were available as feedback. MPO, mean HR (HR_{mean}), and time to complete the simulated 16.1 km TT were recorded.

Mood State

Subjects were asked to complete the RESTQ-76 questionnaire in isolation prior to cycling performance tests at each time-point. Questionnaire responses were analysed as previously described to determine stress score, recovery score, and stress-recovery balance (Coutts and Reaburn, 2008).

All echocardiographic acquisition and analysis of the LV was undertaken as described in chapter 3.

Statistical Analysis

Study data were collected and managed using REDCAP electronic data capture tools hosted at Liverpool John Moores University (Harriss and Atkinson, 2013). All echocardiographic data were presented as mean ± SD. Statistical analyses were performed using the commercially available software package SPSS (SPSS, version 23.0 for Windows, USA). Data were compared using a one-way analysis of variance, with statistical significance set at $P<0.05$.

6.3 Results

Subject Characteristics

Subjects characteristics are summarised in table 11. No significant differences existed between Pre, OL or TA timepoints for weight, BSA or resting HR.

Table 11. Subject characteristics

Variable	*Pre*	*Overload*	*Taper*
Age (years)	23.9 ± 3.1	-	-
$VO_{2\,max}$ ($mL/kg^{-1}/min^{-1}$)	56.7 ± 7.6	-	-
Height (m)	1.76 ± 0.10	-	-
Weight (kg)	68.0 ± 9.7	67.3 ± 9.3	67.4 ± 9.3
BSA (m^2)	1.84 ± 0.17	1.83 ± 0.16	1.83 ± 0.16
Resting HR (beats/min)	52 ± 9	52 ± 9	51 ± 9

* P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

Cycling Training and Performance Variables

Training and performance data are summarised in Table 12. Training hours were greater in the OL period (12.66 ± 1.62 h) compared to PRE (9.13 ± 1.67 h) (P<0.001) Weekly training hours were decreased during TA (6.47 ± 2.04) compared to OL (P<0.001) and PRE (P<0.05).

No changes in WAnT PPO or MPO were observed between PRE and OL training periods. The WAnT HR_{max} achieved by subjects increased at TA compared to OL (189 ± 6 vs 185 ± 5 beats/min, P<0.05). WAnT PPO increased between OL and TA periods (15.64 ± 2.97 and 16.69 ± 3.05 W/kg, P<0.05). No changes in WAnT La_{max} were observed. Time taken to complete the 16.1 km TT was lower at TA compared to OL (23:50 ± 01:35 and 23:57 ± 02:05, P<0.05).

Table 12. Training and performance outcomes

Variable	*Pre*	*Overload*	*Taper*
Mean Weekly Training Hours (h)	9.13 ± 1.67	12.66 ± 1.62 ↑**	6.47 ± 2.04 ↓*, ↓††
RESTQ-76 Stress Score (A.U.)	14.8 ± 7.1	24.8 ± 5.2 ↑**	15.9 ± 7.1 ↓†
RESTQ-76 Recovery Score (A.U.)	28.5 ± 6.3	23.5 ± 5.6 ↓*	27.1 ± 7.1
RESTQ-76 Stress-Recovery Balance Score (A.U.)	13.6 ± 8.9	-1.3 ± 8.9 ↓**	11.1 ±11.8 ↑†
30 s Wingate Peak Power Output (W/kg)	16.00 ± 3.10	15.64 ± 2.97	16.69 ± 3.05 ↑†
30 s Wingate Mean Power Output (W/kg)	9.75 ± 1.85	9.72 ± 2.03	9.95 ± 2.00
30 s Wingate HR_{max} (beats/min)	190 ± 7	185 ± 5	189 ± 6 ↑†
30 s Wingate La_{max} (mmol/L)	12.7 ± 1.4	11.7 ± 1.6	12.0 ± 1.8
16.1 km TT Time (mm:ss)	24:16 ± 2:12	23:57 ± 2:05	23:50 ± 1:35 ↓†
16.1 km TT Mean Power Output (W/kg)	3.73 ± 0.77	3.86 ± 0.77	3.93 ± 0.66
16.1 km TT HR_{mean} (beats/min)	180 ± 6	176 ± 7	180 ± 9

*

P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

RESTQ-76 stress score was higher at OL (24.8 ± 5.2) compared to PRE (14.8 ± 7.1, P<0.001) and TA (15.9 ± 7.1, P<0.05). RESTQ-76 recovery score was decreased at OL compared to PRE (23.5 ± 5.6 vs 28.5 ± 6.3, P<0.05). RESTQ-75 Stress-Recovery Balance was decreased at OL (-1.3 ± 8.9) compared to PRE (13.6 ± 8.9, P<0.001) and TA (11.1 ± 11.8, P<0.05).

Left Ventricular Structure

Left Ventricular Structural data are summarised in Table 13. LVd was greater at OL compared to PRE (51.8 ± 3.2 vs 50.8 ± 2.9 mm, P<0.05). MWT at OL was significantly lower at TA compared to OL (7.7 ± 0.7 vs 7.5 ± 0.7 mm, P<0.05). LV concentricity decreased from OL to TA (5.56 ± 0.83 vs 5.06 ± 0.59, P<0.05).

Table 13. Left ventricular structural parameters

Variable	*Pre*	*Overload*	*Taper*
LVIDd (mm)	50.8 ± 2.9	51.8 ± 3.2 ↑*	50.1 ± 2.8
LV EDV (mm)	133 ± 27	138 ± 25	141 ± 23
MWT (mm)	7.2 ± 0.7	7.7 ± 0.7	7.5 ± 0.7 ↓†
LV Concentricity (g/(ml)$^{2/3}$)	5.08 ± 0.92	5.56 ± 0.83	5.06 ± 0.59 ↓†
RWT	0.30 ± 0.04	0.32 ± 0.03	0.32 ± 0.03
LV Mass (g)	133 ± 33	148 ± 32	137 ± 24

* P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

Left Ventricular Function and Mechanics

Left ventricular functional data are summarised in Table 14, and mechanical data are summarised in Table 15. No changes in conventional measures of LV function were observed between PRE, OL and TA time-points. LV Twist was decreased at OL compared to PRE (15.3 ± 3.3 vs 17.7 ± 4.5 °, P<0.05).

Left Ventricular Exercise Function and Mechanics

Exercise LV function data are summarised in Table 16, and exercise mechanical data are summarised in Table 17. Medial A' was increased at OL (12 ± 2 cm/s) compared to both PRE and TA (9 ± 3 and 10 ± 2 cm/s, both P<0.05). Exercise augmentation of LV Twist was greater at OL compared to PRE (9.2 ± 7.8 vs 2.2 ± 6.5 °, P<0.05). Longitudinal SRS was decreased at OL compared to PRE (-1.16 ± 0.08 vs -1.26 ± 0.12 S^{-1}, P<0.05). In contrast, Circumferential SRE was increased at OL compared to PRE (-2.33 ± 0.40 vs -1.89 ± 0.19 S^{-1}, P<0.05).

Table 14. Left ventricular conventional resting functional parameters

Variable	*Pre*	*Overload*	*Taper*
LV EF (%)	60 ± 6	60 ± 5	60 ± 5
LV SV (ml)	80 ± 16	82 ± 14	84 ± 11
MV E (cm/s)	0.90 ± 0.19	0.92 ± 0.14	0.94 ± 0.13
MV A (cm/s)	0.44 ± 0.11	0.44 ± 0.09	0.40 ± 0.10
MV E:A	2.13 ± 0.56	2.19 ± 0.40	2.48 ± 0.54
Medial S' (cm/s)	10 ± 1	9 ± 1	9 ± 1
Medial E' (cm/s)	17 ± 1	17 ± 1	17 ± 2
Medial A' (cm/s)	7 ± 2	7 ± 2	7 ± 2
Lateral S' (cm/s)	12 ± 2	11 ± 2	12 ± 3
Lateral E' (cm/s)	20 ± 2	21 ± 3	20 ± 2
Lateral A' (cm/s)	6 ± 2	7 ± 1	6 ± 1

* P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

Table 15. Resting Speckle Tracking Echocardiographic parameters

Variable	*Normal Training*	*Overload*	*Taper*
Global Longitudinal Strain (%)	-20.2 ± 1.0	-19.2 ± 1.3	-19.6 ± 0.8
Peak Longitudinal SRS (S^{-1})	-0.95 ± 0.09	-0.89± 0.08	-0.91 ± 0.07
Peak Longitudinal SRE (S^{-1})	1.87 ± 0.12	1.88 ± 0.10	1.90 ± 0.16
Peak Longitudinal SRA (S^{-1})	0.50 ± 0.09	0.53 ± 0.13	0.53 ± 0.10
Peak Global Circumferential Strain (%)	-21.1 ± 2.2	-21.2 ± 1.8	-21.1 ± 1.9
Peak Circumferential SRS (S^{-1})	-1.06 ± 0.20	-1.01 ± 0.13	-1.01 ± 0.09
Peak Circumferential SRE (S^{-1})	1.87 ± 0.42	1.87 ± 0.15	1.82 ± 0.29
Peak Circumferential SRA (S^{-1})	0.36 ± 0.09	0.38 ± 0.08	0.40 ± 0.10
Peak LV Twist (^{o})	17.7 ± 4.5	15.3 ± 3.3 ↓*	17.7 ± 4.3

* P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

Table 16. Conventional functional parameters (50% HR max)

Variable	*Pre*	*Overload*	*Taper*
LV EF (%)	67 ± 5	63 ± 3	64 ± 6
LV SV (ml)	84 ± 14	86 ± 15	85 ± 20
MV E (cm/s)	1.17 ± 0.20	1.12 ± 0.18	1.21 ± 0.20
MV A (cm/s)	0.64 ± 0.20	0.81 ± 0.16	0.79 ± 0.14
MV E:A	2.00 ± 0.64	1.51 ± 0.29	1.54 ± 0.26
Medial S' (cm/s)	11 ± 2	11 ± 1	12 ± 1
Medial E' (cm/s)	19 ± 4	19 ± 2	19 ± 2
Medial A' (cm/s)	9 ± 3	12 ± 2 ↑*	10 ± 2 ↓†
Lateral S' (cm/s)	14 ± 2	15 ± 3	13 ± 3
Lateral E' (cm/s)	21 ± 2	21 ± 3	20 ± 3
Lateral A' (cm/s)	11 ± 3	12 ± 3	12 ± 3

* P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

Table 17. 50% HR max Speckle Tracking Echocardiographic Parameters

Variable	*Pre*	*Overload*	*Taper*
Global Longitudinal Strain (%)	-21.8 ± 1.7	-20.8 ± 1.1	-21.4 ± 1.4
Peak Longitudinal SRS (S^{-1})	-1.26 ± 0.12	-1.16 ± 0.08 ↓*	-1.19 ± 0.09
Peak Longitudinal SRE (S^{-1})	-2.30 ± 0.32	-2.21 ± 0.21	-2.26 ± 0.25
Peak Longitudinal SRA (S^{-1})	-1.03 ± 0.18	-0.86 ± 0.26	-0.99 ± 0.21
Peak Global Circumferential Strain (%)	-20.8 ± 1.9	-21.7 ± 1.5	-21.0 ± 1.4
Peak Circumferential SRS (S^{-1})	-1.22 ± 0.17	-1.19 ± 0.10	-1.22 ± 0.12
Peak Circumferential SRE (S^{-1})	-1.89 ± 0.19	-2.33 ± 0.40 ↑*	-2.17 ± 0.34
Peak Circumferential SRA (S^{-1})	-0.77 ± 0.32	-0.79 ± 0.37	-0.82 ± 0.29
Peak LV Twist (^{o})	17.4 ± 5.4	22.3 ± 7.0	20.1 ± 5.9

* P<0.05 vs Normal Training, ** P<0.001 vs Normal Training
† P<0.05 vs Overload, †† P<0.001 vs Overload

6.4 Discussion

This is the first study to determine the impact of short-term overload endurance training on LV structure, function and mechanics at rest, and under exercise-stress using speckle-tracking echocardiography. Subjects presented with an acute fatigue and subsequent supercompensation response to training, whereby performance was maintained at OL (despite high perceived fatigue), and subsequently improved at TA. The main findings of the present study were; 1) a trend towards reduced systolic function and a shift towards late diastolic filling in-exercise following OL, 2) a trend towards increased LV twist, and decreased GL SRS in-exercise following OL, and 3) a return of all functional and mechanical parameters to baseline following the TA period.

LV Structure

Literature regarding short-term structural adaptation of the endurance athlete's heart is sparse. Although it is generally accepted that high dynamic, high static component sports (such as cycling) result in eccentric hypertrophy of the LV (Brown et al., 2017), the process of ventricular remodelling is less clear. Assumptions that chamber dilatation and wall thickness development take place concurrently are largely based on cross-sectional examinations.

The landmark work of Weiner et al. (2015) described LV remodelling (in response to a HDHS stimulus) to be a phasic process, whereby chamber dilatation precedes development of wall thickness. Subsequently, this short-term adaptive response has been observed in elite soccer athletes, where one month of pre-season (when training hours increase) resulted in an eccentric-type remodelling of the LV (D'Ascenzi et al.,

2015). In contrast, dilatation of the LV chamber with no change in volume or wall thickness was observed in response to OL. It is unclear whether this process of structural remodelling can be attributed to the shorter timescale of the present study (and thus a normal physiological response), or if chamber dilatation represents an adaptation or potential mal-adaptation specific to the OL training stimulus. More detailed information regarding training hours, and rate of progressive overload in particular, are needed to provide context for this disparity in findings.

LV Function

Previous characterisations of resting LV systolic function in response to structured endurance training have described increased systolic tissue velocities (Baggish et al., 2008; Weiner et al., 2015), and increased (Weiner et al., 2010) or unchanged (Weiner et al., 2015; Oxborough et al., 2019) GLS. Particular attention has been paid to endurance training induced alterations in LV twist (Weiner et al., 2010; Aksakal et al., 2013; Weiner et al., 2015), with longitudinal assessments observing significant short-term (3 months) increases in this parameter, followed by a return to baseline levels in the longer-term (3-year) (Weiner et al., 2015). As training-induced increases in blood volume and chamber expansion are proposed as primary mechanisms to facilitate increases in LV twist (through elevated pre-load) (Weiner et al., 2015), it is pertinent to understand why this cohort presented with significant decreases in LV twist following short-term OL. Furthermore, the chamber dilatation observed in this cohort (with no change in wall thickness) would appear to pre-dispose the LV to greater levels of peak twist, as obliquely orientated myocardial fibres are arranged around a larger cavity (at basal level) (van Dalen et al., 2010).

The finding of decreased LV twist, and a trend towards decreased GLS (P=0.063) could therefore be explained in two ways: 1) development of a functional reserve capacity, meaning the LV does not need to contract as forcefully at rest to generate an appropriate SV, or 2) as a mild form of the systolic dysfunction observed in cases of acute EICF (Nottin et al., 2009; Lord et al., 2018b).

As SV remained similar to PRE despite a trend towards greater in-exercise augmentation of LV twist (P=0.056), it appears more likely this mechanical alteration represents a compensatory effect for mild (non-significant) systolic dysfunction in longitudinal and/or radial planes, rather than development of a function reserve capacity. The OL-induced decrease in exercise longitudinal SRS bears close resemblance to acute post-marathon (Oxborough et al., 2010b) and ultra-marathon (Oxborough et al., 2011; La Gerche et al., 2012b) assessments of the LV, providing further support for LV twist acting as a compensatory mechanism.

The trend observed towards a lower exercise LV EF following OL (P=0.056) is in stark contrast to previous descriptions of the athlete's heart phenotype, and does not appear to represent a normal physiological response to training (Millar et al., 2017; Claessen et al., 2018). This mild presentation of reduced LV EF bears closer resemblance to the description of acute cardiac fatigue at rest (Lord et al., 2018b).

Application of exercise stress also revealed specific diastolic alterations which appear at odds with conventional physiological adaptation of the LV. In contrast to the training-induced increases in resting E', and a trend towards increased MV E:A (P=0.06)

reported by (Weiner et al., 2010), an increase in Medial A' and a trend towards increased MV A was observed in the present study. These findings are indicative of a shift towards late diastolic filling, an atypical physiological adaptation for the AH phenotype (Brown et al., 2017). In the case of acute exercise-induced cardiac fatigue, a shift from early- to late-diastolic filling can be accounted for by altered loading conditions or changes in chamber compliance, forcing the atrial component to compensate (Neilan et al., 2006).

As subjects abstained from exercise in the 24 hours prior to echocardiographic assessment, it is unlikely the large changes in loading conditions responsible for diastolic impairment in acute exercise induced cardiac fatigue are relevant to this study. TDI is less load dependent than Doppler (George et al., 2005), and as such, changes are more likely to represent impaired chamber compliance/relaxation.

The compromised exercise augmentation of LV function at 50% HR_{max} observed following short term OL, is likely to be more significant at higher intensities, where diastolic filling time is reduced and a greater ejection fraction is required. The potential negative consequences of OL induced LV dysfunction for endurance performance are therefore clear for road cyclists, who regularly sustain >80 % HR_{max} for extended periods in both training and racing contexts (van Erp, Sanders and de Koning, 2019). Future research should seek to develop an over-reaching (rather than acute fatigue) response to OL, to confirm this.

Limitations

The small sample size utilised in this study means interpretations of outcomes are limited to healthy competitive male and female road cyclists aged 20-26 years. Whether similar outcomes would be achieved with subjects of a different age or training modality requires further study.

In-exercise echocardiographic assessment restricted to 50% HR_{max} to retain image quality in this study. Although data in this study provides new insight into OL-induced mechanical and functional alterations of the LV, the methodological challenge of echocardiographic assessment at intensities >50% HR_{max} mean proposed alterations at maximal exercise intensities remain speculative.

Measurement (and manipulation) of overload in this study was restricted to training hours only, rather than training load as a function of training quantity x intensity. The contribution of training load generated by exercise intensity during overload and taper periods were not controlled in this study, and may have impacted on specific adaptations occuring during these periods (Seiler, 2010).

The use of both male and female subjects is a strength of this study. However, the 9-week protocol employed will have caused laboratory data collection to have occurred at different points within female subjects' menstrual cycles. Although athletic performance and conventional measures (except resting MV E:A) are not affected by the menstrual cycle (Fuenmayor, Ramírez and Fuenmayor, 2000; Kishali et al., 2006) the impact on mechanical measures of the LV are not known.

Conclusions

Short-term OL training elicited acute fatigue and a subsequent supercompensation in endurance performance for competitive level road cyclists. Acute fatigue was associated with dilatation of the LV, and a maintenance of global function (despite decreased LV twist) at rest. Exercise stress revealed mildly reduced LV EF and a shift towards late diastolic filling with altered ventricular mechanics in acutely fatigued subjects. All structural, functional and mechanical adaptations elicited by OL training returned to baseline levels following TA.

These findings suggest a mild form of EICF may be expressed by athletes following the common practice of OL endurance training, even when a performance decrement is not present. As a result, the potential role of LV dysfunction (and thus cardiac output) in performance decrements observed in cases of over-reaching and/or overtraining should be considered. Furthermore, as in-exercise assessment is commonly used to clarify ambiguous echocardiographic parameters in road cyclists, these data highlight the importance of standardising the timing of pre-participation screening to account for OL training induced alterations in LV function/mechanics.

Further work is required to elucidate the mechanisms of OL training-induced alterations in LV structure, function and mechanics. Assessment of the over-reached athlete's LV in response to dobutamine infusion should be considered to exclude beta-adrenergic receptor de-sensitisation. The sensitivity of the RV to acute EICF (Elliott and La Gerche, 2015) also makes it an ideal candidate for assessment in over-reached athletes, to investigate the impact of serial ventricular interaction.

Chapter 7 **General Discussion**

7.1 Aims of Thesis

The work in this thesis enabled the following objectives to be achieved: 1) to establish the impact of moderate and very high training loads on structural, functional and mechanical adaptation of the road cyclist's LV; 2) to determine how the LV responds to variations in training hours across a competitive road cycling season; 3) to assess the impact of short-term overload endurance training on LV structure-function-mechanical relationships of the road cyclist's LV; 4) to evaluate the relationship between LV function and road cycling performance following short-term overload endurance training.

7.2 Brief Summary of Findings

A summary of key findings generated by the work in this thesis is presented in figure 19.

Chapter 4 highlighted that the high dynamic, high static nature of road cycling alone was not sufficient to develop marked structural remodelling of the LV. Significant differences in chamber volume and wall thickness between EC and SEC provide strong evidence for the role of chronic high training hours as a primary driver for development of the AH phenotype. Furthermore, this study demonstrated concentric remodelling does not represent a normal or common physiological adaptation, as previously described (Abergel et al., 2004). In stark contrast, over one-third of this group presented with eccentric hypertrophy. The extent of structural remodelling presented by EC was also associated with reduced conventional measures of systolic and diastolic function, which are suggestive of a considerable functional reserve. Similar GL ε values between

EC, SEC and NA groups provide support for the application of this robust STE measure in a pre-participation screening setting.

Chapter 5 aimed to elucidate alterations in structure-function relationships of the LV in response to varying training hours. In agreement with previous assessments of the LV in rowers (Weiner et al., 2015) and soccer players (D'Ascenzi et al., 2015), a progressive increase in LV mass was observed in parallel with accumulation of training hours. No change in LV MWT or concentricity were observed during this period, suggesting an eccentric-type remodelling precedes hypertrophic adaptation found in athletes with a chronic high training hours. RC presented with a transient decrease in diastolic function at the point training hours increased most sharply (+80%). This appeared similar in nature (but not severity) to previous descriptions of EICF (Lord et al., 2018b).

Chapter 6 aimed to determine the impact of the common overload endurance training model employed in road cycling, on structure-function relationships of the LV. This study revealed significant eccentric-type remodelling of the LV can be expected within 3 weeks, when an overload training model is applied. In contrast to previous descriptions of normal physiological training adaptation (Weiner et al., 2010) where performance is improved, a decrease in LV twist and a trend towards decreased GL ε was observed in acutely fatigued athletes. Furthermore, exercise echocardiography revealed decreased systolic function with altered mechanics, and a shift towards late diastolic filling following OL.

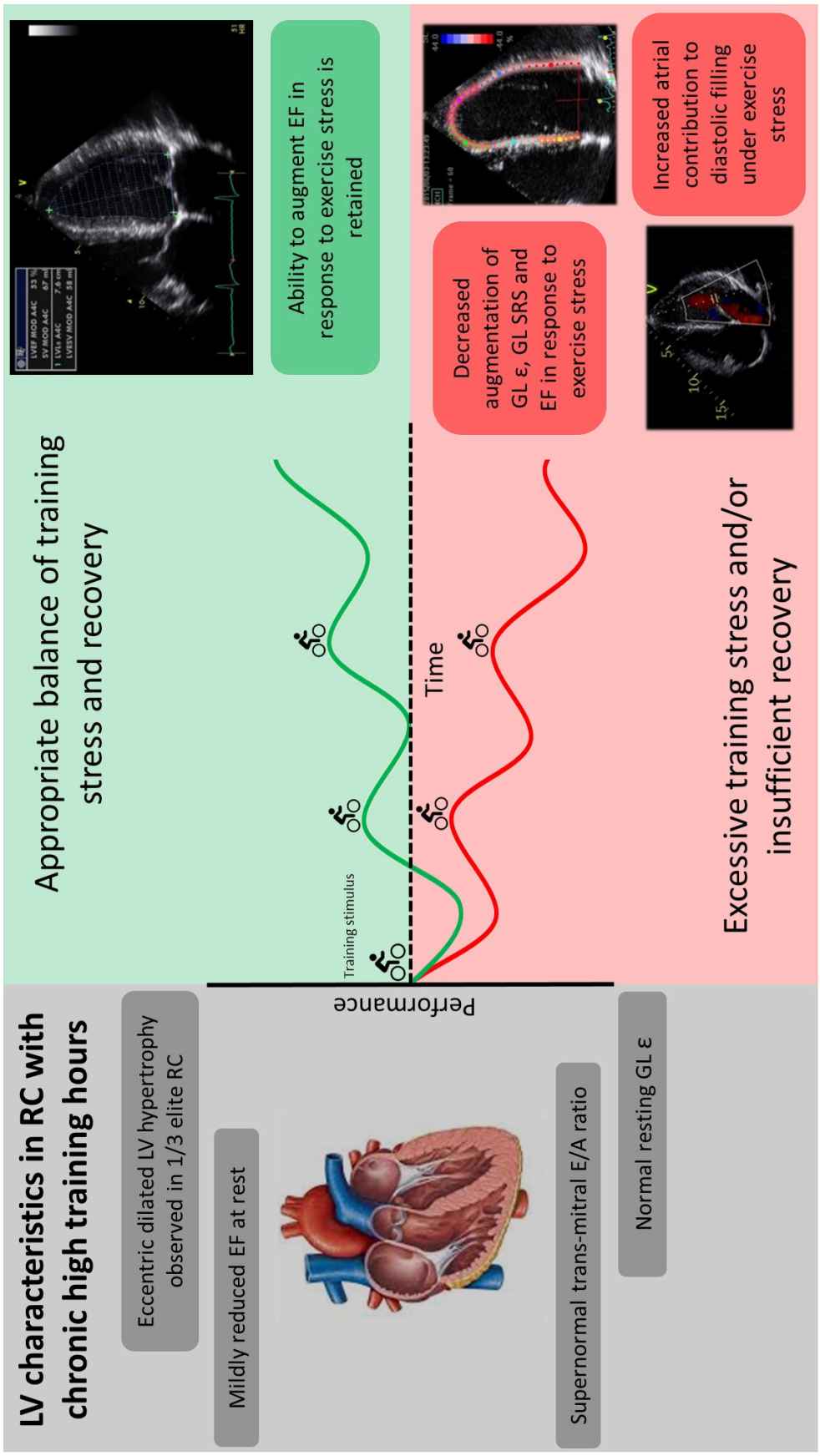

Figure 19. Overview of key findings generated by the work in this thesis

Following a 2-week taper period whereby training quantity was reduced, all functional and mechanical alterations had normalised to baseline values. These findings provide new evidence that mild EICF may be extended from an acute phenomenon to medium-term in the case of overload HDHS training.

Overall, the results from this thesis have developed understanding of how high training quantity impacts LV structure, function and mechanics in short, medium- and long-term timeframes. In addition to resting echocardiographic data, exploratory in-exercise assessment of the LV has provided new insight into the ability of the AH phenotype to augment function (or not) dependent upon previous training quantity. The mechanisms of diminished LV function in response to overload training appear complex and multifactorial.

7.3 Overarching Issues

This thesis aimed to develop understanding of training-load induced adaptation of the LV, and to gain new insight into the timeframe and mechanisms responsible. Current understanding of training-load induced LV adaptation is predominantly based on cross-sectional assessment of athletes presumed to have high chronic training loads (Whyte et al., 2004; Makan et al., 2005; Basavarajaiah et al., 2008; Santoro et al., 2014; Utomi et al., 2014; Caselli et al., 2015), and acute assessment of athletes completing a highly strenuous single exercise bout (Neilan et al., 2006; Hart et al., 2007; Nottin et al., 2009; Oxborough et al., 2010b; Oxborough et al., 2011; La Gerche et al., 2012b; Elliott and La Gerche, 2015; Lord et al., 2015). Of the few studies to consider medium-term adaptation in a longitudinal design, only one has examined athletes from a high dynamic, high static component sport (Weiner et al., 2015).

Chronic Training Load

Studies within this thesis have confirmed the role of training hours in determining the magnitude and nature of structural, function and mechanical adaptation of the LV. Chapter 4 confirmed that there is a predominance of normal LV geometry in high dynamic, high static athletes, as previously suggested (Utomi et al., 2014). However, over one-third of EC (completing very high training hours) presented with eccentric dilated LV hypertrophy. This magnitude of structural remodelling appears to be reserved for athletes completing extreme training hours, as SEC almost exclusively presented with normal LV geometry (96.7%). In agreement with recent cross-sectional analyses of the AH phenotype, concentric hypertrophy was found to be rare in EC (3.3%), and almost exclusively accompanied by chamber dilation (2.7%).

In chapter 5, a progressive eccentric type remodelling between off- and end-season was demonstrated in competitive road cyclists, in tandem with training quantity. These findings support the theory that chronic high training quantity (independent of intensity) forms the principal driver for long-term structural adaptation of the LV. The timeframe of data collection utilised in chapter 5 did not, however, capture the increase in LV concentricity observed between SEC and EC in chapter 4. These findings therefore appear to be in concurrence with the phasic adaptation proposed by (Weiner et al., 2015), but confirm that the initial phase of remodelling may be extended to at least 10 months. The mechanism that initiates the secondary phase of adaptation whereby wall thickening drives further increases in LV mass remains unclear, particularly as a very high proportion (87%) of training carried out by EC is carried out below a heart rate corresponding to LT1 (resting blood lactate + 0.4 mmol/L) (Sanders et al., 2017).

LV systolic function, as measured using EF, was comparable between SEC and NA. However, like Abergel et al. (2004), significantly lower EF was observed in EC. Cases of reduced EF (<52%) were more prevalent in this group (11.6 %) than previously reported (Abergel et al., 2004; Lang et al., 2015). In addition to the marked cavity dilatation presented by these athletes, the potential for misdiagnosis of DCM is increased (Millar et al., 2017). However, in the case of the AH phenotype, this simply reflects the development of a considerable functional reserve whereby lower contractile force is required to generate an appropriate stroke volume at rest (Claessen et al., 2018). The high sensitivity and specificity of exercise echocardiography has been demonstrated in the differentiation between AH phenotype and DCM, via the ability to augment LV EF ≥10% (Millar et al., 2017). Although a small proportion of SEC presented with reduced EF in chapter 4 (6.7%), the process of generating a functional reserve large enough to influence EF appears to take longer than 10 months, or more significant training hours than those recorded in chapter 5.

In agreement with previous assessments of the AH phenotype, GL ε represented a robust measure of LV mechanics, with EC, SEC and NA presenting similar peak values despite considerable differences in chronic training quantity. Furthermore, this parameter remained unchanged despite a sustained period of training quantity elevation in chapter 5. Together, these findings provide additional support for the translation of GL ε into clinical practice, as chronic high training hours do not influence resting measurements in healthy individuals, even in extreme cases (Beaumont et al., 2017).

In contrast, increased GC ε was found to be as a characteristic of the AH phenotype in EC. Increased GC ε appears to represent a compensatory mechanism to develop appropriate SV in the presence of a vastly increased LV EDV and unchanged LV twist for these individuals. The role of GC ε in generating SV is well established, and has a far greater influence on EF compared to GL ε (67% compared to 33%) (MacIver, 2012). Increased basal circumferential ε, but not GC ε has previously been demonstrated for competitive endurance athletes (Beaumont et al., 2017). Whether the increased GC ε we observed can be attributed to the magnitude of functional reserve in EC, or if it is reflective of a different methodological approach (whereby GC ε excludes apical segments) requires further elucidation.

In addition to mild reductions in resting systolic function, structural adaptation of the LV driven by very high chronic training hours elicits reductions in early diastolic filling and early diastolic tissue velocities at rest. The present findings in EC are in agreement with Finocchiaro et al. (2018), who demonstrated a clear association between LVIDd and early diastolic tissue velocities. The mild increase in septal E' observed in SEC (despite increased LVIDd) presents a challenge to this theory. It may be the case that differential acute and chronic adaptation of the LV can be extended to sub-elite and elite development phases. In this example, differing rates of blood volume expansion and adaptive myocyte hypertrophy result in altered mechanics via the Frank-Starling mechanism. The increased pre-load demand in SEC (relative to EC) requires a more compliant chamber in comparison to EC, whereby more pronounced ventricular adaptation can accommodate elevated blood volume without challenge (Weiner et al., 2015).

LV Twist plays a key role in both systolic and diastolic function, due to its “wringing” motion that ejects blood from the ventricle, and subsequent recoil, generating a pressure gradient from the LA to LV (Weiner and Baggish, 2011). The present work observed preserved LV twist, but with a reduced apical contribution in EC, compared to SEC and NA in chapter 4. This finding of reduced apical rotation is in keeping with previous analyses of the high dynamic AH phenotype (Santoro et al., 2014; Weiner et al., 2015), and reflects the aforementioned decrease in resting pre-load associated with profound structural adaptation (Beaumont et al., 2017). As the relationship between peak apical rotation and untwist rate is reciprocal, a diminished recoil carried over to diastole in EC (Burns et al., 2009). In combination, reduced E, E’ and apical rotation could be interpreted as reduced diastolic function, likely driven by impaired chamber relaxation. However, exercise echocardiography reveals superior augmentation in the AH phenotype and acts as confirmation for a functional reserve capacity (La Gerche et al., 2012a).

Acute Overload Training

Previous descriptions of EICF have presented atypical structural, functional and mechanical adaptation of the LV in response to an acute bout of strenuous exercise (Lord et al., 2018b). The atypical adaptations can be expected to return to baseline levels in 2-28 days (Lord et al., 2018b). In chapter 6, the impact of repeated bouts of strenuous exercise with insufficient recovery periods was assessed, to determine whether EICF can be extended from an acute phenomenon, to a short-term (mal-)adaptation.

Upon completion of a 3 week overload training period, a small but significant eccentric type remodelling of the LV was observed. This appeared similar in nature to training-induced increase over a 3 month period reported in chapter 5 and previous literature (Weiner et al., 2015). This structural remodelling is in stark contrast to acute EICF however, where LV EDV can be expected to decrease (Lord et al., 2018b). That said, LV EDV alterations are heavily influenced by altered post-exercise loading conditions (Lord et al., 2018b). Subjects refrained from training for at least 6 hours prior to echocardiographic examination, to minimise the potential for post-exercise loading alterations to confound findings.

In contrast to previous assessments of EICF where systolic and diastolic function are depressed (Middleton et al., 2006; Hart et al., 2007), or short-term training-induced adaptation where systolic and diastolic function are increased (Weiner et al., 2015), no change in resting function was observed upon completion of overload training. Normal function was achieved with altered mechanics however, as resting GL ε and LV Twist decreased.

Pre-load alterations are proposed to play a role in both EICF and short-term training-induced physiological adaptation of the LV (Weiner et al., 2015). In the case of EICF, acute dehydration and decreased chamber volume are associated with reduced LV twist (Lord et al., 2018b), whereas training-induced blood volume and chamber expansion are associated with increased LV twist (Weiner et al., 2010). The observation of decreased twist in a dilated LV therefore represents an atypical adaptation, likely indicated a degree of intrinsic myocardial dysfunction. The trend identified towards

decreased GL ε appears to confirm this, as GL ε is considered a far less load-dependent mechanical measure (Marwick, 2006).

Exercise-echocardiography revealed more significant disturbances in LV function, as a result of overload training. In stark contrast to chapter 5, where a normal augmentation response to exercise is presented, overload training was associated with decreased EF and longitudinal SRS at 50% HR_{max}. These alterations provide further evidence of intrinsic contractile dysfunction. Cardiomyocyte damage (i.e. stunning) (driven by excessive wall stress), oxidative stress signalling (Vitiello et al., 2011) and β-adrenoreceptor desensitisation (driven by an extended period of elevated catecholamine) have been proposed as causative mechanisms in EICF (Nottin et al., 2009; Lord et al., 2018b).

Overload training-induced dysfunction of the HPA-axis, and resultant decreases in systemic adrenaline production has also been proposed as a potential factor driving functional and mechanical dysfunction in the LV (Le Meur et al., 2014). This is particularly prescient, as greater dysfunction becomes apparent as exercise-stress increases (Le Meur et al., 2014). That said, technological and logistical implications mean echocardiographic assessment of maximal exercise (when adrenal insufficiency is most impactful), is not feasible. Furthermore, β-adrenoreceptor desensitisation and/or HPA-axis mediated adrenal insufficiency fail to account for the reduced diastolic function observed in overloaded athletes.

Increases in late diastolic filing and tissue velocity are not characteristics of the AH phenotype. A shift from early to late diastolic filling under normal loading conditions

is indicative of impaired chamber relaxation (Neilan et al., 2006). Failure to generate a pressure gradient through relaxation/suction places a larger emphasis “active” atrial component of diastole (Neilan et al., 2006). Recent work has proposed upstream RV systolic dysfunction is likely to play a central role in EICF mediated LV diastolic dysfunction (Lord et al., 2015). Sustained periods of strenuous exercise (which characterise overload training in RC) place a disproportionate stress on the RV free wall, due to the pulmonary arteries’ inability to dilate (Elliott and La Gerche, 2015). The elevation in stress placed upon the thin RV free wall is proposed to generate myocyte damage, resulting in decreased contractile function (Lord et al., 2015). Consequently, downstream LA pre-load is decreased, and LV filling compromised (Oxborough et al., 2010b).

Decreased contractile capacity in GL ε and LV twist suggest β-adrenoreceptor desensitisation likely plays a role in overload induced LV mechanical alteration. In its dual role as a determinant of LV systolic and diastolic function, LV twist appears to present limited contractile capacity, subsequently resulting in reduced untwisting rate to generate an early diastolic pressure gradient. The additional diastolic dysfunction brought about by exercise stress raises the possibility of serial ventricular interaction, driven by upstream RV systolic dysfunction. It is possible that adrenal insufficiency plays a role in decreasing exercise longitudinal SRS, and warrants further investigation. This may be limited by practicalities of echocardiography imaging, however.

It is important to consider that findings in chapter 6 represent a very mild alteration in LV mechanics and function. This is likely due to the training hours employed, which, while high in relative terms for the subjects enrolled in this study, would not represent

a challenge for elite athletes. Nonetheless, this work provides novel evidence that mechanisms of LV dysfunction in EICF are also present following a short-term overload training structure commonly employed by RC.

7.4 Implications

Clinical/research perspective

The data presented in this thesis provide further evidence that development of the AH phenotype is a phasic phenomenon, whereby initial adaptation is characterised by an eccentric-type chamber remodelling (as shown in chapters 5 and 6), and the secondary stage includes increased chamber concentricity (as in chapter 4). Irrespective of development stage, pronounced concentric hypertrophy is unlikely to represent a physiological adaptation, and warrants investigation.

Athletes with marked structural remodelling are likely to present with depressed systolic and diastolic function at rest. Exercise echocardiography represents a sensitive and specific method to differentiate functional reserve capacity from cardiomyopathy. However, augmentation may be compromised in the case of an acutely fatigued athlete, increasing the likelihood of false-positive identification of pathology. Clinicians should carefully consider the timing and context of screening to ensure appropriate action is taken in this case.

The long-term consequences of repeated overload training periods on the LV remain unknown. The proposed long-term negative impact of repeated assaults on the RV with insufficient recovery suggests LV diastolic function may be challenged via serial

ventricular interaction (Heidbüchel and La Gerche, 2012). The findings in this thesis suggest cardiomyocyte damage is less likely to be the primary cause of intrinsic LV dysfunction, and that EICF-type symptoms should be more reversible as a result.

Performance/athletic perspective

Cardiac output plays a central role in determining endurance capacity (Levine, 2008). Chronic high training hours, and sustained periods of high dynamic, high static training result in marked structural remodelling of the LV to facilitate this. An appropriate balance between training stimulus and recovery opportunity facilitate maintenance of normal in-exercise LV function. In contrast, overload training stimulates a mild presentation of EICF, whereby function and mechanics are compromised. Although no impact on SV was observed at 50% HR_{max} (albeit with reduced EF), the work of Le Meur et al. (2014) suggests this may not be the case at higher intensities. Overload training induced LV dysfunction is therefore likely to have a direct impact on endurance performance.

7.5 Future Research

This thesis presents novel information concerning LV structure-function-mechanics relationships in response to short-term and chronic high training hours. The finding that short-term overload training elicits reductions in LV function during exercise has clear implications for the capacity to generate cardiac output, and by association, endurance performance. Future research should therefore seek to clarify the mechanisms which drive overload induced reductions in LV systolic and diastolic function, and provide practical surrogate measures which provide; 1) applied sport scientists the opportunity

to monitor and manipulate athletes' response to training in the field, and 2) sports cardiologists with context when determining the cause of ambiguous findings.

From a methodological perspective, this would require perturbation of the HPA-axis, oxidative stress, blood volume and biomarkers of cardiomyocyte damage to be measured alongside in-exercise bi-ventricular echocardiographic assessment, likely during an event which pre-disposes athletes to development of an overreached state (i.e. Grand Tour in RC).

This knowledge would provide valuable insight to applied sport scientists, guiding the use of data gathered from practical tests such as plasma adrenaline concentration (Le Meur et al., 2014), oxidative stress index (Lewis et al., 2020), and serum cTnT concentration (Shave et al., 2010) to manage training stress-recovery balance more effectively in the pursuit of endurance performance. Furthermore, generation of normal reference ranges for these parameters could be used by clinicians in a secondary/tertiary care setting to enhance differential diagnosis of pathology/overload induced dysfunction in ambiguous cases.

7.6 Overall Conclusions

The application of conventional and novel echocardiographic techniques provided additional understanding of how short-term to chronic high training quantity influences adaptation of the AH phenotype. Short- to medium- term structural adaptation of the LV is characterised by eccentric type remodelling, likely due to blood volume

expansion (and thus pre-load). Chronic high training quantity is associated with increased chamber concentricity, often resulting in eccentric-dilated LV hypertrophic geometry.

Stable or conservative short-term increases in training hours do not impact LV function or mechanics, whereas athletes undertaking rapid overload training present with decreased function and altered mechanics at rest. Somewhat confoundingly, athletes with very high chronic training hours also present with reduced LV function and mechanics at rest. Exercise echocardiography represents a specific, sensitive means to differentiate reserve capacity (in the case of athletes with very high chronic training hours) and genuine dysfunction in the case of fatigued athletes, where augmentation is compromised.

New insights into the differential responses to short-term and chronic high training quantities are likely to improve the sensitivity of pre-participation in RC. Furthermore, these insights bear importance in the context of athletic overtraining-, overreaching- and/or acute fatigue mediated underperformance.

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Appendices

Appendix 1 – Seasonal Variation in the Cardiac Structure, Function and Mechanics of Competitive Road Cyclists: Sex-specific echocardiographic outcomes.

Table 18. Male and female left ventricular structural parameters

Variable	*Group*	*Off-Season*		*Pre-Season*		*Mid-Season*		*End-Season*	
		M	F	M	F	M	F	M	F
LVd (mm)	RC	53.6 ± 1.4	51.0 ± 3.0	52.8 ± 0.8	50.5 ± 5.5	54.6 ± 1.7	51.5 ± 2.5	54.0 ± 0.9	51.0 ± 3.0
	NA	49.3 ± 3.9	47.3 ± 1.8	47.0 ± 1.6	45.5 ± 4.0	48.0 ± 4.3	46.8 ± 4.1	50.3 ± 1.2	47.8 ± 3.5
LV EDV (ml)	RC	152 ± 12	123 ± 7	168 ± 4	119 ± 1	158 ± 11	123 ± 5	167 ± 9	133 ± 7
	NA	135 ± 33	109 ± 18	135 ± 28	114 ± 12	130 ± 26	118 ± 22	140 ± 16	111 ± 19
MWT (mm)	RC	7.9 ± 0.5	6.9 ± 0.2	8.6 ± 0.2	7.1 ± 0.6	8.9 ± 0.2	7.3 ± 0.4	8.5 ± 0.3	6.8 ± 0.6
	NA	7.2 ± 0.4	6.2 ± 0.2	7.4 ± 0.1	6.4 ± 0.3	7.3 ± 0.5	6.2 ± 0.4	6.8 ± 1.1	6.4 ± 0.3
LV Concentricity ($g/(ml)^{2/3}$)	RC	5.4 ± 0.4	4.8 ± 0.7	5.4 ± 0.3	5.2 ± 0.6	6.1 ± 0.1	5.1 ± 0.1	5.9 ± 0.4	4.5 ± 0.5
	NA	4.6 ± 0.8	4.5 ± 1.1	4.2 ± 0.5	3.7 ± 0.6	4.1 ± 0.3	3.6 ± 0.8	4.1 ± 0.5	4.2 ± 1.1
LV Mass (g)	RC	152 ± 8	118 ± 12	166 ± 10	126 ± 13	178 ± 10	125 ± 6	180 ± 18	117 ± 9
	NA	119 ± 20	99 ± 15	109 ± 8	86 ± 11	104 ± 20	84 ± 12	112 ± 23	95 ± 15

Table 19. Male and female left ventricular conventional resting functional parameters

Variable	*Group*	*Off-Season*		*Pre-Season*		*Mid-Season*		*End-Season*	
		M	F	M	F	M	F	M	F
LV EF (%)	RC	55 ± 4	64 ± 4	56 ± 4	55 ± 2	57 ± 5	62 ± 0	58 ± 2	59 ± 1
	NA	58 ± 4	58 ± 6	57 ± 4	56 ± 6	55 ± 2	57 ± 4	59 ± 4	56 ± 4
MV E (cm/s)	RC	0.93 ± 0.08	0.91 ± 0.01	0.82 ± 0.07	0.84 ± 0.08	0.83 ± 0.08	1.02 ± 0.12	0.94 ± 0.01	0.85 ± 0.03
	NA	0.92 ± 0.06	0.98 ± 0.17	0.92 ± 0.07	1.02 ± 0.12	0.84 ± 0.09	1.00 ± 0.16	0.81 ± 0.05	1.04 ± 0.11
MV A (cm/s)	RC	0.41 ± 0.04	0.40 ± 0.06	0.42 ± 0.01	0.48 ± 0.06	0.42 ± 0.01	0.45 ± 0.01	0.42 ± 0.02	0.43 ± 0.01
	NA	0.44 ± 0.06	0.53 ± 0.08	0.43 ± 0.06	0.57 ± 0.10	0.40 ± 0.03	0.56 ± 0.21	0.39 ± 0.05	0.49 ± 0.09
MV E:A	RC	2.27 ± 0.27	2.32 ± 0.32	1.98 ± 0.16	1.77 ± 0.04	1.97 ± 0.25	2.29 ± 0.24	2.33 ± 0.13	1.96 ± 0.01
	NA	2.13 ± 0.42	1.95 ± 0.66	2.14 ± 0.16	1.82 ± 0.33	2.10 ± 0.35	2.02 ± 0.65	2.07 ± 0.17	2.22 ± 0.62
Medial S' (cm/s)	RC	9 ± 1	8 ± 0	9 ± 1	7 ± 1	9 ± 1	8 ± 1	9 ± 1	8 ± 1
	NA	10 ± 1	9 ± 1	10 ± 1	9 ± 1	9 ± 1	9 ± 2	9 ± 2	9 ± 1
Medial E' (cm/s)	RC	16 ± 3	16 ± 1	15 ± 2	14 ± 0	16 ± 1	16 ± 1	15 ± 2	17 ± 1
	NA	14 ± 1	13 ± 3	15 ± 4	16 ± 1	14 ± 3	16 ± 3	15 ± 2	16 ± 3
Medial A' (cm/s)	RC	7 ± 1	6 ± 0	7 ± 1	6 ± 1	7 ± 2	6 ± 0	8 ± 1	6 ± 0
	NA	9 ± 2	8 ± 2	10 ± 1	8 ± 2	8 ± 1	8 ± 1	8 ± 1	6 ± 0
Lateral S' (cm/s)	RC	12 ± 2	11 ± 2	11 ± 0	10 ± 2	10 ± 1	10 ± 1	11 ± 2	11 ± 2
	NA	13 ± 1	11 ± 1	12 ± 4	11 ± 2	12 ± 2	11 ± 2	13 ± 3	10 ± 1
Lateral E' (cm/s)	RC	19 ± 4	20 ± 1	19 ± 1	21 ± 2	21 ± 1	20 ± 3	19 ± 1	21 ± 1
	NA	23 ± 3	20 ± 4	21 ± 3	19 ± 3	21 ± 2	20 ± 2	21 ± 2	19 ± 4
Lateral A' (cm/s)	RC	6 ± 1	6 ± 0	7 ± 0	7 ± 1	6 ± 1	8 ± 2	6 ± 0	6 ± 0
	NA	8 ± 1	8 ± 3	9 ± 1	7 ± 2	7 ± 1	8 ± 1	7 ± 1	8 ± 2

Table 20. Male and female resting Speckle Tracking Echocardiography (STE) parameters

Variable	*Group*	*Off-Season*		*Pre-Season*		*Mid-Season*		*End-Season*	
		M	F	M	F	M	F	M	F
Global Longitudinal Strain (%)	RC	-18.0 ± 1.8	-21.4 ± 0.4	-19.3 ± 1.6	-19.2 ± 2.5	-18.5 ± 1.9	-20.6 ± 0.1	-18.4 ± 0.7	-20.9 ± 1.4
	NA	-19.1 ± 1.8	-20.9 ± 2.0	-18.7 ± 2.5	-21.1 ± 0.7	-18.4 ± 1.3	-20.2 ± 1.6	-19.4 ± 1.5	-21.6 ± 1.7
Peak Global Circumferential Strain (%)	RC	-19.5 ± 0.9	-19.9 ± 1.3	-19.0 ± 1.5	-19.2 ± 0.6	-19.1 ± 0.5	-18.1 ± 0.2	-19.0 ± 0.9	-18.8 ± 3.4
	NA	-20.8 ± 0.8	-19.9 ± 3.1	-20.1 ± 1.3	-18.8 ± 1.4	-19.6 ± 0.8	-18.1 ± 2.0	-20.7 ± 1.1	-19.6 ± 1.2
Peak LV Twist (º)	RC	16.8 ± 5.5	19.6 ± 3.5	17.6 ± 3.5	9.6 ± 4.5	14.3 ± 2.6	13.2 ± 1.3	18.2 ± 4.8	7.5 ± 2.1
	NA	22.0 ± 2.2	18.8 ± 4.5	19.4 ± 2.4	12.7 ± 1.3	23.7 ± 7.6	17.7 ± 6.8	22.6 ± 3.7	13.4 ± 2.5
Peak Basal Rotation (º)	RC	-5.4 ± 1.0	-9.4 ± 0.4	-4.7 ± 1.5	-4.9 ± 1.0	-5.6 ± 1.5	-8.4 ± 1.7	-6.1 ± 1.2	-4.6 ± 1.6
	NA	-7.7 ± 2.3	-9.3 ± 4.5	-7.2 ± 1.4	-8.6 ± 3.6	-5.9 ± 1.8	-9.7 ± 2.0	-6.6 ± 2.3	-7.0 ± 2.4
Peak Apical Rotation (º)	RC	12.1 ± 5.1	10.2 ± 3.1	14.1 ± 5.1	5.3 ± 3.0	9.9 ± 3.2	5.1 ± 0.6	13.7 ± 6.3	4.1 ± 0.1
	NA	14.4 ± 1.7	9.5 ± 1.0	12.7 ± 1.3	6.7 ± 2.8	17.6 ± 7.7	11.5 ± 3.4	14.5 ± 1.1	7.7 ± 2.3

Table 21. Male and female 50% HR max Speckle Tracking Echocardiography (STE) parameters

Variable	*Group*	*Off-Season*		*Pre-Season*		*Mid-Season*		*End-Season*	
		M	F	M	F	M	F	M	F
Global Longitudinal Strain (%)	RC	-19.9 ± 1.7	-24.3 ± 0.8	-19.3 ± 2.4	-21.7 ± 1.8	-19.0 ± 1.6	-25.1 ± 1.7	-19.9 ± 0.5	-22.7 ± 1.7
	NA	-21.6 ± 1.9	-23.1 ± 2.3	-21.0 ± 1.8	-22.9 ± 1.4	-19.5 ± 1.6	-21.2 ± 1.9	-20.5 ± 1.1	-23.6 ± 1.4
Peak Global Circumferential Strain (%)	RC	-20.2 ± 1.5	-22.6 ± 0.9	-21.8 ± 0.7	-20.0 ± 1.2	-21.3 ± 0.5	-22.9 ± 1.0	-21.5 ± 1.0	-20.7 ± 0.8
	NA	-22.5 ± 1.1	-22.3 ± 2.6	-22.7 ± 0.5	-21.3 ± 2.4	-19.8 ± 3.5	-20.2 ± 3.8	-20.7 ± 2.5	-20.5 ± 1.9
Peak LV Twist (°)	RC	17.3 ± 2.9	9.8 ± 3.2	21.8 ± 4.9	16.0 ± 4.2	20.2 ± 4.5	10.9 ± 3.1	21.2 ± 6.1	12.2 ± 3.6
	NA	31.0 ± 6.6	17.5 ± 1.0	29.7 ± 5.3	21.5 ± 5.8	28.0 ± 6.6	20.8 ± 3.5	30.4 ± 5.7	24.8 ± 4.9

Appendix 2 – Ethical Approval (Seasonal Variation in the Cardiac Structure, Function and Mechanics of Competitive Road Cyclists)

Dear Ben

With reference to your application for Ethical Approval:

17/SPS/006 – Ben Brown, PGR - Changes in Cardiac Structure, Function and Mechanics During the Competitive Road Cycling Season

The University Research Ethics Committee (UREC) considered the above application by proportionate review. 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 e.g. 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/93205.htm

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 March 2022. An application for extension of approval must be submitted if the project continues after this date.

Mandy Williams

Research Support Officer

Appendix 3 – Ethical Approval (The Impact of Short-Term Overload Training on Cardiac Mechanics in Trained Road Cyclists)

Dear Ben

With reference to your application for Ethical Approval:

16/SPS/036 Ben Brown, PGR - Cardiac Adaptation in Response to Short-Term Overload Training (David Oxborough/Keith George)

The University Research Ethics Committee (UREC) has considered the above application by Chairs action and 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 e.g. poster, information sheets, consent forms, questionnaires. The LJMU logo can be accessed at http://www2.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://www2.ljmu.ac.uk/RGSO/93205.htm

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 July 2021. An application for extension of approval must be submitted if the project continues after this date.

Mandy Williams

Research Support Officer

Appendix 4 – Participant Information Sheet (Seasonal Variation in the Cardiac Structure, Function and Mechanics of Competitive Road Cyclists)

LIVERPOOL JOHN MOORES UNIVERSITY
PARTICIPANT INFORMATION SHEET

Title of Project: Changes in cardiac structure, function and mechanics during the competitive road cycling season

Name of Researcher: Mr Benjamin Brown, Research Institute for Sport and Exercise Sciences.

You are being invited to take part in a research study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

1. What is the purpose of the study?

Athletes commonly place large amounts of stress upon the cardiovascular system during training and competition. As a precaution, it is common practice to carry out pre-participation cardiac screening to ensure there are no underlying pathologies which could trigger a cardiac event during exercise. Currently, there is no consensus regarding the time point at which cardiovascular screening should be carried out.

The aim of this study is to investigate how changes in training load over the course of a road cycling season impact on the normal variation of structure and function of the heart.

2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do 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. A decision to withdraw will not affect any future treatment.

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

In total, four testing periods will be carried out (at pre-, mid-, end-, and off-season time points). Each testing period includes the following:

Firstly, you will be required to wear a heart rate monitor during all of your normal training sessions over a 2 week period. The results of each training session will be shared with a researcher, allowing training volume and intensity to be calculated.

Upon completion of this 2 week monitoring period, you will be invited to the Liverpool John Moores University laboratories to complete a health questionnaire, an assessment of height, weight and resting blood pressure, an exercise test to establish fitness (VO2 max test), an assessment of the electrical activity of the heart (12-lead electrocardiogram (ECG)), and an

ultrasound scan of the heart (an echocardiogram) at rest and during a short exercise stimulus. In total, each laboratory visit will take approximately 2.5 hours, and is divided up as follows:

Exercise VO2 max Test

The VO2 max assessment is a test of aerobic fitness. This test will be carried out on a stationary exercise bike in the laboratory. You will be asked to wear a soft rubber mask, from which oxygen and carbon dioxide levels are monitored while you cycle. After a 5 minute period of cycling at moderate intensity,

resistance will be increased every 2 minutes until you are no longer able to continue to cycle. This test will take between 8 and 30 minutes depending on fitness.

12-Lead ECG

The 12-lead ECG will take approximately 10 minutes and requires you to lie still on your back while a researcher places small stickers across your chest, and on your wrists and ankles. The stickers will be connected to a machine via 10 leads. You will be instructed to breathe normally and relax while your heart beat is recorded and a graph will be printed from the ECG machine.

Resting Echocardiogram

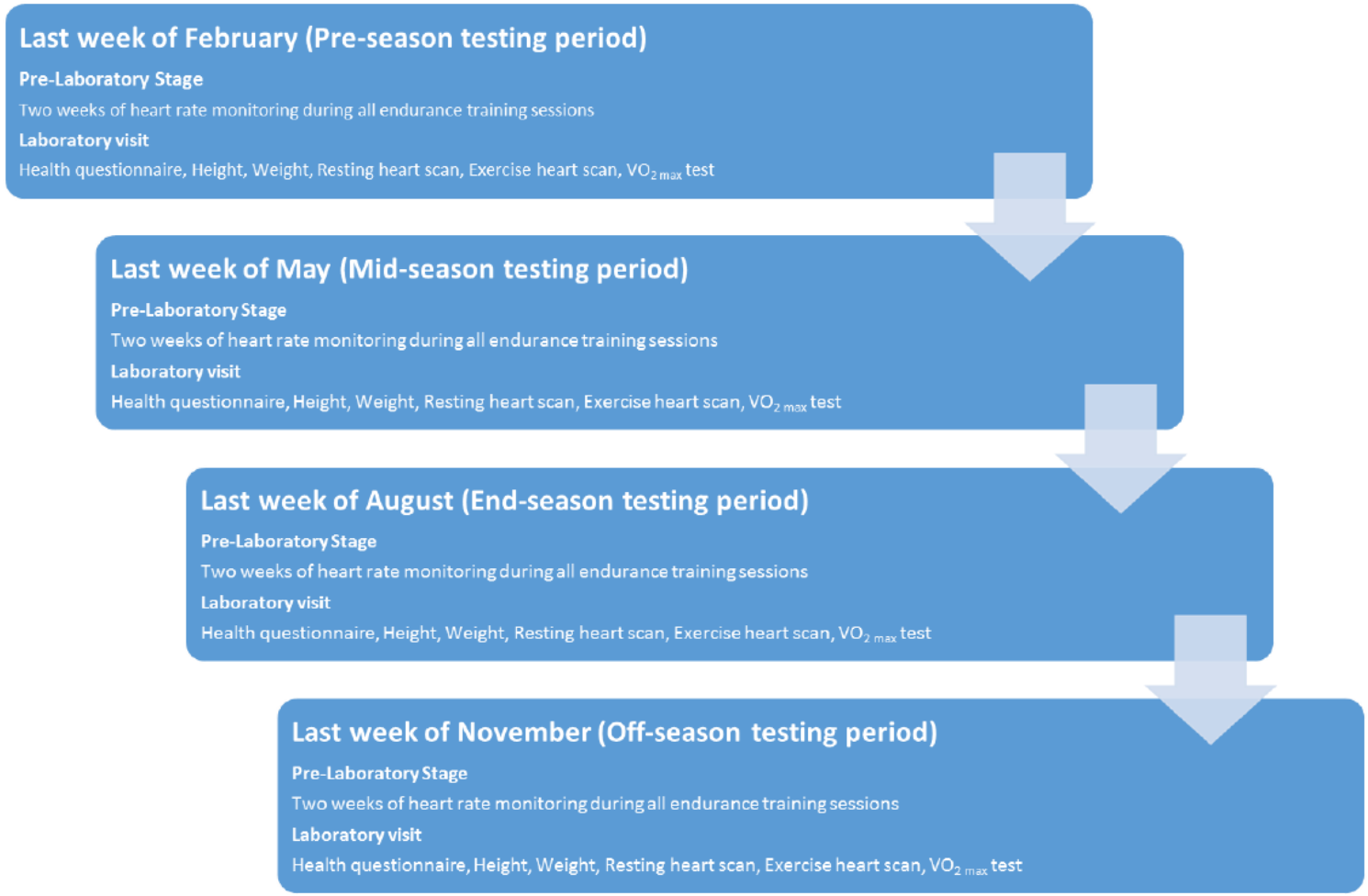
The echocardiographic assessment will take approximately 15 minutes, and requires you to lie on the left side of your body while an ultrasound probe is placed upon your chest. The ultrasound probe will be moved across your chest to take images/video clips of the heart from various angles.

Exercise Echocardiogram

The second part of the echocardiographic assessment will take approximately 20 minutes, and will involve cycling while lying on a specially adapted bed. When your heart rate hits specific targets, the bed will be tilted on an angle, and more images and video clips will be taken as you continue to cycle.

Please arrive for laboratory testing sessions in a well-hydrated state, having abstained from consumption of caffeine and alcohol in the 6 hours prior to testing.

Please see full protocol diagram overleaf


Last week of February (Pre-season testing period)
Pre-Laboratory Stage
Two weeks of heart rate monitoring during all endurance training sessions
Laboratory visit
Health questionnaire, Height, Weight, Resting heart scan, Exercise heart scan, $VO_{2\,max}$ test
Last week of May (Mid-season testing period)
Pre-Laboratory Stage
Two weeks of heart rate monitoring during all endurance training sessions
Laboratory visit
Health questionnaire, Height, Weight, Resting heart scan, Exercise heart scan, $VO_{2\,max}$ test
Last week of August (End-season testing period)
Pre-Laboratory Stage
Two weeks of heart rate monitoring during all endurance training sessions
Laboratory visit
Health questionnaire, Height, Weight, Resting heart scan, Exercise heart scan, $VO_{2\,max}$ test
Last week of November (Off-season testing period)
Pre-Laboratory Stage
Two weeks of heart rate monitoring during all endurance training sessions
Laboratory visit
Health questionnaire, Height, Weight, Resting heart scan, Exercise heart scan, $VO_{2\,max}$ test

4. Are there any risks / benefits involved?

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.

It is important to be aware that this is not a complete cardiac screening and discrete underlying disease or sub-clinical disease that affects the coronary arteries may not be detected.

If at any point during each of these protocols you feel uncomfortable (or unable to continue), testing will be ceased immediately.

The benefits from involvement in this study include a cardiac health check (please be aware that this is not a full cardiac screening) and you will also receive an accurate assessment of your aerobic fitness which may will 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 which only the researcher and academic supervisory team will have access to. Upon 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.

This study has received ethical approval from LJMU's Research Ethics Committee (17/SPS/006)

Contact Details of Researcher
B.Brown@2011.ljmu.ac.uk

Contact Details of Academic Supervisor
D.L.Oxborough@ljmu.ac.uk

If you any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

Appendix 5 – Participant Information Sheet (The Impact of Short-Term Overload Training on Cardiac Mechanics in Trained Road Cyclists)

LIVERPOOL JOHN MOORES UNIVERSITY PARTICIPANT INFORMATION SHEET

Title of Project: Cardiac adaptation to normal and short-term overload training

Name of Researcher: Mr Benjamin Brown, Research Institute for Sport and Exercise Sciences.

You are being invited to take part in a research study. Before you decide it is important that you understand why the research is being done and what it involves. Please take time to read the following information. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to take part or not.

1. What is the purpose of the study?

Athletes commonly place large amounts of stress upon the cardiovascular system during training and competition. As a precaution, it is common practice to carry out pre-participation cardiac screening to ensure there are no underlying pathologies which could trigger a cardiac event during exercise. Currently, there is no consensus regarding the time point at which cardiovascular screening should be carried out.

The aim of this study is to investigate how short-term increases in training load may impact the structure and function of the heart, and whether changes caused by training load could affect diagnostic interpretation of tests undertaken for cardiovascular screening.

2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do 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. A decision to withdraw will not affect any future treatment.

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

Testing will be carried out over a 9 week period, with all subjects completing periods of "normal training", "overload training" and "tapered training" periods.

Firstly, you will be invited to the Liverpool John Moores University laboratories to complete 3 pre-testing sessions. These sessions will consist of:

Pre-Testing

Visit One (approximately 2 hours)

- Health questionnaire
- Assessment of height, weight and resting blood pressure
- A resting heart rate variability assessment

- Exercise $VO_{2\ max}$ test to establish aerobic fitness
- Instructions on how to use an actigraphic wristwatch, which you will use to monitor your sleep for the following week

Session Two (approximately 1.5 hours)
- A resting assessment of the electrical activity of the heart (12-lead ECG)
- A resting ultrasound scan of the heart (resting echocardiogram)
- An in-exercise ultrasound assessment of the heart (exercise echocardiogram)
- A cycling sprint test (30 second Wingate test)

Session Three (approximately 1.5 hours)
- A training and recovery questionnaire
- In-exercise blood pressure assessment
- Simulated 10 mile cycling time trial, including pre- and post- venous blood samples

Normal Training

You will then complete two weeks of normal training. Each training week will consist of high intensity intermittent training (HIIT) sessions, carried out on a Wattbike within the Liverpool John Moores University laboratories, and road based training sessions (on your own bicycle). Heart rate variability will be assessed in the university laboratories three times per week, prior to HIIT sessions. You also will be required to wear a heart rate monitor (provided) for all training sessions. Each "normal training" week will consist of 12 training hours.

Taper Period 1

After three weeks of normal training have been completed, a one week taper period will take place. All procedures carried out in pre-testing will be repeated, to monitor changes which have occurred following three weeks of training. This taper period will consist of 6 training hours.

Overload Training

A three week period of overload training will then commence. Training will consist of the same HIIT and road-based training sessions, and heart rate variability assessment and heart rate monitoring will continue. All procedures carried out in pre-testing (apart from $VO_{2\ max}$) will be re-tested in week 3 of this period. Each overload training week will consist of 19 training hours.

Taper Period 2

Finally, you will undertake a two week taper period. All pre-testing procedures will be re-tested in weeks one and two of this phase (apart from $VO_{2\ max}$).

Each of the testing processes are described below:

Exercise $VO_{2\ max}$ Test

The $VO_{2\ max}$ assessment is a test of aerobic fitness. This test will be carried out on a stationary exercise bike in the laboratory. You will be asked to wear a soft rubber mask, from which oxygen and carbon dioxide levels are monitored while you cycle. After a 5 minute period of cycling at moderate intensity, resistance will be increased every 2 minutes until you are no longer able to continue to cycle. This test will take between 8 and 30 minutes depending on fitness.

30 Second Wingate
The 30 second Wingate test is a test of cycling sprinting ability. This test will be carried out on a stationary exercise bike in the laboratory. After completing a 5 minute period of cycling at moderate intensity, and a 2 minute resting period, you will be asked to sprint as fast as possible against a high resistance for 30 seconds.

Simulated 10 mile Cycling Time Trial
The simulated 10 mile time trial is a cycling specific test of endurance ability. This test will be carried out on a stationary exercise bike in the laboratory. 30 minutes before your structured warm-up commences, a venous cannula will be fitted to your arm by a trained phlebotomist. Immediately prior to your warm-up, a small blood sample will be taken via the cannula. You will then complete a 10 minute, structured warm up, and a 2 minute resting period. You will be asked to complete the 10 mile time trial as quickly as possible. Upon completion of the time trial, a second venous blood sample will be taken via the cannula.

Heart Rate Variability
The heart rate variability assessment will take approximately 15 minutes and requires you to lie still on your back while wearing a heart rate monitor. You will be instructed to breathe normally and relax while your heart beat is recorded.

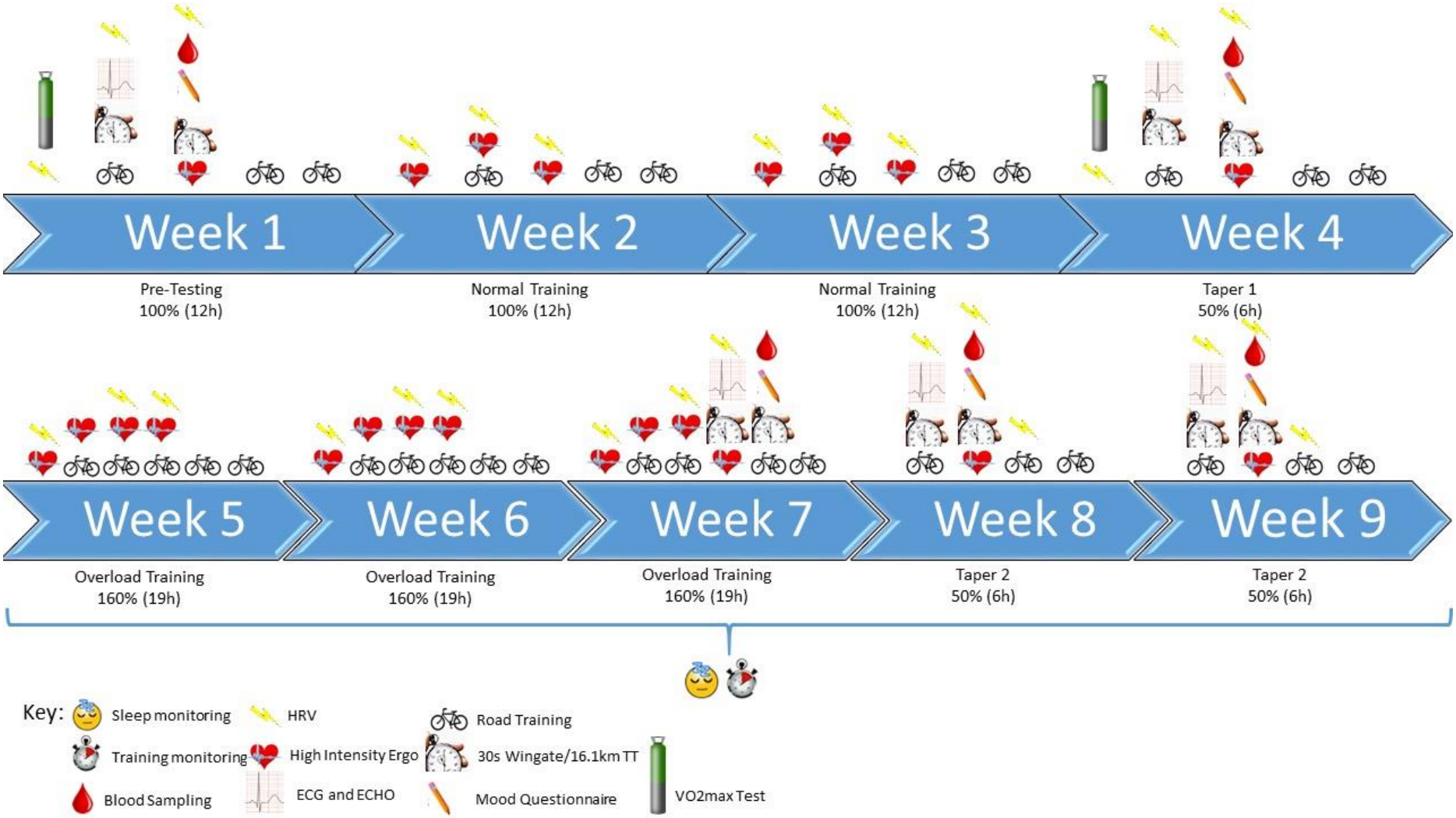
Sleep Monitoring
Upon your first visit to the Liverpool John Moores University laboratories, you will be presented with a wrist-worn actigraphy watch. Once you have been instructed how to use the watch, you will be asked to record each night's sleep for the duration of the study.

12-Lead ECG
The 12-lead ECG will take approximately 10 minutes and requires you to lie still on your back while a researcher places small stickers across your chest, and on your wrists and ankles. The stickers will be connected to a machine via 10 leads. You will be instructed to breathe normally and relax while your heart beat is recorded and a graph will be printed from the ECG machine.

Resting Echocardiogram
The echocardiographic assessment will take approximately 15 minutes, and requires you to lie on the left side of your body while an ultrasound probe is placed upon your chest. The ultrasound probe will be moved across your chest to take images/video clips of the heart from various angles.

Exercise Echocardiogram
The second part of the echocardiographic assessment will take approximately 20 minutes, and will involve cycling while lying on a specially adapted bed. When your heart rate hits specific targets, the bed will be tilted on an angle, and more images and video clips will be taken as you continue to cycle.

Please see full protocol diagram overleaf


Week 1
Week 2
Week 3
Week 4
Pre-Testing
100% (12h)
Normal Training
100% (12h)
Normal Training
100% (12h)
Taper 1
50% (6h)
Week 5
Week 6
Week 7
Week 8
Week 9
Overload Training
160% (19h)
Overload Training
160% (19h)
Overload Training
160% (19h)
Taper 2
50% (6h)
Taper 2
50% (6h)
Key:
Sleep monitoring
HRV
Road Training
Training monitoring
High Intensity Ergo
30s Wingate/16.1km TT
Blood Sampling
ECG and ECHO
Mood Questionnaire
VO2max Test

4. Are there any risks / benefits involved?

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 employed by Liverpool John Moores University, and the appropriate action will be undertaken i.e. referral to secondary or tertiary care.

The high volume of exercise involved in this project is likely to cause fatigue and muscle soreness. In addition to this, previous research suggests this level of intensive training may cause small, short-term decreases in cardiac function. These training induced changes are reversible, and generally return to normal within 1 week. Regular echocardiographic screening will allow us to assess the nature of these changes throughout the training period. In the very unlikely case of a significant cardiac abnormality being presented, we will be able to advise you to make an appointment with your GP, or refer you to secondary/tertiary care if appropriate.

If at any point during each of these protocols you feel uncomfortable (or unable to continue), testing will be ceased immediately.

In addition to receiving free screening for CV disease, participants will be given the VO_{2max} which may will 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 which only the researcher and academic supervisory team will have access to. Upon 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.

This study has received ethical approval from LJMU's Research Ethics Committee (16/SPS/036, 22/07/17)

Contact Details of Researcher
B.Brown@2011.ljmu.ac.uk

Contact Details of Academic Supervisor
D.L.Oxborough@ljmu.ac.uk

If you any concerns regarding your involvement in this research, please discuss these with the researcher in the first instance. If you wish to make a complaint, please contact researchethics@ljmu.ac.uk and your communication will be re-directed to an independent person as appropriate.

Appendix 6 – Participant Consent Form (Seasonal Variation in the Cardiac Structure, Function and Mechanics of Competitive Road Cyclists)

LIVERPOOL JOHN MOORES UNIVERSITY CONSENT FORM

Changes in cardiac structure, function and mechanics during the competitive road cycling season

Mr Benjamin Brown
Research Institute for Sport and Exercise Sciences

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 take part in the above study ☐

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

Appendix 7 – Participant Consent Form (The Impact of Short-Term Overload Training on Cardiac Mechanics in Trained Road Cyclists)

LIVERPOOL JOHN MOORES UNIVERSITY CONSENT FORM

Cardiac Adaptation to Normal and "Short-Term Overload Training

Mr Benjamin Brown
Research Institute for Sport and Exercise Sciences

5. 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 ☐

6. 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. ☐

7. I understand that any personal information collected during the study will be anonymised and remain confidential ☐

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

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

Appendix 8 – Cardiac Screening Questionnaire

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

Cardiac Screening Health Questionnaire

Full Name *(Include parents names if under 16)*:	**Date of Screening:**

Personal Details

Home *(correspondence)* address:			Doctors name and Address:
POSTCODE:			**POSTCODE:**
Phone Number:			Phone Number:
E-mail:			
Date of Birth:	Age:	Gender:	Have you had Heart tests before?
Do you have any illnesses?			List illnesses
Are you taking any medication:			List medications

Which country were you born in? …….

Ethnicity *(please tick the appropriate box)*

White	**Mixed**	**Black**	**Asian**	**Other**
British ☐	White and Black Caribbean ☐	Caribbean ☐	Indian ☐	Chinese ☐
Irish ☐	White and Black African ☐	East African ☐	Pakistani ☐	Filipino ☐
European ☐	White and Asian ☐	West African ☐	Bangladeshi ☐	Vietnamese ☐
Turkish /Cypriot ☐		North African ☐		Other ☐
Greek /Cypriot ☐				
If other, please state your ethnic origin:				

Height …….cm	**Weight ……..Kg**	**Blood Pressure ……/…….mmHg**

1. Have you ever fainted?

During Exercise	Yes / No	How recently did this occur?	If yes, please describe the circumstances
Following Exercise	Yes / No	How recently did this occur?	
Unrelated to exercise	Yes / No	How recently did this occur?	

2. Do you experience dizzy turns?

During Exercise	Yes / No	How recently did this occur?	If yes, please describe the circumstances
Following Exercise	Yes / No	How recently did this occur?	
Unrelated to exercise	Yes / No	How recently did this occur?	

Based on CRY (Cardiac Risk in Young) questionnaire 2010 Prof John Somauroo 16.8.12

Professor John Somauroo

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3. Do you experience palpitations? *(palpitations are a fluttering in your chest that you can notice whilst resting)*

Yes / No	If yes, how recently and please describe the circumstances

4. Do you experience chest pain, heaviness or tightness?

During Exercise	Yes / No	If yes, please describe the circumstances
Following Exercise	Yes / No	
Unrelated to exercise	Yes / No	

5. Do you feel that you are more breathless or more easily tired than your team mates?

Yes / No	If yes, please describe the circumstances

6. Is there a family history of (please tick):

High Blood Pressure ☐ High Cholesterol ☐ Diabetes ☐

7. Is there a family history of heart disease in anyone under the age of 50?

Yes / No	If yes, please state the age of onset

8. Has anyone died suddenly in your family under the age of 50?

Yes / No	If yes, please describe the circumstances and at what age did the death occur

9. Approximately, how many days per week are you physically active (playing sport)? .....

10. On average, how many hours per week are you physically active (playing sport)? .....

11. If you are competitive athlete what sports do you play and at what level?

e.g. International, National, County, Club, Other

A (main sport)..... Level: .....

B. ..... Level: .....

C. ..... Level: .....

12. How long (for how many years) have you been participated in sport? .....

13. Do you agree for the results of your screening including investigations to be discussed with the Club doctor? Yes / No

14. Do you agree for your results to be kept on a database for research in the future? Your personal identity will never be disclosed to anyone if your data is used for research. Yes / No

Signature..... **Date.....**

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Appendix 9 – RESTQ-76 Sport Questionnaire

R E S T Q - 76 Sport

Single Code: _____ Group Code: _____

Name (Last): _____ **(First):** _____

Date: _____ Time: _____ Age: _____ Gender: _____

Sport/Event(s): _____

This questionnaire consists of a series of statements. These statements possibly describe your mental, emotional, or physical well-being or your activities during the past few days and nights.

Please select the answer that most accurately reflects your thoughts and activities. Indicate how often each statement was right in your case in the past days.

The statements related to performance should refer to performance during competition as well as during practice.

For each statement there are seven possible answers.

Please make your selection by marking the number corresponding to the appropriate answer.

Example:

In the past (3) days/nights

... I read a newspaper

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In this example, the number 5 is marked. This means that you read a newspaper very often in the past three days.

Please do not leave any statements blank.

If you are unsure which answer to choose, select the one that most closely applies to you.

Please turn the page and respond to the statements in order without interruption.

In the past (3) days/nights

1) ... *I watched TV*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

2) ... *I did not get enough sleep*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

3) ... *I finished important tasks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

4) ... *I was unable to concentrate well*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

5) ... *everything bothered me*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

6) ... *I laughed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

7) ... *I felt physically bad*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

8) ... *I was in a bad mood*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

9) ... *I felt physically relaxed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

10) ... *I was in good spirits*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

11) ... *I had difficulties in concentrating*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

12) ... *I worried about unresolved problems*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In the past (3) days/nights

13) ... *I felt at ease*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

14) ... *I had a good time with friends*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

15) ... *I had a headache*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

16) ... *I was tired from work*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

17) ... *I was successful in what I did*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

18) ... *I couldn't switch my mind off*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

19) ... *I fell asleep satisfied and relaxed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

20) ... *I felt uncomfortable*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

21) ... *I was annoyed by others*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

22) ... *I felt down*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

23) ... *I visited some close friends*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

24) ... *I felt depressed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In the past (3) days/nights

25) ... *I was dead tired after work*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

26) ... *other people got on my nerves*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

27) ... *I had a satisfying sleep*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

28) ... *I felt anxious or inhibited*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

29) ... *I felt physically fit*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

30) ... *I was fed up with everything*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

31) ... *I was lethargic*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

32) ... *I felt I had to perform well in front of others*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

33) ... *I had fun*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

34) ... *I was in a good mood*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

35) ... *I was overtired*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

36) ... *I slept restlessly*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In the past (3) days/nights

37) ... *I was annoyed*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

38) ... *I felt as if I could get everything done*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

39) ... *I was upset*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

40) ... *I put off making decisions*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

41) ... *I made imporiant decisions*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

42) ... *I felt physically exhausted*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

43) ... *I felt happy*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

44) ... *I felt under pressure*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

45) ... *everything was too much for me*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

46) ... *my sleep was interrupted easily*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	alwayss

47) ... *I felt content*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

48) ... *I was angry with someone*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In the past (3) days/nights

49) ... *I had some good ideas*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

50) ... *parts of my body were aching*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

51) ... *I could not get rest during the breaks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

52) ... *I was convinced I could achieve my set goals during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

53) ... *I recovered well physically*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

54) ... *I felt burned out by my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

55) ... *I accomplished many worthwhile things in my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

56) ... *I prepared myself mentally for performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

57) ... *my muscles felt stiff or tense during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

58) ... *I had the impression there were too few breaks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

59) ... *I was convinced that I could achieve my performance at any time*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

60) ... *I dealt very effectively with my teammates' problems*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In the past (3) days/nights

61) ... *I was in a good condition physically*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

62) ... *I pushed myself during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

63) ... *I felt emotionally drained from performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

64) ... *I had muscle pain after performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

65) ... *I was convinced that I performed well*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

66) ... *too much was demanded of me during the breaks*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

67) ... *I psyched myself up before performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

68) ... *I felt that I wanted to quit my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

69) ... *I felt very energetic*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

70) ... *I easily understood how my teammates felt about things*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

71) ... *I was convinced that I had trained well*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

72) ... *the breaks were not at the right times*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

In the past (3) days/nights

73) ... *I felt vulnerable to injuries*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

74) ... *I set definite goals for myself during performance*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

75) ... *my body felt strong*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

76) ... *I felt frustrated by my sport*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

77) ... *I dealt with emotional problems in my sport very calmly*

0	1	2	3	4	5	6
never	seldom	sometimes	often	more often	very often	always

Thank you very much!