

The Effects of Running, Cycling, and Duathlon Exercise Performance on Cardiac Function,
Haemodynamics and Regulation

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated in the following sections. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Abstract

This thesis examined the effects of prolonged exercise, specifically Olympic Distance (OD) duathlon upon ultrasound derived indices of cardiac function, cardiac autonomic regulation measured via heart rate variability (HRV), and high-sensitivity cardiac troponin T (hs-cTnT) release.

The primary aims were to (1) ascertain the influence of Olympic distance (OD) duathlon performance on cardiac function; (2) to investigate potential relationships between autonomic regulation, hs-cTnT release, and cardiac function, and (3) to investigate the effect of the individual legs of an OD duathlon on post-exercise cardiac function and to quantify the potential performance reserve of highly-trained endurance athletes when completing standalone legs of the duathlon. Findings from a systematic review and meta-analysis (Chapter 1) on research that performed serial echocardiographic and troponin measurements before and after exercise, intensity predicted changes in post-exercise cardiac troponin release and diastolic function. The findings agreed with previous meta-analyses using a more recent sample of studies; however, the recommendation for future studies to implement advanced cardiac imaging techniques, such as myocardial speckle tracking into their data collection would provide a more sensitive measure of post-exercise cardiac function. Whilst a large degree of heterogeneity in the results exists, this was in part explained by study exercise heart rate, participant age, and the prevalence of cardiac troponin release above the clinical detection threshold.

The study performed in Chapter 3 was the first to investigate the effects of OD duathlon exercise on immediate and 24 hours post-exercise cardiac function. Additionally, a second OD duathlon was performed by participants with intra-leg measurements of cardiac function. In a highly trained cohort, there was evidence of transient post-exercise reductions in cardiac function and elevated serum high-sensitivity cardiac troponin T (hs-cTnT) above the clinical reference value, which was largely resolved within 24h of recovery. This study also demonstrated the reliability of lab-based duathlon exercise in a highly trained cohort and

identified the pacing features of experienced multi-sport athletes that partially explained the different findings between the running and cycling legs of the duathlon.

By investigating each leg of the duathlon individually (10k run, 5k run, 40k cycle), both at duathlon race-pace (DM) and maximal (Max) intensity on separate occasions, the performance reserve of the highly-trained cohort was quantified and further explored. The studies presented in Chapters 4 and 5 revealed that experienced duathletes were able to improve their speed across each leg by between 5-15% in a laboratory setting, compared to the duathlon effort. Additionally, the maximal effort 10k run leg provoked the most persistent changes to cardiac function that were present at 6h of recovery. Changes in cardiac function post DM 10k confirmed the findings of Chapter 3 that the greatest magnitude of cardiac perturbations occur following the initial 10k run leg. Aside from the Max 10k run and 40k cycle trials, all perturbations had resolved within 6h of recovery after each bout of exercise, highlighting the importance of recovery following maximal intensity efforts.

The lack of 6h and 24h recovery data in Chapter 4, and Chapters 5 and 6, respectively, is a shortcoming of these findings and therefore limits interpretation in the context of providing athletic guidance. Future research in this area should endeavour to include 6h and 24h recovery measures as standard, as multi-sport athletes typically perform multiple daily training sessions. The implications of substantial cardiac fatigue accumulation over many years of endurance training history are still unclear, and athletes may benefit from preventing its occurrence.

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Abbreviations

A Peak late transmitral filling velocity

A' Peak late diastolic mitral annular tissue velocity

ANOVA Analysis of variance

ANS Autonomic nervous system

BP Blood pressure

Ca²⁺ Calcium

CI Confidence interval

CoV Coefficient of variation

cTn Cardiac troponin

cTnT Cardiac troponin isoform type T

cTnI Cardiac troponin isoform type I

dBp Diastolic blood pressure

E Peak early transmitral filling velocity

E' Peak early diastolic mitral annular tissue velocity

ECG Electrocardiogram

EDV End diastolic volume

ESV End systolic volume

ET Ejection time

FAC Fractional area change

HF High frequency

HR Heart rate

HRV Heart rate variability

hs High sensitivity

IVSd Interventricular septal diameter

LF Low frequency

LFnu Low frequency normalised units

LV Left ventricle

LVEF Left ventricular ejection fraction

LVIDd Left ventricular internal diameter diastole

LVIDs Left ventricular internal diameter systole

LVLS Left ventricular longitudinal strain

LVSRA Left ventricular late diastolic longitudinal strain rate

LVSRE Left ventricular early diastolic longitudinal strain rate

LVSRS Left ventricular systolic longitudinal strain rate

LVPWd Left ventricular posterior wall thickness diastole

mBP Mean blood pressure

MV Mitral valve

PSAX Parasternal short axis

PSE Prolonged strenuous exercise

PSD Power spectral density

PW Pulsed wave

PWd Diastolic left posterior wall thickness

PWs Systolic left posterior wall thickness

\dot{Q} Cardiac output

RA Right atrium

ROI Region of interest

RV Right ventricle

RVLS Right ventricular longitudinal strain

RVSR A Right ventricular late diastolic longitudinal strain rate

RVSR E Right ventricular early diastolic longitudinal strain rate

RVSR S Right ventricular systolic longitudinal strain

RVOT Right ventricular proximal outflow tract dimension

sBP Systolic blood pressure

SD Standard deviation

ε Strain

SR Strain Rate

SR A Peak late diastolic strain rate

SR E Peak early diastolic strain rate

SR S Peak systolic strain rate

SV Stroke volume

TAPSE Tricuspid annular plane systolic excursion

TDI Tissue Doppler imaging

TFM Task Force® Monitor

$\dot{V}O_2$ Oxygen consumption

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Chapter 1 Literature Review

1.1 Introduction

Prolonged exercise causes functional and structural changes (Hellsten and Nyberg, 2015b) in the human heart that we widely perceive as being beneficial to our health (Franklin et al., 2020a). Decades of research in the field has established that regular endurance exercise is a stimulus for beneficial cardiovascular adaptations such as improved vagal tone and vascularisation, strengthened cardiac contractility, and reduced blood pressure (Hermansen et al., 1970, Rowell, 1974, Douglas et al., 1990, Millard-Stafford et al., 1991, Cheng et al., 1992, Eysmann et al., 1996, Wannamethee et al., 2000, Shave et al., 2004, La Gerche et al., 2004, Seiler et al., 2007, La Gerche and Prior, 2007, Hellsten and Nyberg, 2015a, O'Keefe et al., 2020). These positive adaptations contribute towards a lower overall risk of acute coronary syndrome and coronary heart disease, proven by physically active populations demonstrating reduced incidence of cardiovascular disease in comparison to sedentary individuals (Wannamethee et al., 2000). Regular physical exercise also reduces the chance of developing unstable angina, non-ST segment elevation myocardial infarction, and ST-elevated myocardial infarction (Kumar and Cannon, 2009).

However, as the occurrence of exercise induced sudden cardiac death has become more widely noticed, research groups have explored the theory that adverse cardiac adaptations (Lindsay and Dunn, 2007) or events (Harris et al., 2017) may take place in response to 'excessive' levels of exercise (O'Keefe et al., 2020). Accordingly, there is a large body of research, which has explored the relationship between exercise characteristics and measures of resulting cardiac damage or dysfunction. Endurance sport performance requires continuously elevated heart rates (HR), aerobic metabolism, and musculoskeletal and thermoregulatory strain (González-Alonso et al., 2008), and the prevalence and potential causes of adverse cardiac events during and following bouts of prolonged strenuous exercise (PSE) have been carefully studied (Franklin et al., 2020a, O'Keefe et al., 2012, Yankelson et al., 2014, Belonje et al., 2007). Generally, the most at-risk individuals for adverse cardiac

events during PSE are seemingly those who are unaccustomed to PSE, those with underlying heart health conditions, and events that are held in conditions where heat-stroke is more likely to occur (Belonje et al., 2007). The body of research has not yet established a causal link between potential adverse cardiac adaptations and exercise history, or to specific exercise characteristics such as mode, intensity, or duration.

Such bouts of PSE have previously been shown to perturb normal cardiac performance, influence autonomic regulation, influence biomarker release, and myocardial membrane health, as well as impact cardiac function (Stewart et al., 2014, Stewart et al., 2015, Stewart et al., 2016). A large body of research exists, which documents diminished cardiac performance following ‘ultra’ endurance exercise events such as a marathon or Ironman triathlon (La Gerche et al., 2008, Lord et al., 2018). Most of the research has previously focused on very long-duration exercise, and the findings of Middleton et al’s (2006) meta-analysis concluded that exercise duration was the strongest predictor of exercise induced cardiac fatigue (EICF) (Middleton et al., 2006). Recently however, the effects of short duration, high intensity bouts of exercise have been investigated and there is strong evidence for the role of exercise intensity in determining cardiac fatigue and biomarker release. Evidence shows that bouts as short as 45 – 60 minutes of high-intensity exercise results in marked cardiac fatigue and biomarker release (Kleinnibbelink et al., 2021b, Stewart et al., 2015).

Acute reductions in left and right ventricular mechanics (Oxborough et al., 2006, Oxborough et al., 2011), elevations in plasma levels of cardiac biomarkers (Legaz-Arrese et al., 2015) and disruption of cardiac parasympathetic/sympathetic innervation (Aagaard et al., 2014, Seiler et al., 2007), have all occurred following PSE of varying modes, durations and intensities. Despite this, most research suggests that the consequences of PSE are often transient, with post-exercise measurements showing a return to sub-clinical levels within 24-72 hours of rest (Shave et al., 2010a, Oxborough et al., 2010, Shave and Oxborough, 2012). Furthermore, several studies have demonstrated that PSE can leave the right ventricle more vulnerable to episodes of arrhythmia (Oxborough et al., 2011, Lord et al., 2015). To assess

the reversibility of EICF, most study designs involve post-exercise measurements following 24 hours of rest. One potential flaw in such a methodology may be the inclusion of a 24-hour period of rest, as this may not exist within a typical athlete's training schedule. This is especially true for elite-endurance and multisport athletes who will often include multiple high-intensity sessions within a 24-hour period (Millet et al., 2011).

Accordingly, of great interest is whether the temporary alterations in cardiac performance arising from PSE are cumulative, ultimately leading to pathological adaptations such as arrhythmogenic right ventricular cardiomyopathy and hypertrophic cardiomyopathy (O'Keefe et al., 2012). These are the two leading pathologies that are present in victims of sudden cardiac death (Franklin et al., 2020a). Wilson and colleagues (2011) have previously hypothesised that myocardial fibrosis is mediated by the multiple loading of high intensity exercise, which, followed by insufficient recovery, causes scar tissue to form. Evidence exists to support this claim, including high coronary artery calcium scores and evidence of late gadolinium enhancement in lifetime masters (age >50 years) endurance athletes (Lindsay and Dunn, 2007, Wilson et al., 2011a, La Gerche et al., 2012a, O'Keefe et al., 2012, Schnell et al., 2016, van de Schoor et al., 2016, Pujadas et al., 2018). It is therefore possible that endurance training may lead to fibrosis of the myocardium and the development of these cardiomyopathies (Wilson et al., 2011a), which can lead to life-threatening arrhythmia (O'Keefe et al., 2020).

Therefore, the long-term effects of PSE on cardiovascular health are less certain, and the specifics of the stimulus-response mechanism for exercise induced cardiac dysfunction remains to be fully elucidated. Additionally, the role that the mode of exercise plays in potential cardiac dysfunction and injury is still unclear and requires further investigation. For example, the characteristics of upright vs. seated exercise influence cardiac loading conditions. Elevated preload has been shown to increase cardiac troponin (cTn) release (Feng et al., 2001), therefore the lower peak exercise HRs, SV and reduced efficiency of the peripheral muscular pump during cycling exercise may cause smaller perturbations following cycling exercise when compared to running exercise (Millet et al., 2009). Additionally, the

work of Wilson and colleagues (2011), found evidence of fibrotic infiltration of the myocardium, in a cohort of runners (Wilson et al., 2011a). Therefore, it may be the case that running exercise is the most damaging and requires a relatively greater amount of recovery. The meta-analysis of exercise induced cTn release by Shave et al. (2007) has shown that heavier individuals are more likely to demonstrate an elevation in cTn following strenuous exercise, which may be explained due to the eccentric nature of weight bearing exercise, such as running (Shave et al., 2007). Unfortunately, no studies to date have investigated potential intra-individual differences in the extent of cardiac fatigue between weight-bearing i.e., running, and non-weight bearing exercise, such as cycling. The majority of primary evidence indicates that the magnitude of EICF and cTn release appears to be related to exercise HR and duration (Donaldson et al., 2019, Lord et al., 2018, Middleton et al., 2006, Shave et al., 2010a, Shave et al., 2007, Shave and Oxborough, 2012). Whole-body dynamic exercise such as running, and cycling are the most widely studied modes and are the most participated in endurance sports globally (Hulsteen et al., 2017). While several previous studies have investigated EICF following triathlon races, there have been no similar studies on duathlons (run-bike-run). Duathlon is a popular alternative to triathlon during the off-season and are completed by athletes at amateur to the elite level. Standard or Olympic distance (OD) triathlon consists of a 1500m swim, 40k cycle and 10k run, and the OD duathlon involves a 10k run, 40k cycle and 5k run. It remains to be determined whether duathlon competitions and training potentially incurs a greater level of cardiac fatigue due to the additional run leg. The purpose of the following chapter will be to review the literature and to provide an overview and assess current methodologies used to assess cardiac function and mechanics following exercise. Further, it will review the current literature with reference to the effects of exercise intensity and duration, and the effect of differing exercise modalities.

1.2 Cardiac Function

1.2.1 Normal Cardiac Function

The normal cardiac cycle is composed of the two separate phases of diastole (cardiac relaxation), and systole (cardiac contraction). The right atrium (RA) receives deoxygenated blood from the systemic circulation, which fills the right ventricle (RV) and is ejected into the pulmonary circulation. Passing through the lungs, the oxygenated blood returns to the left atrium (LA) and into the left ventricle (LV) to be pumped into the systemic circulation (Herring, 2018). The pulmonary circulation is under lower pressure than the systemic, and therefore the RV is required to do less work to achieve the same output as the LV. As a result of this, RV mass is approximately one quarter of the LV (Herring, 2018). The cardiac cycle involves four separate phases: diastole (early filling, diastasis, atrial contraction), and systole.

The components of diastole form two thirds of a normal cardiac cycle (see figure 1.1.1). During diastole, both atria and ventricles are relaxed and blood flows passively through the atria from the superior and inferior vena cava into the right heart, and from the four pulmonary veins into the left heart, past the interventricular valves into the relaxed ventricles. Relaxation of the ventricles causes ventricular pressure to drop rapidly, creating suction, which leads to rapid filling of the ventricle during the early filling phase (E) (Matsuo et al., 1977). The rate of filling is decreased (diastasis) as the ventricles reach their relaxed volume and further volume and pressure increase is driven by venous pressure (Fleming et al., 1994). Atrial contraction (A) is the final phase of diastole where further blood is pumped into the ventricle (Matsuo et al., 1977). The amount of blood added during atrial systole increases with age from around 10-20% in young adults up to 46% by the age of 80 (Herkner et al., 2000). Additionally, elevated heart rates during exercise cause reduced passive filling time and atrial contribution augments cardiac function to meet metabolic and functional demands (Faulkner et al., 1971). The volume of blood in the ventricle at the end of diastole is the end diastolic volume (EDV), which serves as a measure of ventricular sarcomere length (also known as preload). Left ventricular EDV ranges from 100–200 mL in males aged 20-80 in the supine position (Kawel-Boehm et al., 2015). The Frank-Starling mechanism describes

how increased preload, caused by the stretching of the myocardium during diastole, improves cardiac contractility and subsequent systolic function as an outcome of the elastic properties of stretched cardiac myocytes (Delicce and Makaryus, 2021). When supine, equivocal gravitational forces on upper and lower bodily segments increase venous return, thus EDV is increased approximately 30% over standing. The corresponding pressure (EDP) is slightly higher in the LV than the RV as the LV wall is generally thicker (6-12mm LV wall thickness vs. 3-5mm RV wall thickness) and therefore requires higher pressure to cause deformation (Vizza et al., 1998).

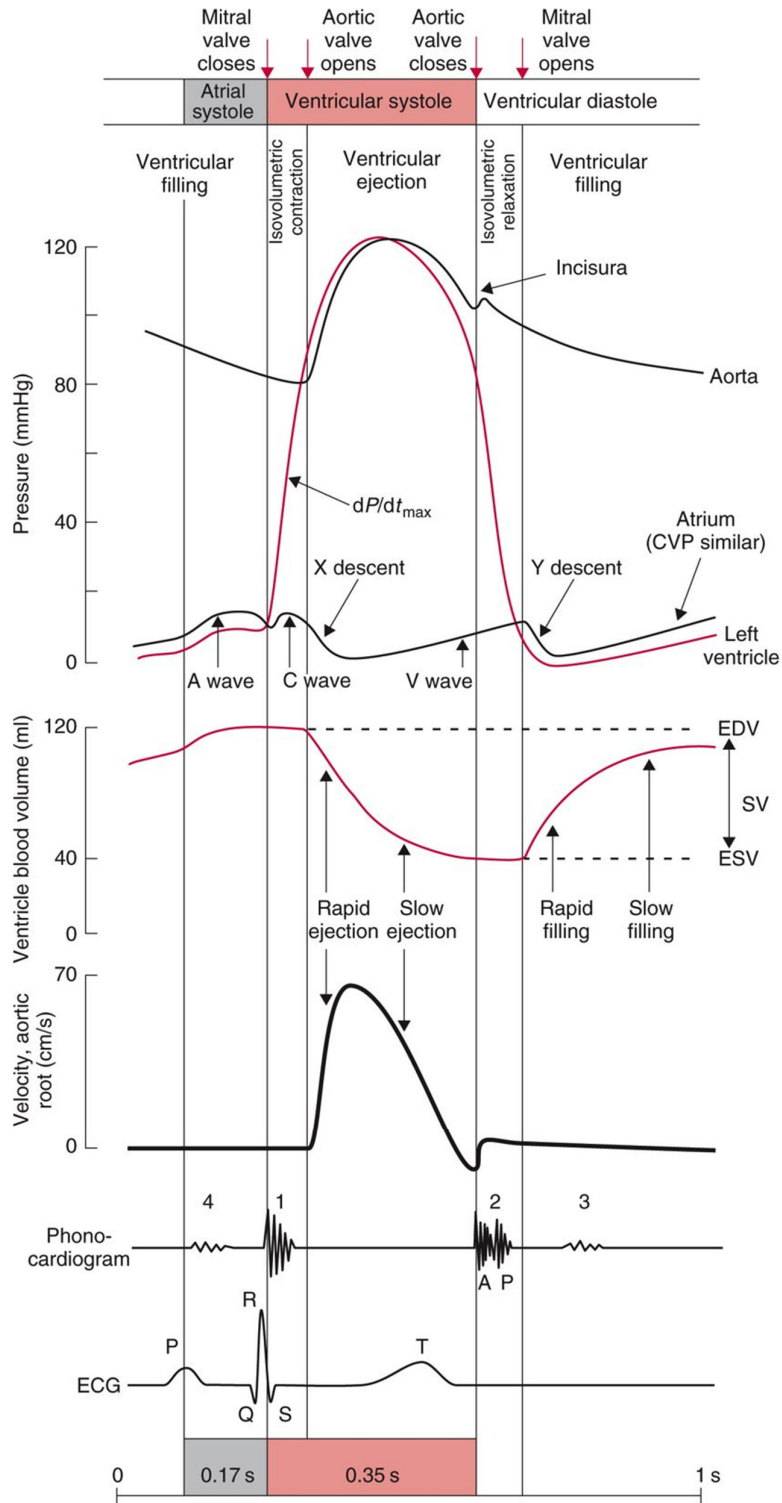


Figure 1.1 Changes in pressure, volume and flow in the aorta, LV, and LA during the human cardiac cycle.

Note: Reproduced with permission from Herring et al. 2018.

Ventricular systole occupies the remaining third of the cardiac cycle and follows the A phase of diastole. Systole involves a brief isovolumetric phase that begins when the atrioventricular valves close following the increase in ventricular pressure over atrial pressure (Pollock and Makaryus, 2021). With all heart valves closed and the myocardium contracting, pressure within the ventricle rises rapidly until it exceeds arterial pressure and causes the pulmonary and aortic valves to open and ejection to commence. The pressure that the ventricle must overcome to initiate ejection is known as afterload, or systemic vascular resistance (SVR) and is closely related to aortic pressure. Most of the blood is ejected during the rapid ejection phase and is accommodated temporarily in the distended aorta or pulmonary arteries, resulting in peak systolic BP (Rehman and Nelson, 2021). The ejected volume of blood is known as the stroke volume (SV) and the volume remaining in the LV is the end systolic volume (ESV), which is approximately 1/3rd of the EDV. In a healthy adult individual SV ranges from 70-80 mL and ESV is ~50 mL (Pollock and Makaryus, 2021). The ejection fraction (EF) is the SV divided by the EDV, which ranges from 50-70% at rest. The total work performed by the heart is known as cardiac output (\dot{Q}) and is calculated as a product of SV and HR and is expressed in litres per minute ($L \cdot \text{min}^{-1}$). In healthy adults, \dot{Q} is normally ~5.0 $L \cdot \text{min}^{-1}$ at rest and can increase by up to 3-5-fold during exercise, whilst increases can be greater in athletes (Pollock and Makaryus, 2021, Hellsten and Nyberg, 2015b).

1.2.2 Cardiac Function During Exercise

When dynamic exercise begins the cardiac response involves a rise in HR and SV to meet the need for increased O₂ delivery to the active muscles. Additionally, skeletal muscle O₂ extraction also increases, from ~4.5 mL·100 mL⁻¹ at rest to ~16 mL·100 mL⁻¹ at maximal exercise (Herring, 2018). Therefore, the ability to increase work rate is provided by a combination of both improved O₂ delivery by the CV system and extraction at the skeletal muscle cell level. The rise in HR is the primary driver behind the increased \dot{Q} , which is also modulated by preload, afterload, and contractility (Rowell, 1974). Systolic BP (SBP)

increases in response to the rise in \dot{Q} while diastolic BP (DBP) remains consistent between rest and exercise, and may fall slightly (~ 10 mmHg), which results in an overall rise in mean arterial pressure (MAP) (González-Alonso et al., 2008). Systemically, increased regional dilatation in the active skeletal musculature and sympathetic vasoconstriction in less active tissues facilitates reduced total peripheral resistance (TPR) and increased muscle blood supply (Trammel and Sapro, 2021). Prolonged aerobic exercise requires consistently elevated \dot{Q} to maintain energy and thermoregulatory demands; the magnitude of the CV response depends on exercise intensity, environmental conditions, and fitness level (Crandall and González-Alonso, 2010). During sub-maximal exercise the CV system supplies adequate blood flow to maintain work rate and maintains a steady state where cardiac parameters reach a plateau, typically around 45-70% of maximal aerobic capacity $\dot{V}O_{2\max}$ (Rowland, 2009). During exercise intensities approaching $\dot{V}O_{2\max}$, SV has been shown to plateau at 40-50% of the peak work rate (Stöhr et al., 2011b), although in some studies SV has been shown to continue increasing until $\dot{V}O_{2\max}$ (Vella and Robergs, 2005). In trained individuals, the sustained rise in SV has been attributed to improved cardiac function resulting from training adaptation, which confers benefits such as diastolic filling, enhanced contractility, greater blood volume and reduced afterload (Travers et al., 2020).

1.2.3 Cardiac Function in Endurance Athletes

The performance of regular intensive exercise stimulates several structural and functional cardiac adaptations (Rowell, 1974, Hellsten and Nyberg, 2015b). Adaptive electrical remodelling is reflected by increased vagal tone and the presence of sinus bradycardia, which is a resting heart rate lower than $60 \text{ beats}\cdot\text{min}^{-1}$ (Hellsten and Nyberg, 2015b). Structurally, the increased SV that facilitates improved exercise performance is known to occur by increased EDV reduced ESV and increased ventricular filling (Douglas et al., 1990). Left ventricular cavity sizes are markedly increased in endurance-trained athletes, with values up to 70 mm being demonstrated vs. ~ 45 mm in healthy non-athletes (Herring, 2018). Figure

1.1.2 demonstrates the marked cavity dimension differences between a 23 year-old non athlete and 23 year-old professional cyclist (Prior and La Gerche, 2012). Ventricular cavity size is proposed to be increased as an adaptive response to the repeated pressure overload caused during high-intensity exercise (Beaumont et al., 2017). This mechanism also stimulates myocardial hypertrophy, which results in improved myocardial contractility (Shave et al., 2019).

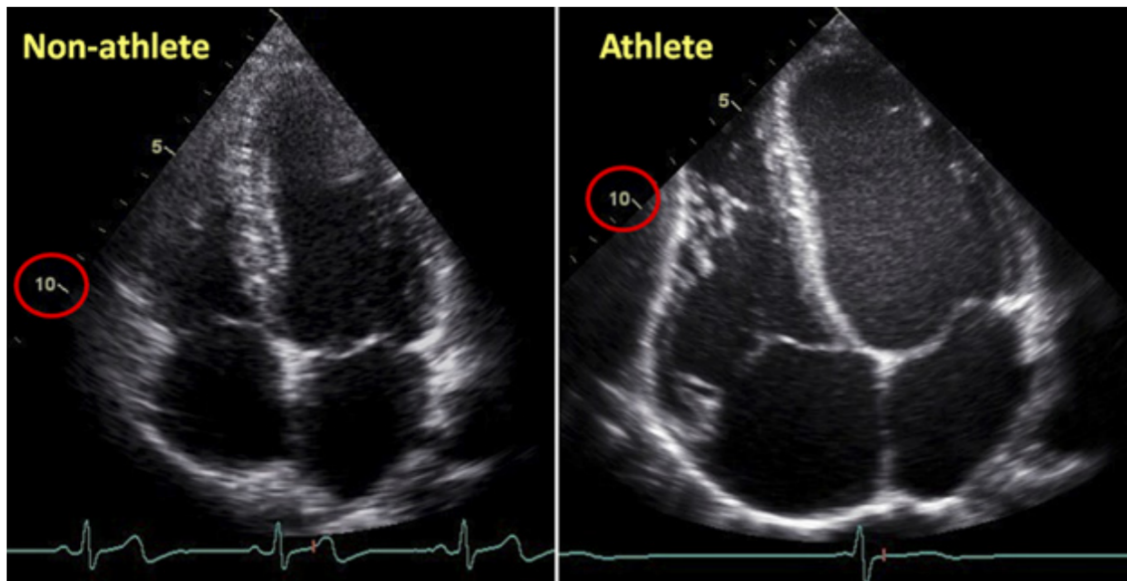


Figure 1.2 An apical 4 chamber view of the heart of 2 age-matched participants, one a non-athlete and the other a professional cyclist.

The effects of endurance training result in dilatation of all four cardiac chambers and a notable increase in SV at rest in the athlete's heart. The 10cm echocardiographic field depth is marked in red to highlight the differences in cardiac size. Reproduced with permission from (Prior and La Gerche, 2012).

1.3 Echocardiography

1.3.1 Conventional 2D Echocardiography

Ultrasound imaging of the heart uses sound frequencies in the range of 4-7 MHz to non-invasively capture information about cardiac function and health. Sound frequencies are

created within ultrasound transducers by striking piezo-electric crystals with electrical pulses, which causes the release of sound waves (Bom et al., 1973). Most tissues in the body absorb the sound waves; however, at the boundaries between tissues, some of these sound waves are reflected. These returning waves are also detected by the transducer and converted to images by the ultrasound machine. In modern echocardiography, several modes are used: conventional 2D echocardiography, M-mode imaging, Doppler imaging, and myocardial speckle-tracking (MST).

The earliest form of echocardiography made use of an A (amplitude) mode that generated reflected signals of varied amplitudes, which were caused by acoustic differences between different tissue boundary densities in the heart (Singh and Goyal, 2007). This technique was refined to produce various points or ‘dots;’ the brightness of which were dependent on their amplitude and the mode was appropriately named B (brightness) mode. Following on from B-mode, M-mode was created by sweeping the dot across the screen at a fixed point. M-mode is used for analysing moving anatomical areas within the heart by providing a 1D view, which is a function of depth and time (Edler and Lindström, 2004). This is useful to assess motion and distance within the movement of a cardiac structure e.g., the mitral valve leaflets.

Following development of the B-mode and M-mode techniques the next step utilised several beams to generate B-mode signals across an imaging sector, allocating depth and brightness. This was originally termed ‘multiscan echocardiography,’ and today is known as 2D echocardiography (Bom et al., 1973). 2D echocardiography enabled sonographers to clearly visualise all the cardiac chambers and greater vessels and to comprehensively assess their structure and function. There are three standard windows for conducting echocardiographic assessments: the parasternal (long axis and short-axis views), subcostal, and apical windows. For the purposes of this review the parasternal and apical views will be briefly summarised below.

The parasternal long axis (PLAX) view (see figure 1.4 below) provides information on the morphology and motion of the intraventricular septum (IVS), the left atrium (LA), the aortic root, and the dimensions of the LV.

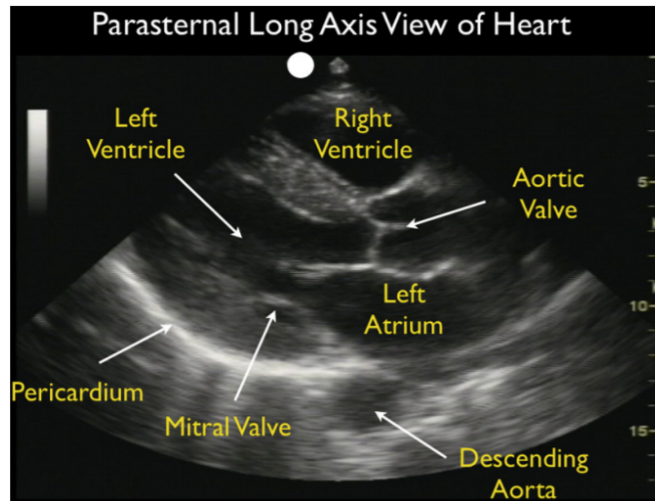


Figure 1.3 The parasternal long-axis (PLAX) view.

Adapting to the short axis (PSAX) view (see figure 1.4) allows for 4 conventional levels of the LV: the base, the mitral valve, the papillary muscle, and the apex. The movement and morphology of the IVS and the MV are easily viewed from these positions.

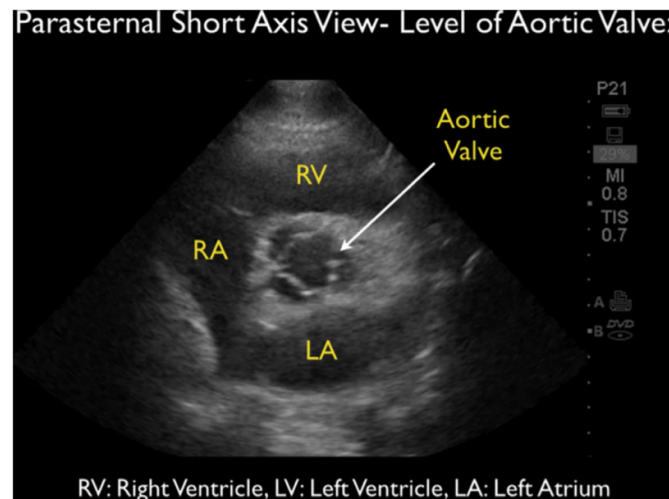


Figure 1.4 The parasternal short-axis (PLAX) view at the basal level.

Moving to the apical window (see figure 1.1.2), the heart can be viewed from several angles that provide information on the different chambers and their walls. For assessment of the LV, the apical 4 chamber (AP4CH), 2 chamber (AP2CH), and long axis (APLAX) views are commonly used to assess cardiac function (Lang et al., 2015).

In addition to chamber sizes, it is also possible to calculate LV volumes from a 2D approach using biplane methodology (Wahr et al., 1983) and the Simpsons rule. This provided a much

more accurate assessment of volumes compared to the previous M-mode technique, which were validated in vitro (Helak and Reichek, 1981) and in vivo (Erbel et al., 1983). The Simpsons biplane approach has been shown to correlate very well with cardiac magnetic resonance imaging (cMRI) (Gutiérrez-Chico et al., 2005), although the limitation of this technique is the disposition to underestimate absolute chamber volume due the nature of biplane calculation. Despite this, Simpson's biplane is the recommended method for LV chamber quantification by the American Society of Echocardiography (ASE) (Lang et al., 2015, Lang et al., 2005). This method yields the LV EF, SV, EDV, ESV and Q and is the recommended approach for assessing these variables (Lang et al., 2015). The key limitations of 2D imaging are its reliance on image quality. The derived measures are useful for global function and structure assessment but may not be appropriate for detecting smaller subtle changes in cardiac morphology (Thomas and Popović, 2006).

It is also possible to use 2D imaging to assess RV function (Foale et al., 1986) et al., 1986). However, the assessment of RV function utilising a 2D or M-mode technique has been challenging for researchers and clinicians due to excessive trabeculation and the non-uniform geometry of the RV. Comparison with RV data obtained *via* cMRI has provided a more accurate assessment and highlighted the limitations of using echocardiography to estimate RV volume (Helbing et al., 1995) et al., 1995). Most commonly, a simple measure of RV area from a single plane acquisition is used to calculate the fractional area change (RVFAC). This measurement does not imply any mathematical assumptions of geometry, as the Simpsons method for LV volume does, yet the technique has been proven to correlate well with RV EF (Anavekar et al., 2007) and is currently recommended by the ASE (Lang et al., 2015). Additionally, the m-mode measurement TAPSE (tricuspid annular plane systolic excursion) is a parameter of global RV function which describes apex-to-base shortening and correlates closely with RV EF and RVFAC (Alam et al., 1999). TAPSE has been used clinically and results of <18mm have been associated with increased morbidity (Schmid et al., 2015).

1.3.2 Doppler Imaging

Doppler echocardiography is based on principles from the works of Austrian physicist Christian Doppler. Doppler was the first to analyse the phenomenon of perceived changes in wave frequency in relation to an observer who is moving relative to the wave source, known as the Doppler effect (Edler and Lindström, 2004). The development of the method of Doppler echocardiography paralleled the 2D and M techniques but it was not used in a clinical setting until the 1970s (Matsuo et al., 1977, Baker, 1970). One of the most important developments in Doppler echocardiography was the ability to evaluate LV filling using a combined 2D and pulsed wave (PW) ultrasound system (Kitabatake et al., 1982). This yields the early (E) and late (A) diastolic components of LV filling, and their ratio (E/A ratio). Normal and pathological LV diastolic function can be assessed and graded according to the specific patterns of flow obtained from the PW Doppler signal (Lang et al., 2005). However, there are several limitations to consider when interpreting this data. Trans-mitral flow measured by PW Doppler signal is dependent on several factors, including LV relaxation (Garcia et al., 1998) and compliance (Khouri et al., 2004), LA pressure and compliance (Ommen et al., 2000), LV systolic function (Saraiva et al., 2010), and HR (Cheng et al., 1992). Therefore, it is important to remember that the trans-mitral Doppler measures LV filling and not just LV diastolic function (Nagueh et al., 2016).

Tissue Doppler imaging (TDI) is the result of the concept of using a Doppler technique to evaluate myocardial velocities and was introduced by Isaaq and colleagues (Isaaq et al., 1989), who utilised standard PW Doppler to assess movement of the myocardium instead of blood flow. When the PW sample is applied to the annulus of the mitral valve the returning Doppler shifted signal is related to longitudinal movement of the myocardium. Assessment of myocardial function in the longitudinal plane has been suggested to be more sensitive to discrete changes caused by pathology (Fraser et al., 1999) primarily because longitudinal dysfunction precedes circumferential dysfunction (Kang et al., 2006, Vinereanu et al., 2003). Numerous clinical studies have demonstrated the important role of TDI when assessing pathology (Kang et al., 2006, Yu et al., 2007) and longitudinal movement has been shown to

be directly related to global LV and RV function (Alam et al., 1992). TDI is particularly useful in the assessment of exercise responses, as it demonstrates good reproducibility and the ability to assess regional function with less reliance on 2D image quality. Systolic (S'), early diastolic (E') and late diastolic (A') myocardial velocities are parameters obtained from TDI measurement at different myocardial wall segments and provide excellent temporal and spatial resolution.

While TDI has been shown to be accurate and reproducible in the assessment of systolic and diastolic LV function (Ommen et al., 2000, Fleming et al., 1994) there are several limitations. While TDI was originally proposed as a load-independent technique, research has demonstrated that TDI is more load-dependent than originally thought (Andersen et al., 2004). Andersen et al. (2004) utilised the Trendelenburg position for preload augmentation and demonstrated significant variation in TDI variables when compared to supine values. Additionally, Hart et al. (2007) demonstrated similar findings using leg elevation pre- and post-marathon run (Hart et al., 2007). Nevertheless, TDI has been widely utilised in a range of clinical and exercise focused research and generally efforts are made to statistically correct for the effects of changes in loading conditions by using the average HR as a covariant (Serrano Ostariz et al., 2013, Passaglia et al., 2013).

The ratio of E/E' , has been employed as a surrogate for LA pressure and LV filling pressure (Nagueh et al., 2016) as it correlates very well with invasive measurement of pulmonary wedge pressure in patients with some degree of diastolic dysfunction. This is based on the principle that E' is a more sensitive measure of LV relaxation and less influenced by LA pressure. Finally, TDI is also influenced by the rotation, translation, and tethering of adjacent myocardial segments (Sutherland et al., 1999).

1.3.3 2D Strain Measurement

Myocardial speckle tracking (MST) provides an echocardiographic technique that allows the assessment of myocardial deformation (strain) (ϵ). Myocardial ϵ describes the shortening and

lengthening of the myocardium, as opposed to the movement depicted by TDI data, and is given as a dimensionless parameter that defines the percentage change in myocardial length (Sutherland et al., 2004). Strain rate (SR) is the rate of deformation or change and is expressed in terms of length per second (l/s) (Yip et al., 2003). MST is considered a breakthrough in the echocardiographic assessment of ventricular function, as the derived parameters represent myocardial contractility and relaxation more closely than previous methods allowed (Sutherland et al., 2004).

Myocardial speckle tracking technology has been integrated into commercially available analysis software and been validated against cMRI (Amundsen et al., 2006). LV ϵ has been shown to agree strongly with cMRI data across radial, longitudinal, and circumferential planes, therefore the MST approach to assessment of LV ϵ is considered superior to TDI derived ϵ (Geyer et al., 2010). Myocardial speckle tracking accuracy is dependent on several factors including image quality (Geyer et al., 2010), depth, and frame rate (Sivesgaard et al., 2009). Lower frame-rates create unstable speckle patterns and reduce the accuracy of peak values, whilst high frame-rates cause signal disruption and reduce image resolution (Sutherland et al., 2004). MST has been shown to be angle independent (Sivesgaard et al., 2009), though aligning the transducer with myocardial movement parallel to the ultrasound beam has been shown to provide the greatest accuracy (Grabskaya et al., 2010). Additionally, this method provides more valid and reproducible data from the longitudinal plane compared to radial and to a lesser extent the circumferential plane (Grabskaya et al., 2010, Mor-Avi et al., 2011). The use of LV ϵ as a prognostic indicator in hypertensive patients has associated reductions in LVLS of ~18% with poor exercise tolerance and a higher presence of cardiac complications during follow-up measures (O'Connor et al., 2010).

Use of MST imaging to evaluate RV ϵ was pioneered by the work of Oxborough and colleagues (Oxborough et al., 2012, Oxborough et al., 2011). The researchers demonstrated excellent reliability of their method using an altered AP4CH view to assess RV LS (RV LS), while SR was less reliable generally. Their key finding when interpreting and utilising the general application of RV ϵ was the magnitude in change between physiology and pathology

is less than in LV function and that good reproducibility is essential to differentiate any intra-individual changes. They concluded that changes of ~7% in RV ϵ and 15 to 20% in SR data represent meaningful changes. When assessing the RV, a modified AP4CH view that clearly depicts the RV free wall is currently recommended by the ASE, and either the free wall, or the free and septal walls should be analysed (Burns et al., 2009, Lang et al., 2015).

Importantly, it is important to identify the methodology employed when interpreting such data as the RV free wall produces higher ϵ values compared to the combined free and septal wall measurements, due to septal tethering (Lang et al., 2015).

1.4 Autonomic Regulation and Control of the Heart

The autonomic nervous system (ANS) is an efferent neural pathway transporting information from the brain to central and peripheral tissues. The ANS is composed of the sympathetic and parasympathetic branches, which are responsible for ‘fight or flight’ and ‘rest and digest’ functions of maintaining homeostasis (Tortora and Derrickson, 2008). Therefore, ANS control mechanisms are fundamental to both the regulation of the cardiovascular system and maintenance of homeostasis. Impaired ANS function is associated with an increased risk of all-cause mortality (Gerritsen et al., 2001) and is implicated in the development of several cardiovascular diseases (Schroeder et al., 2003).

Neural input from the sympathetic and parasympathetic nervous systems influences the rate (chronotropic state), speed of electrical impulse conduction (dromotropic effect), force of LV contraction (inotropic effect) and relaxation (lusitropic effect), independent of preload (fibre length) and afterload (Calvert and Lefer, 2012). At rest, the sinoatrial node prompts a calcium ion induced release of calcium ions from the sarcoplasmic reticulum. The calcium ions interact with troponin C of the troponin-tropomyosin complex, resulting in a cross-bridge formation and force generation/myocyte contraction (Takimoto and Kass, 2012). Relaxation involves the removal of calcium ions from troponin C and subsequent extrusion from the cell via the sodium/calcium exchanger, ATPase pump or intracellular uptake into the sarcoplasmic reticulum or mitochondria (Takimoto and Kass, 2012). While resting, the

parasympathetic nervous system releases acetylcholine from postganglionic fibres, which slows the intrinsic rate of depolarisation the myocardium (Sam and Bordoni, 2021). To produce an increased cardiac output during submaximal exercise, an increased HR and SV must occur. A withdrawal of parasympathetic tone and an increase in sympathetic activity via the neurotransmitters adrenaline and noradrenaline stimulate the β -adrenergic receptors in the myocardium, resulting in an increase in chronotropic, dromotropic, inotropic and lusitropic effects (Opie, 2004) from a greater release and uptake of calcium into the sarcoplasmic reticulum. Sympathetic overactivity, i.e. following periods of extensive exercise training with inadequate recovery (Belonje et al., 2007), places excessive demand on the cardiovascular systems, causing premature degradation, which can lead to adverse cardiac and vascular remodelling (La Gerche and Prior, 2007).

The ANS is acutely controlled by the release of catecholamines following stimulation of the sympathetic cardioaccelerator nerves. Catecholamines contribute to the regulation of blood vessels and blood flow distribution. Adrenaline reacts with adrenoreceptors (α - and β -) to cause vasoconstriction and vasodilation. Higher levels of adrenaline result in vasoconstriction whereas lower levels of adrenaline result in the predominance of β -adrenoceptor stimulation causing vasodilation and reduced TPR. In the heart, catecholamine induced β -stimulation causes positive inotropy, lusitropy, and chronotropy. High levels of catecholamines in a resting state indicates sympathetic overactivity; however, circulating plasma noradrenaline levels constitute only a fraction of the amount secreted from sympathetic nerve terminals (Esler et al., 1990). Autonomic adjustments in parasympathetic and sympathetic activity have been widely reported following exercise training and it is thought that such neural control may be responsible for mediating vascular and cardiac responses to exercise training (Fisher et al., 2015).

Although invasive measures of ANS activity are preferable, heart rate variability (HRV) is a commonly used measure advocated by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Malik, 1996). Heart rate variability is calculated by analysing the oscillations and interval times between consecutive

R-R intervals on the ECG and is considered as a measure of neurocardiac function, reflecting heart-brain interactions and ANS dynamics (Shaffer et al., 2014b). Non-invasive measures of HRV include frequency domain analysis, time domain analysis, rhythm pattern analysis and non-linear methods (Gronwald and Hoos, 2020). Spectral frequencies of ECG data can be separated into their component parts and an indication of the dominant nervous activity can be established. The frequency domain method of establishing HRV, measures overall power spectral density (PSD) to provide information of how power variance distributes as a function of frequency. Frequency parameters are classified as very low frequency (VLF) low frequency (LF) and high frequency (HF) power, which change in relation to autonomic modulations of the heart period. Although there is some contention surrounding HRV frequency domain analysis (Reyes del Paso et al., 2013), LF has been associated with sympathetic activity while HF is associated with parasympathetic activity (Pomeranz et al., 1985). The ratio of low and high frequencies (LF:HF ratio) is generally considered a representation of sympathovagal balance (Malliani, 1999), while expression of LF and HF in normalised units (LFnu and HFnu, respectively), represents the relative value of each power component (Borchini et al., 2018).

1.5 Cardiac Biomarkers

Through meta-analyses (Shave et al., 2007, Middleton et al., 2006), and systematic reviews (Shave et al., 2010a, Oxborough et al., 2010), of the body of research, it is known that significant elevations in cardiac biomarkers, which are associated with myocardial damage are present following exercise bouts ranging from 30 minutes to 24 hours duration. The presence of cardiac biomarkers associated with ACS in the blood of clinical patients and research participants following endurance exercise demonstrates the potential cellular disruption caused by PSE (Shave and Oxborough, 2012). The mechanisms by which cardiac biomarkers are released during, or post PSE are currently unclear and there are several credible theories that explain the release patterns and mechanisms of specific biomarkers

(Shave and Oxborough, 2012). In clinical and research settings, the most widely measured and specific biomarker for cardiac damage is cardiac troponin (cTn).

The troponin complex forms part of the myocardial sarcomere, and is formed of troponin T (TnT), troponin I, (TnI) and troponin C. Separate isoforms of TnT and TnI exist, which are found in cardiac (cTnT, cTnI) and skeletal muscle. Cardiac troponin (cTn) is predominantly located within the myofibril bound to tropomyosin, and there exists a smaller pool of unbound cTn in the cytoplasm, which accounts for roughly 10% of cTn (Shave et al., 2010a). Cardiac troponin T and I are highly specific cardiac biomarkers shown to be released in a biphasic pattern, with initial early release that potentially signals the leakage of unbound cTn from the cytosol, followed by a secondary larger release of bound cTn following approximately 12-24 hours later (Baker et al., 2019).

The second, larger release of bound cTn is likely caused by proteolytic degradation and potentially indicates myocardial necrosis, though this is debated (White, 2011). Repeated blood sampling has shown that post-exercise cTn release is usually greatest 0-1 hours following exercise, with values returning to near-resting levels typically within 24 hours; which suggests that cardiomyocyte necrosis may not occur due to myocardial infarction (Wilson et al., 2011b, White, 2011). Indeed, the half-life of cTn in the blood stream is only ~2 hours, therefore it is believed that when no second peak in cTn is present cardiomyocyte necrosis is less likely (Shave et al., 2010). Cardiac troponin levels of $>0.1 \text{ ug}\cdot\text{L}^{-1}$ are associated with acute MI and endurance athletes often present with values more than this threshold following PSE bouts lasting from 30 minutes to 24 hours, at average HRs in the moderate to high intensity domains (typically $>150 \text{ beats}\cdot\text{min}^{-1}$) (Donaldson et al., 2019, Shave and Oxborough, 2012, Shave et al., 2007). Interestingly, there has been little evidence to demonstrate a relationship between elevated post-exercise serum cTn concentrations and echocardiography-derived parameters of EICF (Wilson et al., 2011b, Lord et al., 2018, Donaldson et al., 2019).

1.6 Changes in Cardiac Function Following Prolonged Strenuous Exercise – Meta-Analysis and Systematic Review

Prolonged strenuous endurance exercise is associated with both transient disruptions in cardiac function and detectable levels of cTn in the peripheral circulation (Stewart et al., 2016). In some instances, systolic and diastolic left ventricular (LV) function following PSE, as measured via echocardiography, has been shown to be comparable to patients with acute heart failure (Franklin et al., 2020b). This exercise induced cardiac fatigue (EICF) typically persists for 12-48 hours post-exercise before the heart recovers and functional measures return to baseline levels (Dawson et al., 2003, McGavock et al., 2002, Middleton et al., 2007). Likewise, cTn levels in the blood of endurance athletes have been reported to exceed clinical detection thresholds for the diagnosis of acute MI (Rifai et al., 1999, Whyte et al., 2000, Neumayr et al., 2002, Shave et al., 2002), and typically return to baseline within 72 hours post-exercise (Shave et al., 2010a). The influence of exercise intensity and duration on cTn release and EICF is unclear, as significant effects have been recorded at both ends of the intensity/duration spectrum (Stewart et al., 2015, Serrano-Ostariz et al., 2009). There is evidence of EICF and cTn release following exercise bouts as short as 30 minutes at high relative intensities (Middleton et al., 2008), and from exercise bouts lasting as long as 10-24 hours (Tulloh et al., 2006, Whyte et al., 2000, Passaglia et al., 2013).

To explore the variability of both EICF and troponin release in the body of literature, separate meta-analyses have previously been conducted (Middleton et al., 2006, Shave et al., 2007, Gresslien and Agewall, 2016). In their meta-analysis of 23 studies, Middleton and colleagues (2006) investigated the degree of echo-assessed EICF, following exercise bouts ranging from 60 to 640 minutes, involving running, cycling, or triathlon exercise (Middleton et al., 2006). The authors calculated an overall mean reduction in LV E/A ratio of 0.45 (95%CI -0.39 to -0.51) (normal range: 1.53 +/- 0.40, 95% CI 0.73 to 2.33) and a mean reduction in LVEF of 1.95% (95% CI -1.03 to -2.88) (normal range: >55%) (Middleton et al., 2006). A meta-analysis of troponin positive event rates by Shave and colleagues (2007) suggests that cardiac

myocyte damage, measured by serum cTn before and after exercise occurs following endurance exercise bouts of similar duration and mode (Shave et al., 2007). The authors calculated that 47% of participants, from a sample of 26 studies totalling 1120 cases, exceeded the troponin assay detection limit of $0.01 \text{ ug}\cdot\text{L}^{-1}$ (95% CI 39-56%), indicating that exercise stimulates cTn release when sufficiently strenuous. It is currently debated whether intensity, duration, or a specific volume of the exercise contributes to cardiac dysfunction, and the previous meta-analyses (Middleton et al., 2006, Shave et al., 2007) have reported significant ($P > 0.05$) levels of heterogeneity in their effect sizes, which may be explained by study-level variables (Thompson and Higgins, 2002).

Moderator analyses via meta-regression are a useful tool to explore heterogeneity among the combined effect sizes (Thompson and Higgins, 2002). Participant variables such as body-mass and sex have been shown to influence the rate of cTn release (Shave et al., 2007) and exercise factors such as duration, and intensity account for the heterogeneity in both Shave and Middleton's meta-analyses. Interestingly, Shave and colleagues reported a negative correlation of duration and event rate in their meta-analysis, and a positive correlation between participant mass and event rate (Shave et al., 2007). Conversely, there was significant effect of exercise duration on reduced EF, but not E/A ratio, in Middleton et al's (2006) meta-analysis. Together, these findings suggest that cTn release, and diastolic dysfunction are triggered by shorter, more intense exercise, while reduced systolic function may be the result of long-duration exercise at lower relative intensities, such as during an Ironman triathlon or ultra-marathon races (Shave and Oxborough, 2012). Unfortunately, exercise heart rates were not assessed as potential moderators in previous meta-analyses and the influence of exercise intensity has not previously been meta-analysed. There is also a need for further investigation of the role of exercise mode in eliciting cardiac fatigue and damage as cardiac demands vary between running and cycling exercise (Millet et al., 2009). Shave and colleagues concluded in their meta-analysis that running exercise elicited the greatest post-exercise positive cTn response, compared to cycling and triathlon exercise (Shave et al., 2007). A meta-analytical model that made use of meta-regression methods

would allow for clearer definitions of specific exercise stimuli that elicit the release of cardiac troponin, and/or EICF (Baker et al., 2019). While often measured together (Middleton et al., 2007, Dawson et al., 2005, George et al., 2004, Scharhag et al., 2008, Stewart et al., 2014, Stewart et al., 2016, Stewart et al., 2015, Wyatt et al., 2011, Tian et al., 2012, Shave et al., 2004, Chan-Dewar et al., 2013a, Chan-Dewar et al., 2013b, Aagaard et al., 2014, Neilan et al., 2006, Leetmaa et al., 2008, Lucia et al., 1999, Serrano Ostariz et al., 2013, Nie et al., 2011, La Gerche et al., 2008, Wilson et al., 2011b, Shave et al., 2002), the extent of a possible relationship between cTn release and cardiac dysfunction is still unclear, with few studies that measure both variables having reported any meaningful correlations between the two (Rifai et al., 1999, Whyte et al., 2000). It is still unclear from individual studies whether the release of cTn indicates functional damage to the myocardium following these types of exercise (Shave et al., 2010a). A meta-analysis of the findings from studies measuring both cTn and EICF variables would add to the separate findings of transient cardiac dysfunction and damage following exercise lasting between 60 and 640 min in the previous meta-analyses in this field (Middleton et al., 2006, Shave et al., 2007). A practical method to meta-analyse the relationship between cardiac damage and dysfunction, while accounting for study-level differences, is to use the prevalence of a positive troponin response as a continuous moderator variable when comparing the differences in pre- to post-exercise cardiac functional measures. Additionally, the inclusion of exercise characteristics; mode, duration and intensity as moderators would add to the mechanistic explanation of EICF and cTn release. Therefore, the aim of this study was to employ a meta-analytical approach on studies that measured both cardiac dysfunction and cTn following endurance exercise, to elucidate the influence of both exercise and participant factors.

1.6.1 Methods

The meta-analysis was performed according to PRISMA guidelines. Accordingly, a literature search for peer-reviewed, English-language journal articles examining the effects of exercise on both cardiac troponin release and cardiac function, as measured by echocardiography was conducted.

1.6.1.1 Data Sources

Sources used for this search were SPORTDiscus, PubMed Central, Science Direct and MEDLINE. The key words were cardiac troponin, endurance exercise, and echocardiography. The reference lists from published papers were also searched for relevant studies that did not appear in the database search, and also papers that cited the selected studies were reviewed for inclusion.

1.6.1.2 Study Selection and Data Abstraction

The initial database search yielded 223 articles; these were further refined via the following inclusion criteria (in no particular order):

- 1) Blood and echo measurements taken prior-to exercise, and within 1 hour of cessation of exercise
- 2) two-dimensional M-mode echocardiographic measurement of either E/A ratio or Ejection fraction or provided data from which they could be calculated
- 3) taken venous blood samples for cardiac troponin T (cTnT) or high-sensitivity cTnT,
- 4) Reporting the number of positive troponin tests pre- and post-exercise
- 5) No pharmacological or dehydration interventions
- 6) Exercise duration of 30 minutes or greater
- 7) Available information on exercise mode and participant training status
- 8) For inclusion into the regression analysis, studies must have reported average exercise heart rate (HR), exercise duration, participant age, and mass.

Following the refinement process, nineteen studies were eligible for inclusion into the meta-analysis. A further 3 studies were obtained from the reference lists of these studies, for a total of 22. Eight of these studies employed repeated bouts of exercise in their methodologies; each bout was treated as a separate study if the participants received at least 24 hours rest,

and full pre-post data were reported for each bout. This resulted in 32 sets of pre-post troponin and echocardiographic data. Total n was 607 cases of echocardiographic data; of these there were 604 troponin samples collected and 3 were lost due to haemolysis of the samples (Shave et al., 2004). For the regression analysis, 31 sets reported participant mass and age, 29 reported exercise duration (in minutes) and 23 sets contained average exercise HR (Figure 1.1)

1.6.1.3 Statistical Analysis

Derivation of outcome statistics

Data were extracted from the studies independently by the lead author. The key variables were E/A ratio and ejection fraction (EF), with inter-individual SDs, as well as total troponin samples and positive troponin responses in each study. The dichotomous event rate for troponin response was defined as the number of participants exceeding the assay detection limit for the specific assay reported in the study. If available, the average exercise HR, exercise duration, participant age and mass were also extracted. If these data were not available, efforts were made to contact the authors of the paper to obtain them.

Pooling of Results

Data were analysed using the statistical software CMA (Biostat, Englewood, NJ). Standardised mean differences (Cohen's d) with 95% CI's were computed individually for E/A ratio, EF, and event rate was calculated for the troponin positive. The effect sizes were weighted according to the intra-study variability (calculated from reported means and SDs), with larger, more precise studies having a greater weighting on the overall effect size (Tawfik et al., 2019). Separate random-effects meta-analysis were run for each variable using CMA's meta-regression function. This yielded the summary measures, significance levels (P), and

the between-study heterogeneity (Cochran's Q and I^2) and variability (τ^2) (Tawfik et al., 2019).

Exploration of Publication Bias

Publication bias was assessed via funnel plot inspection (Thornton and Lee, 2000), with the expectation that studies would be evenly distributed about both sides of the average effect size, with high-precision studies close to the mean. Quantitative assessments of Kendall's τ and Egger's regression are also reported for all three effect sizes.

Exploration of Heterogeneity and Meta-Regression Analyses

Heterogeneity of the 3 effect sizes were assessed by Cochran's Q and I^2 values calculated in the random-effects model. Statistical significance for Q was $p < 0.01$ and I^2 of 25, 50 and 75% were interpreted as small, medium and large degrees of heterogeneity (Higgins and Thompson, 2002). In the event of significant levels of heterogeneity in any of the meta-analyses, exploratory meta-regression analyses were conducted to determine how participant and exercise characteristics accounted for heterogeneous effect sizes. The residual plots were inspected for suitability before reporting the R^2 value of the fitted models. In the event of non-random residual plots, further variables were added to the model to capture possible significant interactions (Thompson and Higgins, 2002). A method of moment's estimator for the between-study covariance matrix in the random effect model's meta-regression was used (Thompson and Higgins, 2002). A sub-group analysis was conducted based on study exercise-mode, the 3 groups formed were Running, Cycling and Multisport. Following the meta-analysis of troponin event-rate (ER), the ER from each study was matched as a separate continuous moderator variable in the meta-analysis of E/A ratio and EF.

Sensitivity Analysis

A sensitivity analysis was carried out by removing outlying studies (Tawfik et al., 2019). The duration of exercise in the study by Passaglia et al (Passaglia et al., 2013) was twice that of

the next longest study and was removed from the analysis during the sensitivity analysis. No changes were noted following its exclusion, so it was left in the final analysis.

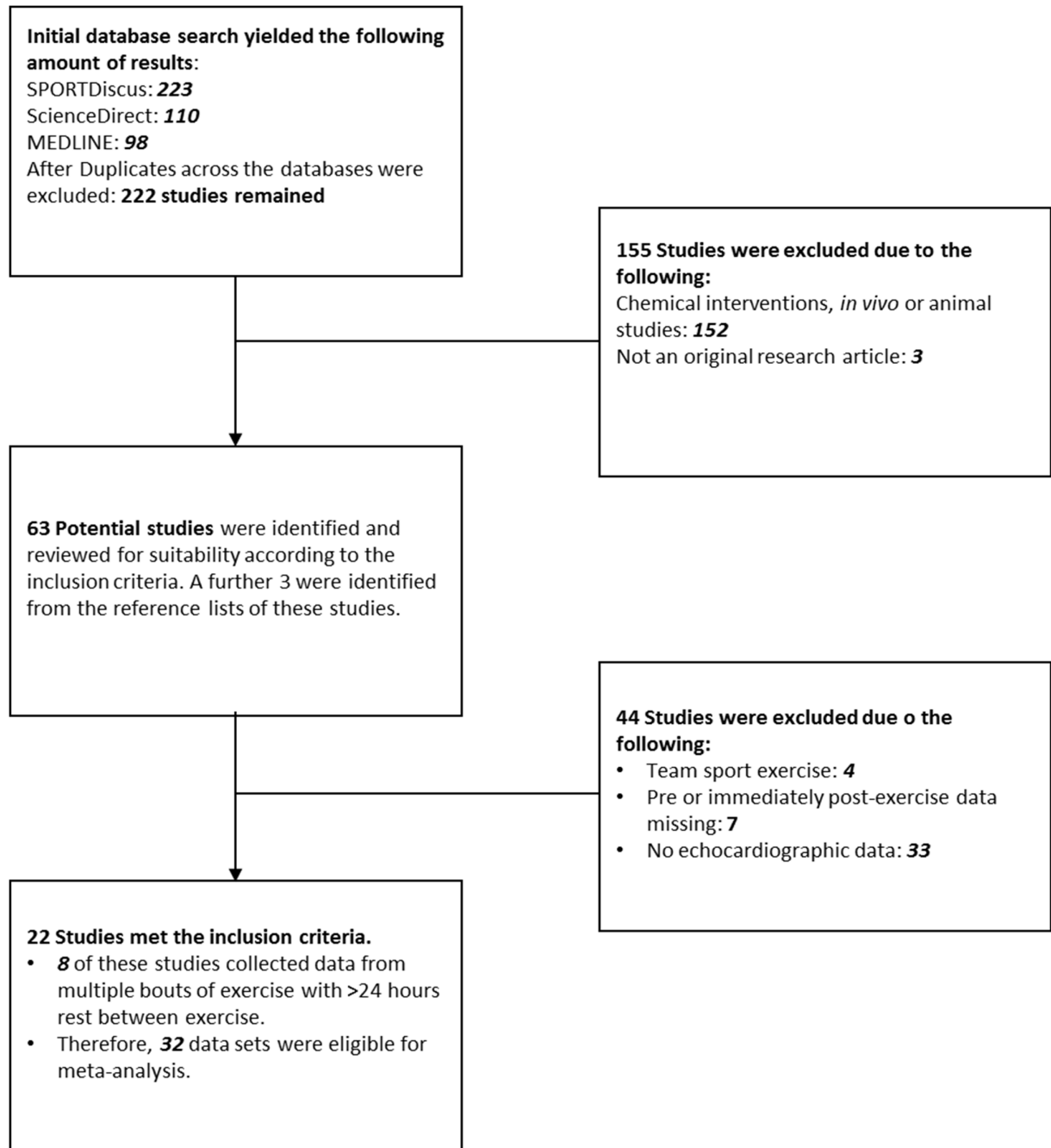


Figure 1.5 Flow diagram of study selection and exclusion stages

1.6.2 Results

1.6.2.1 Publication Bias

Publication bias analysis demonstrated a symmetrical distribution of SE about the mean that closely followed along the guidelines printed by the CMA software. The analysis highlighted the absence of clustering around the mean shown in more precise studies, and an absence of publication bias among the studies meta-analysed can be inferred (Thornton and Lee, 2000). Further tests to explore publication bias also confirmed this was absent; Egger's regression intercept was insignificant (-0.60 [-2.12, 0.92], $P > 0.1$), and Kendall's Tau reported a non-significant negative correlation of study size on ES ($\tau = -0.024$, $P > 0.1$)

1.6.2.2 Troponin Event Rates

The overall event rate, as calculated by the random-effects meta-analysis, for the detection of cardiac troponin T or I was 45.6% (95% CI = 33.6 – 58.2%) (Figure 1.6). There was evidence of large, significant heterogeneity between the 32 studies that were meta-analysed ($Q = 147.7$, $df = 31$, $I^2 = 79.0$, $P < 0.001$), highlighting the appropriateness of the random-effects model. Further, it was calculated that 79% of the between study variance ($T^2 = 1.4$) was due to real differences in effect size and not sampling or random error, therefore a meta-regression analysis was also performed.

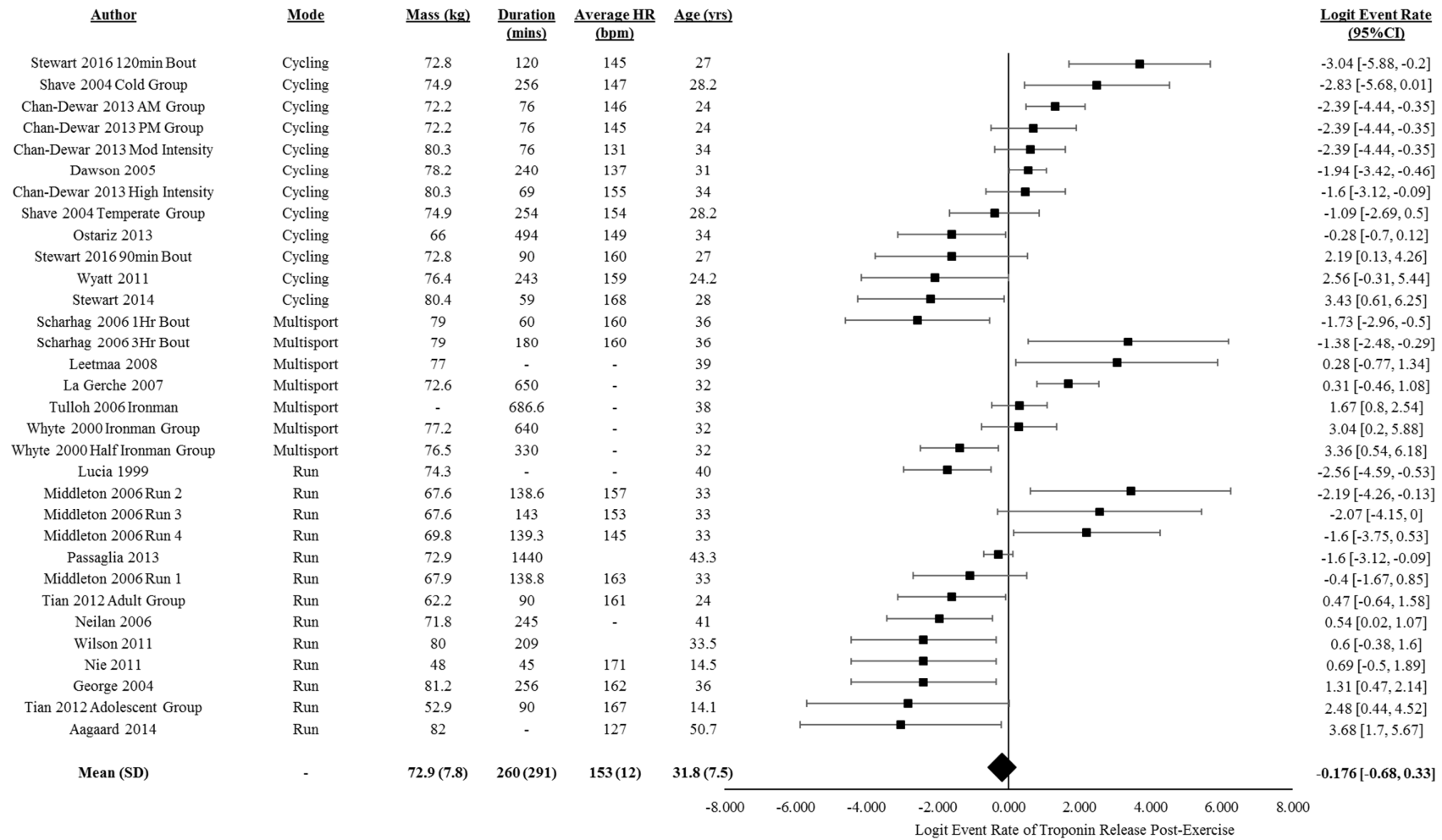


Figure 1.6. Forest plot showing logit event rate (ER) and 95%CI limits for each study. Studies have been ranked with ascending magnitude of actual event rate by exercise mode sub-groups. The overall random-effects logit ER and 95%CI are shown in the bottom row. – indicates data were unavailable. A logit ER of 0 corresponds to an event rate of 50%, a negative logit ER indicates an increased E

Subgroup Analysis

Studies were divided into those involving running (13), cycling (12), and multisport (7) exercise. In the mixed-effects model, overall, there was no significant differences between exercise modes on the troponin positive response to exercise ($Q = 3.85$, $P = 0.146$). At the subgroup level, there were no significant effects of exercise on troponin response for any exercise mode. Pooled event rates and 95% CIs were running 51.7%, [33.0, 69.0], cycling 28.8% [13.5, 51.1], or multisport 61.3% [33.4, 83.3], although cycling did approach significance for a reduced event rate over running and multisport ($P = 0.061$). The fixed-effects analysis reported a significant overall effect of exercise mode ($Q = 18.08$, $P < 0.001$). Additionally, a significant effect of exercise on troponin release was present for cycling ($P = 0.001$) and running ($P = 0.014$) exercise. However, this was accompanied by significant heterogeneity overall ($Q = 147.7$, $I^2 = 79.0$, $P < 0.001$) and within groups (running $Q = 51.2$, $I^2 = 76.6$, $P < 0.001$, cycling $Q = 41.1$, $I^2 = 73.2$, $P < 0.001$), indicating the true effect sizes differed within the subgroups as well as between them, and that the fixed-effects model is unlikely to be true.

Table 1.1. Meta-regression for between-study variance

	Troponin Event Rate	E/A Ratio	Ejection Fraction
I^2 total	75.48***	52.18**	83.42***
τ^2 total	1.4280***	0.1963**	0.7256***
Q-total	85.63***	35.55**	114.58***
df	21	17	19
n	22	18	20

Note: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Meta-Regression Analyses

Of the 32 data sets, 22 contained exercise HR and duration data, as well as participant age and mass for inclusion into the regression analyses. All covariates were analysed separately, and then together, to assess the individual contribution of each variable. For the reduced sample size of $n = 22$, between study variance was similar to the original data set (see Table 1.1).

The multivariate model significantly related to troponin event rate ($Q = 29.85$, $df = 4$, $P < 0.001$), however this was accompanied by significant variance amongst studies of similar levels for all covariates ($\tau^2 = 0.5343$, $I^2 = 44.08$, $Q = 30.40$, $P = 0.0236$), indicating variation in effect sizes at similar values among studies for the 4 covariates. Troponin ER was significantly influenced by participant age, exercise HR, and duration (Table 1.2). This model accounted for 63% of the total between-study variance ($R^2 = 0.63$), which was a high proportion of the total variance of 75% explainable by study-level covariates, as measured by the between study I^2 value (Table 1.1).

Table 1.2. Multivariate regression model for troponin event rate.

Covariate	Coefficient	Standard Error	95% Lower	95% Upper	Z-value	2-sided P-value
Intercept	-22.84	5.651	-33.90	-11.8	-4.04	0.0001***
+Age	-0.16	0.065	-0.29	-0.034	-2.48	0.013*
+Heart Rate	0.13	0.028	0.075	0.19	4.65	0.0000***
+Duration	0.0061	0.0023	0.0017	0.011	2.72	0.0066**
+Mass	0.080	0.0447	-0.007	0.17	1.80	0.072

Note: * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

To assess the impact of individual study level variables, separate meta-regression analyses were also performed for the 4 covariates. The results of these single covariate analyses can be seen in Table 3 and showed that post-exercise positive troponin response rate was influenced by increased exercise HR and reduced age. The regression coefficient for HR predicted a 0.118 increase in logit event rate per 1 $\text{beat}\cdot\text{min}^{-1}$ increase in HR, which predicted a 90% positive troponin event rate at a HR of 180 $\text{beats}\cdot\text{min}^{-1}$ (Figure 1.7).

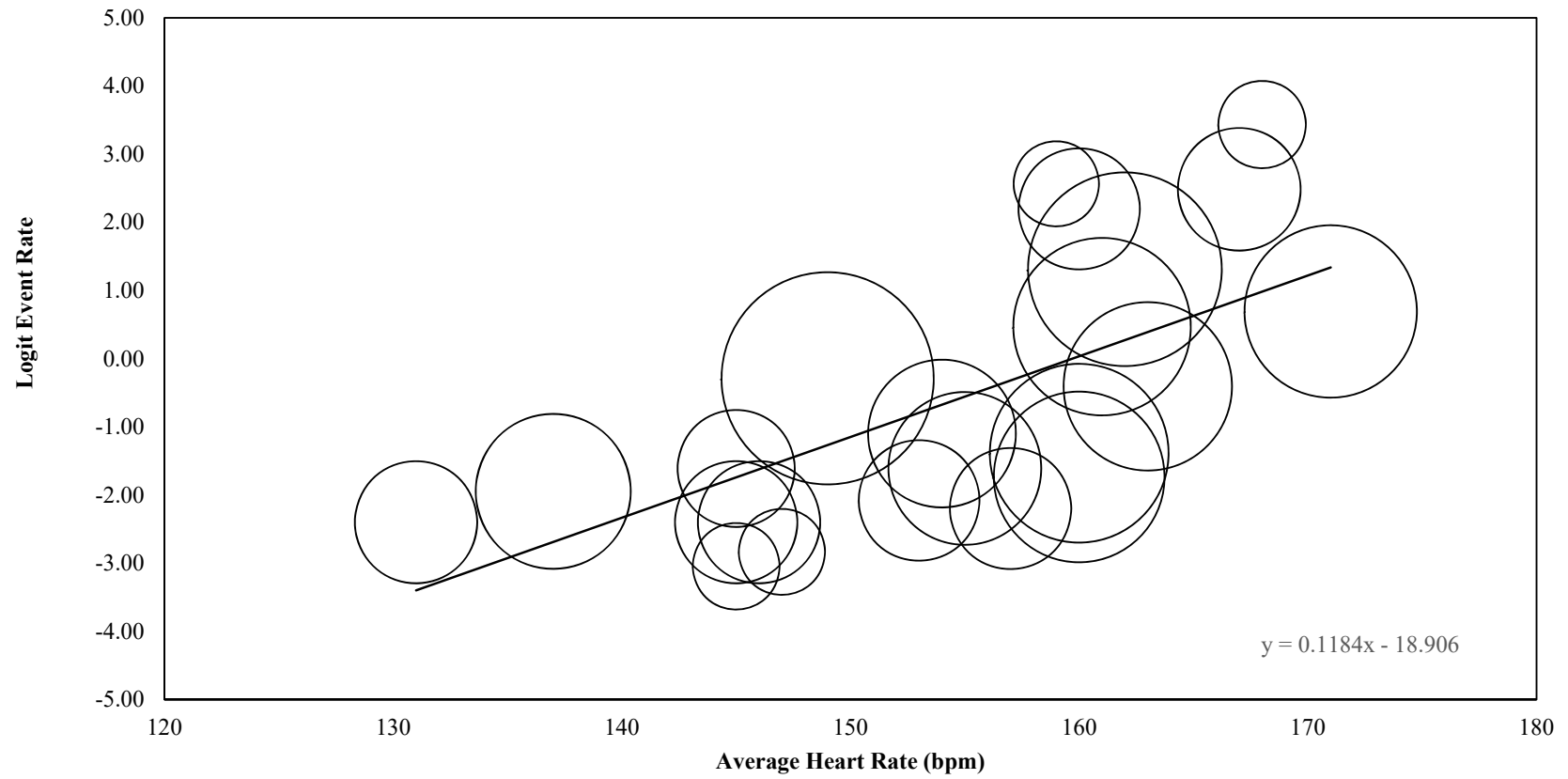


Figure 1.7. Regression plot of average HR vs troponin ER.

Participant age was negatively associated with logit ER. The regression coefficient was -0.1035 per increased year of age, which predicted a 75% event rate at age 10, and 5% event rate at age 40 when remaining covariates are kept equal.

Tests for remaining unexplained variance were significant for each covariate ($P < 0.001$), highlighting that troponin ER still differed among studies reporting similar participant age and HR.

1.6.2.3 E/A Ratio Standardised Mean Difference (SMD)

Post-exercise E/A values were significantly ($P < 0.001$) reduced over pre-exercise levels ($d = -1.197$ (95% CI [-1.401, -0.993])), as calculated by the random-effects meta-analysis (figure 1.8). There was evidence of large, significant heterogeneity between the 28 studies that were meta-analysed ($Q = 56.61$, $df = 27$, $I^2 = 56.61$, $P = 0.001$), highlighting the appropriateness of the random-effects model. Further, it was calculated that ~57% of the between study variance ($\tau^2 = 0.143$) was due to real differences in effect size and not sampling or random error, therefore a meta-regression analysis was also performed for the SMD of E/A ratio.

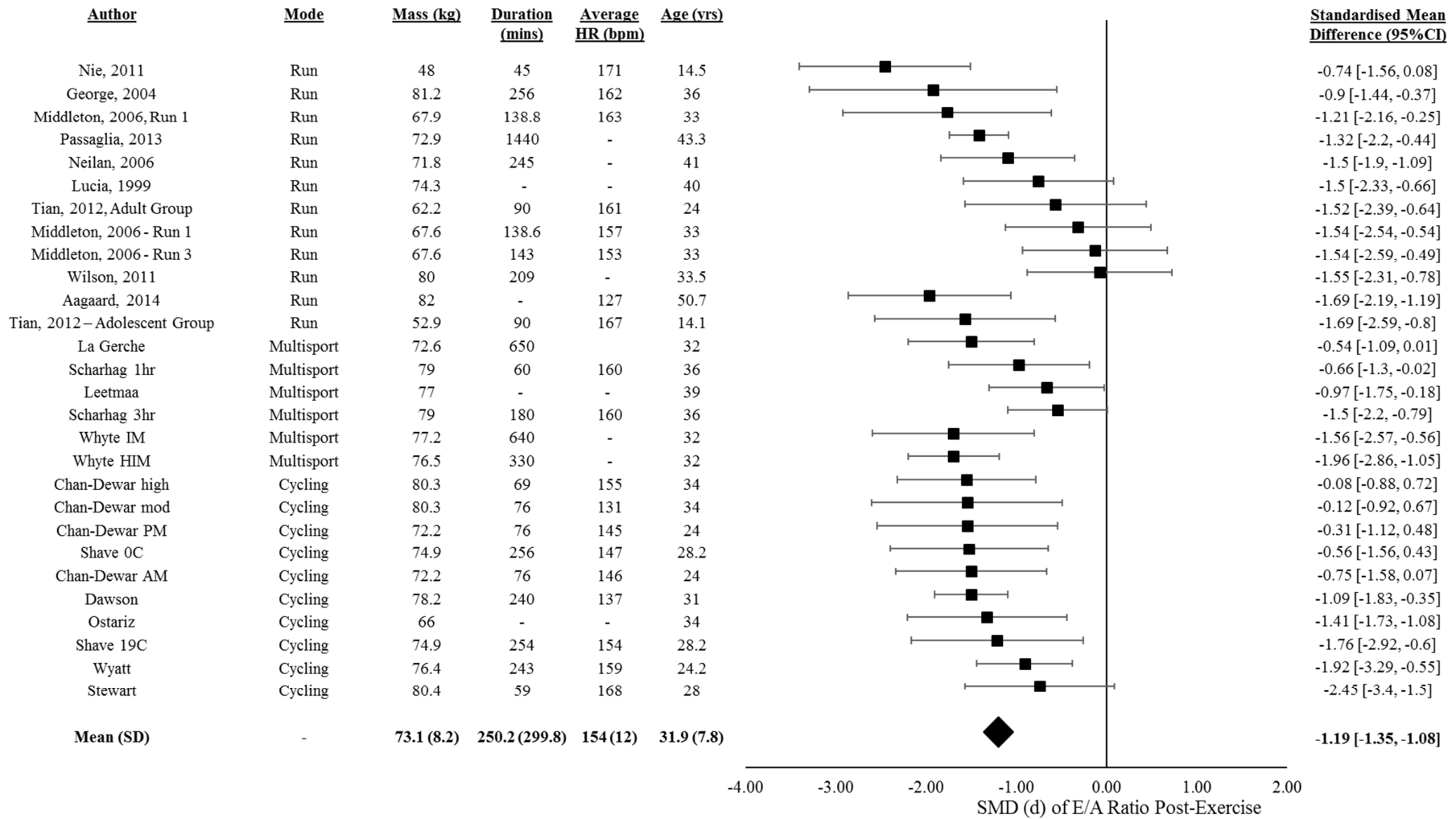


Figure 1.8. Forest plot showing SMD (Cohen's d) of E/A ratio after exercise.

Subgroup Analysis

Studies were divided by exercise mode, into running (12), cycling (10), and multisport (6) exercise. In the mixed-effects model, overall, there were no significant differences between exercise modes on E/A ratio SMDs following endurance exercise ($Q = 3.074$, $P = 0.215$). At the subgroup level, E/A ratio was significantly reduced post-exercise in each exercise mode. Pooled SMDs and 95% CIs were running -1.40 [-1.60, -1.20], cycling -1.00 [-1.47, -0.54], multisport -1.13 [-1.58, -0.68].

The fixed-effects analysis reported a non-significant effect of exercise mode ($Q = 5.72$, $P = 0.057$). Additionally, a non-significant Q test for Running was demonstrated ($Q = 8.29$, $P = 0.687$), indicating that there was a common effect of exercise on E/A ratio amongst running studies. However, this was accompanied by significant heterogeneity overall ($Q = 56.6$, $I^2 = 52.3$, $P = 0.001$), indicating the true effect sizes differed in the remaining 2 subgroups, and the pooled dataset.

Meta-Regression Analyses

Of the 28 data sets containing E/A data, 18 reported participant mass, age, exercise HR, and duration. Additionally, troponin ER (%) was included as a covariate in the regression analyses. Between-study variance was similar to the original data set (see Table 1.1). A multivariate model containing age, HR, and duration accounted for the greatest proportion of between-study variance ($R^2 = 0.29$), but this was also not significant ($P = 0.061$) and inclusion of the 2 additional covariates reduced R^2 and increased P values further ($Q = 7.14$, $df = 5$, $P = 0.21$).

Separate meta-regression analyses were also performed for the 5 covariates. The results of these single covariate analyses can be seen in table 4 and showed that post-exercise positive troponin response rate was influenced by increased exercise HR and increased Troponin Event Rate. The regression coefficient for HR predicted a 0.029 reduction in E/A SMD per 1 $\text{beat} \cdot \text{min}^{-1}$ increase in HR, which predicted the mean reduction of -1.12 at an average exercise

HR of $160 \text{ beat} \cdot \text{min}^{-1}$. Troponin event rate was negatively associated with E/A ratio. The regression coefficient was -0.010 for every 1 percent increase in event rate, which predicted the mean change in E/A ($d = -1.197$) at a 50% event rate, when other covariates were kept equal.

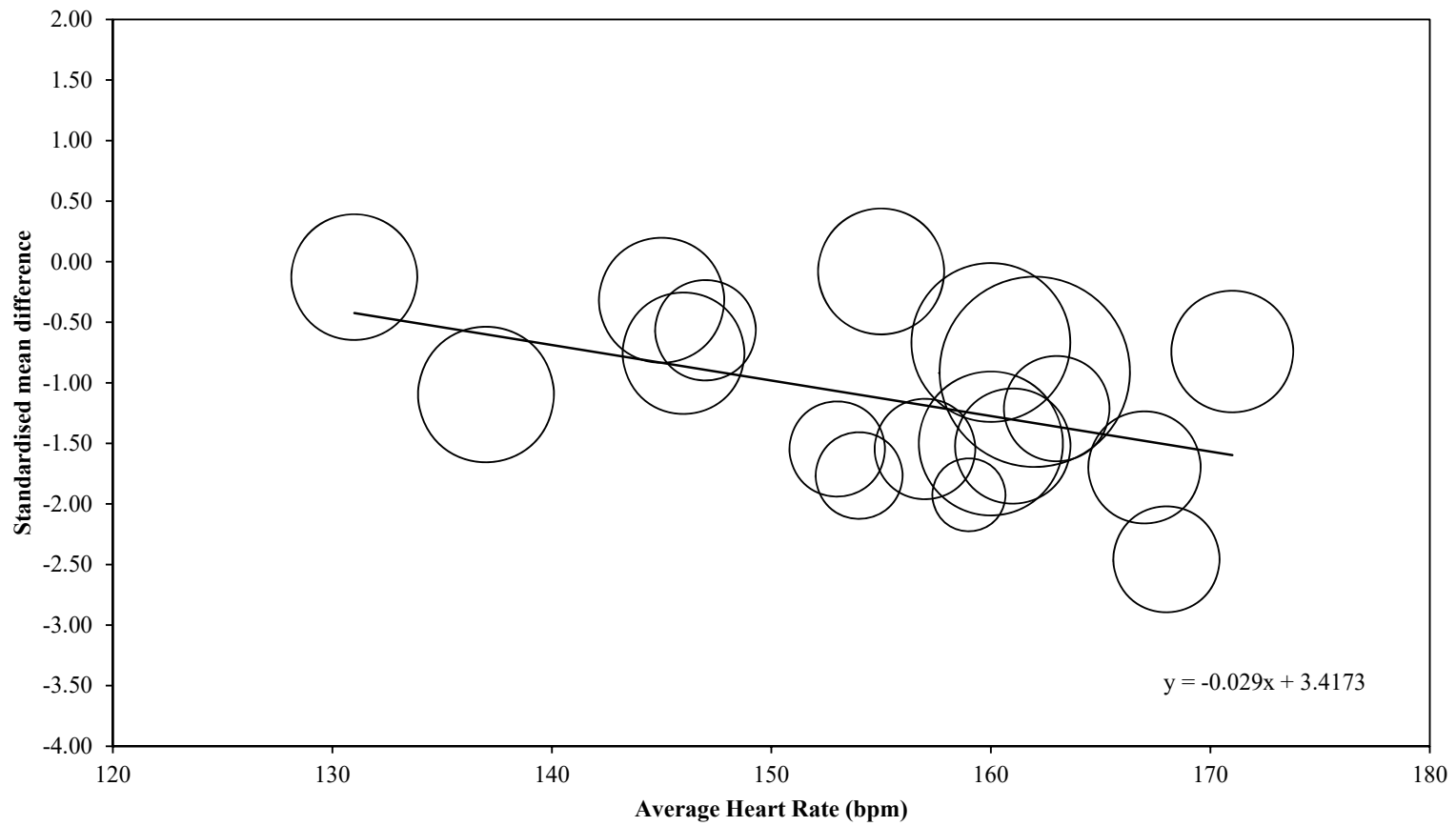


Figure 1.9. Meta regression of exercise HR vs. SMD of E/A ratio pre-post exercise.

The tests for remaining unexplained variance were significant for each covariate, with the exception of duration ($P < 0.001$), highlighting the changes in E/A ratio differed among studies reporting similar participant age, mass, exercise HR and troponin ER .

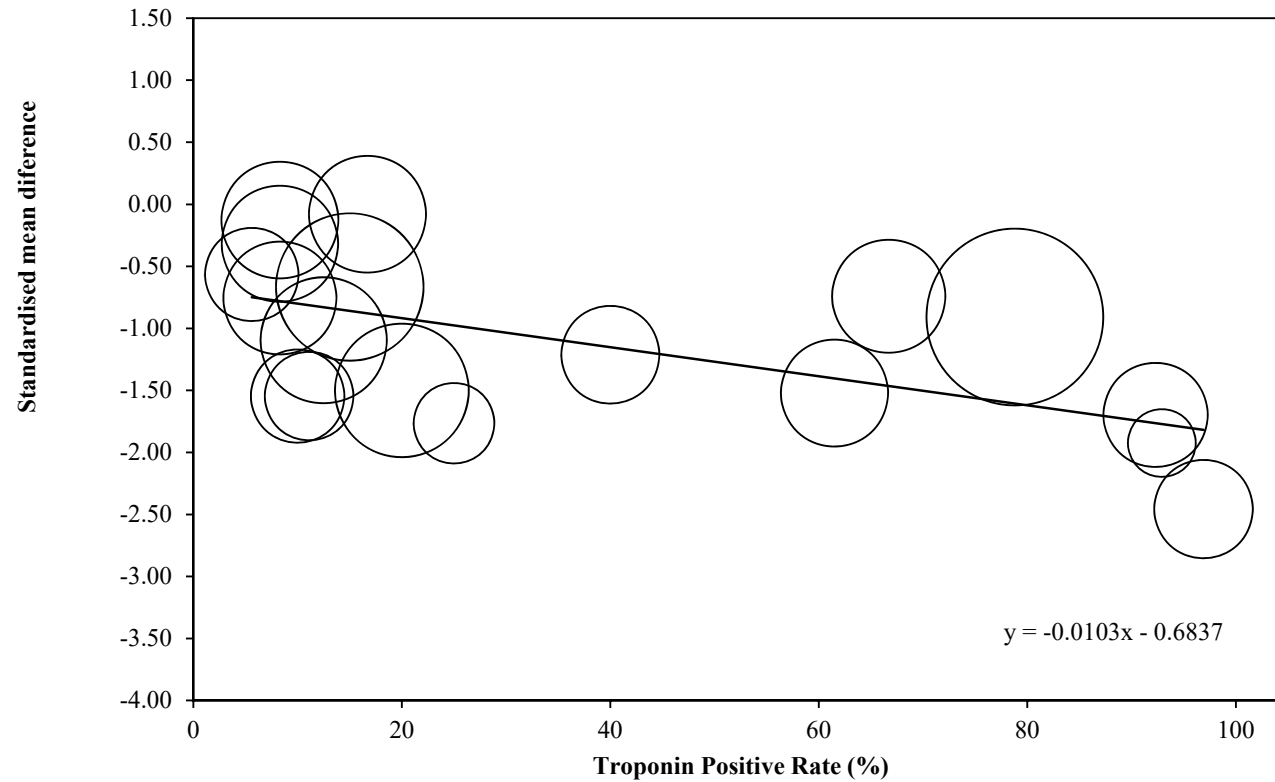


Figure 1.10. Meta regression of the percentage of participants per study exceeding the assay LOD (troponin positive rate) vs SMD of E/A ratio following exercise

1.6.2.4 Ejection Fraction Standardised Mean Difference

The mean difference in LVEF between pre- and post-exercise values was -2.02% (95%CI [-3.14, -0.91]). The standardised mean difference (Cohen's d) was -0.437 (95%CI [-0.74, -0.14]) (figure 1.11). There was evidence of large, significant heterogeneity between the 30 studies that were meta-analysed ($Q = 147.0$, $df = 29$, $I^2 = 80.27$, $P < 0.001$), highlighting the appropriateness of the random-effects model. A meta-regression was therefore conducted. The I^2 value of 80.27 highlighted that a large percentage of the between study variance ($\tau^2=0.516$) could potentially be explained by real differences in effect size and not sampling or random error.

Subgroup Analysis

Studies were divided into those involving running (11), cycling (12), and multisport (7) exercise. In the mixed-effects model, overall, there was no significant differences between exercise modes on the troponin positive response to exercise ($Q = 1.89$, $P = 0.39$). There was a significant effect of exercise on EF in all 3 modes. Pooled event rates and 95% CIs were running -0.37 [-0.73, -0.01], cycling -0.95 [-1.80, -0.09], and multisport -0.32 [-0.59, -0.04]. This was accompanied by significant heterogeneity overall ($Q = 147.0$, $I^2 = 80.3$, $P < 0.001$) and within Running ($Q = 33.5$, $I^2 = 70.2$, $P < 0.001$) and Cycling ($Q = 104.8$, $I^2 = 89.5$, $P < 0.001$), but not Multisport ($Q = 8.0$, $I^2 = 25.0$, $P = 0.24$), indicating the true effect sizes differed within the run and cycle subgroups, whereas the effect sizes were consistent across multisport studies.

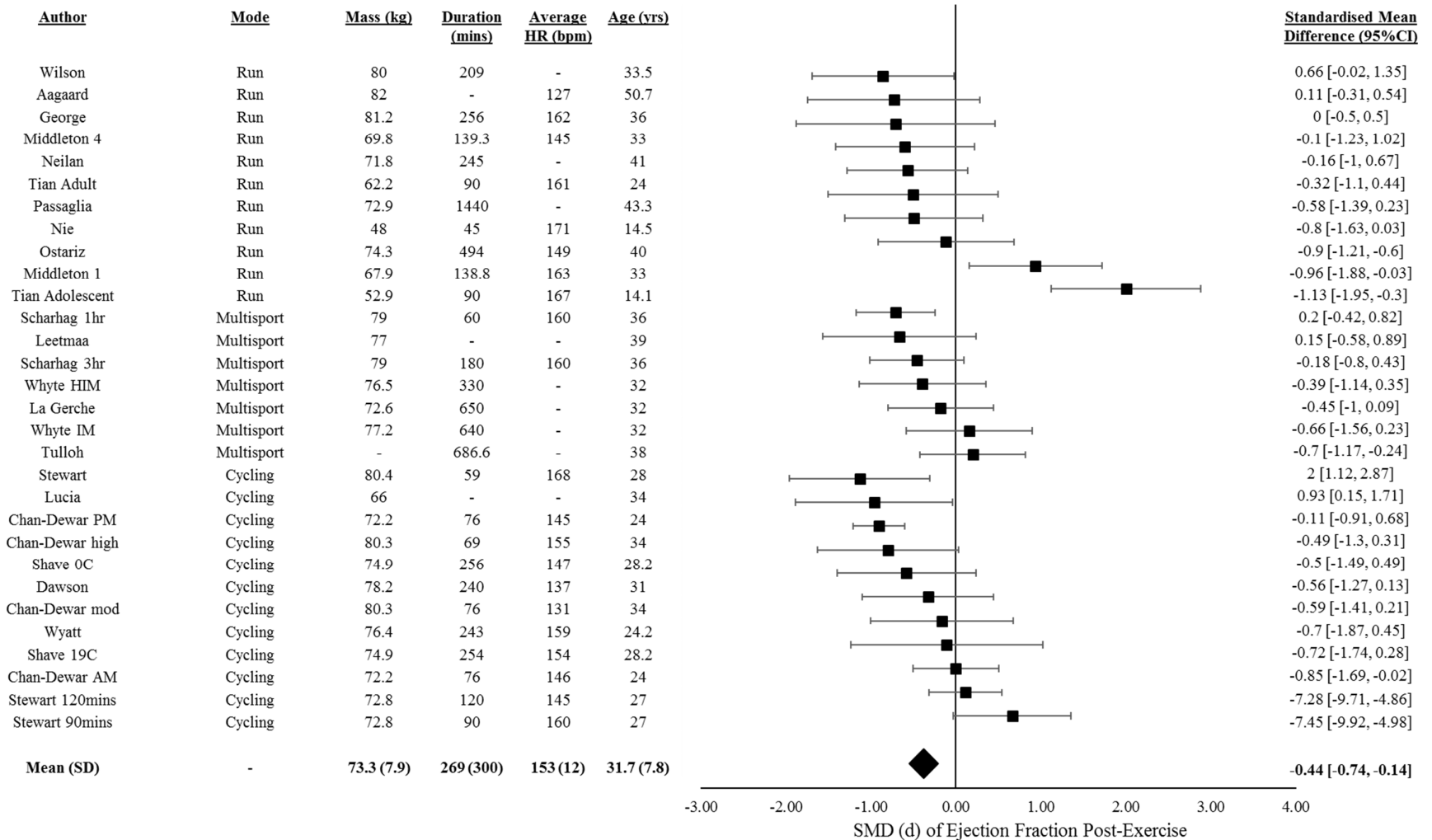


Figure 1.11. Forest plot of ascending post-exercise standardised mean difference (SMD) (Cohen's d) in ejection fraction.

Meta-Regression Analyses

A meta-regression was conducted using exercise HR and duration data, and participant age and mass, from 20 of the 30 data sets. All covariates were analysed separately, and then together, to assess the individual contribution of each variable. For the reduced sample size of $n = 20$, between study variance was similar to the original data set (see Table 1.1). We found no significant interaction of any of the moderator variables on effect size. Despite there being potentially a large proportion of the variance explainable by study-level covariates, as indicated by I^2 of 80.3, the data available failed to account for this. Even when combined, a method that usually produces more substantial interactions, the meta-regression model did not explain any of the between study variance witnessed.

1.6.3 Discussion

This was the first meta-analysis to focus on the relationship between functional and biochemical indices of cardiac fatigue and damage. Studies using the mixed methods of echocardiography and cardiac biomarkers were exclusively analysed to attempt to elucidate the relationship between them. Further to this, the hypothesis that cTn is released from the myocardium following PSE and this brings about functional decrements was also investigated (Wilson et al., 2011b, Shave and Oxborough, 2012). To better inform endurance athletes, coaches, and sports physicians the relationship between exercise duration, mode, and intensity in eliciting EICF and troponin release was explored. The outcomes of this study demonstrate a detrimental effect of endurance exercise on cardiac diastolic and systolic function, and confirm Shave and colleagues' finding of a ~50% event rate of troponin release (Shave et al., 2007). Endurance exercise of durations ranging from 45 to 1440 minutes caused significant reductions in E/A Ratio of -0.34 ($d = -1.20$, $P < 0.001$), and ejection fraction of -2.0% ($d = -0.44$, $P = 0.004$). These findings agree with the previous meta-analysis performed by Middleton et al. (2006), who reported mean reductions in E/A ratio of 0.45 (-0.39, -0.51) and EF of -1.95% (-1.03, -2.88%) following endurance exercise of similar durations.

The novel findings were that exercise bouts causing the greatest amount of positive troponin responses resulted in the largest reductions in left ventricular diastolic filling (E/A ratio). The current study also established exercise intensity, measured via average exercise HR, as the predominant factor in causing EICF and troponin release. Therefore, together these findings suggest that higher participant exercise HRs elicits both increased troponin release and reduced diastolic function. At the study level, reduced diastolic function is also related to troponin positive ER. It was possible to explain a portion of the variance in troponin ER, and diastolic, dysfunction using meta-regression. However, it is important to also recognise that the low to moderate R^2 values related to exercise HR and troponin ER (0.32, 0.33 respectively) leave a remaining portion of the variance requiring further exploration.

1.6.3.1 Effect of Exercise Intensity

Troponin

Novel to this meta-analysis was exploring the role of exercise intensity. Previous meta-analyses have not reported this variable, citing lack of availability of data. Interestingly, exercise HR was the strongest predictor of troponin event rate ($R^2 = 0.31$) though, unfortunately the relative intensities were not widely available to allow for a more accurate comparisons of intensities. However, as all studies that reported HR data recruited trained participants only, it is reasonable to assume that the mean HRs reported were reflective of the intensity demanded by the duration of the exercise bout, which goes some way towards normalising the HR data.

The current finding of increased likelihood of blood cTn levels exceeding the troponin assay detection limit with increasing HR supports the emerging theory that troponin release is intensity dependent. In contrast to prior research that proposed exercise duration was the determining factor, more recent studies have measured appreciable rises in cTn following exercise bouts as short as 30 minutes at 85-90% $\dot{V}O_{2max}$. If thresholds of exercise intensity,

rather than duration, at which cTn is released exist, these may be altered depending on fitness level.

Unfortunately, it was not possible to separate the cases based on training status in the present study owing to a lack of data. Whether troponin release following exercise is an adaptive response that elicits improved cardiac function and a reduced threshold for future cTn release remains to be thoroughly examined.

The findings support the notion that the rate and force of myocyte contraction influences cTn release. It has been suggested that post-exercise troponin release occurs due to membrane damage caused by increased mechanical force of cardiac myofibrillar contraction. In the absence of ischemia, the transport of intact troponin molecules is potentially mediated by excessive stretch of myofibrils stimulating integrin-mediated transport (Hessel et al., 2008). Troponin I degradation in the absence of ischemia has been shown to increase with LV preload in the rat model (Feng et al., 2001). Additionally, membrane disruption resulting from oxidative damage and inflammation subsequent to intense exercise may allow cTn release (Scherr et al., 2011). Whether this cTn is bound to the myocardium or stored in the cytoplasm remains to be determined. It is therefore plausible that both intact and degraded cTn products found in serum post-exercise originate from viable cardiomyocytes, due to the increased preloads during exercise.

E/A Ratio

Higher exercise HRs increased the magnitude of the post-exercise decline in LV diastolic filling. In most cases, this was due to reduction in the early (E) mitral wave and maintenance of the late (A) wave. This is associated with increased stiffness of the left ventricle due to inadequate myocardial relaxation and unpaired ventricular twisting (Shave and Oxborough, 2012). Potential mechanisms responsible for this include downregulation of cardiac β -adrenoceptors mediated by elevated catecholamines during exercise, which has been demonstrated in both athletes and healthy sedentary individuals (Ashley et al., 2006, Douglas et al., 1990, Eysmann et al., 1996). Circulating catecholamines are responsible for

maintaining tachycardia during endurance exercise, and previous works have shown concentrations of plasma catecholamines to increase with exercise HR (Breuer et al., 1993). The data bear out this hypothesised relationship between impaired myocardial relaxation, and decreased sensitivity of β -adrenoceptors induced by increasing circulating catecholamines ubiquitous to higher exercise HRs during prolonged strenuous exercise (Douglas et al., 1998, Welsh et al., 2005).

1.6.3.2 Effect of Exercise Mode

The subgroup analyses did not find any stark differences between exercise modes in the random effects analysis, which was not what was hypothesised. While the fixed effects model returned a significant effect of exercise mode, the accompanying test of heterogeneity did not fulfil the assumptions of the FE model. It was expected that this study would yield similar results to Shave et al's finding that running exercise stimulated the greatest amount of troponin release compared to cycling exercise (Shave et al., 2007). It has been hypothesised that running exercise elicits higher HRs than cycling due to greater $\dot{V}O_2$ requirements, recruitment of the upper body musculature and accessory muscles, and the lack of postural support evident during cycling (Millet et al., 2009). In the data average cycling HR was 150 vs. 156 in running. Unfortunately, it is not possible to compare these with the previously published meta-analysis as HR data were unavailable. Additionally, modal differences in troponin release may be explained by attaining higher absolute cardiac and metabolic work rates during running (Schneider and Pollack, 1991, Millet et al., 2009), and evidence of increased SV (preload) and HR during running vs. cycling at a given submaximal $\dot{V}O_2$ and \dot{Q} (Hermansen et al., 1970). Despite evidence of a potential physiological mechanism to explain increased cTn release during running exercise, the data do not suggest this is the case as there was no effect of exercise mode. Exercise duration was 171 vs. 267 minutes in running, but this was heavily skewed by the inclusion of the 1440 min bout of exercise in the study by Passaglia et al (2013). Removal of this from the subgroup analysis greatly increased the disparity between the subgroups and approached significance ($P=0.068$ vs. $P=0.112$). It is

possible that the cardiac demands of the exercise bouts employed in both the running and cycling studies were of sufficient intensity and duration to hide any modal differences that might be shown at lower exercise intensities.

1.6.3.3 Effect of Exercise Duration

There was no significant effect of exercise duration on either cTn release, LV systolic, or diastolic function. When the exercise protocols employed in PSE studies typically involve a race-effort at maximal efforts for the duration, exercise duration dictates exercise intensity. Previous meta-analyses have failed to investigate the role of exercise HR and have concentrated on duration; therefore, no comparable data exists. The absence of a significant role of exercise duration on either cTn release or EICF contradict the prior works by Shave et al. (Shave et al., 2007) and Middleton et al (Middleton et al., 2006), despite inclusion of a similar amount of studies, cases and participant characteristics. Further, the previous meta-analysis of troponin ERs failed to report regression coefficients for significant moderators and used a fixed-effects meta-regression. This may not have been appropriate, depending on the level of heterogeneity between studies in the model as significant heterogeneity at the study level for all MVs in all 3 meta-analyses was encountered. In the current review, this meant that the true effect size likely differed between studies at similar levels of any MV and use of the fixed-effects model would not be appropriate. This finding of a significant influence of exercise HR on cTn ER concomitant with an absence of significant effect of duration refutes the dominant theory that cTn release is duration dependent.

1.6.3.4 Troponin Event Rate Affects Diastolic Function

Cardiac biomarkers and non-invasive imaging techniques are used together to explore whether endurance exercise produces persistent cardiac dysfunction. Most of the research refutes this possibility, as most post-exercise cardiac dysfunction is typically resolved within 24h to 1 week of the exercise. The current data demonstrated a significant relationship between cardiac diastolic dysfunction and cTn release, whereby the degree of dysfunction was related to the magnitude of troponin positive ERs. This finding is contradictory to most

of the research as few individual studies have report correlations between any LV functional indices and troponin release. While the possibility of statistical error cannot be ruled out, it is interesting that the meta-regression failed to find a similar link between systolic function (EF) and cTn event rates. When loading conditions are controlled for, exercise induced diastolic dysfunction is characterised by reductions in myocardial relaxation. While the mechanistic basis for exercise induced diastolic dysfunction are yet to be fully determined, cardiomyocyte membrane disruption has been suggested as a factor in this, as well as troponin release. Though individual studies rarely find significant correlations between the two (George et al., 2004), it may be the case that this was due to insufficient sample sizes to bear out the underlying relationships. The larger sample sizes meta-analysed in this study allowed us to determine this possibility. While the current study found evidence of a significant effect of troponin release on diminished diastolic filling, these data are merely correlational. There is insufficient physiological data present in the original studies to determine a causal relationship. More sensitive measures of diastolic filling function such as tissue Doppler, twist/untwist velocities, and global strain rate imaging(Stewart et al., 2016). While the data are suggestive of the theory that exercise induced cardiomyocyte membrane disruptions transiently decrease membrane permeability and chronotropic sensitivity, thus effecting greater troponin release, this finding has not been demonstrated in the individual studies (George et al., 2004) and must be interpreted with caution.

1.6.3.5 Troponin Event Rate Associated with Participant Age

Participant age was significantly negatively associated with troponin ER. As age increases the risk of troponin positive response was diminished. There also was evidence of a significant negative correlation between age and heart rate, indicating that older participants do not achieve higher HR's. This, with the finding that troponin ER increases with HR can rule out other physiological mechanisms that prevent cTn release in older athletes. It may be that lifelong athletes develop greater thresholds to cTn release than younger, less-trained

counterparts. Without training history data this phenomenon cannot be investigated via meta-analytical methods.

1.6.3.6 Limitations

The studies included in this meta-analysis were limited by several methodological factors. Inconsistent timing of the post-exercise echocardiographs and blood samples across the range of studies may have had severe effects on the values analysed. Mechanisms responsible for this include the time-course of cardiac troponin release, which is thought to peak at approximately 3hrs post-exercise (George et al., 2004). To control for this, only studies that obtained blood values within 1hr of the cessation of exercise were selected; however, no studies recorded the time delay between finishing exercise and completion of blood draws for individual participants. The effects on troponin levels could be severe in field in studies with large numbers of participants finishing at varying times. Additionally, the present study used the assay LOD as a requirement to count as a troponin positive event. This was an attempt to standardise the wide intra individual levels of cTn release that have been previously reported but it must be recognised that patients presenting with a cTn level of >0.01 ug/L are of a vastly different clinical population than patients >0.1 ug/L (Kumar and Cannon, 2009). In terms of echocardiography the timing factor is relevant as changes in loading conditions altered by blood plasma shifts during and after exercise significantly influence echocardiographic variables. Further, the influence of post-exercise hypotension and augmented autonomic modulation during the recovery from exercise should not be overlooked. Stewart et al (2016) attempted to normalise cardiac work rates during the recovery from moderate and heavy intensity exercise by employing a standardised low-intensity exercise challenge (individual work rate to achieve $100 \text{ beats} \cdot \text{min}^{-1}$) in their methodology. There were demonstrably greater differences in LV and RV function post-exercise when loading and functional requirements are equal to pre-exercise levels (Stewart et al., 2016). An important limitation to the current meta-analytical approach was the inclusion of studies that measured both troponin I and T. Troponin I is thought to be less sensitive than

troponin T due to several manufacturers producing assays (Shave et al., 2007). Sub-group analysis to determine a significant effect of assay type on troponin event rate did not find a significant difference between assays measuring troponin T and I, and this is supported by previous work finding comparable diagnostic performance of hs-cTn I and T assays in a pathological population (Sou et al., 2016).

1.6.4 Conclusion & Summary

In summary, the current meta-analytical approach determined that endurance exercise lasting >45 minutes has a significant detrimental effect on diastolic filling, systolic performance, and increases troponin levels. Further, it was found that exercise intensity, and not duration or mode is the strongest influence over both reduced diastolic filling and troponin release. These findings also raise the possibility of a correlational relationship between diastolic function and troponin release. Future work should aim to investigate this relationship further, using more specific tissue Doppler measurements of diastolic function, and potentially by employing standardised exercise challenges to normalise cardiac function before and after PSE (Stewart et al., 2016). Finally, the finding of a significant negative effect of participant age on troponin event rates indicates that longer-term athletes may be less prone to troponin release as a result of cardiac adaptations pursuant to life-time training volumes (Scharhag et al., 2002). Future studies research into the effects of age on cardiac adaptation to exercise may wish to investigate the effects of troponin release following a detraining period on life-long endurance athletes. The theory that troponin release is related to permanent cardiac injury is also challenged by this finding (Wilson et al., 2011a). In conjunction with a recent report on exercise induced sudden cardiac death that found morbidity increased with age, the finding of reduced troponin release with age suggests the two are not causally linked (Harris et al., 2017).

The findings of previous meta-analyses that investigated modern echocardiographic techniques indicate that after exercise bouts lasting at least 120 minutes, LV LS is reduced by

-0.9 % (-1.0 to -0.5 range) and systolic SR is concomitantly reduced by -0.9 (-1.3 to -0.5) (Lord et al., 2018). Published meta-analysis studies in EICF have involved a diverse range of exercise protocols in terms of their durations, modes, and intensities (Donaldson et al., 2019, Shave et al., 2007, Middleton et al., 2006). The study of Lord and colleagues (2018) was the first to meta-analyse MST parameters and the authors noted that there were similar responses for LV longitudinal and radial strain following a marathon run (26.2 miles) and 100-mile ultramarathon run (Lord et al., 2018). However, similar reductions in LV LS have been shown following comparatively short bouts of cycling exercise (60 minutes) (Stewart et al., 2015). Further work on short-duration exercise by Kleinnibbelink and colleagues (2021) has demonstrated marked reductions in LV and RV ϵ following just 45 minutes of high intensity running exercise, which also elicited mean HRs of ~ 170 beats \cdot min $^{-1}$ (Kleinnibbelink et al., 2021b). In their meta-analysis, Lord and colleagues calculated a similar level in systolic twist reduction of -1.0 (confidence interval (CI) -1.6 to -0.3) (Lord et al., 2018). The amount of LV twist is reflective of the stored energy within the myocardium at the end of systolic contraction, prior to its release during diastole (Stohr et al., 2012). Therefore, a reduction in LV twist is indicative of both reduced systolic and diastolic performance. The authors noted that this finding was unanimous across the 5 studies that were included in the analysis, and that the reduction in LV twist was reflected in their findings of significantly reduced early diastolic filling velocity (E) in the PW Doppler data (Lord et al., 2018).

The potential mechanistic basis for the exercise-induced reduction in myocardial strain has been proposed to be a result of either, or a combination of: alterations in loading and HR, sub-clinical levels of myocyte damage, β -adrenergic desensitisation, serial or parallel ventricular interaction (Lord et al., 2018, Oxborough et al., 2010). The concept of LV and RV interaction has emerged from research that identified the relative weakness of the RV free wall in comparison to the LV, rendering it unable to compensate for the increase in afterload during PSE (Lewicka-Potocka et al., 2020). This could result in reduced RV contractility and RV SV and eventually a diminished LA preload and LV filling (Oxborough et al., 2010). Additionally, evidence of RV dilatation caused by consistently high afterload pressures and

concomitantly reduced RV ϵ and LV EDV following ultra-endurance running exercise (Lord et al., 2015, Oxborough et al., 2011, La Gerche et al., 2008) may have resulted in IVS displacement. It has been proposed that septal displacement results in impaired LV twisting mechanics and longitudinal contractility (Lord et al, 2018).

Of great interest is whether the temporary alterations in cardiac performance arising from PSE are cumulative, ultimately leading to pathological adaptations such as arrhythmogenic right ventricular cardiomyopathy, and hypertrophic cardiomyopathy; the two leading pathologies that are present in victims of SCD (O'Keefe et al., 2020). Evidence exists to suggest a lifetime of endurance training may lead to fibrosis of the myocardium and the development of these cardiomyopathies, which can lead to life-threatening arrhythmia (Oxborough et al., 2010, Wilson et al., 2011a). The cumulative effect of increased ventricular wall stress, alongside sustained catecholamine release and reactive oxygen species is thought to cause myocardial scarring (Shave and Oxborough, 2012). Additionally, coronary artery calcification is higher in lifelong endurance athletes, which is hypothesised to be a result of arterial wall injury and recovery calcification resultant from the haemodynamic and mechanical stress of endurance training (Baggish and Levine, 2017). However, the clinical relevance of this data is not certain as it has been demonstrated that atherosclerotic plaques in masters athletes are less prone to disruption and therefore less likely to cause a myocardial infarct (O'Keefe et al., 2020). A protective, rather than pathological effect of a lifetime of exercise is supported in part by evidence from Mehta and colleagues (2011) who demonstrated a significant negative relationship between training history and cTnI release following the London Marathon (Mehta et al., 2012).

Therefore, while the long-term effects of PSE on myocardial health and performance are less certain there is still a need to assess the cardiac strain of commonly participated in sporting events on cardiac function, with a view to determine the presence of transient and/or persistent EICF (Franklin et al., 2020b, O'Keefe et al., 2020). To assess the potential cumulative effects of EICF, previous works have included post-exercise cardiac measurements, which have ranged from 1 hour to several days of rest after the exercise bout

(see Table 1). Typically, researchers will conduct further measurements at 24 hours post-exercise; however, this is not currently a standardised method within the body of research. There is also a large inter-individual variation in post-exercise recovery kinetics (Mehta et al., 2012), which is further confounded by variations in exercise duration, intensity, and mode. The inclusion of a 24h period of rest may not be typical for many athletes, especially those competing in multisport endurance events such as the triathlon and stage events like the Tour de France (Rifai et al., 1999, Millet and Bentley, 2004, La Gerche et al., 2004, Tulloh et al., 2006, Leetmaa et al., 2008, Nottin et al., 2009, Park et al., 2014). Research by Williams et al (2009), was conducted to assess the impact of repeated endurance exercise on cardiac function for 22 days of cycling activity (Williams et al., 2009). The authors found an initial decline in EF and E/A ratio after each stage; however, there was limited evidence of progressive declines over the 22-day period. Additionally, there was evidence of spontaneous appearance of cTnI, though this was not sustained across all stages and likely had limited clinical relevance. Interestingly, while systolic function returned to baseline (BL) within 2 days of the end of the tour, diastolic function was significantly reduced at the same timepoint (TP), which was attributed to a reduction in E and a slight increase in A velocity (Williams et al., 2009). These findings are similar to the work of Middleton et al (2007), who investigated the effects of repeated strenuous running bouts on cardiac function and noted reductions in E/A ratio that persisted beyond the completion of the final exercise bout (Middleton et al., 2007).

Whether there are any modal differences in the magnitude of EICF has been investigated but there is insufficient evidence to draw many conclusions to date (Legaz-Arrese et al., 2015, Le Goff et al., 2020, Franklin et al., 2020a). The work of La Gerche and colleagues (2007), which investigated the level of fibrotic infiltration of the myocardium, was conducted on a cohort of trained runners (La Gerche and Prior, 2007). Additionally, most of the research to date has been focused on athletes competing in triathlon or marathons, which both involve extensive running training (Suriano and Bishop, 2010, Millet et al., 2011), with several meta-analyses determining a greater rate of myocardial fibrosis in lifelong athletes than in the

general population (La Gerche et al., 2012a, Schnell et al., 2016, Pujadas et al., 2018, Wilson et al., 2011a, van de Schoor et al., 2016, Zhang et al., 2020). Therefore, while still unclear, the wealth of evidence would suggest that lifelong running is associated with myocardial fibrosis, and it may be the case that running exercise is the most damaging and requires the greatest amount of recovery. Additionally, the meta-analysis by Shave and colleagues (2007) has shown that relatively heavier individuals are more likely to demonstrate an elevation in cTn following strenuous running exercise (Shave et al., 2007), which may be explained due to the eccentric nature of weight bearing exercise, such as running (Millet et al., 2009). Unfortunately, no studies to date have investigated potential differences in the extent of cardiac functional changes between weight-bearing i.e., running, and non-weight bearing exercise, such as cycling. It is, however, currently accepted that the magnitude of the exercise induced cardiac functional change appears to be related to exercise HR and modality, with running and cycling exercise eliciting the highest exercise HRs and subsequently generating the largest elevations in cardiac biomarkers and temporary reductions in diastolic function (Shave and Oxborough, 2012, George et al., 2012). Additionally, Kreider et al (1988) demonstrated that running preceded by cycling exercise results in greater exercise HR, heat stress and reduced SV and MAP compared to standalone running at the same speed (Kreider et al., 1988). However, in this study resting cardiac function was not assessed and no biomarkers were measured. Several previous studies have investigated the degree of cardiac fatigue following triathlon races, these have mainly focused on ultra-distance events, such as the half or full Ironman (Douglas et al., 1998, Rifai et al., 1999, La Gerche et al., 2004, Tulloh et al., 2006, Shave et al., 2004). There is substantial evidence to indicate reduced systolic and diastolic function following events of these durations (6-12 hours), indicated by significant reductions in EDV, FAC and LVEF that have been attributed to impaired myocardial contractility and reduced preload (McGavock et al., 2002).

However, more people take part in shorter, more intense bouts of exercise, and they also perform them more frequently than ultra-distance events. Single sport exercise bouts lasting 45-60 minutes have been shown to cause transient cardiac perturbations (Kleinnibbelink et

al., 2021a, Stewart et al., 2015, Chan-Dewar et al., 2013a, Shave et al., 2010b) and cardiac assessment following the individual legs of a short-format bike-run transition lasting 120 minutes has demonstrated changes in LV function during each leg (McGavock et al., 2003). The key findings were that FAC increased with concomitant reductions in EDV and ESV, but the authors concluded that there was no systolic dysfunction present during exercise or after. However, this research study made use of older echocardiographic techniques and disrupted the cycling and running legs every 30 minutes to conduct the measurements. Park et al (2014) have also demonstrated significant elevations in cardiac biomarkers, including cTnT, following short-format triathlon lasting up to 120 minutes (Park et al., 2014). In this study, cardiac perturbations were greatest in those who elicited the highest exercise work rates and HRs. This finding corroborates more recent research by Richardson et al (2018), who noted that post-marathon cTnT was associated strongly with exercise intensity, measured by peak HR and average percentage of max HR during exercise (Baker et al., 2019).

While there is a growing number of research studies that have investigated EICF and shorter duration exercise bouts, research into duathlon is currently scarce. Duathlons are a popular alternative to triathlon during the off-season and are completed by athletes at amateur to the elite level (Sparks et al., 2005, Alvero-Cruz et al., 2011, Rust et al., 2013, Berry et al., 2016, Nikolaidis et al., 2019, Tsuzuki et al., 2019, Nikolaidis et al., 2021). As it remains to be seen whether running exercise potentially elicits greater cardiac strain and fatigue than cycling and swimming exercise, duathlon competitions and training may incur a greater level of cardiac fatigue due to the additional run leg. Further, it remains to be seen if exercise modality affects the magnitude of the transient decline in cardiac function and health following PSE in an endurance-trained population. Investigation of exercise induced cardiac functional changes (perturbations) in athletes who are both cycling and running entrained would allow for a fairer comparison of the two modes, with reduced intra-individual variation in responses (see figure 1.6 below) (Millet et al., 2009, Millet et al., 2011).

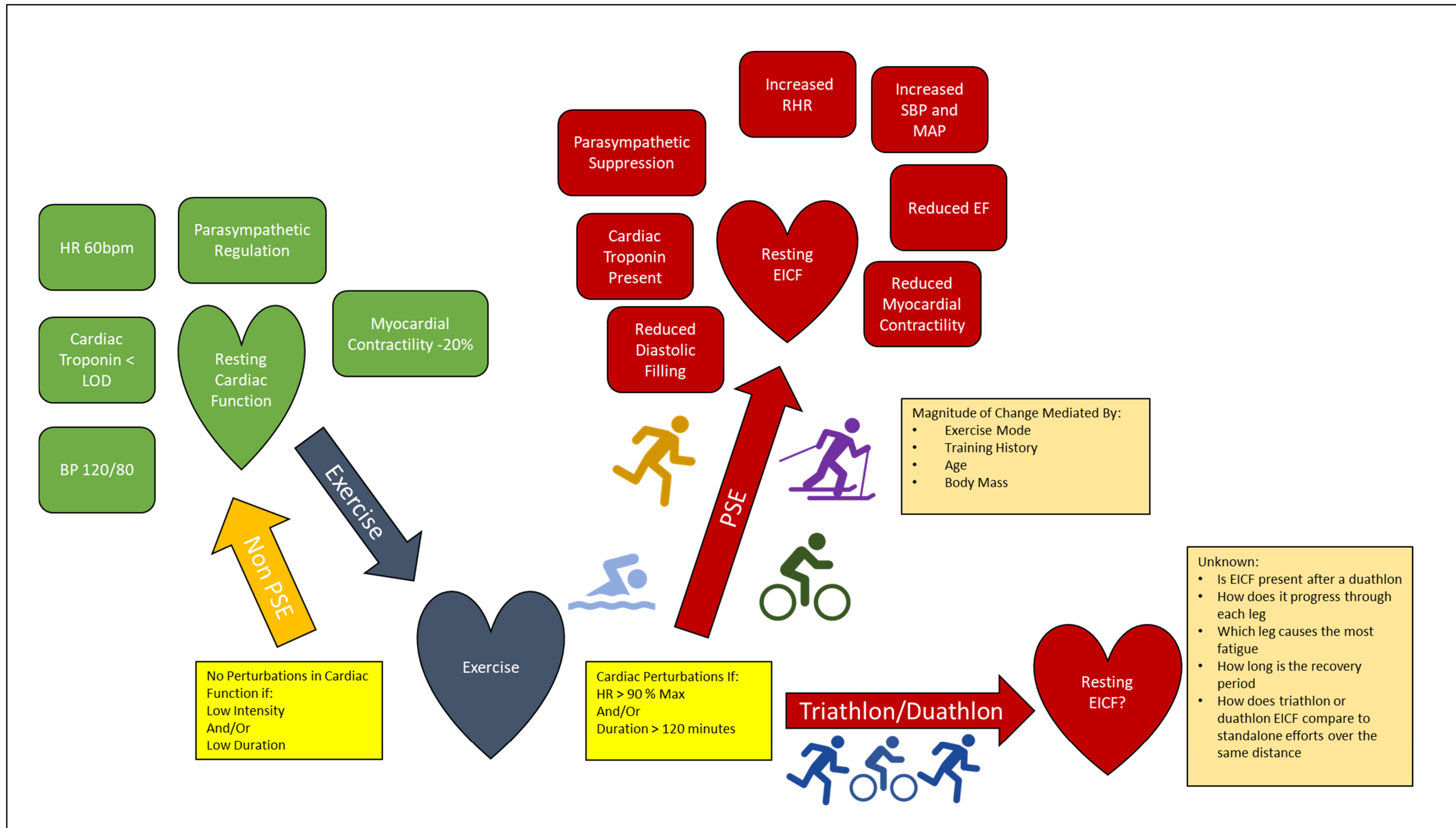


Figure 1.12 Outline of changes in cardiac function following exercise of varied intensity, mode, and duration.

Note: LOD = Assay limit of detection.

1.7 Thesis Aims

Therefore, the purposes of this thesis were to provide an overview and assess current methodologies used to assess cardiac function and mechanics following exercise, and to systematically review and meta-analyse the current literature with reference to the effects of exercise intensity, mode, and duration on potential EICF and cTn release. Additionally, there was the aim to perform a research study that investigated the effects of duathlon exercise on cardiac function, as assessed by standard measures of systolic function such as Simpson's biplane analysis, measures of myocardial longitudinal function using MST to determine ventricular longitudinal strain (LS), and Doppler indicators of diastolic function. This thesis first aimed to assess the effect of exercise across each stage of an OD duathlon in highly trained athletes. Further, a secondary study was performed to explore the individual effects of each leg at duathlon race intensity. Further, due to duathlon pacing strategies, experienced duathletes aimed to maintain work rate across the 3 legs to achieve the best overall time and distribute their effort accordingly. Therefore, athletes may be able to increase their performance in maximal effort time trials across each of the separate duathlon legs. Accordingly, to quantify any additional effect of increased work rate, this thesis also aimed to investigate the effects of a separate maximal effort trial over each duathlon leg.

1.8 Hypotheses

1. There will be significant alterations from pre-exercise resting measures in cardiac autonomic regulation, and echocardiographic measures of ventricular LS, systolic function, and diastolic function that will be acutely present following an OD duathlon.
2. These changes from baseline will reverse within 24 hours of passive recovery to near pre-exercise values.

3. In the OD duathlon, the initial 10k running leg will be the most strenuous leg in the duathlon and cause the initial alterations in measures of cardiac function and regulation.
4. Experienced, fit multisport athletes will be able to significantly increase their work rate and performance during maximal effort standalone time trials over 5k and 10k of running, and 40k of cycling exercise.
5. Standalone, maximal effort performances of each duathlon component will produce greater effects on post-exercise cardiac function than consecutive bouts.
6. Increased run distance during the 10k run trials will cause a greater magnitude of cardiac perturbations than 5k of running.
7. Increased cycling work rates will result in greater perturbations to normal cardiac function.

Chapter 2 General Methods

2.1 Overview

The aim of this chapter is to provide an understanding of the overall research approach adopted, the research methodology, the participants studied, the variables measured, and to describe the common procedures used throughout the studies described in this thesis. Details of measurement and recording will be provided with regards to the following variables: HR, BP, \dot{Q} , SV, heart rate variability (HRV), $\dot{V}O_{2\max}$, cardiac function as well as information regarding the validity and reliability of the variables.

2.2 The Research Approach

A quantitative, experimental approach was adopted for the research conducted in this thesis. Chapter 4 and 5 examined the effects of PSE on cardiac function, defined as biomarker release, echocardiographic derived functional indices, and autonomic regulation. Echocardiography was performed prior to, and after each bout of exercise within a 35-minute window. This was followed by cardiac autonomic function measurements, which were performed continuously at rest and during recovery to ascertain sympathovagal modulatory responses. Finally, a venous blood sample was taken prior to the beginning of exercise and following the cardiac autonomic measurements during recovery. Chapter 4 explores the effects of OD duathlon exercise on cardiac function, in which participants performed 2 self-paced duathlon trials on separate occasions. Chapters 5 and 6 investigated the contribution of the cycling and running legs, respectively, of the duathlon to changes in cardiac function, and compared the effects of varied exercise intensities, modes, and durations. All experimental procedures were conducted in a laboratory environment within the Section of Sport and Exercise Sciences at Canterbury Christ Church University. All studies were approved by the Canterbury Christ Church University Ethics Committee and all procedures were conducted

according to the Declaration of Helsinki (2013). A copy of the letter of approval is presented in Appendix 1.

2.3 Participant information

2.3.1 Inclusion criteria

Highly trained (>3 years triathlon/duathlon training and competition experience) male duathletes aged 18-35 were recruited from local triathlon, cycling, and running clubs. Recruitment was also subject to achieving a $\dot{V}O_{2\max}$ of at least 60 mL·kg⁻¹ during a running or cycling cardiopulmonary exercise test (CPET), to indicate a highly aerobically trained participant (Albouaini et al., 2007). Prior to main study inclusion, all participants performed both running and cycling CPET tests with echocardiographic and ECG measurements prior to and immediately following exercise. Participants were also excluded if any incidental structural or functional abnormalities were identified on baseline transthoracic echocardiography determined by an experienced clinical echocardiographer or if resting blood pressure was outside normal limits. Participants were referred to a general practitioner for further investigation if deemed necessary. All participants were non-smokers and free from injury or disease that could conceivably affect their wellbeing or the results of the study during the experimental period. Participants were screened using a self-reported standardised health and medical questionnaire (see appendix 2), including details of personal and family heredity health. Participants were free from any clinically diagnosed cardiovascular condition/disorder and were not taking any medication and had no immediate family history of CVD (See Appendix 2: Physical activity readiness questionnaire). Females were excluded from the research due to known confounding variation in cardiovascular and BP variables during the menstrual cycle (Chapman et al., 1997, Sato et al., 1995).

2.3.2 Recruitment

All participants were either members of triathlon, cycling, running, or swimming clubs local to the Canterbury area, or were associated contacts that gained knowledge of the research through word of mouth. Participants who met the inclusion criteria based on the results of their health and training history check were given a participant information sheet detailing the research and were invited to take part. The participant information sheets can be found in Appendix 3. The participant information sheets for both studies contained a written explanation of the testing protocols, testing requirements, and purpose of investigation. It was explained that participants should expect to endure the typical amount of discomfort they would normally experience during competition. Participation was voluntary and included no monetary or material reward.

2.3.3 Testing requirements

Prior to each visit to the laboratory, participants were requested to maintain an abstinence from alcohol and caffeine for 24 hours, and from food for 4 hours, as these factors have previously been shown to influence HR and/or BP measurements (James, 2004, Potter et al., 1986, Shapiro et al., 1996). Participants were also required to avoid involvement in physical activity for 48 hours prior to each visit, due to possible enduring recovery effects of an acute exercise session (Rezk et al., 2006). Throughout the testing period, participants were required to maintain their pre-participation dietary habits and routine level of physical activity (other than the inclusion of the exercise testing protocols). Adherence to these requirements was verbally confirmed with each participant prior to the start of each laboratory visit before any measures were taken.

2.3.4 Familiarisation

Following recruitment and consent, all testing protocols and measurement procedures

were verbally explained to each participant. Participants were familiarised with the experimental procedures and performed a standard CPET test on a treadmill (Woodway, USA) and cycle ergometer (SRAM, Germany) on 2 separate occasions.

2.4 Measurement of the variables studied

This thesis includes the measurement of several cardiovascular and haemodynamic variables, and this section will outline the techniques used to measure these parameters. The validity and reliability of each measure will be discussed with reference to relevant previous research.

2.4.1 Cardiac autonomic assessment

The Task Force® Monitor (TFM) (CNSystems, Graz, Austria) is a multi-use device employed in this thesis for recording a range of measures at rest and in recovery, as well as intra-exercise. The validated monitoring system provides non-invasive and continuous evaluation of the cardiac ANS. In this thesis, the TFM was used for the continuous non-invasive beat-to-beat monitoring and automatic online calculation of all haemodynamic and HRV parameters (Fortin et al., 1998, Valipour et al., 2005). The TFM is shown in Figure 2.1 (CNSystems, 2021).



Figure 2.1 The Task Force[®] monitor.

The TFM enables the continuous measurement of BP using the vascular unloading technique (Fortin et al., 1998, Gratze et al., 1998), which is automatically corrected to oscillometric BP values obtained at the brachial artery of the contralateral arm. A 6-channel ECG is included for R-R interval determination (Valipour et al., 2005) and the beat-to-beat values are used for the real-time calculation of HRV by an autoregressive model (Bianchi et al., 1997) and are displayed as 3-dimensional sliding power spectra. The TFM meets the requirements of the CE mark (CE 0408, TUV Austria, Vienna) and the Food and Drug Administration (FDA) clearance 510(k) no:K014063). A selection of haemodynamic parameters measured are indexed to participant body surface area (BSA). Participant stature in centimetres (cm) using a stadiometer (Seca 213, Seca GmbH & Co. Kg., Hamburg, Germany) and body mass in kilograms (kg) using mechanical column scales (Seca 710, Seca GmbH & Co. Kg, Hamburg, Germany) were measured during the first lab visit, and each subsequent laboratory visit commenced with the measurement of participant body mass.

2.4.2 Continuous blood pressure monitoring

Single BP measurements using a sphygmomanometer are deemed sufficient in clinical practice and can provide adequate assessment of seated resting BP (Williams et al., 2004). However, this method does not account for sudden transient changes in the circulation, such as in response to an exercise stimulus. The recording of beat-to-beat fluctuations in arterial pressure enable evaluation of cardiovascular control mechanisms (Benditt et al., 1996, Low, 1996), and non-invasive methods are preferential to invasive intra-arterial measurement due to potential complications and the effect on autonomic tone (Harms et al., 1999, Stevens, 1966). Compared with the Finapres[®], the TFM employs an improved version of the vascular unloading technique to measure continuous BP at the proximal limb of the index or middle finger (Hirschl et al., 1996, Parati et al., 1989, Parati et al., 2003). Within the finger cuff, blood flow is detected by infrared light sensors and pressure is exerted by inflation or deflation of the cuff to keep blood flow and pulsation constant. The pressure required to maintain constant blood flow corresponds to real arterial pressure (CNSystems, 2014). Multiple digital feedback loops and high-fidelity signal processing exert system control and ensures artefact and vasomotor activity rejection. An algorithm is used to translate plethysomographic signals into BP information based on the changes in blood volume (Fortin et al., 2006).

The arteries in the fingers are responsible for thermoregulation and therefore possess a heightened susceptibility to vasoconstriction and vasodilation in accordance with environmental temperature and blood volume of the participant. Although arterial pressure in the fingers may not correspond with pressure in the larger arteries, continuous BP is automatically corrected to oscillometric BP values obtained at the brachial artery of the contralateral arm, providing true arterial BP values as opposed to finger arterial pressure (Fortin et al., 1998, Fortin et al., 2006). Oscillometric BP monitoring is explained in 2.4.3. The continuous BP device of the TFM has been systematically tested against intraarterial BP monitoring, and the Finapres[®]. All methods

reveal comparable results during both rest and autonomic function testing. The TFM is advantageous as it offers continuous, non-interrupted BP recording while the Finapres[®] requires recalibration during testing.

2.4.3 Oscillatory blood pressure monitoring

Oscillometric BP monitoring methods are employed during continuous (TFM) resting measurement within this thesis. An automated oscillometric BP monitor consists of a pneumatic upper arm cuff containing a transducer, an air hose, and a monitor. Manual or automatic activation of the BP monitor initiates a sequence of cuff inflation and deflation controlled by a microprocessor, in a cycle which takes ~20-30 seconds. The cuff initially inflates to suprasystolic pressure, high enough to occlude the underlying brachial artery. The transducer in the upper arm cuff detects oscillations of the arterial wall. The amplitude of minute pressure oscillations within the cuff are measured and inflation ceases when oscillations are no longer detected. The cuff then deflates progressively in increments of 5 mmHg, every time two pressure pulsations of equal amplitude are detected, known as stepped deflation (GE Medical Systems, 2002). Systolic BP is detected where oscillation amplitudes increase most rapidly, at the point where blood begins to pass once again down the previously occluded artery. Diastolic BP (dBP) is detected where oscillation amplitudes decrease most rapidly. The cuff fully deflates when oscillation amplitudes cease to exist below dBP (Rehman and Nelson, 2021). An appropriately sized cuff is important for accuracy in BP measurement, and the margin of error tends to be larger when the cuff used is too small (Bovet et al., 1994). A cuff width of 46% of the participants' upper arm is the ideal size (Marks and Groch, 2000) and the length to width ratio should be 2:1 (Pickering et al., 2006). In addition, postural factors have also been shown to affect BP readings. Supine dBP tends to be 5 mmHg lower than when a person is seated (Pickering et al., 2005), BP readings may be higher if the back is not supported (Cushman et al., 1990) or if the legs are crossed (Peters et al., 1999), and if the arm is hanging down below the level of the right atrium (Pickering et al., 2006). It is important to note that the brachial BP measurements were not

used in the context of this thesis but required to acquire the accurate continuous BP measurement described in 2.4.2.

2.4.4 Heart Rate Variability

Heart rate variability is widely accepted as a non-invasive marker of cardiac autonomic activity (Blaber et al., 1995, Malik, 1996), reflecting heart-brain interactions and ANS dynamics (Shaffer et al., 2014b). Both ECG and power spectral analysis methods provide an index of autonomic modulation (Akselrod et al., 1981). A healthy heart is characterised by significant beat-to-beat variability, symbolising its capacity to adapt to transient changes in autonomic input (Pagani et al., 1997). Heart rate variability reflects sympathetic and parasympathetic input and demonstrates the end organ response in the SA node, which is determined by nerve firing, cardiac adrenergic receptor sensitivity, and post-synaptic signal transduction (Sandercock et al., 2005). However, HRV cannot quantify the intensity of a stimulus, as it is a marker of neural efferent activity (Floras, 2009).

To maintain homeostasis, neural hierarchy elicits modifications in myocardial performance, vascular tone and R-R intervals (Lanfranchi and Somers, 2002). The oscillating changes in R-R intervals are the result of continuous changes in sympathetic and vagally mediated impulses. The frequency and amplitude of impulses can be assessed to distinguish sympathetic and parasympathetic nervous activity. It is widely accepted that the LF component represents sympathetic outflow to the heart, while the HF component represents parasympathetic outflow via the Vagus nerve (Shaffer and Ginsberg, 2017). It is proposed that the ratio of LF:HF components provides a measure of cardiac sympathovagal balance (Pomeranz et al., 1985). There are several limitations to this framework; the LF and HF components of HRV are divided at 0.15 Hz which is based on the work of Berger et al (1989), which was performed on canine sympathetic and vagal nerves and is limited in its applicability to humans in this regard (Berger et al., 1989). Additionally, during periods of slow respiration rates (< 8.5 breaths per minute, or during sighing or deep breathing), vagal

activity can be observed to affect heart rhythms that cross into the LF band (Shaffer et al., 2014a). Therefore, participants were advised to control their breathing rates during the measurement periods.

The TFM uses power spectral analysis to measure HRV. Each R-R interval is degenerated into a sum of waves (sinusoidal) of different amplitudes and frequencies.

The results are displayed with the magnitude of variability as a function of frequency (power spectrum) (Ditor et al., 2005). The power spectrum reflects the amplitude of RR interval fluctuations at different oscillation frequencies, allowing frequency specific oscillations to be studied (Akselrod et al., 1981). The TFM uses a combination of published detection algorithms (Pumprla et al., 2002), and uses the output data to calculate real-time HRV by an autoregressive model (Gratze et al., 1998). Data is displayed as three-dimensional sliding power spectra (Gratze et al., 1998). The total power of the LF, HF and an additional very low frequency (VLF) band is expressed in absolute values (ms^2) and the cumulative values are expressed as power spectral density (PSD). The physiological correlates of the VLF component remain mostly unknown and require further investigation (Malik, 1996, Heathers, 2014). Therefore, to assess the distribution of power spectral variance, the VLF component is discarded, as this is considered unnecessary noise and the remaining LF and HF measures can be expressed in normalised units (nu), by dividing the power of every LF and HF component by the total power and multiplying the ratio by 100 (Sharma et al., 2015). The QRS-algorithm used by the TFM has been evaluated with the MIT/BIH databases, containing 24-hours of real-world ECG data, including the broadest possible range of waveforms, achieving a detection rate of all included data of 98.87%, demonstrating the reliability of the device (Fortin, 2001).

2.4.5 Task Force® monitor reliability

The most accurate measurements of autonomic function are invasive procedures; however, these are impractical in non-clinical and outpatient settings and as such are reliant on small

sample sizes. Measuring HRV non-invasively can provide a good index of cardiac autonomic modulation; however, some early methods of measuring HRV non-invasively returned poor reproducibility, with co-efficient of variation ranging from 1-235% (Sandercock et al., 2005). Measurement of HRV requires participants to be stationary and for recording periods to be free from ectopic beats and irregularities. Inter-individual and intra-individual reliability of the TFM has been previously performed over four separate trials at 2-week intervals, demonstrating low intraindividual and moderate intra-individual CoV (Goswami et al., 2009).

2.4.6 Transthoracic echocardiography

Echocardiography is the practice of imaging the heart and the great vessels using ultrasound. Since ultrasound was first published for cardiac investigation (Edler and Lindström, 2004) the rapid improvement in technology has led to echocardiography being widely used in the assessment and management of the cardiac patient (Douglas et al., 1990) as well as in the assessment of acute cardiovascular response to exercise (Middleton et al., 2006).

Transthoracic echocardiography (TTE) is the standard approach to imaging the heart and involves the insonation of the cardiac chambers from the anterior surface of the patient's chest. Using this approach, 2D, M-mode, Doppler, TDI and MST echocardiography provide comprehensive quantitative assessment of cardiac function. This work focuses on utilising these techniques to provide morphological and functional information pertaining to the LV and RV.

Echocardiography is a skilled technique and highly operator dependant. Image quality can vary between individuals due to differences in anatomical orientation of the myocardium, acoustic windows, body composition, and/or patient disease state (Lang et al., 2005). These indiscriminate physical characteristics cause changes in tissue density and the degree of ultrasound penetration, which may introduce error into measurements.

To gain images of cardiac structures, the participants lay in the left lateral decubitus position. Echocardiographic images were acquired using a systematic approach (Henry et al., 1980).

Images were recorded during apnoea at end expiration, and a minimum of 3 cardiac cycles stored digitally in raw DICOM format to USB flash archive. Off-line analysis was undertaken by the same single experienced sonographer using commercially available software (Echo-pac, GE Medical Systems, Horten, Norway, version 113.0.x) and all measurements were averaged over a minimum of 3 consecutive cardiac cycles. The modality specific methods for image acquisition and analysis are further sub-divided and are as follows.

2.4.7 Standard 2D and M-mode Echocardiography

2D and M-mode echocardiography was performed using harmonic imaging and images optimised to maximise spatial and temporal resolution. This involves the adjustment of various ultrasound parameters including gain, dynamic range, depth, angle width, frame rate and frequency. 2D images were obtained in accordance with the ASE (Lang et al., 2005, Lang et al., 2015) and a systematic approach was adopted, initially a 2D parasternal long axis (PLAX) orientation was acquired (see Figure 1.1.4). From this orientation an M-mode of the LV at the level of the minor axis (Schiller et al., 1989) was acquired to allow the offline measurement of diastolic and systolic LV septal (IVSd and IVSs) and posterior wall thicknesses (PWd and PWs), cavity dimensions (LVIDd and LVIDs), the calculation of LV mass (LVM) and relative wall thickness (RWT).

This thesis adopts the approach to LV M-mode quantification using the leading-edge method as well as using the thinnest continuous echo lines as recommended by the ASE (Henry et al., 1980). When measuring the LV from M-mode, end diastolic measurements were made when the chamber was at its largest whilst the systolic dimension of the LV was made when the cavity was at its smallest (Sahn et al., 1978). Standard 2D echocardiography images were also used to assess LV volumes utilising standard AP4CH and AP2CH orientations. A range of methods for the calculation of LV volumes have been published, including linear, ellipsoid, area length and biplane methodology (Edler and Lindström, 2004). This thesis adopts Simpson's biplane

methodology as recommended by the ASE (Lang et al., 2015), which has been shown to be the most accurate method (Ujino et al., 2006) and specifically when compared to conventional 3D reconstruction (Khankirawatana et al., 2004), cinecomputed tomography (Vandenberg et al., 1995) and cMRI (Rodevan et al., 1999). This approach involves LV planimetry at end-systole, as defined by the frame immediately before mitral valve opening (Abhayaratna et al., 2006) from both apical orientations. The area is then subsequently divided into a series of stacked oval disks. The volume of the LV is calculated as the sum of the volume of all the disks using the equation ($\text{Volume} = \pi / 4 (h) \Sigma (D1) (D2)$) where h is the height of each disk and D1 and D2 are the orthogonal minor and major axis of each disk (Lang et al., 2015). The endocardial border is traced at end diastole, as defined by the frame immediately following mitral valve closure and at end systole as defined as the frame immediately prior to mitral valve opening. The impact of image quality is extremely important, particularly when defining the endocardial/blood pool interface (Lang et al., 2015). Care was taken to maximise contrast and spatial resolution. The imaging depth and sector width were minimised to image only the LV. Gain, dynamic range and frequency were adjusted while achieving the minimum target frame rate of 67 frames per second (FPS). These changes in conjunction with careful transducer movements provided optimal image quality (Lang et al., 2005). LV systolic functional data was calculated from the 2D volume data, LV ejection fraction (EF) is a frequently used parameter for the assessment of global LV function (Pombo et al., 1971) and is derived using the following equation ($(\text{EF}) (\%) = (\text{end diastolic volume (EDV)} - \text{end systolic volume (ESV)} / \text{EDV})$).

Apical and parasternal 2D images were also used to provide structural and functional data from the RV. Due to the lack of uniformity to the shape of the RV, three different measurements were taken in accordance with ASE guidance (Rudski et al., 2010). These guidelines suggest measurements to be made at end diastole at the proximal RV outflow tract (RVOT), the RV inflow (RV1) and the RV length (RV3). From a standard AP4CH, the transducer is moved laterally providing a clearer image of the RV free wall and the endocardial border. RVFAC is one of the few measurements of RV function that has been used clinically (Haddad et al., 2008). RV area is obtained by planimetry of the RV wall and

septum in end diastole (RVDa) as defined by the frame immediately following tricuspid valve closure and end systole (RVSa) as defined by the frame immediately prior to tricuspid valve opening (see Figures 2.10 and 2.11). RVFAC is calculated using the following equation: $(RVFAC (\%) = (RVDa - RVSa) / RVDa)$ (Lang et al., 2015).

2.4.8 Standard Doppler Echocardiography

Global LV diastolic function was assessed using standard PW Doppler from the flow across the mitral valve in line with guidance from the Canadian Consensus on Diastolic Dysfunction (Rakowski et al., 1996). The AP4CH orientation was used, and a 4 mm sample volume was placed at the tips of the mitral valve in diastole, parallel to mitral inflow (Garcia et al., 1998). The subsequent spectral signal was optimised to maximise signal to noise ratio, which was achieved by careful adjustment of the Doppler gain, pulse repetition frequency (PRF), the baseline, and the high-pass filter. The mitral inflow signal (Figure 3.2) allowed the measurement of peak flow velocities in early diastole (E) and late diastole following atrial contraction (A) and the ratio E/A was calculated.

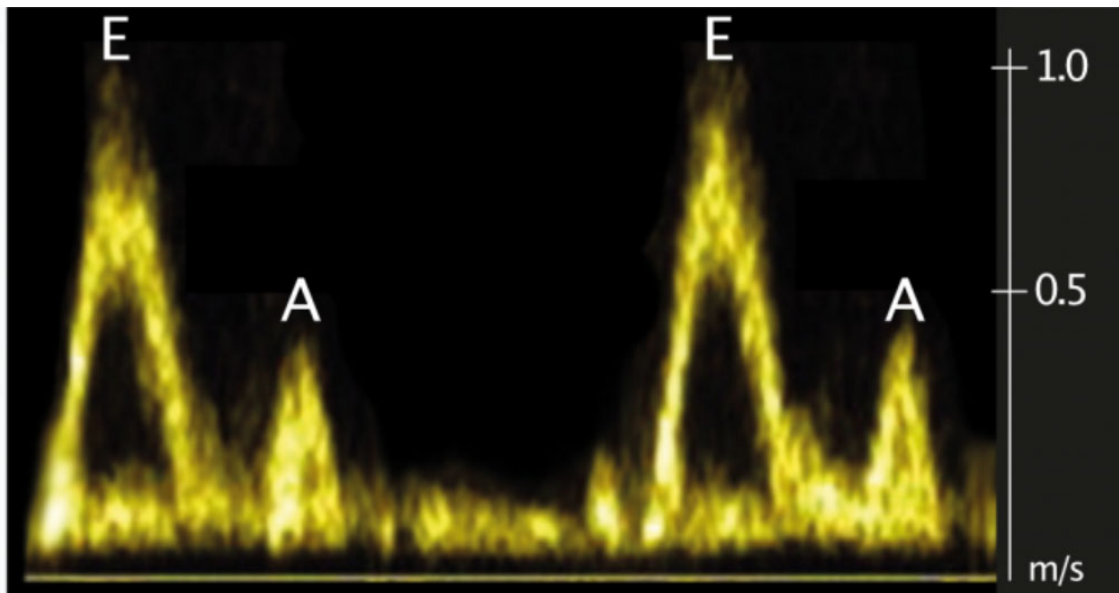


Figure 2.2 Normal PW Doppler trace showing E and A velocities.

2.4.9 Tissue Doppler Imaging

TDI was performed during the same examination and involved a standard PW modality only to assess LV longitudinal diastolic function. Whilst imaging from a standard apical acoustic window the TDI mode was activated. This subsequently bypasses the high pass filter, reduces the pulse repetition frequency and reduces the overall amplitude of the returning Doppler signals (Sutherland et al., 1999). From an AP4CH orientation, a PW sample volume of 2 mm axial length (Waggoner and Bierig, 2001) was placed within the inferoseptal and anterolateral mitral annulus. Optimisation of the resultant Doppler signal was achieved with careful adjustment of the gain, PRF, sweep speed and baseline. This signal (Figure 2.3) provides peak myocardial velocities and was utilised to obtain S', E' and A' and subsequently E/E' was derived from an average of the septum and lateral myocardial velocities. The peak velocity of the systolic myocardial wave (S'), peak early diastolic (E') and late diastolic (A') velocities were recorded, with LV values averaged. The LV mitral E/E' ratio was used to estimate LV filling pressure (Ommen et al., 2000). Validation studies using simultaneous catheterisation and Doppler echocardiography to assess myocardial function, filling pressures and LVEF have reported moderate correlations ($R=0.539-0.842$, $p<0.001$) (Tei et al., 1997).

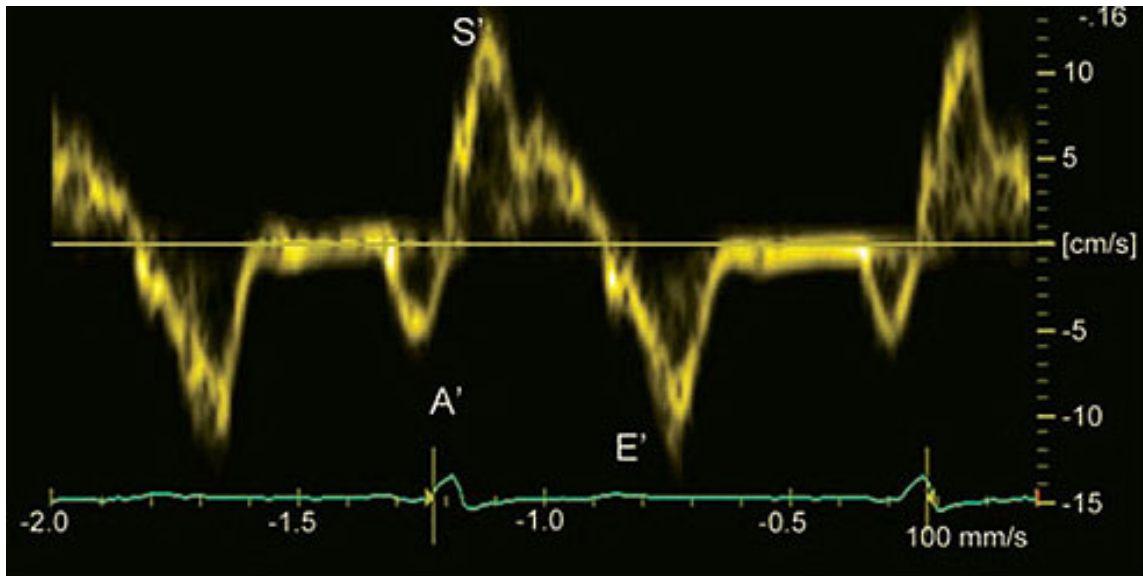


Figure 2.3 Normal TDI signal trace showing S', E' and A' velocities.

2.4.10 Myocardial Speckle Tracking

Myocardial speckle tracking was performed during the same analysis and involves the use of standard 2D apical image acquisitions. All images for subsequent MST analysis were acquired at end expiration. To assess LV longitudinal function, the AP4CH orientation was acquired providing the LV inferoseptum and lateral walls. When imaging a standard AP4CH, lateral sliding of the transducer provided optimal imaging of the RV. In all the apical acoustic windows the focal point was positioned at the mitral or tricuspid valve and images were optimised as described above.

During the analysis process, a region of interest (ROI) was allocated to the whole of the LV in all the views ensuring alignment with the endo and epicardium. Myocardial deformation was determined from continuous frame-by-frame tracking of the 'natural acoustic markers' (Korinek et al., 2005) and ϵ and SR were calculated from the displacement and rate of displacement (Hein and Brien, 1993). The analysis software includes increased averaging capabilities that improve signal to noise ratio (Modesto et al., 2006) as well as automatic grading of the tracking quality as either acceptable (V) or unacceptable (X).

Segments were excluded from the analysis if the software considered them unacceptable (X) or the operator observed inappropriate tracking during the analysis process. The basal PSAX of the LV was divided into 6 myocardial segments: basal inferoseptum, basal anteroseptum, basal anterior, basal anterolateral, basal inferolateral and basal inferior walls (Lang et al., 2015).

Each AP4CH, AP2CH and APLAX image provided peak basal, mid, and apical LS, SRS, SRE and SRA from each myocardial wall. All regional values were recorded (Figure 3.4), and a mean value of all analysable segments was presented as a global parameter of LV longitudinal function (Amundsen et al., 2006). The focal point was positioned at mid RV cavity and the RV lateral wall was traced from base to apex (Sutherland et al., 2004). The subsequent analysis provided data of RV ϵ , SRS, SRE and SRA.

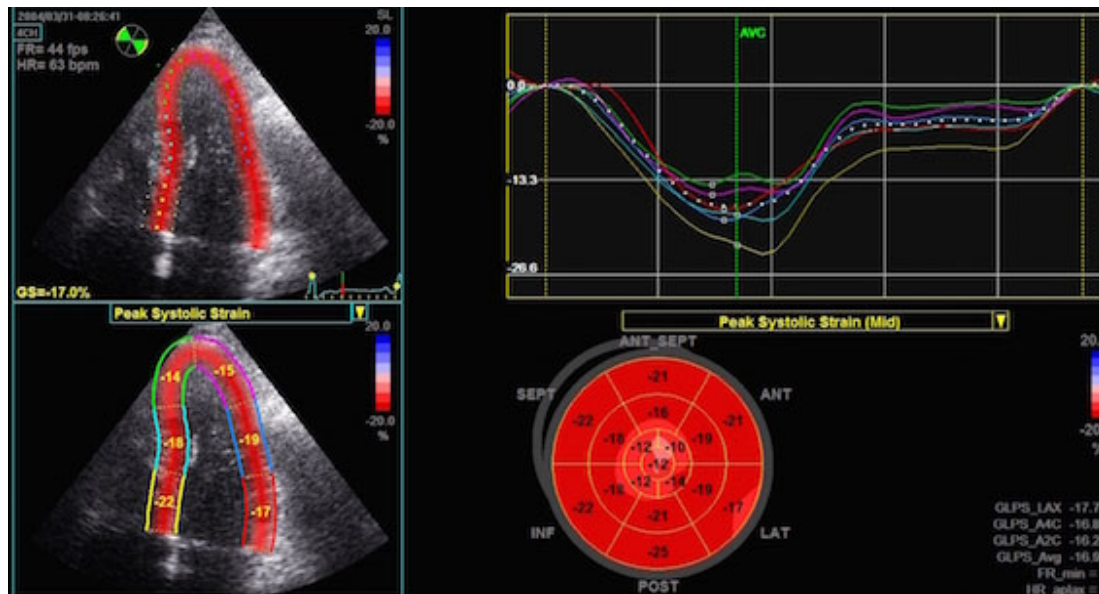


Figure 2.4 Myocardial speckle tracking in Echo-pac showing longitudinal strain in AP4CH view.

Following approved tracking by the system and the operator, raw data was exported to a spreadsheet (Excel, Microsoft Corporation, Seattle, Washington) to allow temporal assessment of all the indices throughout the cardiac cycle. To account for inter and intra-

individual differences in HR, data were then normalised to the percentage of systolic and diastolic duration (Burns et al., 2009, Stöhr et al., 2011b). Normalisation was achieved by exporting the raw data to the Python package NumPy (Harris et al., 2020) and interpolating the data to 100 points in both systole and diastole using a cubic spline method, resulting in a total of 200 points per cardiac cycle. The end of systole was defined as aortic or pulmonary valve closure (AVC / PVC) (Lang et al., 2005), which was automatically determined by the analysis software based on the onset of the QRS complex on the integrated electrocardiogram (ECG). TDI of the mitral annulus was used to confirm timings for end systole, defined as the time from the onset of the QRS to the end of the S' waveform (Lang et al., 2015).

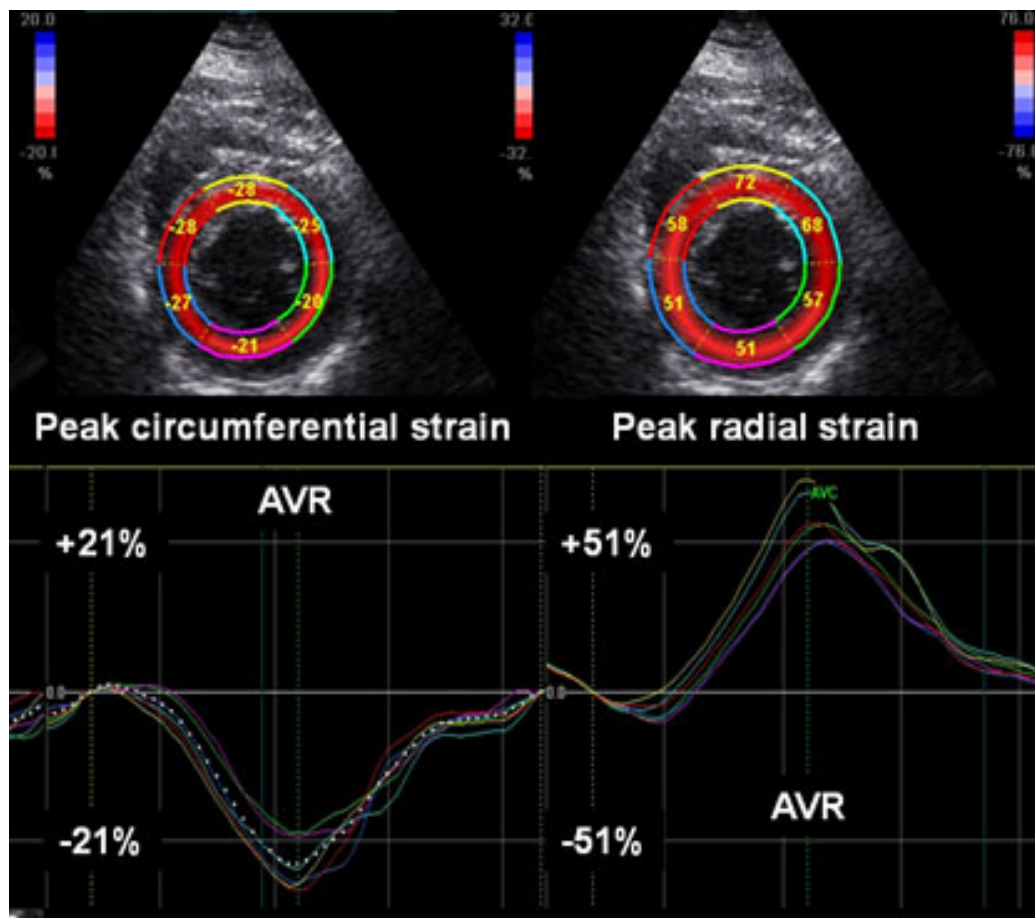


Figure 2.5 Myocardial speckle tracking in Echo-pac showing short axis ϵ traces in the PSAX view at the mitral valve level.

2.5 Blood Sampling

Venous cannulation was performed by a trained experimenter in adherence with World Health Organisation Guidelines (Bitmead and Oliver, 2018). Cannulation was performed immediately after the echocardiographic assessment, immediately prior to exercise to reduce any confounding influence on autonomic function and resting BP. Three 5 mL samples of venous blood were withdrawn into heparinised vacutainers *via* cannulation of the cephalic vein. Samples were then centrifuged at 15000 rpm for 15-minutes to separate the blood plasma (Giavarina and Lippi, 2017). Plasma was carefully extracted via pipet into 2 ml storage vials, which were labelled with a code to protect the sample identity and placed in purpose designed plastic storage containers within a securely padlocked freezer at -80°C at Canterbury Christ Church University. The remaining cell matter was disposed of *via* incineration at a biomedical waste centre. Once all samples had been collected, plasma vials were transported via specialist courier to St George's University Hospital NHS Foundation Trust, London, where they were subsequently analysed for hs-cTnT (Siemens)

2.6 Preliminary Study to Determine Reliability of Measurements

2.6.1 Participants

Twelve healthy male participants volunteered to participate in the preliminary study (Age 27 ± 4 , height 177 ± 4 cm, weight 73 ± 9 kg). All participants provided written informed consent and the study received ethical approval from the University ethics committee.

2.6.2 Procedure

Participants attended the laboratory on two days of the week, with each visit separated by 48 hours. All visits commenced at the same time on each day, following a >4 hour fast and a >24-hour abstinence from caffeine or alcohol. The visit protocol was precisely replicated on each occasion. Participants rested in a supine position for 15 minutes, before which, height was measured in centimetres (cm) using a stadiometer (Seca 213, Seca GmbH & Co. Kg.,

Hamburg, Germany) and body mass was measured in kilograms (kg) using mechanical column scales (Seca 710, Seca GmbH & Co. Kg, Hamburg, Germany). Once the TFM was set up, participants were asked to remain as still, quiet and relaxed as possible and rested in a supine position with the lights turned off, a 5-minute recording period was used for analysis following 15 minutes of supine rest. Following the TFM measures, a transthoracic echocardiogram was performed to establish the above mentioned cardiac systolic and diastolic functional measures. Participants then performed either a cycle ergometer or treadmill cardiopulmonary exercise test (CPET) to measure $\dot{V}O_{2\max}$, HR and maximal work rate.

2.6.3 Data Analysis

Prior to analysis the data was checked for the assumptions using parametric tests. Mean values were calculated for all test variables for each pre-exercise portion of the trial. Within-subject variation was derived by log-transformed two-way analysis of variance and expressed as a CoV as described by Atkinson and Nevill (2001), together with the 95% confidence intervals for a normal distribution (Field, 2010).

2.6.4 Results

On each of the two laboratory visits, cardiac autonomic function was recorded for 5-minutes. The reliability as CoV, SEM, and CI of all measures taken are shown in Table 2.1 overleaf.

2.6.5 Discussion

The results of this preliminary study show that the TFM provides a reliable measure of cardiac autonomic and haemodynamic function in a non-clinical population, a finding in line with those previously demonstrated in a clinical population (O'Driscoll, 2009).

These reliability findings support the use of the selected measures for the intended research purposes of this thesis.

Table 2.1 Reliability of cardiac measurements

	CoV (%)	SEM	95% CI Lower	95% CI Upper
Echocardiography				
<i>Pulse Wave and Tissue Doppler</i>				
Mitral E (cm·s ⁻¹)	5.41	1.35	4.06	6.76
Mitral A (cm·s ⁻¹)	4.27	0.45	3.82	4.72
Mitral E/A Ratio	5.42	3.15	2.27	8.57
Septal e' (m·s ⁻¹)	4.69	2.25	2.44	6.94
E/e' (m·s ⁻¹)	6.15	1.8	4.35	7.95
Tricuspid E (cm·s ⁻¹)	6.97	1.35	5.62	8.32
Tricuspid A (cm·s ⁻¹)	7.02	0.9	6.12	7.92
Tricuspid E/A Ratio	6.78	2.75	4.03	9.53
TAPSE (cm)	5.16	3.6	1.56	8.76
<i>Simpsons Biplane</i>				
Q (L·min ⁻¹)	5.66	2.25	3.41	7.91
EF (%)	2.89	1.35	1.54	4.24
EDV (mL)	2.64	1.8	0.84	4.44
ESV (mL)	4.58	3.6	0.98	8.18
SV (mL)	2.74	1.35	1.39	4.09
<i>MST</i>				
LV LS (%)	-4.7	0.38	-5.08	-4.32
RV LS (%)	-5.07	0.49	-5.56	-4.58
<i>Structure</i>				
IVSd (mm)	4.7	1.35	3.35	6.05
IVSs (mm)	3.47	1.35	2.12	4.82
LVIDd (mm)	2.68	0.45	2.23	3.13
LVIDs (mm)	4.98	1.35	3.63	6.33
LVPWd (mm)	4.3	1.8	2.5	6.1
LVPWs (mm)	3.95	2.25	1.7	6.2
<i>Haemodynamics</i>				
sBP (mmHg)	4.21	2.06	3.26	6.27
dBp (mmHg)	3.09	1.4	2.33	4.49
mBP (mmHg)	5.66	2.61	4.3	8.27
HR (beats·min ⁻¹)	5.1	3.02	4.19	8.12
<i>HRV</i>				
PSD (ms ²)	3.92	2.01	2.99	5.93
HF (ms ²)	11.12	6.53	8.87	17.65
LF (ms ²)	9.97	6.15	8.24	16.12

Note: TDI, tissue doppler imaging; PWD, pulse wave doppler; MST, myocardial speckle tracking; sBP, systolic blood pressure; dBp, diastolic blood pressure; mBP, mean blood pressure; HR, heart rate; PSD, power spectral density; VLF, very low frequency; HF, high frequency; LF, low frequency.

Chapter 3 The Effects and Validity of Laboratory Olympic Distance Duathlon Performance on Myocardial Function, Biomarker Release and Cardiac Autonomic Modulation

3.1 Abstract

Background: While there exists a large body of research into the effects of triathlon exercise on post-exercise cardiac function, research into duathlon is relatively sparse. Substituting the swim for an additional run may increase the overall metabolic and cardiovascular demand compared with triathlon. The sequential nature of multi-sport events allows for intra-exercise measurements between legs, to track any progression of changes in cardiac function. It is also currently unclear whether lab-based multisport performances are reproducible in a trained cohort, and whether there would be confounding effects due to the added rest during the time taken between legs to complete the cardiac measurements.

Methods: Participants (n=10) completed two lab-based OD duathlons (10km run, 40km cycle, 5km run), with pre and post exercise cardiac measurements of autonomic function, echocardiography, and plasma hs-cTnT concentration. In the broken duathlon trial (BD), intra-leg cardiac measurements and blood samples were taken, and in the unbroken duathlon (UD) participants performed each leg sequentially.

Results: Mean duathlon completion times (UD 128.3 ± 6.0 , BD 131.5 ± 7.0 minutes) and individual leg times were not different between UD and BD. Additionally, there were no differences in mean HR or work rates between trials. Duathlon exercise resulted in significant elevations in serum hs-cTnT in each trial (UD $F = 15.51$, $P < 0.001$; BD $F = 11.00$, $P < 0.001$). In addition, there was an effect of exercise on mBP (UD $F = 4.61$, $P < 0.05$) and dBP (UD $F = 5.87$, $P < 0.05$). There were also significant effects of exercise on \dot{Q} (UD $F = 9.30$, $P < 0.01$; UD $F = 4.62$, $P < 0.01$), mitral E/A ratio (UD $F = 15.00$, $P < 0.01$; BD $F = 7.60$, $P < 0.01$) and tricuspid E/A ratio (UD $F = 18.10$, $P < 0.001$; BD $F = 7.72$, $P < 0.01$). The results of MST analysis found reductions in left and right ventricular longitudinal strain (LS) in both

duathlons (BD $F = 4.41$, $P < 0.01$), RVLS (BD $F = 5.91$, $P < 0.01$). Analysis of autonomic function revealed an effect of exercise in all time and frequency parameters of HRV including total PSD (UD $F = 5.62$, $P < 0.05$; BD $F = 5.75$, $P < 0.05$), RMSSD (UD $F = 39.26$, $p < 0.01$; BD $F = 5.92$, $P < 0.01$), and LF/HF ratio (UD $F = 15.31$, $P < 0.001$);

Conclusions: Olympic Distance duathlon performance results in similar elevations in cardiac biomarkers, autonomic regulatory changes, and transient reductions in cardiac systolic and diastolic functional measures as seen in triathlon and standalone running or cycling ultra-endurance competitions.

3.2 Introduction

Transient perturbations in normal cardiac function measured by echocardiography, autonomic regulation, and cardiac biomarker release following exercise have been observed in athletic and non-athletic populations (Shave et al., 2007, Middleton et al., 2006, Shave et al., 2004, Tulloh et al., 2006, Neilan et al., 2006, Oxborough et al., 2006, La Gerche and Prior, 2007, Belonje et al., 2007, Leetmaa et al., 2008, Nottin et al., 2009, Wyatt et al., 2011, George et al., 2012, Stewart et al., 2014, Stewart et al., 2016, Eijsvogels et al., 2016, Donaldson et al., 2019). These exercise induced cardiac functional changes (EICF) closely resemble those observed in cardiac disease patients at risk of heart failure, therefore it could be argued that PSE causes long term damage to the heart and increases life-time risk of cardiomyopathy (Franklin et al., 2020b). Alternatively, the physiological and mechanical stress caused by PSE may provide stimulus for cardiomyocyte adaptation, similar to the changes seen in skeletal muscle following exercise training (Clarkson et al., 1992, Shave et al., 2004). Previously reported measures used to assess EICF have included PW sonography to measure transmitral diastolic blood flow, B-mode measurement of cardiac chambers, and M-mode tracking of tissue displacement (Shave et al., 2007). More recent developments in speckle tracking technology (Yip et al., 2003) allow for the measurement of echocardiograph-derived myocardial strain (ϵ), which is a sensitive index of contractility (Dandel et al., 2009, Beaumont et al., 2017). The use of speckle tracking in the short axis and long axis planes enables researchers to quantify and compare the longitudinal and rotational mechanics of the heart following bouts of PSE. In favour of the adaptive model, Stohr and colleagues (2012) demonstrated improved cardiomyocyte efficiency, and greater mechanical reserve in individuals with a greater aerobic fitness (Stohr et al., 2012); and Oxborough and colleagues (2006) reported a positive association with an athlete's training history and post-exercise cardiac function (Oxborough et al., 2006). This has been further supported by meta-analysis of the MST values of trained athletes, which demonstrated a 1% increase in resting LV LS (LS) compared to non-athlete counterparts (Beaumont et al., 2017).

There has been substantial investigation into the impact of the duration and the intensity of exercise that provokes transient reductions in myocardial systolic and diastolic function, and the release of cardiac biomarkers, such as cardiac troponin (cTn) (Whyte et al., 2000, George et al., 2004, Dawson et al., 2005, Middleton et al., 2006, Middleton et al., 2008, Scharhag et al., 2008, Wilson et al., 2011a, George et al., 2012). Previously, most research has focused on long-duration (>4 hours) 'ultra' endurance exercise, however, it has recently been demonstrated that both LV and RV ϵ reductions, and a substantial release of cTn have been observed following bouts of shorter duration (30-60 minutes), high intensity (mean HR > 170 beats·min⁻¹) exercise (Shave et al., 2010b, Stewart et al., 2015, Kleinnibbelink et al., 2021b)

In addition, there is evidence that links the impact of EICF with time and frequency domain indices of heart rate variability (HRV). Stewart and colleagues (2014) have found evidence of increased LF/HF ratio and reduced RMSSD following high intensity cycling exercise with concomitant EICF, though there is currently no correlational evidence to suggest a link between HRV and EICF metrics both are transiently affected following PSE (Stewart et al., 2014). It has been postulated that the onset of EICF is the result of exceeding a tolerable sympathetic load (Stewart et al., 2016), therefore changes in cardiac function may reflect the underlying autonomic balance in control of the heart and may be quantified by HRV (Seiler et al., 2007, Legaz-Arrese et al., 2015, Shaffer and Ginsberg, 2017, Gronwald and Hoos, 2020, Bechke et al., 2020). Use of HRV parameters is more readily available to athletes and coaches, with most GPS watches having a HRV feature built-in and HRV analysis is currently widely applied among professional and amateur athletes.

In terms of the real-world application of this area of research, evidence of EICF acutely following PSE (Stewart et al., 2016) has relevancy to athletes training and competing multiple times within a 24h period, such as triathletes (Millet et al., 2011), swimmers competing in galas and heptathletes/decathletes (Wilson et al., 2011a). Furthermore, evidence from several meta-analyses suggests increased average exercise HR and duration contribute to greater levels of EICF during endurance cycling, running and multi-sport exercise (Middleton et al., 2006, Shave et al., 2007, Donaldson et al., 2019). Therefore, understanding

the extent and potential implications of EICF following repeated bouts of exercise such as in training and competition, will be of benefit to multisport triathletes and coaches. Duathlon (run, cycle, run) is a popular off-season alternative to triathlon competition (Nikolaidis et al., 2021) and may potentially incur greater levels of skeletal muscle and cardiac fatigue due to the substitution of the non-weight bearing swim for an additional run leg (Millet et al., 2009). The thermoregulatory (Sparks et al., 2005), metabolic (Berry et al., 2016) and endocrine (Alvero-Cruz et al., 2011) physiological responses to duathlon performance have been previously investigated; however, to date there has been no substantial investigation into the effects of duathlon performance on cardiac function, and even less research into the effects of PSE on ventricular longitudinal function. Additionally, the distances of each leg in the Olympic distance (OD) duathlon (10k for the first run, 40k for the bike, and 5k for the second run) are also popular standalone competitive distances (Myburgh et al., 2018). Therefore, to provide an insight into the development of exercise induced cardiac functional changes and the exercise factors associated with it, we investigated cardiac function, biomarker release, and regulation following each leg of an OD duathlon, and following an uninterrupted OD duathlon.

3.3 Methods

3.3.1 Study Protocol

Participants were recruited through social media and word of mouth at triathlon, running, swimming, and cycling clubs local to the Canterbury area. Requisites for recruitment to the study were at least 3 years of multisport (triathlon, duathlon) training experience, to be under 40 years of age, and to achieve a $\dot{V}O_{2\max}$ (relative) of at least $60 \text{ mL}\cdot\text{kg}\cdot\text{min}^{-1}$ during either a run or cycle CPET. Participants reported no medication use and were actively training for and competing in triathlon and duathlon competitions at the time of participation in the study. In addition, pre-participation health screening according to BASES guidelines ensured that participants were apparently healthy, non-smokers and had no history of potential CPET contraindications. Participant demographic and exercise characteristics are displayed in Table 3.1 below.

Table 3.1 Participant characteristics

Measure	Unit	Mean	SD
Stature	cm	179.1	4.5
Mass	Kg	73.4	6.9
Age	Years	32.6	5.9
History _{TX}	Years	10.6	6.0
Bike			
$\dot{V}O_{2\max}$ (Relative)	mL·kg ⁻¹ ·min ⁻¹	58.3	3.70
$\dot{V}O_{2\max}$ (Absolute)	L·min ⁻¹	4.18	0.35
$\dot{V}E_{\max}$	L·min ⁻¹	160.7	17.4
$P\dot{V}O_{2\max}$	W	368.5	28.8
HR _{MAX}	Beats·min ⁻¹	177.9	7.61
Run			
$\dot{V}O_{2\max}$ (Relative)	mL·kg ⁻¹ ·min ⁻¹	59.2	5.89
$\dot{V}O_{2\max}$ (Absolute)	L·min ⁻¹	4.34	0.38
$\dot{V}E_{\max}$	L·min ⁻¹	153.8	15.5
$v\dot{V}O_{2\max}$	Kilometres·hour ⁻¹	18.8	0.79
$t_{\text{Lim}}v\dot{V}O_{2\max}$	s ⁻¹	49.3	10.2
HR _{MAX}	Beats·min ⁻¹	183.8	10.8

Note: History_{TX} = number of years training for endurance sports, VEMAX = maximum minute ventilation ($\dot{V}E$), $P\dot{V}O_{2\max}$ = power output (cycling) at $\dot{V}O_{2\max}$, $v\dot{V}O_{2\max}$ = running velocity at $\dot{V}O_{2\max}$, $t_{\text{Lim}}v\dot{V}O_{2\max}$ = time spent at $\dot{V}O_{2\max}$ at the end of the CPET test.

3.3.2 Study Design

All participants reported to the laboratory at Canterbury Christ Church University Section of Sport and Exercise Sciences in Canterbury, Kent, UK on 6 separate occasions, and abstained from caffeine before each visit, and exercise for a minimum of 48h before each experimental trial. During the first 2 visits, participants performed pre-participation health screening and

exercise testing which involved a run or bike CPET, the order of which was randomised between participants. The CPET trial consisted of baseline measurements for HRV, echo and demographic characteristics followed by an incremental exercise test. The bike CPET was performed on an SRM cycling ergometer (SRM, Germany) with increments of 25W from a 100W starting point. The run CPET was performed on a motorised treadmill (Woodway ELG, Woodway, Wisconsin, USA) with increments of 1.0 kilometres·hour⁻¹ from a 10 kilometres·hour⁻¹ initial stage. $\dot{V}O_2$, carbon dioxide output ($\dot{V}CO_2$) and $\dot{V}E$ were measured breath-by-breath using an automated metabolic measurement system (Jaeger Oxycon Pro, Carefusion, Pennsylvania, USA), and were subsequently averaged over 30-s intervals. Peak exercise values were calculated as the average of the highest 30-s value attained during the CPET. Following the CPET, resting echocardiogram, HRV and body mass measurements were immediately recorded.

During the subsequent visits (see Figure 3.1), participants reported to the lab for baseline measurements (HRV, echocardiography, and anthropometry) and venous blood samples were also taken. Participants then performed either a continuous unbroken duathlon (UD) trial or discontinuous broken duathlon (BD) trial, the order of which was randomised between participants using an online random number generator (Haahr, 2021). Each duathlon trial involved a 10k run, 40k cycle, and 5k run using the same equipment as during the CPETs. In the UD trial participants were given a controlled 5 minutes 'transition' time between legs to change clothing and footwear as needed and between legs in the BD trial passive HRV, echo and BM measurements were performed, and blood samples were taken. The average time for the intra-leg measurements was 35 ± 10 minutes between each leg and at the end of the trial following the second run. Participants were encouraged to replenish lost bodily fluids at an assumed sweat-loss rate of 1 L·hour⁻¹ to ensure euhydration throughout the trials (González-Alonso et al., 2008). Following 24 hours of passive recovery, participants returned to the lab for subsequent baseline measurements and blood sampling.

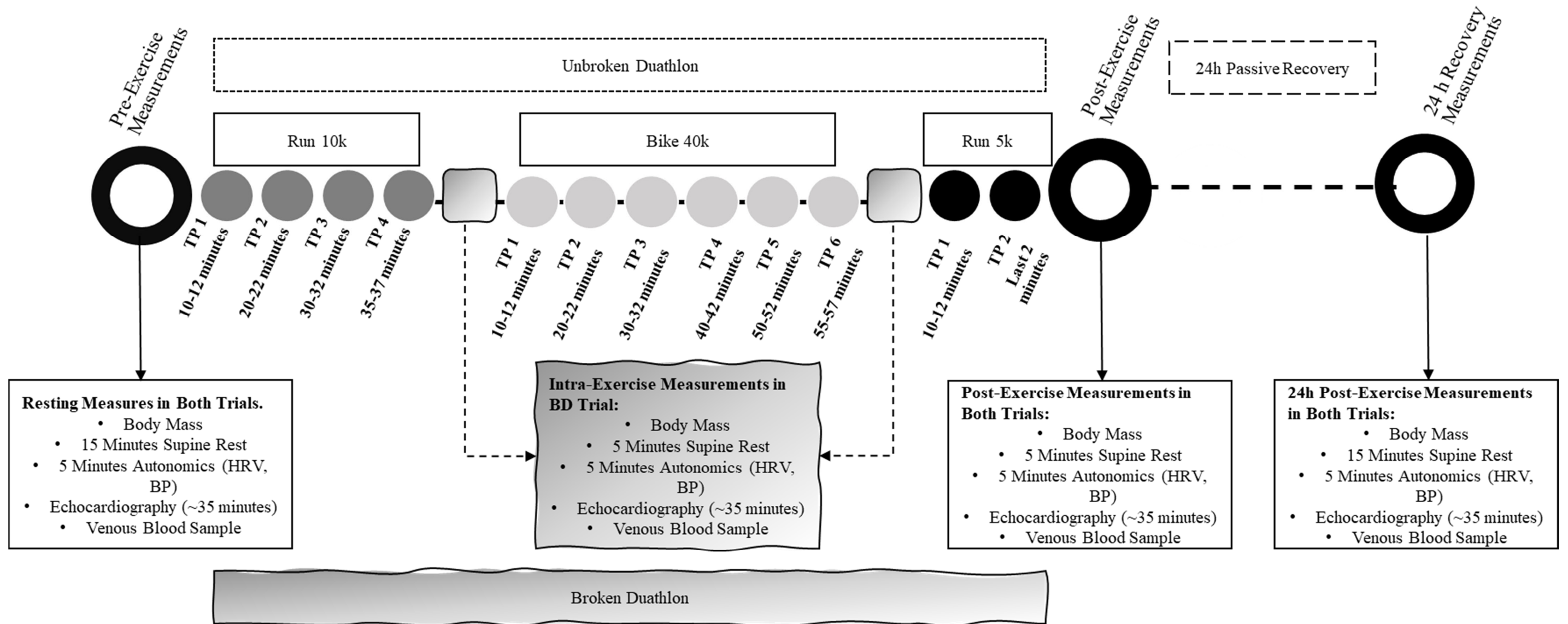


Figure 3.1 Experimental visit protocol. In the Unbroken trial, participants were given 5 minutes to transition between the different legs of the duathlon. In the broken format, intra-exercise cardiac functional measures and blood samples were taken.

3.3.3 Echocardiography

To gain images of cardiac structures, the participants lay in the left lateral decubitus position. Echocardiographic images were acquired using a systematic approach (Henry et al., 1980). Images were recorded during apnoea at end expiration, and a minimum of 3 cardiac cycles stored digitally in raw DICOM format to USB flash archive. Off-line analysis was undertaken by the same single experienced sonographer using commercially available software (Echo-pac, GE Medical Systems, Horten, Norway, version 113.0.x) and all measurements were averaged over a minimum of 3 consecutive cardiac cycles.

3.3.4 Autonomic Function

The Task Force® Monitor (TFM) (CNSystems, Graz, Austria) is a multi-use device employed in this thesis for recording a range of measures at rest and in recovery, as well as intra-exercise. The validated monitoring system provides non-invasive and continuous evaluation of the cardiac ANS. In this study, the TFM was used for the continuous non-invasive beat-to-beat monitoring and automatic online calculation of all haemodynamic and HRV parameters (Fortin et al., 1998, Valipour et al., 2005). The TFM enables the continuous measurement of BP using the vascular unloading technique (Fortin et al., 1998, Gratze et al., 1998), which is automatically corrected to oscillometric BP values obtained at the brachial artery of the contralateral arm. A 6-channel ECG is included for R-R interval determination (Valipour et al., 2005) and the beat-to-beat values are used for the real-time calculation of HRV by an autoregressive model (Bianchi et al., 1997) and are displayed as 3-dimensional sliding power spectra. To obtain autonomic functional measures, participants lay in a quiet, darkened room for 15 minutes before a 5-minute data recording was captured on the TFM.

3.3.5 Blood Sampling

Venous cannulation was performed by a trained experimenter in adherence with World Health Organisation Guidelines (Bitmead and Oliver, 2018). Cannulation was performed immediately after the echocardiographic assessment, immediately prior to exercise to reduce any confounding influence on autonomic function and resting BP. Three 5 mL samples of venous blood were withdrawn into heparinised vacutainers *via* cannulation of the cephalic vein. Samples were then centrifuged at 15000 rpm for 15-minutes to separate the blood plasma (Giavarina and Lippi, 2017). Plasma was carefully extracted *via* pipet into 2 ml storage vials, which were labelled with a code to protect the sample identity and placed in purpose designed plastic storage containers within a securely padlocked freezer at -80°C at Canterbury Christ Church University. The remaining cell matter was disposed of *via* incineration at a biomedical waste centre. Once all samples had been collected, plasma vials were transported *via* specialist courier to St George's University Hospital NHS Foundation Trust, London, where they were subsequently analysed for hs-cTnT (Siemens).

3.3.6 Statistical Analysis

Unless otherwise stated, continuous variables are expressed as mean \pm standard deviation (SD). All data were analysed using the statistical package for social sciences (SPSS 22 release version for Windows; SPSS Inc., Chicago IL, USA). Data were assessed for conformity with parametric assumptions (Field, 2000). A two-way repeated measures analysis of variance (ANOVA) was performed, to assess the effects of exercise, followed by Bonferroni post hoc tests for multiple comparisons (Field, 2000). A *p* value of <0.05 was regarded as statistically significant. Following tests for normality and significant interactions for main effects following the ANOVA, paired samples T-tests were used to compare baseline and post exercise measurements.

3.4 Results

3.4.1 Exercise Results

During both exercise trials there was no significant effect of trial on overall time, individual leg performance or the physiological demand of exercise measured by HR and gas exchange for any leg (Table 3.2). Total exercise time and individual leg time were similar between trials. Additionally, cycling power, exercise heart rate, run speed, and $\dot{V}O_2$ were also similar at every time point between legs (Table 3.3). Between legs and both trials, average heart rate and peak heart rate were significantly higher in both run legs than the bike legs (Table 3.2). There were no differences in any exercise performance variables between 5k and 10k run legs within or between trials (Table 3.3).

Table 3.2. Exercise performance and HR data for each duathlon leg

Trial	Broken Duathlon			Unbroken Duathlon		
	Run 1 (10k)	Bike (40k)	Run 2 (5k)	Run 1 (10k)	Bike (40k)	Run 2 (5k)
Average HR beats·min ⁻¹	171.9 (11.7)	154.7 (11.5)	172.9 (9.3)	169.3 (12.7)	151.5 (11.9)	168.8 (11.4)
HR Peak	179.3 (8.7)	159.6 (11.7)	177.9 (8.7)	175.8 (11.4)	158.4 (11.5)	172.8 (11.0)
Run Speed (km·hour ⁻¹)	14.4 (0.6)	-	14.2 (1.0)	14.3 (0.9)	-	14.0 (1.3)
$\dot{V}O_2$ (L·min ⁻¹)	4.01 (0.33)	3.34 (0.42)	3.91 (0.36)	4.04 (0.32)	3.22 (0.51)	3.93 (0.42)
$\dot{V}O_2$ (mL·kg·min ⁻¹)	53.3 (4.4)	44.0 (6.0)	52.1 (5.4)	54.0 (4.8)	43.6 (6.6)	52.7 (5.4)
Watts	-	210.6 (38.2)	-	-	200.8 (32.7)	-
Fluid (L)	0.45	1.07	0.35	0.36	1.015	0.18
Time (minutes)	41.9 (1.9)	65.1 (4.2)	21.3 (1.6)	42.0 (2.8)	67.8 (3.9)	21.7 (2.1)
Total Time (minutes)	128.3 (6.0)			131.5 (7.0)		

Note: n = 10 participants. Significant differences from the paired-samples t-tests are displayed as * = P<0.05 within duathlon trials, ** = P<0.001 within trials, § p<0.05 between trials.

Table 3.3 Exercise data at each timepoint within the BD (top) and UD (bottom) trials.

Broken Duathlon														
	Leg				Run1 (10k)				Bike (40k)				Run2 (5k)	
Timepoint	1	2	3	4	1	2	3	4	5	6	1	2		
HR beats·min ⁻¹	165.4 (13.1)	171.2 (13)	174.2 (9.4)	176.9 (9)	150.4 (10.2)	153.3 (12.3)	154.5 (12.6)	156.5 (12.3)	156.4 (12.3)	157.2 (10.6)	170.1 (9.8)	175.8 (8.4)		
Run Speed (km·hour ⁻¹)	14.3 (0.7)	14.3 (0.7)	14.4 (0.8)	14.5 (0.9)	-	-	-	-	-	-	14.1 (1)	14.4 (1.5)		
$\dot{V}O_2$ (L·min ⁻¹)	3.9 (0.3)	3.9 (0.3)	4 (0.3)	4 (0.4)	3.2 (0.4)	3.3 (0.5)	3.2 (0.5)	3.3 (0.4)	3.3 (0.5)	3.3 (0.3)	3.8 (0.3)	3.9 (0.4)		
$\dot{V}O_2$ (mL·kg·min ⁻¹)	52.6 (4.5)	53.2 (4.7)	53.5 (4.3)	53.8 (4.7)	42.8 (6.1)	44.5 (5.8)	43.6 (6.9)	44.3 (6.2)	44.5 (6.9)	44.6 (5.6)	51.4 (4.9)	52.8 (6)		
Watts	-	-	-	-	205.1 (41.3)	216 (39.4)	208.7 (42)	213.3 (39.2)	208.6 (38.1)	211.9 (38.5)	-	-		
Unbroken Duathlon														
	Run1 (10k)				Bike (40k)				Run2 (5k)					
Timepoint	1	2	3	4	1	2	3	4	5	6	1	2		
HR beats·min ⁻¹	159.5 (9.8)	169.3 (10.6)	173.9 (13)	174.6 (12.6)	153.5 (12.7)	152.2 (13.5)	150.2 (14)	149 (12.1)	151.1 (11.4)	152.9 (9.7)	166.8 (13)	170.9 (9.7)		
Run Speed (km·hour ⁻¹)	14.3 (0.9)	14.5 (0.9)	14.4 (1.1)	14.3 (1.1)	-	-	-	-	-	-	14.1 (1.1)	11.1 (6)		
$\dot{V}O_2$ (L·min ⁻¹)	3.9 (0.3)	4 (0.3)	4 (0.4)	3.9 (0.4)	3.1 (0.5)	3.1 (0.5)	3.1 (0.5)	3.2 (0.5)	3.3 (0.4)	3.3 (0.5)	3.8 (0.4)	3.9 (0.3)		
$\dot{V}O_2$ (mL·kg·min ⁻¹)	53.8 (4.5)	54.2 (5)	54.7 (5.4)	53.4 (5.1)	42.4 (7.2)	42.5 (6.9)	43.1 (6.7)	43.8 (7.2)	44.8 (6.4)	45.4 (6.4)	52.3 (5.4)	53 (5.6)		
Watts	-	-	-	-	194.1 (35.5)	195.7 (29)	196.9 (33.9)	201 (33.4)	205.2 (33.2)	211.6 (35.7)	-	-		

Note: n = 10 participants. All timepoints in each leg occurred at 10-minute intervals, starting after the first 10-minutes had elapsed. Work rate is expressed in Watts for the cycling legs and km·hour⁻¹ for the run legs.

3.4.2 Echocardiographic Results

3.4.2.1 Systolic and Diastolic Function

In both trials there were significant effects of exercise on \dot{Q} (UD F = 9.30, P < 0.001; BD F = 4.62, P = < 0.001), mitral E/A ratio (UD F = 15.02, P < 0.001; BD F = 7.59, P = < 0.001), and tricuspid E/A ratio (UD F = 15.29, P < 0.001; BD F = 7.72, P < 0.001) (Table 3.4). There were also significant changes in HR (UD F = 3.71, P < 0.001; BD F = 2.89; P < 0.001).

In the BD trial there were also significant effects of exercise on EDV (F = 3.69, P = 0.01) and SV (F = 4.54, P < 0.05). Following Run 1 in the BD trial, the mitral and tricuspid E/A ratio became significantly lower than baseline and remained until the end of the duathlon (Table 3.4). Left ventricular EDV and \dot{Q} were also significantly lower and higher, respectively, than baseline following Run 1; however, following the bike leg, these effects were ameliorated.

Following Run 2, the changes in LV EDV and \dot{Q} were again significantly different than baseline (see Figure 3.2). Additionally, individual repeated measures correlation analyses found significant, moderate to strong correlations (Pearson's r) to HR for \dot{Q} (UD P = < 0.01, r = 0.71, 95%CI [0.40,0.87]; BD P = < 0.001, r = 0.53, 95%CI [0.27,0.72]) and E/A ratio (UD P = < 0.01, r = -0.80, 95%CI [-0.91,-0.56], BD P = < 0.01, r = -0.48, 95%CI [-0.69,-0.2]).

Table 3.4 Left and right ventricular PW Doppler and TDI measures at each timepoint.

	Broken Duathlon					Unbroken Duathlon		
	Pre	Run 1	Bike	Post	24hPost	Pre	Post	24h Post
Left Ventricle Function								
E (cm/s)	0.70 (0.12)	0.66 (0.09)	0.64 (0.11)	0.59 (0.13)	0.7 (0.15)	0.75 (0.17)	0.70 (0.1)	0.76 (0.11)
A (cm/s)	0.40 (0.07)	0.49 (0.12)	0.47 (0.09)	0.45 (0.06)	0.39 (0.11)	0.42 (0.07)	0.55 (0.11)*	0.43 (0.07) ‡
E/A Ratio	1.78 (0.23)	1.43 (0.39)*	1.44 (0.34)	1.34 (0.35)*	1.93 (0.59)	1.80 (0.38)	1.31 (0.23)*	1.79 (0.22) ‡
E/E'	4.12 (1.0)	4.0 (0.72)	4.21 (0.9)	3.88 (1.03)	4.38 (0.79)	4.35 (0.97)	4.54 (1.1)	4.39 (1.18)
Right Ventricle Function								
TAPSE (cm)	2.8 (0.3)	2.6 (0.3)	2.7 (0.4)	2.6 (0.4)	2.8 (0.3)	2.9 (0.3)	2.8 (0.3)	2.7 (0.4)
E (cm/s)	0.68 (0.06)	0.52 (0.06)*	0.54 (0.07)	0.51 (0.03)*	0.62 (0.10)	0.72 (0.11)	0.58 (0.12)*	0.69 (0.11)
A (cm/s)	0.32 (0.01)	0.31 (0.02)	0.33 (0.03)	0.39 (0.08)*	0.32 (0.03) ‡	0.36 (0.08)	0.44 (0.13)*	0.33 (0.03)
Tricuspid E/A Ratio	1.8 (0.3)	1.6 (0.3)*	1.6 (0.4)	1.3 (0.2)*	1.9 (0.4) ‡	2.1 (0.3)	1.3 (0.2)*	2.0 (0.3) ‡

Note: n = 10 participants. Paired-samples T-tests results * = significantly (P = 0.05) different from within trial baseline measurement, ‡ significantly (P<0.05) different from within trial post exercise measurement

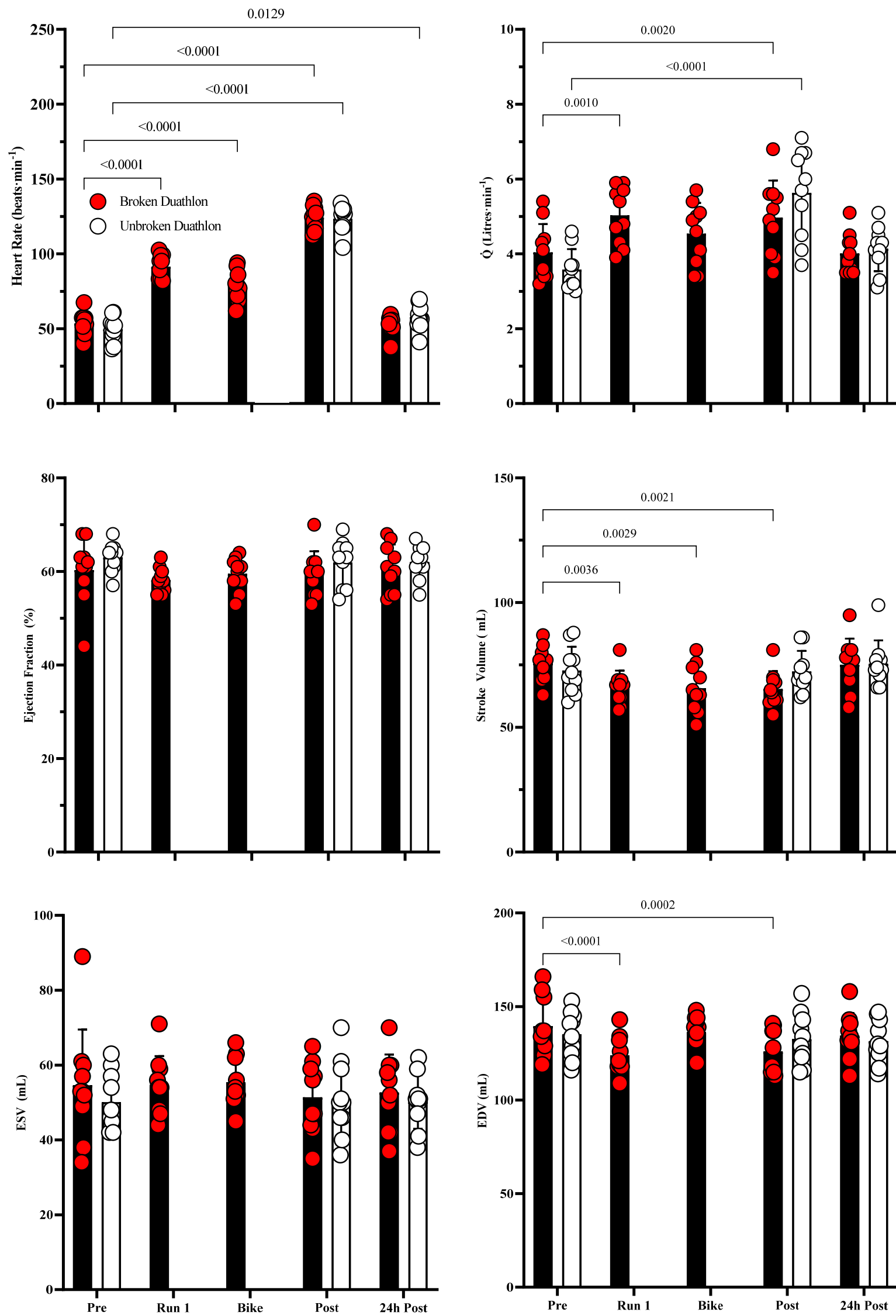


Figure 3.2 Plot of left ventricular systolic functional parameters following each stage of the BD (closed bars) and UD (open bars) trials.

Note: $n = 10$ participants. Results of the paired-samples T-tests results are presented on the figure where significant ($P = 0.05$) differences from the pre-exercise values within each trial occurred. Individual data points are represented by red circles for the BD trials and open circles for the UD trials.

3.4.2.2 Longitudinal Strain and Strain Rate

There was a significant effect of exercise in both duathlons on left (UD $F = 3.92$, $P < 0.05$; BD $F = 4.41$, $P < 0.01$) and right ventricular LS (UD $F = 4.23$, $P < 0.01$; BD $F = 5.91$, $P < 0.01$), as well as late diastolic SR in the LV (UD $F = 17.70$, $P < 0.01$; BD $F = 4.94$, $P < 0.01$). During the UD trial, there were significant reductions in left and right ventricular LS across each leg were following Run 1, but this recovered during the bike leg, before becoming significantly lower than baseline again after Run 2 (Figure 3.2).

In the UD trial, the baseline to immediate post-exercise changes in RVLS in both duathlon trials were correlated with alterations in LV diastolic and systolic SR. Numerical data of the correlation analyses are displayed in Table 3.5 below.

Table 3.5 Correlation coefficients for LV SR parameters vs. RV LS.

LV Variable	RV Variable	r	P	CI 95%	β
Early Diastolic SR	LS	-0.44	0.001	[-0.64, -0.19]	0.91
Systolic SR	LS	0.41	0.003	[0.15, 0.61]	0.86

Note: Pearson's correlation results; r = Pearson's r , β = beta-coefficient.

Table 3.6 Left and right ventricle longitudinal strain rate data from each trial.

Phase	Broken Duathlon					Unbroken Duathlon		
	Pre	Run1	Bike	Post	24h Post	Pre	Post	24h Post
Left Ventricle								
Systolic SR (%/sec)	-1.05 (0.08)	-1.03 (0.09)	-1.05 (0.12)	-1.04 (0.09)	-1.01 (0.09)	-1.17 (0.11)	-1.23 (0.12)	-1.12 (0.1)
Early Diastolic SR (%/sec)	1.79 (0.26)	1.75 (0.22)	1.79 (0.33)	1.73 (0.27)	1.69 (0.18)	1.81 (0.44)	1.87 (0.37)	1.76 (0.14)
Late Diastolic SR (%/sec)	0.68 (0.16)	0.87 (0.24)*	0.95 (0.31)*	0.99 (0.34)*	0.72 (0.18)†	0.78 (0.19)	1.04 (0.28)*	0.75 (0.17)
Right Ventricle								
Systolic SR (%/sec)	0.88 (0.19)	1.03 (0.37)	0.89 (0.32)	1.04 (0.33)	0.80 (0.23)	0.96 (0.22)	1.01 (0.32)	0.85 (0.25)
Early Diastolic SR (%/sec)	1.37 (0.38)	1.25 (0.25)	1.34 (0.43)	1.22 (0.23)	1.27 (0.18)	1.41 (0.30)	1.38 (0.26)	1.25 (0.47)
Late Diastolic SR (%/sec)	-1.07 (0.18)	-1.1 (0.22)	-1.09 (0.13)	-1.05 (0.16)	-1.08 (0.14)	-1.13 (0.13)	-1.05 (0.07)	-1.33 (0.22)

Note: n = 10 participants. Global LV LS parameters (LVLS), peak longitudinal systolic ϵ (LVLS S), longitudinal SR for peak late diastole (LVSR A), early diastole (LVSR E), and systole (LVSR S). Paired sample T-test results * = significantly different from baseline levels within each trial (P < 0.05). † Significantly (P < 0.05) different from post exercise measurement within each trial.

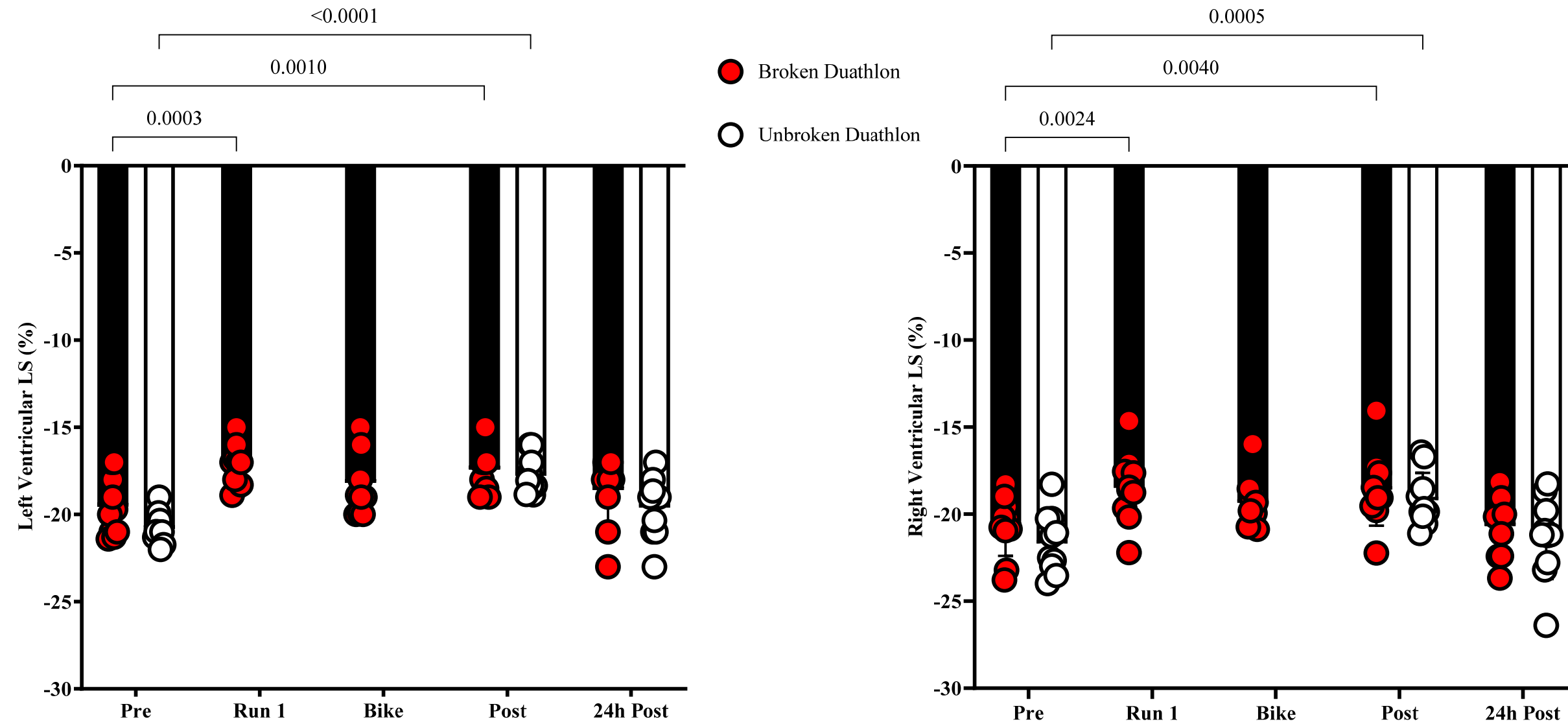


Figure 3.3 Plot of left and right ventricular LS at each time point in both trial

Note: $n = 10$ participants. Results of the paired-samples T-tests results are presented above the figures where significant ($P = 0.05$) differences from the pre-exercise values within each trial occurred. Individual data points are represented by red circles for the BD trials and open circles for the UD trials.

3.4.3 Haemodynamics and Heart Rate Variability

In the UD trial there was a significant effect of exercise on the LF/HF ratio ($F = 15.31$, $P < 0.01$) RMSSD ($F = 39.30$, $P < 0.01$) and total PSD ($F = 5.62$, $P < 0.05$). In addition, mean arterial pressure ($F = 4.61$, $P < 0.05$) and diastolic BP ($F = 5.85$, $P < 0.05$) were also affected. Following the BD trial, RMSSD ($F = 5.92$, $P < 0.01$) and total PSD ($F = 5.76$, $P < 0.01$) were affected by exercise. Table 3.7 displays the timepoints at which these significant differences occurred. Both mBP and dBP were significantly higher than the post exercise value following 24h of recovery in the UD trial. In both trials, RMSSD and total PSD were significantly lower than baseline at the end of the duathlon, and LF/HF ratio was also lower than baseline in the UD trial, but this was reversed following 24h of rest. In the BD trial, RMSSD and PSD were reduced following Run 1 and remained significantly lower than baseline following each leg of the duathlon until the 24h recovery point.

Table 3.7 Haemodynamic and log-transformed HRV variables for each duathlon trial and timepoint.

	Broken Duathlon					Unbroken Duathlon		
	Pre	Run 1	Bike	Post	24h Post	Pre	Post	24h Post
Haemodynamics								
sBP (mmHg)	115.5 (11.4)	103.7 (9.6)	107.0 (9.4)	121.2 (9.3)	117.0 (6.5)	115.7 (15.4)	122.2 (9.6)	111.6 (16.7)
mBP (mmHg)	86.5 (10.0)	77.8 (29.6)	83.2 (8.0)	91.9 (6.3) [*]	86.2 (4.6)	86.3 (12.6)	91.1 (7.4)	79.7 (11.0) [‡]
dBP (mmHg)	68.2 (8.0)	60.3 (12.9)	67.6 (7.7)	73.0 (5.1)	67.6 (5.0)	68.0 (10.9)	72.1 (6.6)	61.8 (8.2) [‡]
Heart Rate Variability								
RRI (ms)	7.04 (0.14)	6.49 (0.09)	6.45 (0.69)	6.55 (0.1)	7.03 (0.13)	7.11 (0.18)	6.58 (0.17) [*]	6.97 (0.16) ^{*‡}
RMSSD (ms)	60.31 (16.39)	28.71 (23.88) [*]	32.86 (18.04) [*]	32.96 (29.69) [*]	55.58 (22.06) [‡]	82.35 (22.36)	17.41 (9.71) [*]	49.67 (19.26) ^{*‡}
PSD (ms)	8.18 (0.73)	5.25 (1.67) [*]	7.03 (1.04) [*]	5.96 (1.77) [*]	7.93 (0.89) [‡]	8.72 (0.81)	5.33 (1.97) [*]	7.68 (0.91) [‡]
Hfnu (ms)	56.58 (11.53)	64.41 (18.36)	66.74 (20.98)	63.97 (24.52)	57.6 (13.33)	51.58 (10.37)	80.85 (10.12)	54.15 (11.79)
Lfnu (ms)	43.46 (11.5)	29.48 (18.5)	31.65 (21.16)	27.01 (12.01)	42.4 (13.33)	48.42 (10.37)	18.7 (10.24)	45.72 (11.9)
LF/HF	7.2 (0.7)	4.47 (1.5)	6.3 (0.98)	5.18 (1.67)	6.96 (0.85)	7.69 (0.78)	4.86 (2.05) [*]	6.68 (0.85) [‡]

Note: n = 10 participants. mBP = mean arterial blood pressure, dBP = diastolic blood pressure, sBP = systolic blood pressure. Paired samples T-test ^{*} = significantly (P <0.05) different from baseline measurement within each trial, [‡] = significantly different from post exercise measurement within each trial

3.4.4 Cardiac Troponin

The hs-cTnT was significantly higher at the end of exercise in both trials (Figure 3.4). In the BD trial, mean hs-cTnT was significantly elevated above baseline after Run1 and the concentration increased over the subsequent 2 legs . At the 24h recovery timepoint there were still significant elevations above baseline in hs-cTnT in both trials (Figure 3.4)1).

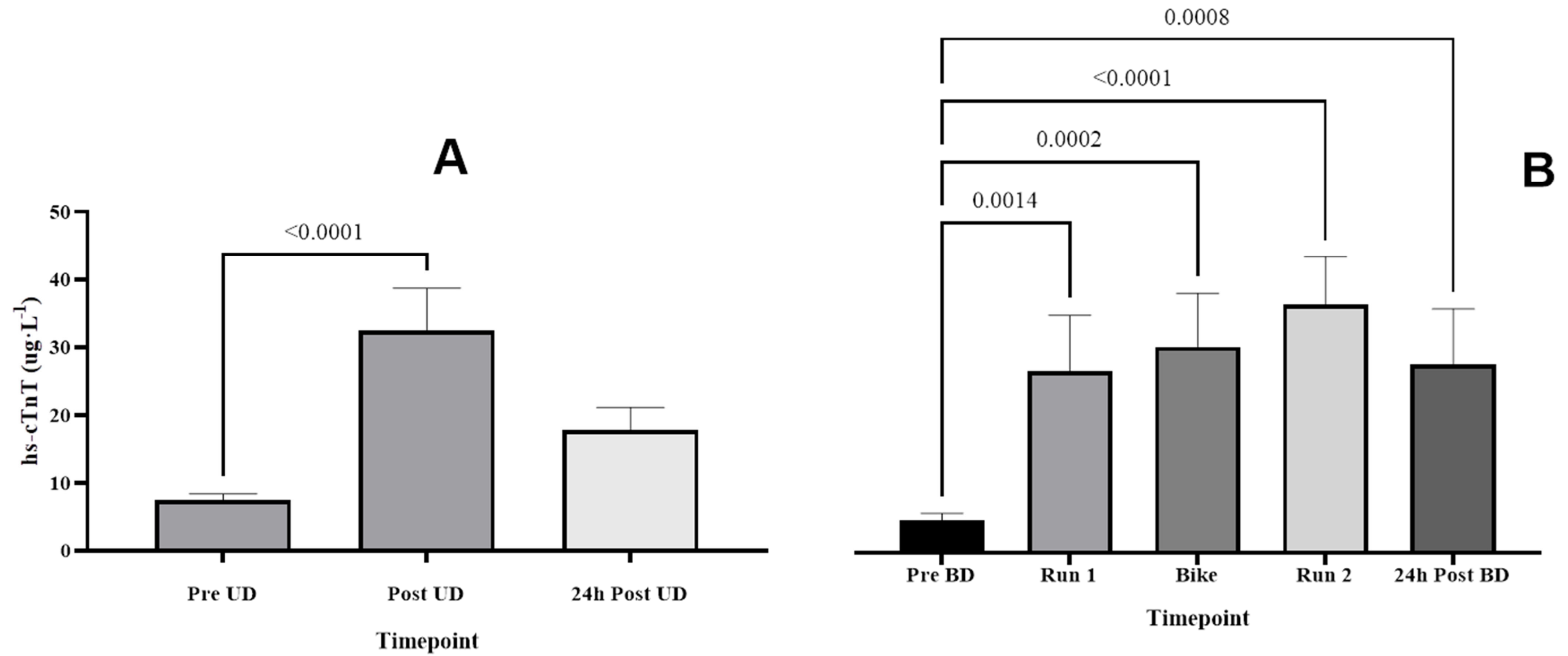


Figure 3.4 Bar plot of mean hs-cTnT concentrations after each leg of the UD trial (A) and the UD trial (B),

Note: n = 10 participants. P values are the results of paired-samples t-tests and displayed compared to baseline levels within each trial.

3.5 Discussion

3.5.1 Key Findings

This study was the first to examine cardiac function and regulation following duathlon exercise. The key findings were:

1. Olympic distance duathlon performance resulted in transient reductions in echocardiographic derived indicators of cardiac systolic, diastolic, and longitudinal function, as well as release of hs-cTnT and cardiac autonomic regulation perturbations.
2. Highly trained triathletes were capable of reproducible self-paced race-effort performances in the lab environment.
3. During the OD duathlon, transient reductions in cardiac longitudinal function and autonomic regulation begin following the first 10k run.
4. Cardiac troponin was released throughout the OD duathlon, with the measured concentration increasing over time between each leg.
5. Functional and regulatory alterations in the cardiovascular system resulting from the strenuous duathlon exercise were mostly ameliorated or reversed within 24h of recovery.

3.5.2 Exercise Results

The participants in this study were highly trained and their physiological characteristics were similar to previous works on sub-elite endurance athletes. Additionally, the exercise performances achieved by the participants were similar to those achieved in previous works investigating the physiological and metabolic effects of duathlon performances (Sparks et al., 2005, Berry et al., 2016).

In each leg of both duathlon trials, participants were able to sustain a high percentage of their $\dot{V}O_{2\max}$ and max HRs in both sports, as they would under typical race conditions (Millet et al.,

2011). It was also found that there were no significant differences within participants between duathlon trials for the times to complete each leg, average heart rates, gas exchange data, cycling power outputs and run speed at each of the inter-leg measurement TPs. It was found that in each trial, in both run legs participants demonstrated higher $\dot{V}O_2$, mean HRs, and peak HRs than during the bike legs. This finding corroborates the work of Kohrt and colleagues, who demonstrated significantly higher $\dot{V}O_{2max}$ in treadmill vs. cycling ergometry (Kohrt et al., 1987). However, in amateur level triathletes, $\dot{V}O_{2max}$ tends to be similar between the two exercise modes (Miura, 1994), which suggest that the cycling leg in the current study were completed at lower relative intensities. In contrast to this, Schabert and colleagues found that running $\dot{V}O_{2max}$ was significantly higher in national-level triathletes (68.9 vs. 65.6 mL·kg⁻¹·min⁻¹) (Schabert et al., 2000). Based on $\dot{V}O_{2max}$ data the participants in the current study were more typical of national-level triathletes and this may explain the disparity in the strenuousness of the running vs. cycling legs (Millet et al., 2011).

Furthermore, there were no significant differences in fluid consumption, or BM change between trials, and participants did not demonstrate significant changes in BM at any timepoint (see figure 4.1). Therefore, it can be said that the physiological costs of the exercise were similar between trials for participants, and that they maintained a euhydrated state throughout the exercise and subsequent recovery periods. Additionally, by maintaining a euhydrated state, it is more likely that any of the confounding effects of dehydration were less likely to affect the measured cardiovascular variables in the current study (Watanabe et al., 2020, Montain and Coyle, 1992). Recent work by Watanabe and colleagues demonstrated that maintaining euhydration during prolonged cycling with heat stress mitigates reductions in EDV and SV that contribute to impaired cardiac filling and reduced \dot{Q} (Watanabe et al., 2020).

At the time of writing, this was the first study conducted that examined EICF following OD duathlon performance. The results have demonstrated that reproducible duathlon performances in the laboratory environment are possible by highly trained, experienced triathletes, and that the physiological demands of these lab trials are also similar to outdoor

competitions (Nikolaidis et al., 2019, Berry, 2012, Rust et al., 2013, Sparks et al., 2005, Nikolaidis et al., 2021). The absence of any significant differences in exercise $\dot{V}O_2$ and HR between trials, despite the additional 30-minute measurement period between legs in the BD trial, has demonstrated the feasibility of future “broken” multisport trials to investigate the development of EICF.

3.5.3 Echocardiographic Results

The left ventricular dimensional characteristics reported in this study were in alignment with previously published works on similar populations to this cohort (Utomi et al., 2013). The participants demonstrated above-average left ventricular internal dimensions indicative of the influence of many years of endurance exercise training. These data also demonstrated good reliability as there were no intra-participant variations in LV dimensions. As participant position, transducer alignment and transducer settings were standardised between trials and refined during the CPET pilot study it is unlikely that a type 1 error was committed in the analysis of these findings (Lang et al., 2015). cMRI has been suggested to be the gold standard measurement to obtain more reliable quantification of cardiac size and function (Helbing et al., 1995) and La Gerche and colleagues (2012) previously demonstrated good levels of agreement between ultrasound and MRI in measures of cardiac function in endurance athletes (La Gerche et al., 2012b). However, in comparison to echocardiography, cMRI is more time consuming, expensive and cannot perform diastolic measurements (Rodevan et al., 1999). Additionally, repeat measures of the same participants were accounted for with Bonferroni post-hoc corrections (Field, 2000) and still found no significant differences in intra-participant measurements.

Similarly, the RV dimensions reported in this cohort were similar to previously reported values in competitive endurance athletes; however, from these data it appears that the athletes in the current study have undergone slightly less physiological RV remodelling than those reported in professional endurance athletes (RVOT = 34mm vs 41mm, RVD₁ 36mm vs 49,

RVD₂ 29mm vs 44, RVD₃ = 90mm vs 92, RVFAC = 43.2% vs 49.3%) (D'Andrea et al., 2013, Oxborough et al., 2011).

3.5.3.1 Systolic and Diastolic Function

The immediate effects of PSE in the during the duathlons were an increased HR, increased \dot{Q} and reduced mitral and tricuspid E/A ratio due to reductions in early and increases in late trans-mitral filling velocities. These findings are in agreement with many other published findings in this area (Middleton et al., 2006, Donaldson et al., 2019, Lord et al., 2018), alongside changes in systolic function (Lucia et al., 1999, Dawson et al., 2005, George et al., 2005, Whyte et al., 2005, Neilan et al., 2006b, Alshaher et al., 2007, Hart et al., 2007), and also with no changes in systolic function (Whyte et al., 2000, Shave et al., 2004a, Shave et al., 2004b, Middleton et al., 2007).

Also in line with previous works was the finding that the post-exercise alterations in LV diastolic variables were strongly associated with changes in HR (Middleton et al., 2007). Elevated HR is thought to play a potentially confounding role in diagnosing changes in measures of LV diastolic function due to reductions in ventricular relaxation time (Oniki et al., 1992). This was supported by the findings of reduced E velocity and significant correlations between HR and PW variables. However, there is evidence to suggest that reductions in diastolic function occur in the absence of positive correlations to HR and preload (Middleton et al., 2006). Diastolic dysfunction following exercise has been previously reported in several studies (see (Oxborough et al., 2010), and may be due to impaired relaxation, reduced preload, or changes in HR. Preload augmentation through the Trendelenberg position has been shown to restore post-exercise E and increase E/A ratio compared to the supine position, indicating that exercise induced reductions in venous return or filling may be responsible for reports of reduced measures of diastolic function (Hart et al., 2007). In this study, the data demonstrated increased post-exercise HR likely played a role in the apparent reduction in diastolic function indicated by reduced E/A ratios (Shave and Oxborough, 2012). This is supported by the recovery data as there was no persistent effect of

exercise at the 24h timepoint, when HR and E/A ratio had both returned to baseline levels. Finally, the E/E' ratio has been suggested as a more sensitive measure than E/A ratio in assessing diastolic performance as a marker of preload (Ommen et al., 2000), and in the present study there were no significant alterations in E/E' at any timepoint in either study. In the BD trial, the magnitude of the change in E/A ratio increased with each leg of the duathlon due to progressively lower E velocities. A possible explanation for this may be that the total cumulative duration of the exercise was the cause of altered diastolic function. This would support the findings of previous meta-analyses that determined impairments in diastolic function become more prolific following longer-duration exercise (Middleton et al., 2006, Oxborough et al., 2010, Shave and Oxborough, 2012). Additionally, the clinical use of the TV E/A ratio is highly limited, due to it being highly influenced by respiration and significant variation across the cardiac cycle (Alam et al., 1999). This was accounted for by taking measurements at end expiration but cannot rule out the influence of post-exercise hyperventilation on RV doppler indices. The finding of no significant changes in RV TDI measurements is also consistent with previous studies investigating the effects of high intensity exercise on RV function (Kleinnibbelink et al., 2021b).

In terms of systolic function, the athletes in this study were able to maintain or significantly increase \dot{Q} , SV and ejection fraction following each leg of the duathlon in the BD trial. Despite being able to increase overall cardiac output, both run legs yielded small reductions in EDV. This finding of a return to pre-exercise levels in EF and \dot{Q} despite reduced EDV and SV shows that HR increased to meet \dot{Q} demands during the measurement period. The return to baseline systolic function suggests that participants were able to recover somewhat on the bike, or that running yielded higher post-exercise HRs and reduced LV filling times.

In summary, immediately following exercise in both duathlon trials there was evidence of altered diastolic filling and systolic emptying kinetics. Systolic function was altered via reductions in EDV and elevations in HR to maintain \dot{Q} , and this occurred following the first and second runs. However, the bike leg enabled athletes to recover despite the relatively high exercise intensities achieved. Though mitral and tricuspid E/A ratios were reduced following

exercise, the obtained values never approached clinical levels, and all values had returned to baseline levels following 24 hours of recovery. It is suggested that this could be attributed to the positive cardiac remodelling these athletes have undergone due to years of endurance training, thus giving them a greater cardiac resilience to the transient perturbations caused by the exercise.

3.5.3.2 Longitudinal Strain

The finding of decreased left and right ventricular LS following each duathlon trial is in line with previous research and the transient decline in strain shown in the study is not uncommon among well-trained and novice cohorts alike (Lord et al., 2018). During the BD trial, LV ϵ was significantly lower than baseline following the first run then recovered after the bike to near pre-exercise values. During the bike leg, participants averaged lower exercise HRs and $\dot{V}O_2$, therefore despite the pre-completed run exercise, the multisport-habituated cohort in this study were able to recover from pre-existing cardiac fatigue during a sub-maximal steady state bike trial. The steady state nature of the cycling exercise may have facilitated this recovery. Previous research by Stewart et al. (2015) demonstrated transient reductions in LV ϵ to a similar level (~1%) following a criterium style race of equal time to the bike leg in this study (~60 minutes), which required a stochastic power output demand from the participants (Stewart et al., 2015). The bike leg in the present study was steady state in nature to replicate the non-drafting demands of duathlon racing, which may have placed less overall demand on the myocardium, but this is not clear. The finding of a return to below baseline levels in LVLS following the second run adds further support to the emerging theory that running exercise places a greater level of cardiac demand than does cycling exercise. Unfortunately, measurements of atrial strain were not possible in this study, however the effect of a disproportionate level of atrial strain over global ventricular SR cannot be ruled out (Saraiva et al., 2010, Sareban et al., 2018).

Our findings of reduced RV strain add to the body of evidence that suggests RV mechanics are impeded to an equal or greater extent following exercise than the LV. This mechanism is

potentially mediated by several factors including increased afterload during exercise on the RV, resulting in disproportionate haemodynamic loading for the myocardium of the right ventricle (Lord et al., 2015, Lewicka-Potocka et al., 2020). Additionally, LV deformation has been shown to impact RV strain (Oxborough et al., 2011), and previous research has demonstrated an increased incidence of septal bounce secondary to RV dilatation following PSE (Dawson et al., 2005, La Gerche et al., 2012a, Oxborough et al., 2011, Oxborough et al., 2012). Some of the data from this study supports this potential mechanism, as there were moderate correlations between RV strain and LV diastolic and systolic strain rates in the UD trial. However, there are limitations to this data as there were no significant correlations between these variables in the BD trial, and the chosen variables demonstrated co-linearity in the direction of the change from pre- to post-exercise. A more robust method of determining this relationship in future studies may be to investigate changes in segmental longitudinal function, as opposed to global LS (Stewart et al., 2016). Alongside this, similar to the work of La Gerche et al. (2012) there was concomitant reductions in LVIDs, which may indicate a left to right septal shift causing lower LV filling pressures (La Gerche et al., 2012b). Such findings have previously occurred alongside significant post-exercise RV dilation, following ultra-endurance running exercise lasting >7hrs (Oxborough et al., 2011, La Gerche et al., 2012a). Therefore, the lack of post-exercise RV dilatation in the current study may be due to the shorter (~2hrs) duration of the exercise protocol.

Our finding that exercise intensity measures ($\dot{V}O_2$, HR) were significantly associated with the post-exercise decline in RV LS adds support to the emerging concept that high intensity exercise can cause significant EICF (Donaldson et al., 2019, Kleinnibbelink et al., 2021b, Stewart et al., 2015, Stewart et al., 2016). Previous research has shown disparate LV and RV functional perturbations following moderate and high intensity cycling exercise (Stewart et al., 2016). In this study, LV and RV strain were both decreased from baseline following each exercise leg in the BD trial, but ϵ mechanics recovered somewhat following the bike in both ventricles. Though there were reduced RV myocardial mechanics during passive recovery, the extent of any transient impairment may have been further improved through use of MRI

to examine RV stroke volumes, and invasive measures such as PCWP to measure pulmonary afterload.

3.5.4 Haemodynamics and Heart Rate Variability

Like the findings of Stewart et al. (2014) and Seiler et al. (2008) there was evidence of sustained sympathetic nervous system outflow and parasympathetic tone suppression during the acute recovery phase from both duathlons, measured by perturbations in LF/HF balance (Seiler et al., 2007). At rest, the LF/HF ratio, PSD and RMSSD were similar in the participant cohort to previously reported data in similar demographics (Stewart et al., 2014). In the present study, the lower RMSSD and total PSD of RR intervals during immediate recovery reflects reduced parasympathetic cardiac modulation, while the increased LFnu and LF:HF ratio is reflective of sustained sympathetic outflow during the acute recovery phase from the duathlon exercise. In the BD trial, parasympathetic modulation was significantly reduced from baseline following the first run and remained suppressed until the 24h recovery point.

The overall findings of significant reductions in n post-exercise parasympathetic modulation, myocardial longitudinal function, alongside post-exercise troponin release further links the relationship between that exercise induced biomarker release and autonomic modulation (Stewart et al., 2016). The mechanism behind this response is still unclear; however, Stewart and colleagues have proposed that the upper limit of the moderate-intensity exercise domain (given as the gas exchange threshold (GET) in their study) demarcates a threshold for EICF and represents an intensity boundary that influences both cardiac health and adaptation (Pringle et al., 2003). In their study, the participants exercised at two intensities, above or below the GET for 90 and 120 minutes, respectively (Stewart et al., 2016). The majority of cardiac autonomic and functional perturbations occurred following the high-intensity 90-minute bout of exercise, above the proposed intensity boundary. In the present study, average relative HR and work rates were similar to the 'high-intensity' bout in the study of Stewart

and colleagues (Stewart et al., 2016), despite participants self-selecting their effort levels. The finding of an association between exercise work rates and cTn release with RMSSD supports the potential existence of a threshold which demarcates the onset of EICF and demonstrates the potential usefulness of HRV as a non-invasive assessment of EICF.

3.5.5 Cardiac Troponin

In this study it was found that RVLS changes were more significantly associated with troponin release than were LV LS changes, as indicated by a greater effect size, lower SE and increased R^2 ($T = 5.2$, $SE = 0.34$, $R^2 = 0.59$ in the RV vs 4.94, 0.44, and 0.56 in the LV). This finding further supports the hypothesis that the RV is subjected to disproportionate wall stress during PSE and experiences greater exercise induced fatigue than the LV, owing to geometrical and wall-thickness differences between the two chambers (Oxborough et al., 2011, Oxborough et al., 2012, La Gerche et al., 2012a, Lord et al., 2015, Elliott and La Gerche, 2015). Additionally, the theory that mechanical strain on myocyte membranes is responsible for exercise induced troponin release does explain these findings (Shave et al., 2010a). While not novel, the investigation of the stress caused to the RV free wall by PSE was lacking from earlier, formative studies into the area of EICF. Combining investigation of the RV with the use of high-sensitivity troponin assays, future research in this area will be more likely to demonstrate significant relationships between myocardial function and biomarker release.

3.5.6 Conclusion

When interpreting the data from this study it is important to distinguish that the reductions in EDV and LS that occurred during Run 1, were not present following the bike leg and later re-emerged after the final run. These findings suggest that there may have been a mode-dependent feature of the running exercise in these trials that contributed to diminished systolic function and that participants were able to recover during the bike leg. Exercise demand was higher in both runs (as measured by average HR and $\dot{V}O_2$) and it is possible that the upright, weight-bearing nature of running places a greater burden on the cardiovascular

system and contributes to reductions in venous return that persists into the immediate post-exercise recovery phase (Millet et al., 2009). Unfortunately, there is no pre-existing data to compare these findings to and this informs the aims of the subsequent chapters wherein future research should be conducted to investigate the extent of cardiovascular functional changes following running and cycling exercise in duathlon trained athletes.

To summarise, this study was the first to investigate the effect of duathlon exercise on cardiac function. Further, this study investigated the progression of cardiac functional changes throughout each leg of an OD duathlon performance, which has been lacking in the literature. There were no significant differences in exercise performance or the acute magnitude of transient cardiac functional, biomarker and regulatory perturbations between continuous (UD) and discontinuous (BD) exercise trials. This chapter also confirmed the findings of most research into the area of exercise induced cardiac functional changes, in that the majority of diastolic functional changes would appear to be load dependent, and that the changes are transient and reverse within 24h of recovery.

Chapter 4 The Effects of 40 Kilometre Cycling Time Trial Performances at Duathlon and Maximal Race Paces on Myocardial Function, Biomarker Release and Heart Rate Variability

4.1 Abstract

Background: Research into the effects of strenuous cycling exercise has demonstrated transient reductions in cardiac function, as seen following triathlon and running exercise. Additionally, it is well known that during triathlon performance, the gross efficiency and work rates achieved during the bike leg are reduced by prior swimming (Millet, 2011). It is currently unclear whether the cycling leg is similarly affected by prior running during duathlon competitions. Lastly, there is a lack of data that compares duathlon-paced 40k cycling time trial performance with a ‘standalone’ (no prior or post exercise legs) maximal performance over the same distance. Therefore, this chapter aimed to assess the effects of standalone 40k cycling time trials on cardiac function at duathlon-matched intensity (DM), and at maximal sustained effort (MAX).

Methods: Participants (n=10) completed two lab-based 40k cycling efforts at different intensities (DM and Max), with pre and post exercise cardiac measurements of autonomic function, echocardiography, and plasma hs-cTnT concentration.

Results: Exercise performance variables were significantly higher in the Max compared to the DM trials. The Max cycling bouts resulted in significant post-exercise elevations in HR and all blood pressure parameters. In addition, following the Max bike left and right ventricular longitudinal strain, LV longitudinal SR, EF, TAPSE, and tricuspid E/A ratio were significantly lower at the post-exercise timepoint than baseline. In addition, LV twist, peak untwist, and twist velocities were also significantly reduced. In the DM trial, LV twist and basal CS were significantly increased following the exercise bout. Analysis of HRV data revealed significant reductions in RMSSD, total PSD, and elevations in the LF/HR ratio in

the Max trials. Blood analysis revealed significant release of hs-cTnT in each condition, which were still elevated above pre-exercise levels at 6h post-exercise in the Max trial.

Conclusions: In duathlon competition the 10k run leg prior to the cycling leg results in significantly elevated HR for the same power output as shown by the lower HR data during the DM trial compared to the duathlon bike legs. Standalone duathlon-paced 40k cycling effort resulted in fewer transient cardiac function alterations and augmentation of left ventricular short axis motion compared to a maximal 40k cycling effort. Both DM and Max cycling intensities (approximately 74 and 86% of $\dot{V}O_{2max}$, respectively) resulted in significant hs-cTnT release, that was still significantly elevated above baseline following 6h of recovery in the Max trial. The data from this study demonstrate an approximate 50W (20%) performance reserve in the cycling ability of highly-trained duathletes, when not affected by a prior 10k run. This research also adds to the body of research investigating the effects of strenuous cycling activity on cardiac function and troponin release.

4.2 Introduction

The findings of the previous chapter confirmed that duathlon exercise causes substantial cardiac perturbations, and that the running legs appeared to cause the initial biomarker release and cardiac functional changes. The weight-bearing nature, higher \dot{Q} (Hermansen et al., 1970), greater lower-limb blood flow distribution (Matsui et al., 1978), and $\dot{V}O_2$ (Hermansen and Saltin, 1969) requirements of strenuous running may be a factor in EICF (Millet et al., 2009). In contrast, during cycling exercise the athlete's weight is supported by the bike, and skeletal muscle activation and blood flow is distributed mainly to the lower body (Laaksonen et al., 2006, Millet et al., 2009). Additionally, data on triathletes indicates that the HR corresponding to anaerobic threshold is always higher in running than cycling, indicating a potentially greater cardiac stress at similar exercise intensities (Hue et al., 1998, Hue et al., 1999, Hue et al., 2000, Miura, 1994). It has been previously demonstrated that exercise mode is not a factor in the release of cTn following PSE over 60 minutes of swimming, cycling and

running (Legaz-Arrese et al., 2015). However, the exercise bouts in the previous study elicited different peak and mean HRs and though not significant, cycling exercise yielded the lowest absolute post-exercise hs-cTnT concentrations and % increase from BL. Additionally, according to the serial hs-cTnT measurements during recovery from exercise, the return to below the upper reference limit (URL) values was achieved after 3hrs following cycling, compared with 12hrs following swimming and running (Legaz-Arrese et al., 2015).

There is a wealth of evidence that suggests, in triathletes, running performance is limited by prior cycling exercise due to a multitude of factors that influence ventilatory efficiency (Hue et al., 1999), running biomechanics (Bonacci et al., 2013), metabolism (Millet and Vleck, 2000), and thermoregulation (Baillot and Hue, 2015). Conversely, there has been little investigation into the influence of running on cycling performance. If running exercise is accepted to be more physiologically demanding than cycling, cycling performance during the duathlon trials in the previous chapter may have been markedly limited. Therefore, fatigue caused by the prior 10k run may explain the lower HR and $\dot{V}O_2$ observed during the duathlon cycling leg, and in EICF measurements between legs in the BD trial. Accordingly, this chapter sought to examine the effects of standalone 40k cycling performance at duathlon intensity on cardiac function, regulation, and biomarker release. Additionally, participants performed a maximal effort 40k cycling trial to quantify their performance reserves and to examine whether any additional cardiovascular functional alterations would occur following a higher-intensity bout of cycling exercise.

4.3 Methods

4.3.1 Participant Characteristics

Participants were recruited by invitation from the study in the previous chapter after being advised of the study protocol and requirements. In total, 6 participants agreed to continue the study and their details and characteristics are displayed in Table 4.1 below.

Table 4.1 Participant characteristics

Characteristic	
Age	33.0 (5.7)
Cycling HR _{MAX} (beats·min ⁻¹)	175.7 (8.0)
Training History (years)	10.5 (5.5)
Mass (kg)	76.2 (7.21)
P $\dot{V}O_{2max}$ (W)	377.0 (32.1)
Stature (cm)	180.0 (5.0)
Cycling $\dot{V}E_{MAX}$ (L·min ⁻¹)	169.4 (18.7)
Cycling $\dot{V}O_{2max}$ (L·min ⁻¹)	4.4 (0.3)
Cycling $\dot{V}O_{2max}$ (mL·kg·min ⁻¹)	58.1 (4.5)

4.3.2 Testing Protocol

On separate days, participants performed 2 bike time trials over 40k (Figure 4.1). One trial was performed at maximal (Max) effort and another at duathlon matched (DM) work rate. The order of trials was randomised for each participant to prevent the influence of any possible order effects. The tests were performed at the same time of day and the same day of the week to minimize the influence of the effects of personal training on the study. The participants were asked to maintain their own training schedule for the duration of the study but were not allowed to compete during this period. On experiment days, the participants were asked to abstain from training for 48 hours before their visit and abstain from caffeine for 24 hours prior to the visit.

Participants reported to the laboratory and underwent baseline (BL) measurements identical to those described in the previous study and general methods. Following each exercise trial, post-exercise resting measurements were taken, also identically to the previous chapter. Participants then underwent 6 hours of passive recovery, where they were encouraged to feed and rehydrate according to their typical post-training routines and undertake no further exercise. Participants were instructed to adhere to this routine following each trial and to also abstain from caffeine. All participants were office workers and did not undertake any undue strenuous manual labour between trials on non-workdays. Following 6 hours of passive recovery the baseline measurements were taken again as previously described.

As the same cohort returned for this study, it was possible to perform pairwise comparisons of the broken duathlon trials. Prior to the Max trials participants were advised to complete the distance as quickly as possible. Work rate was self-selected and recorded at each change. During the DM cycling trial, participants were encouraged to achieve the average power outputs corresponding to the BD trial cycling leg at 10-minute time points (TPs), which are labelled from timepoint 1 to timepoint 6 (see Figure 5.1).

During exercise, HR and cycling power was recorded continuously on a GPS watch (Garmin, France) and transcription. Breath by breath gas exchange and ventilation was recorded using a metabolic cart (Oxycon, Jaeger, Germany) at matching timepoints (TPs) to the BD trial. Figure 5.1 shows a schematic of the experimental procedures. Participants were encouraged to consume fluids throughout exercise using their own preferred beverage choice at a rate to maintain euhydration, as during the duathlon trials.

4.3.3 Statistical Analysis

Unless otherwise stated, continuous variables are expressed as mean \pm standard deviation (SD). All data were analysed using the statistical package for social sciences (SPSS 22 release version for Windows; SPSS Inc., Chicago IL, USA). Data were assessed for conformity with parametric assumptions (Field, 2000). Two-way repeated measures analysis of variance (ANOVA) were performed on the recorded values at each timepoint to assess the effects of exercise, and one-way ANOVA were performed on the standardised mean

difference (SMD) between the pre and post-exercise measurements to assess the effects of trial. A p value of <0.05 was regarded as statistically significant. Following tests for normality and significant interactions for main effects following the ANOVA, Bonferroni post hoc tests were used to compare intra-trial baseline and post-exercise measurements, and the inter-trial pre to post-exercise SMDs (Field, 2000). To determine significant correlations between the large amount of echocardiographic, autonomic, exercise and biochemical variables, a repeated methods correlation was performed using the pre- and post-exercise timepoint data. Any significant ($P > 0.05$) correlations were filtered out from the correlation matrix and incorporated into linear regression models where appropriate. Linear regression was performed on the Python Pingouin package (Vallat, 2018) using the ordinary least squares (OLS) method. Multivariate regressions were also considered if the individual contribution to the linear model increased the R^2 or significance value without diluting the model's power (Alexopoulos, 2010).

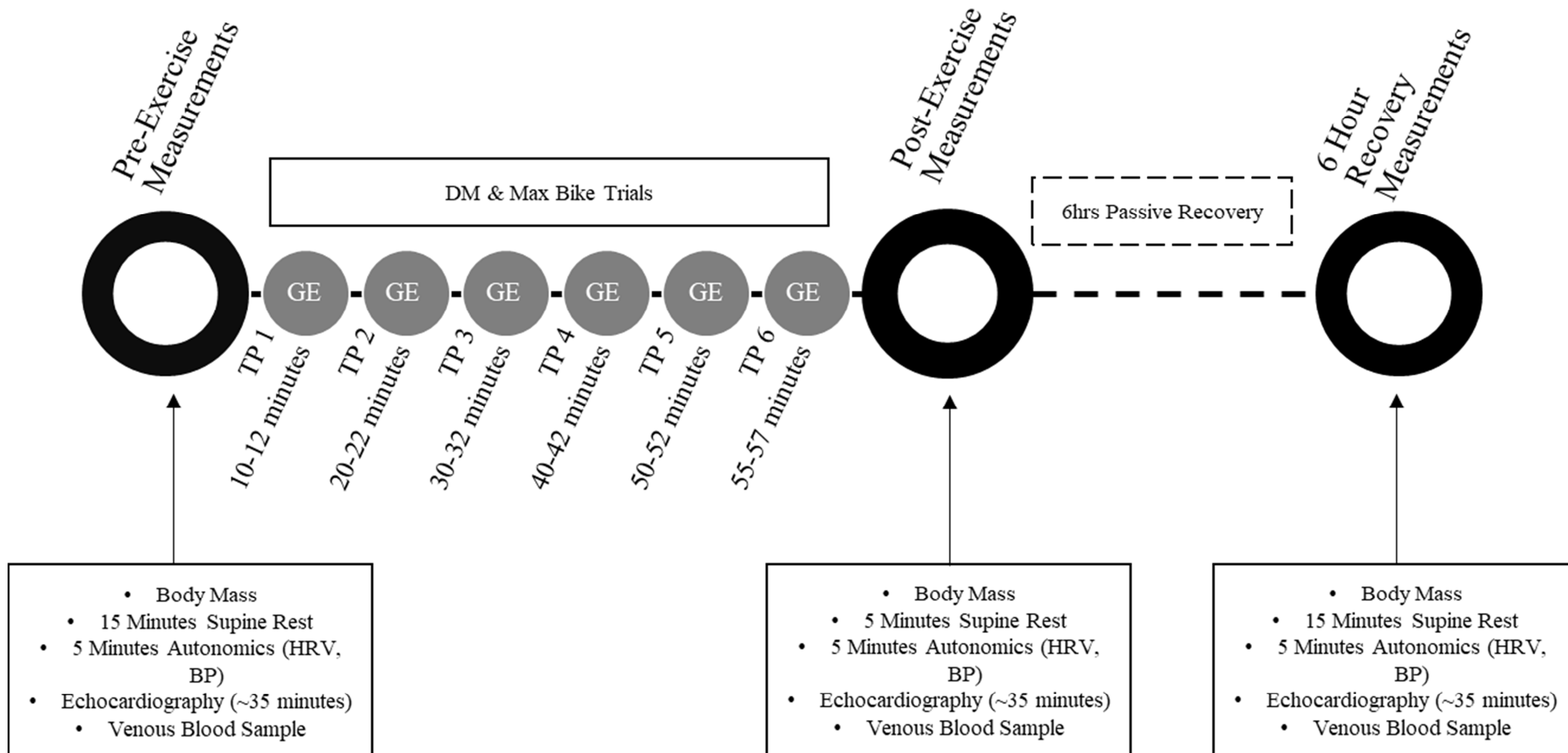


Figure 4.1 Experimental testing protocol for the DM and Max 40k cycling trials.

4.4 Results

4.4.1 Exercise Results

In the DM Bike trials, there were significant differences in HR variables from the counterpart trials performed in the duathlon study (Tables 4.2 and 4.3). The HR_{Peak} was higher in the DM bike than the BD bike leg ($F=5.04$ and $P<0.01$), and average HR was higher in both duathlon bike legs compared with average HR during the DM trial (BD: $F=4.01$ and $P=0.01$; UD: $F=3.01$ and $P=0.03$).

In the Max bike trial, average power output ($F = 8.40$, $P = <0.05$), average HR ($F = 23.60$, $P = <0.01$), and HR_{Peak} ($F = 54.6$, $P = <0.001$) were higher than the DM bike trial. Absolute ($F = 11.2$, $P = <0.05$) and relative ($F = 10.3$, $P = <0.05$) mean $\dot{V}O_2$ were also significantly higher in the Max Bike trial vs. the DM bike legs. Participant mass pre- and post-exercise, and fluid consumption during exercise were similar between DM and Max trials.

Table 4.2 Average exercise data from all bike trials

	DM	Max	Broken Duathlon
Average HR (beats·min ⁻¹)	141.7 (12.3) ^{^*}	161.4 (14.7) [†]	154.7 (11.5)
HR _{Peak} (beats·min ⁻¹)	149.2 (13.4) [*]	178.0 (6.3) [†]	159.6 (11.7)
Power Output (W)	219.5 (38.1)	253.6 (58.9) [†]	210.6 (38.2)
$\dot{V}O_2$ Absolute (L·min ⁻¹)	3.30 (0.3)	3.70 (0.4) [†]	3.30 (0.4)
$\dot{V}O_2$ Relative (mL·kg·min ⁻¹)	43.0 (5.4)	49.6 (6.4) [†]	44.0 (6.0)
BM Δ (kg)	0.99 (0.31)	0.89 (0.42)	0.27 (0.03)
Fluid (L)	0.35 (0.11)	0.60 (0.03)	1.07 (0.31)
Time (minutes)	65.1 (4.2)	56.3 (5.3) [*]	64.3 (4.9)

Note: n = 6 participants. Data from the bike legs of the same participants in Chapter 4 are presented for comparison to the standalone trials. Paired samples T-test results * = significantly (P<0.05) different to BD, ^, † = significantly different to DM.

Table 4.3 Exercise data at each timepoint during Max and DM bike trials.

TP	Bike DM						Bike Max					
	1	2	3	4	5	6	1	2	3	4	5	6
HR (beats·min ⁻¹)	134.7 (13.5)	139.3 (13.0)	141 (12.8)	143.8 (11.8)	145.6 (12.2)	145.6 (11.7)	148.4 (16.0)	154 (14.0)	158.4 (13.2)*	162.3 (12.1)*	169.1 (8.7)*	175.9 (6.9)*
Power Output (W)	213.2 (46.8)	221.2 (40.4)	223.8 (38.5)	224.8 (40)	225.8 (33.9)	219.8 (35.6)	245.7 (44.5)*	243.6 (45.5)*	247.6 (42.8)*	251.1 (40.4)*	266.4 (34.4)*	267.4 (119.8)*
$\dot{V}O_2$ Relative (mL·kg·min ⁻¹)	40.8 (8.9)	42.6 (6.2)	43.4 (4.9)	43.9 (4)	44.1 (3.9)	43.5 (4.5)	46.9 (7.1)	47.1 (6.6)	48.7 (7)	48.7 (5.4)	51.2 (4.7)	54.8 (6.1)
$\dot{V}O_2$ Absolute (L·min ⁻¹)	3.1 (0.6)	3.2 (0.4)	3.3 (0.3)	3.3 (0.2)	3.3 (0.2)	3.3 (0.2)	3.5 (0.5)	3.5 (0.5)	3.6 (0.5)	3.6 (0.4)	3.9 (0.3)	4.1 (0.2)
RER	0.89 (0.04)	0.87 (0.05)	0.87 (0.05)	0.87 (0.04)	0.86 (0.05)	0.86 (0.05)	0.91 (0.04)	0.9 (0.02)	0.89 (0.03)	0.89 (0.04)	0.91 (0.03)*	0.95 (0.06)*

Note: n = 6 participants. Paired samples t-test results at each timepoint compared to the DM trial. * = significantly (P<0.05) different than the same time point in the DM trial

4.4.2 Echocardiographic Results

4.4.2.1 Systolic and Diastolic Function

There was a significant ($P < 0.05$) effect of exercise on LV systolic (Figure 4.2) and diastolic functional measures within each trial (Table 4.4). In the Max trial, there was a significant effect of cycling exercise on EF ($F = 19.8$ and $P < 0.001$), and SV ($F = 7.03$ and $P < 0.01$). In the DM bike trial, there was a significant effect of exercise on SV ($F = 5.02$ and $P < 0.05$). Mitral E/A ratio and E/E' were significantly lower at 6h post than baseline in the Max bike trial. Following the Max trial, LVEF, SV, and EDV were significantly ($P < 0.05$) lower immediately post exercise than pre-exercise, however these were not different from baseline after 6h of recovery. Additionally, \dot{Q} was significantly ($P < 0.05$) higher than baseline immediately after exercise in the Max trial and was significantly ($P < 0.05$) lower than baseline in the DM trial. However, this had also reversed in both trials at the 6h recovery measurement. There was also a significant effect of exercise on TAPSE, resulting in lower post-exercise values in the Max ($F = 5.40$ and $P < 0.05$) and DM ($F = 5.84$ and $P < 0.05$) trials. Between trials, the post exercise change was significantly higher in HR ($F = 9.97$, $P = 0.01$) and \dot{Q} ($F = 3.32$, $P = 0.04$) after the Max bike than the DM bike trial.

Table 4.4 Left and right ventricular PW Doppler and TDI measures at each timepoint from each bike trial.

	DM Bike			Max Bike		
	Pre	Post	6h Post	Pre	Post	6h Post
Left Ventricle Function						
E (m·s ⁻¹)	0.67 (0.05)	0.62 (0.04)	0.65 (0.04)	0.68 (0.07)	0.61 (0.06)	0.64 (0.05)
A (m·s ⁻¹)	0.38 (0.02)	0.4 (0.02)	0.4 (0.04)	0.37 (0.02)	0.41 (0.04)	0.45 (0.04)
E/A Ratio	1.81 (0.19)	1.58 (0.12)	1.73 (0.22)	1.8 (0.12)	1.59 (0.19)	1.47 (0.13)
E/E'	3.70 (0.17)	3.61 (0.19)	3.57 (0.2)	3.86 (0.15)	3.61 (0.27)	3.56 (0.15)
Right Ventricle Function						
TAPSE (cm)	2.97 (0.06)	2.62 (0.07)*	2.77 (0.12)	3.00 (0.11)	2.56 (0.14)*	2.77 (0.08)
E (m·s ⁻¹)	0.55 (0.05)	0.47 (0.02)	0.54 (0.02)	0.55 (0.06)	0.47 (0.04)	0.53 (0.03)
A (m·s ⁻¹)	0.30 (0.02)	0.28 (0.01)	0.32 (0.01)	0.31 (0.02)	0.33 (0.02)	0.34 (0.04)
E/A Ratio	1.83 (0.11)	1.67 (0.06)	1.69 (0.12)	1.81 (0.1)	1.49 (0.16)	1.67 (0.12)

Note: n = 6 participants. Paired sample t-test results* = significantly different from baseline within each trial, ‡ = significantly different from post-exercise measurement within each trial.

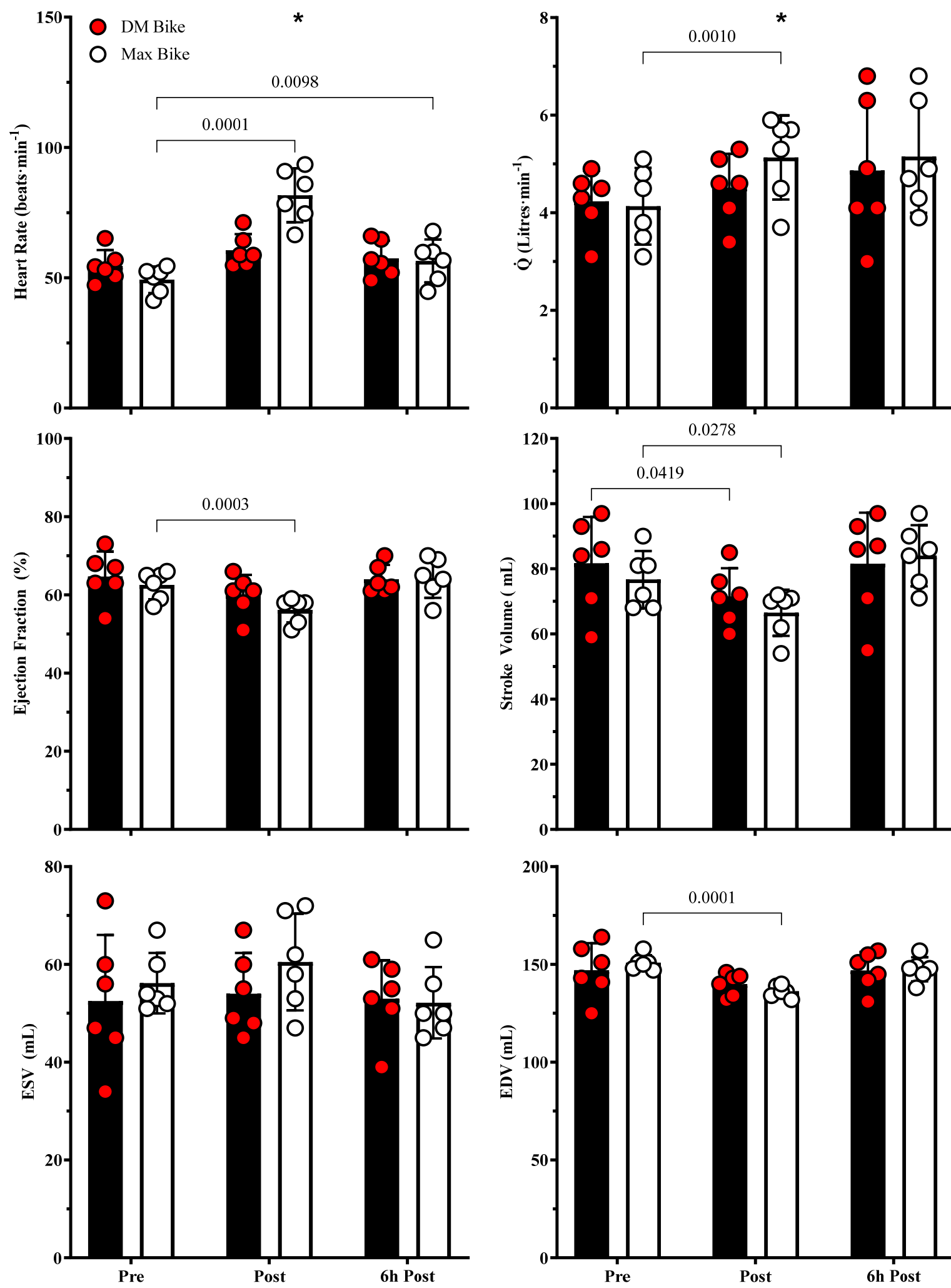


Figure 4.2 Plot of left ventricular systolic functional parameters following each stage of the DM (closed bars) and Max (open bars) bike trials.

Note: $n = 6$ participants. Results of the paired-samples T-tests results are presented on the figure where significant ($P = 0.05$) differences from the pre-exercise values within each trial occurred. Individual data points are represented by red circles for the DM trials and open circles for the Max trials. * = significantly ($P < 0.05$) different change in post-exercise value than the DM trial

4.4.2.2 Longitudinal Strain and Strain Rate

In the Max trial there was a significant effect of exercise on LV LS ($F = 7.31$ and $P < 0.01$), early diastolic SR ($F = 5.33$ and $P < 0.05$), and late diastolic SR ($F = 17.90$ and $P < 0.001$). Left ventricular LS was higher than baseline post Max cycle (Figure 4.3). There was a significant effect of exercise on LV late diastolic SR in the DM trial ($F = 6.50$ and $P < 0.05$), and Max trial ($F = 5.48$ and $P < 0.05$), which was higher than baseline following 6h of recovery. All other values had returned to baseline levels after 6h of recovery. In the Max trial, there was also a significant effect of exercise on right ventricular LS ($F=5.33$ and $P=0.02$), which was higher than baseline after exercise, and in the DM trial there was a significant effect of exercise on RV late diastolic strain rate ($F=5.59$ and $P=0.02$), which was also higher than baseline after exercise (Table 4.5). Between trials, the post-exercise change in LV early diastolic SR was significantly ($F = 6.33$, $P = 0.03$) higher in the DM trial than the Max trial (Table 4.5).

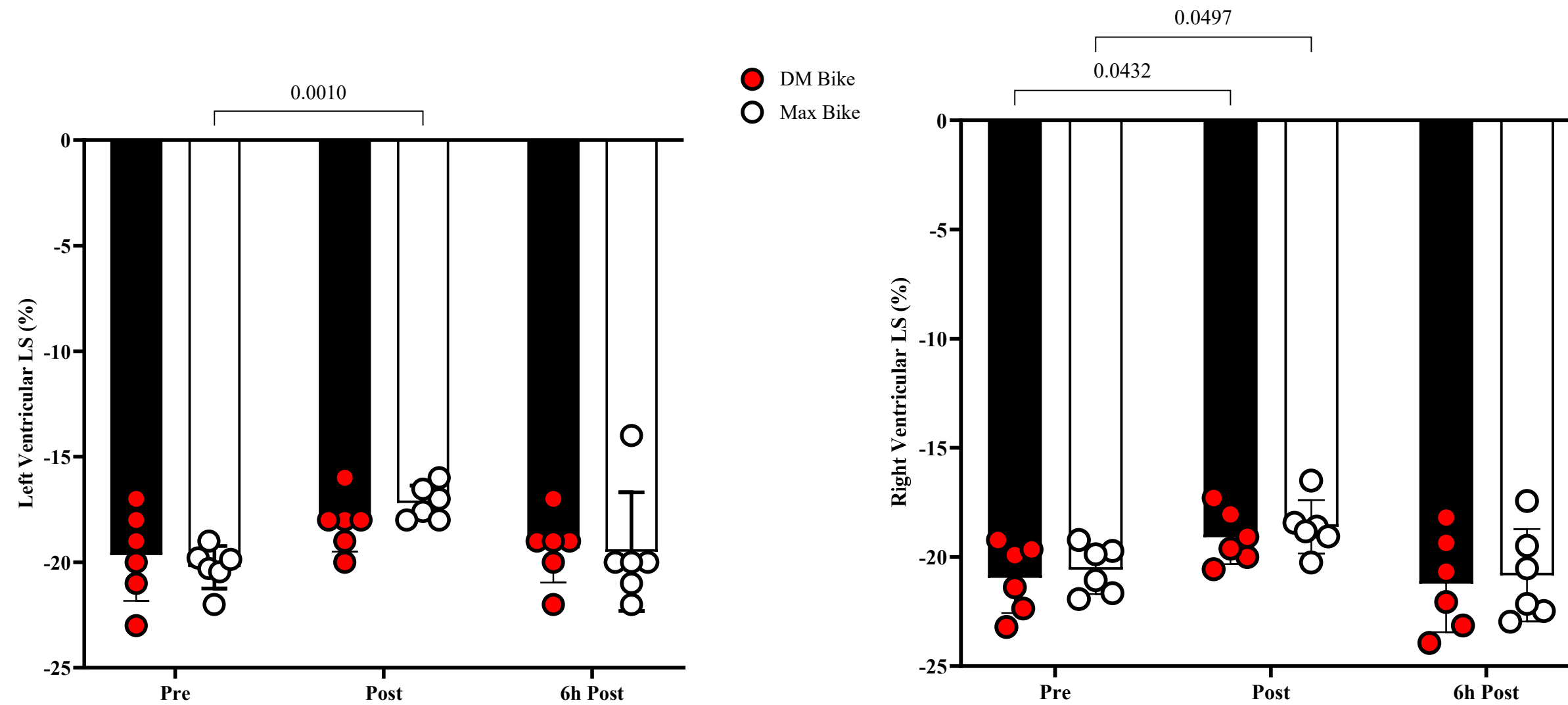


Figure 4.3 Plot of left and right ventricular LS at each time point in DM(closed bars) and Max (open bars) trials.

Note: $n = 6$ participants. Results of the paired-samples T-tests results are presented above the figures where significant ($P = 0.05$) differences from the pre-exercise values within each trial occurred. Individual data points are represented by red circles for the DM trials and open circles for the Max trials.

Table 4.5 Left and right ventricular longitudinal SR variables in DM and Max bike trials.

Phase	DM Bike			Max Bike		
	Pre	Post	6h Post	Pre	Post	6h Post
Left Ventricle						
Early Diastolic SR (%/sec)	1.73 (0.2)	1.68 (0.17) [‡]	1.74 (0.13)	1.77 (0.23)	1.55 (0.18)*	1.67 (0.24)
Late Diastolic SR (%/sec)	0.65 (0.15)	0.69 (0.15)	0.76 (0.2)*	0.66 (0.14)	0.75 (0.17)*	0.79 (0.18)*
Right Ventricle						
Early Diastolic SR (%/sec)	1.31 (0.21)	1.16 (0.22)	1.32 (0.1)	1.37 (0.15)	1.22 (0.37)	1.29 (0.22)
Late Diastolic SR (%/sec)	0.79 (0.17)	0.87 (0.18)*	0.94 (0.28)	0.79 (0.10)	0.83 (0.22)*	0.95 (0.26) [‡]

Note: n = 6 participants. Paired samples t-test results * = significantly (P = 0.05) different from baseline in each trial, ‡ = significantly different from post-exercise measurement in each trial, [‡] = significantly different change from baseline compared to the Max bike trial.

4.4.3 Haemodynamics and Heart Rate Variability

Within Trial Differences

In the Max trial there was a significant effect of exercise on LF/HF ratio ($F = 4.11$, $P < 0.05$), PSD ($F = 6.67$, $P < 0.05$), RMSSD ($F = 9.66$, $P < 0.01$), sBP ($F = 4.84$, $P < 0.05$), mBP ($F = 6.90$, $P < 0.05$), dBP ($F = 4.92$, $P < 0.05$), and RRI ($F = 170$, $P < 0.001$). In the DM trial, there was no effect of exercise on any variable.

In the Max trial, the LF/HF ratio was significantly higher than baseline, while RMSSD and PSD were lower than baseline at the post exercise measurements. The LF/HF ratio and PSD had returned to baseline levels after 6h of recovery, however RMSSD was still significantly lower than baseline (Table 4.6). Post exercise HR, mBP, sBP and dBP were all higher than baseline, but returned to similar levels at the 6h recovery point, except for HR which was still elevated.

Table 4.6 HRV and haemodynamic data from all bike trials.

	DM Bike			Max Bike		
	Pre	Post	6h6h Post	Pre	Post	6h6h Post
Haemodynamics						
HR (beats·min ⁻¹)	52.58 (7.25)	66.74 (7.97)*	57.47 (7.18)‡	50.31 (5.49)	85.7 (11.0)*	58.55 (9.06)*^
sBP (mmHg)	103.77 (28.67)	100.87 (30.05)	112.66 (14.31)	117.43 (9.67)	125.44 (13.32)*	115.26 (13.06)^
mBP (mmHg)	77.76 (21.64)	76.02 (21.68)	82.65 (9.75)	86.47 (7.8)	93.59 (9.0)*	83.4 (9.19)^
dBP (mmHg)	61.05 (17.36)	59.69 (17.32)	65.17 (7.49)	67.78 (6.2)	73.47 (6.83)*	64.75 (7.43)^
Autonomics						
RMSSD (ms)	60.22 (20.93)	43.0 (24.76)	56.15 (26.05)	67.47 (20.91)	27.05 (30.12)*	43.98 (13.29)*
PSD (ms)	8.1 (0.86)	7.34 (1.47)	7.65 (1.17)	8.26 (1.11)	5.86 (1.98)*	7.34 (0.9)
LF/HF	2.03 (1.8)	2.78 (1.44)	1.45 (0.84)	1.12 (0.52)	3.99 (3.47)*	1.49 (1.07)

Note: n = 6 participants. HRV frequency data are given in log₁₀ units. Paired samples t-test results * = significantly (P = 0.05) different from baseline in each trial, ‡ = significantly (P = 0.05) different from post-exercise within each trial.

4.4.4 Cardiac Troponin

All participants were below the LOD for the assay at BL. Exercise resulted in a significant increase in hs-cTnT in the Max (Figure 4.4 A), and DM (Figure 4.4 B) trials, which was still significantly elevated above baseline at 6h post exercise in both trials.

4.4.4.1 Linear Regression

In the linear regression model the magnitude of troponin release was explained by exercise intensity and duration. In both cycling trials, average exercise performance (power output) was negatively related to troponin release and exercise duration was positively related to troponin release (Table 4.9) The same relationships were found for average exercise heart rates and troponin release, with a strong negative relationship between HR and troponin release in the cycling trials. Exercise variables that correlated significantly with hs-cTnT release are shown in Table 4.9.

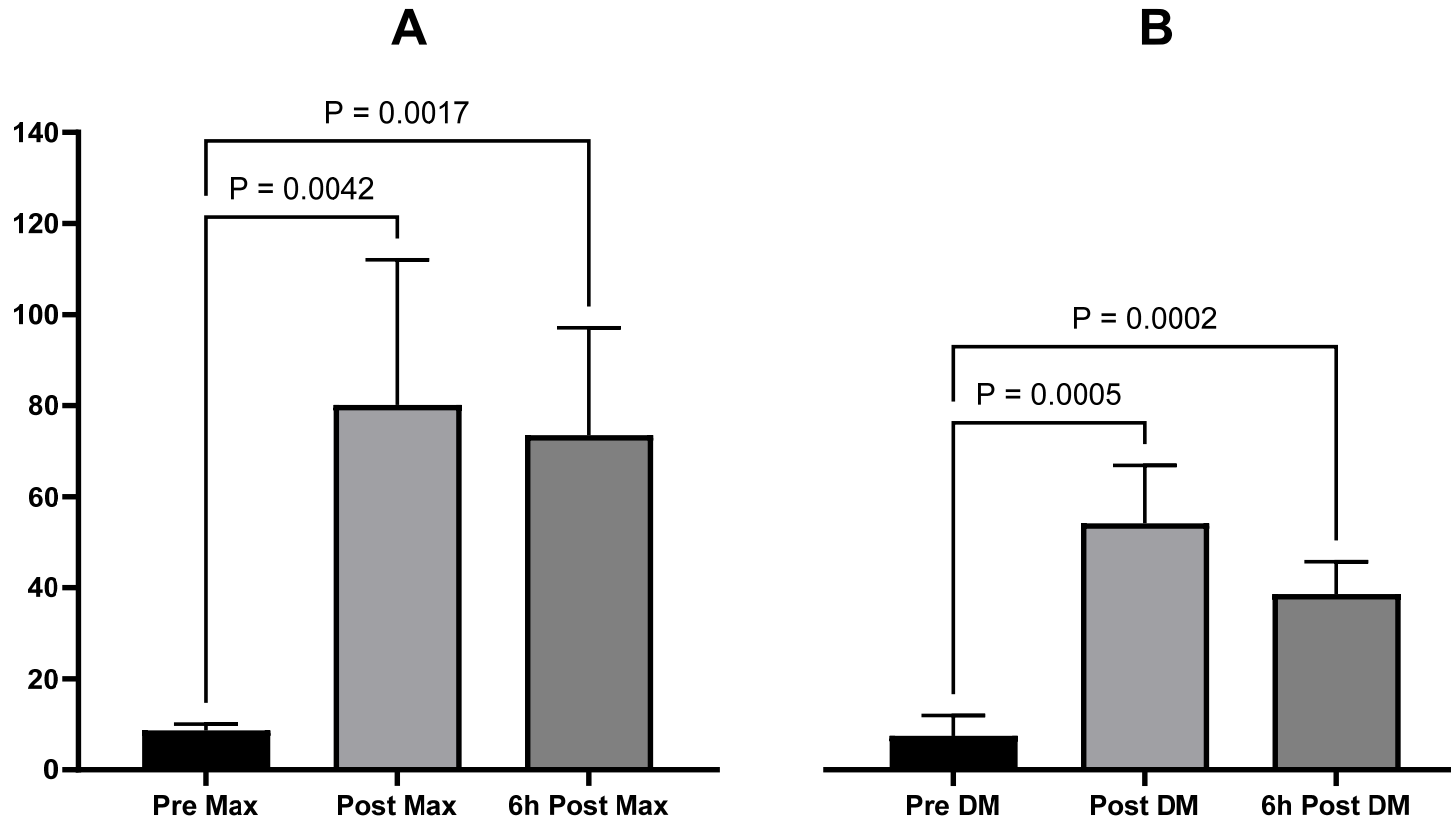


Figure 4.4. Bar plot of hs-cTnT concentrations in the Max (A) and DM (B) trials at each timepoint.

Note: $n = 6$ participants, P values given are pairwise comparisons from pre-exercise levels and the two post-exercise timepoints in each trial.

Table 4.7 Results of the linear regression on hs-cTnT release and exercise variables in the bike trials.

	β	SE	T	P	R ²	CI[2.5%]	CI[97.5%]
Mean HR	-0.45	0.06	8.08	0.000	0.84	0.33	0.58
HR _{Peak}	-0.41	0.05	8.09	0.000	0.85	0.30	0.52
Duration (mins)	1.14	0.11	10.20	0.000	0.90	0.90	1.38
Power Output (W)	-0.28	0.04	7.19	0.000	0.81	0.19	0.36

Note: n = 12 observations, 6 participants. Univariate linear regression results; β = beta coefficient, CI = confidence interval, SE = standard error. HR_{PEAK} = peak HR during the duathlon.

4.5 Discussion

4.5.1 Key Findings

Whilst there have been many previous studies to investigate the extent of EICF following cycling (Koller et al., 1998, Neumayr et al., 2002, Serrano-Ostariz et al., 2009, Nottin et al., 2009, Williams et al., 2009, Stöhr et al., 2011b, Stöhr et al., 2011a, Chan-Dewar et al., 2013a, Chan-Dewar et al., 2013b, Serrano Ostariz et al., 2013, Legaz-Arrese et al., 2015, Thomson et al., 2016, Watanabe et al., 2020, Travers et al., 2020, Stewart et al., 2014, Stewart et al., 2015, Stewart et al., 2016), and triathlon performance (Rifai et al., 1999, La Gerche et al., 2004, Tulloh et al., 2006, Leetmaa et al., 2008, Nottin et al., 2009, Park et al., 2014, Whyte et al., 2000), this study was the first to assess the influence of prior running on cycling performance, and the first to quantify the cycling performance reserve, i.e. when not impaired by prior running, of highly-trained triathletes. This study also determined that the higher work rate achieved during the Max cycling trial resulted in a greater number of cardiovascular functional changes, compared to when cycling at a lower intensity, with or without prior running. The following key findings were observed:

- Participants were able to improve their cycling performance by ~15% over the duathlon bike performances.
- Mean and peak HRs were significantly lower in the DM trial than the BD and UD bike legs in Chapter 4, indicating significant cardiac drift caused by the prior 10k run in these trials.
- Increased mean cycling intensity resulted in higher mean and peak HRs, but despite the higher mean PO and $\dot{V}O_2$ in the Max trial HR was lower than in both duathlon bike legs.
- Increased cycling intensity in the Max trial caused a greater mean absolute concentration of plasma hs-cTnT that remained elevated following 6h of recovery.

- Autonomic function is altered to a greater extent following higher-intensity cycling compared with standalone, lower-intensity cycling.
- Less than 60 minutes of high intensity cycling in the Max trial caused transient reductions in ventricular strain, and temporary reductions in LV filling and ejection that were compensated for by increased heart rate.
- Cycling at ~75-86% of $\dot{V}O_{2\max}$ resulted in significant hs-cTnT release.
- Following strenuous cycling exercise, LV twist was augmented by reduced basal rotation and increased apical rotation, though the magnitude of this effect was greatest in the lower-intensity trial.
- Six hours of passive recovery was sufficient for the majority of alterations in cardiac function and regulation to reverse to pre-exercise levels.

4.5.2 Exercise Performance

When compared to the power outputs achieved during the duathlon bike legs in the previous chapter, the participants in this study were able to increase their work rates by ~15% during the Max cycling leg. This indicates the participants possessed a greater exercise capacity, when not impacted by the fatigue of a previous 10k run, or limited by the constraints of the duathlon pacing strategy (Nikolaidis et al., 2019). These findings are important to note as though they are unsurprising, little empirical evidence is available on the performance reserves of triathletes (Millet et al., 2009, Millet et al., 2011). The mean POs achieved in the DM and Max trials (~220 and ~260W in the DM and Max trials, respectively) were comparable with those reported in studies investigating duathlon performance (Berry et al., 2016, Berry, 2012) and high-intensity cycling exercise (Stewart et al., 2015, Stewart et al., 2016), respectively. However, one methodological difference in this study compared with previous works is that participants self-regulated their effort and PO during the cycling legs. In contrast, in the study of Berry et al. (2016), participants cycled at a constant PO that corresponded to their ventilatory threshold (~80% $\dot{V}O_{2\max}$). Additionally, in separate trials,

participants cycled at a fixed PO corresponding to 90% and 120% of the GET in an EICF study that investigated the effect of exercise intensity by Stewart and colleagues (Stewart et al., 2016). Conversely, Stewart et al (2014) utilised a ‘criterium’ style cycling bout which was performed on an outdoor circuit, where participants cycled in a group and performed a more stochastic PO distribution (Stewart et al., 2015). In the current study, participants were free to select their own pacing strategies and PO distributions during the Max trial but were encouraged to perform at their best effort. The absence of any significant differences in PO at the 6 TPs suggests that they adopted an even-pacing strategy, which has been shown to be the most efficient in time-trials over 4km (Ham and Knez, 2009). In terms of relative exercise performance, it should be noted that in the Max trial participants achieved work rates corresponding to ~85-90% of $\dot{V}O_{2max}$ using their self-selected pacing strategies, which is more representative of real racing performance (Schabert et al., 2000, Suriano and Bishop, 2010, Millet et al., 2011) than prior studies using fixed intensities ranging from 60-75% $\dot{V}O_{2max}$, which were representative of the DM trial mean PO (Kreider et al., 1988, Guezennec et al., 1996, Hue et al., 1998, Millet and Bentley, 2004). One explanation for this difference in exercise intensity is that the majority of previous research into triathlon has been concerned with measures of efficiency changes. Measures of efficiency, such as running economy, become unreliable beyond the AT (Williams and Cavanagh, 1987, Bonacci et al., 2010, Bonacci et al., 2013). Additionally, this does not represent racing intensity for OD triathlon or duathlon race intensity, which is reportedly performed at 85-95% of $\dot{V}O_{2max}$ (Nikolaidis et al., 2021, Millet et al., 2011). Therefore, it may be the case that the triathletes who performed in the current study were less accustomed to the run-cycle transition and their cycling performance was impacted by the prior 10k run in the duathlon bike legs, resulting in a lower intensity requirement for the DM bike trial.

4.5.3 Effect of Prior Running

To assess the effects of the 10k prior running in the BD and UD trials in Chapter 4, the participants performed a matched standalone bike leg. The objective of the DM trial was to

eliminate impact of the physiological alterations that occurred during the 10k run. The findings of lower HR for the same PO and $\dot{V}O_2$ in the DM trial than the duathlon bike legs indicates that the 10k of running caused substantial cardiac drift in the duathlon. Participants were encouraged to achieve the best performance during the duathlons, yet were able to improve their mean PO, HR, and $\dot{V}O_2$ by >10% in the Max trial and generate a higher hs-cTnT release. Unfortunately, at the time of writing there does not appear to be any similar previous research on the impact of running upon cycling performance amongst triathletes to compare these findings to. However, research investigating the impact of prior swimming on cycling performance (Kreider et al., 1988, Peeling et al., 2005), and cycling on running performance (Hue et al., 1998, Millet and Bentley, 2004, Olcina et al., 2019) has shown that the performance decline in the transition leg ranges from 6-12%, which is in line with the performance improvement reported in the Max vs. DM and duathlon bike legs. Physiological alterations to explain the impaired performance following a swim-cycle transition have been attributed to reduced metabolic disturbance measured by lower blood lactate ($La_{[B]}$), increased skeletal muscle acidosis, increased rate of perceived exertion (RPE). One area of weakness in the current study design is that there were no measurements of $La_{[B]}$ or RPE due to methodological limitations. However, the finding of increased RER values during the Max bike trial are indicative of elevations in $La_{[B]}$, (Goedecke et al., 2000), which is a contributing factor in metabolic acidosis (Kraut and Madias, 2014). While there has been no research into the run-cycle transition, impaired exercise performance during the cycle-run transition has been correlated to multiple factors including cycling pedalling mechanics and PO distribution (Bonacci et al., 2013), cycling cadence (Olcina et al., 2019), running cadence and biomechanical alterations (Bonacci et al., 2010), and running economy changes (Guezennec et al., 1996). In the duathlon bike legs, there was evidence of cardiac drift as HR increased throughout and mean HR was higher than during the DM trial. This may indicate cycling performance was limited due to increased cardiovascular strain to cope with thermoregulatory and exercise demands (Rowell, 1974). In all trials there was no evidence of dehydration, as no significant loss of body mass was reported, and to mitigate this, participants were encouraged to hydrate at a rate to maintain body mass. It is possible that

participants may have been hypohydrated, which has been shown to cause cardiac drift in temperate conditions (Heaps et al., 1994). In the duathlon trials, the initial 10k run leg resulted in substantial reductions in ventricular longitudinal function that were reversed following the bike leg and were also not present following the DM bike leg. An approximate 15% increase in cycling power output resulted in significant reductions in longitudinal function, which indicates reduced intensity during the bike leg may be optimal for pacing and recovery when transitioning immediately to another bout of exercise, such as during the triathlon. However, despite the reductions in function, longitudinal function was restored to near baseline levels following 6h of recovery. The use of the bike as a potential recovery period in duathlon warrants further investigation and may prove to be a useful pacing strategy. Also of interest was the absence of reduced longitudinal function in the DM trial, alongside increased circumferential and rotational mechanics that were not present following the duathlon bike legs. To conclude this section, the results of the DM bike leg demonstrate that following ~65 minutes of moderate intensity ($\sim 75\% \dot{V}O_{2\max}$) without prior running, cardiac contractility and systolic function were maintained and autonomic function was also unaltered.

4.5.4 The Effects of Maximal Effort Cycling

In contrast to the DM bike leg, the increased work rates achieved during the Max trial resulted in functional impairments (reduced EDV, SV and EF that were compensated for by increased heart rates and \dot{Q}) and reductions in LV and RV longitudinal contractility, which agrees with previous studies that have demonstrated the effects of high intensity, short duration exercise on transiently reduced cardiac function (Banks et al., 2010). These findings are comparable with those reported following 60 minutes of high intensity cycling (Stewart et al., 2015, Chan-Dewar et al., 2013a). Reductions in LV contractile reserve following PSE are thought to occur due to desensitisation of β -receptors (Hart et al., 2006) alongside enhanced sympathetic innervation (Cheng et al., 1992). Additionally, PSE is known to impair cardiomyocyte membrane permeability (La Gerche et al., 2015), potentially causing the

release of cTn, reducing autonomic responsiveness (Stewart et al., 2014) and impairing cardiac contractility (Stewart et al., 2016). Increased exercise intensity yielded sustained hs-cTnT release at the 6h post-exercise measurement that was not present in the DM trial, alongside sustained reductions in RMSSD. Additionally, in the Max trial it was observed that some participant's hs-cTnT continued to increase over the immediate post-exercise concentrations. Elevated and sustained cTn release may indicate a more serious cardiac perturbation, and the increase over 6h of recovery in the Max trial is a similar release pattern to that observed following AMI (Aengevaeren et al., 2021). The initial release is considered to originate in the cytoplasmic stores of cTn and is dubbed the 'early releasable pool' (Shave and Oxborough, 2012). It is theorised that this store is released through bleb formation (Aengevaeren et al., 2021), transient imbalance of O₂ supply and demand (Wu, 2017), apoptosis (Le Goff et al., 2020), myocardial stretching (Shave and Oxborough, 2012), and the release of degraded cTn peptides (Wu, 2017). While there was no evidence of concomitant haemodynamic dysfunction that might explain the sustained elevation in hs-cTnT in the Max trial, there was evidence of sustained reductions in RMSSD, which may indicate continued parasympathetic suppression occurred during the 6h recovery period (Stewart et al., 2014, Aagaard et al., 2014, Bjorkavoll-Bergseth et al., 2020, Aengevaeren et al., 2021). This sustained reduction of RMSSD adds credence to the theory that there is a tolerable boundary of exercise that provokes significant cardiac perturbations when exceeded (Pringle et al., 2003, Shave et al., 2010a). In the present study such a boundary would appear to be exceeded following prolonged cycling at >85% of $\dot{V}O_{2max}$, in addition to OD duathlon performance, as shown in Chapter 3.

Despite reduced longitudinal contractility and LV filling, there was augmented LV diastolic short-axis function that was indicated by increased apical and basal diastolic rotation rates. Previous works have determined that reductions in systolic function following PSE are attributed to impaired filling and venous return, as opposed to impaired ventricular function (Doucende et al., 2010, Stöhr et al., 2011b, Watanabe et al., 2020). In addition, similarly to previous findings, (Baggish et al., 2008, Stöhr et al., 2011a) CS was maintained alongside

reductions in CSR following the Max trial. The mechanism behind enhanced contractility has been previously attributed to augmented sympathetic excitation (González-Alonso et al., 1999), which was supported by the finding of concomitantly increased LF/HF ratio at the post-exercise timepoint (Akselrod et al., 1981, Fisher et al., 2015).

Therefore, the post-exercise reductions in systolic and diastolic function after the Max trial can be attributed to post-exercise alterations in loading conditions, in the absence of dehydration. Longitudinal contractility was impaired across both ventricles; however, this was compensated for by increased heart rate, increased diastolic short-axis function, augmented LV twist and potentially elevated sympathetic tone. . The RMSSD was the most sensitive HRV-derived marker that detected the influence of exercise up to 6 hours later in the Max bike trial and should be considered an important measure of autonomic balance which may potentially indicate the relative strenuousness of exercise and signal other functional cardiac changes (Stewart et al., 2016).

4.5.5 Conclusions

To summarise, it may be concluded that standalone cycling efforts at OD duathlon pace stimulate hs-cTnT release, but do not cause substantial short-term cardiac functional changes, while also potentially being optimal for training and health (Eijsvogels et al., 2016, O'Keefe et al., 2020, Franklin et al., 2020a). The imposition of running on subsequent cycling results in increased HR and oxygen consumption, however ventricular longitudinal function is still apparently preserved during cycling at lower intensities with or without prior running. Additionally, maximal effort cycling time trials elicit more temporary cardiac perturbations, in terms of function, biomarker release and regulation.

Chapter 5 The Effects of 5- and 10-Kilometre Running Performances at Duathlon and Maximal Race Paces on Myocardial Function, Biomarker Release and Heart Rate Variability

5.1 Abstract

Background: The previous chapter demonstrated that highly trained triathletes are able to improve their cycling power output by approximately 15% in a standalone time-trial compared to when during a duathlon. The outcomes of this increased performance were a greater level of cardiac and autonomic functional changes, which reversed within the same time period as the lower-intensity DM cycle. There is a lack of data on the equivalent running performance reserve within a highly trained cohort. Furthermore, to date there has been no comparison of the effects of 5k and 10k running on cardiac functional changes.

Methods: Participants (n=7) completed on separate days two lab-based 5k running trials, and two 10k running trials at duathlon-matched (DM) and maximal effort (Max). The pre and post measurements of cardiac function taken were identical to the previous chapters.

Results: All trials resulted in significant levels of hs-cTnT release, and transiently increased left and right ventricular LS. Run speed was significantly higher in the Max compared to the DM trials (~6% over the 10k and 15% over the 5k runs). All trials resulted in similar levels of transient cardiac functional changes, which reversed after 6h of passive recovery. The DM 5k trial was apparently the least strenuous and resulted in fewer cardiac functional changes.

Conclusions: In duathlon competition the 10k run leg prior is paced slightly under maximal effort, which may be a part of the pacing strategy for the cycling leg. The athletes in this study were slightly slower over the final run of the duathlon, potentially due to fatigue, and as a result they were able to increase their standalone maximal 5k speed by approximately 15% vs. the duathlon 5k results in significantly elevated HR for the same power output as shown by the lower HR data during the DM trial compared to the duathlon bike legs. Both DM and Max trials resulted in significant hs-cTnT release. Despite the lower intensity and duration in the run trials, all run trials resulted in significant hs-cTnT release. The data from this study demonstrate an approximate 2 kilometre·hour⁻¹

(15%) performance reserve in the 5k running ability of highly trained triathletes, when not affected by a prior 10k run and 40k cycle. Additionally, this study has demonstrated that the preferred pacing strategy appears to involve running the first 10k run at close to maximal speed, and this in turn causes the majority of the initial cardiac functional changes.

5.2 Introduction

During multi-sport competition such as triathlon and duathlon, the performances of the individual legs that comprise the event are typically reduced when compared against stand-alone events (Kohrt et al., 1987, Kreider et al., 1988, Suriano and Bishop, 2010, Millet et al., 2011). The results of the previous chapter have demonstrated that in cycling this reduction is approximately 15%. This is due to numerous factors including pacing strategies, cumulative muscular, nervous, and mental fatigue from the preceding events, dehydration (Park et al., 2014), hypoglycaemia (Tulloh et al., 2006) and biomechanical dysfunction (Millet and Bentley, 2004). According to the (unpublished) data from Chapter 3 it is possible that transient reductions in cardiac function may also play a role. The previous chapters demonstrate that even maximal effort cycling results in lower HR and $\dot{V}O_2$ than running. Additionally, while cardiac troponin increased cumulatively with each leg of the broken duathlon in Chapter 3, measures of cardiac and autonomic function were substantially closer to resting levels following the bike leg than at the post-run measures. TPs. Additionally, in prior studies investigating the physiological impact of duathlon exercise heart rates during the run legs were significantly higher than during the bike, and the duration of the bike leg was approximately equal to the combined duration of the run legs, which is similar to the data presented so far in this thesis (Tsuzuki et al., 2019, Nikolaidis et al., 2021).

Running is thought to be more strenuous than cycling exercise in similarly trained individuals due to increased skeletal muscle recruitment, and the effect of having to support one's body mass during running, (Millet et al., 2009). Further, running exercise may be more strenuous on the heart due to increased \dot{Q} induced by a higher SV during running and greater physiological strain overall (Faulkner et al., 1971, Kohrt et al., 1987, Kreider et al., 1988, Hue et al., 1999, Millet and Bentley, 2004, Suriano and Bishop, 2010, Dalla Vecchia et al., 2019).

Therefore, the increased intensity of the run legs may be responsible for eliciting the increased myocardial and cardiac autonomic fatigue during the duathlon, or conversely the

bike leg may have provided an opportunity for recovery of these functions. Accordingly, the aim of this Chapter was to examine the myocardial, cardiac autonomic, and biochemical consequences of maximum effort (Max) “standalone” running time trials over 5k and 10k. The research hypothesis was that participants would perform at a higher work rate during the Max trials than during the duathlon, and that the magnitude of cardiac fatigue elicited would be elevated.

Additionally, as multisport athletes typically train at the intensities at which they intend to race (Seiler and Kjerland, 2006), to see the impact of training at duathlon-pace, it is important to quantify the effects of separate 5k and 10k run trials without the influence of the preceding exercise. This methodology also would allow assessment of the acute recovery from the first 10k run without the influence of the subsequent bike and 5k run legs. Therefore, participants performed duathlon-matched (DM) standalone time trials at the mean running velocities performed in the duathlons over the 5k and 10k distances. As multisport athletes typically train more than one time in a diurnal period, the potential for the development of cumulative cardiac fatigue because of race-pace training has been suggested to also cause longer-term cardiac dysfunction (La Gerche and Prior, 2007). Therefore, the recovery from EICF over the medium-term period of 6 hours post exercise was also investigated alongside the immediate post exercise effects.

5.3 Methods

5.3.1 Participant Characteristics

Participants were recruited by invitation from the study in the previous chapter after being advised of the study protocol and requirements. In total, 6 participants agreed to continue the study and their details and characteristics are displayed in Table 5.1 below.

Table 5.1 Participant characteristics

Characteristic	
Age	33.0 (5.7)
Running HR _{MAX} (beats·min ⁻¹)	181.3 (8.4)
Training History (years)	10.5 (5.5)
Mass (kg)	76.2 (7.21)
Stature (cm)	180.0 (5.0)
Running $\dot{V}_{E_{MAX}}$ (L·min ⁻¹)	157.3 (12.5)
Running $\dot{V}O_{2max}$ (L·min ⁻¹)	4.5 (0.3)
Running $\dot{V}O_{2max}$ (mL·kg·min ⁻¹)	59.3 (6.3)
tLimv $\dot{V}O_{2max}$ (s)	50.0 (10.2)
v $\dot{V}O_{2max}$ (km·hour ⁻¹)	19.2 (0.70)

5.3.2 Testing Protocol

Each participant was tested in a five-phase protocol, which took place over five consecutive weeks. The protocol involved both running and cycling exercise bouts, but this chapter will focus on the running trials only (Figure 5.1). The order of trials was randomised for each participant to prevent the influence of any possible order effects. The tests were performed at the same time of day and the same day of the week to minimize the influence of the effects of personal training on the study. The participants were asked to maintain their own training schedule for the duration of the study but were not allowed to compete during this period. On experiment days, the participants were asked to abstain from training for 48 hours before their visit and abstain from caffeine 24 hours prior to the visit.

Participants reported to the laboratory and underwent baseline (BL) measurements identical to those described in the previous study and general methods. Following each exercise trial,

post-exercise resting measurements were taken, also identically to the previous chapter. Participants then underwent 6 hours of passive recovery, where they were encouraged to feed and rehydrate according to their typical post-training routines and undertake no further exercise. Participants were instructed to adhere to this routine following each trial and to also abstain from caffeine. All participants were office workers and did not undertake any undue strenuous manual labour between trials on non-workdays. Following 6h of passive recovery the baseline measurements were taken again as previously described.

Each participant completed 2 maximum effort trials (Max) over 5k and 10k of running, and 1 duathlon-matched (DM) trial over 5k of running. As the same cohort returned for this study, it was possible to perform pairwise comparisons of the broken duathlon trials. Additionally, as no exercise succeeded the first 10k in the BD trial, the pre/post data from the first 10k run of the BD was used as the DM 10k trial. Prior to the Max trials participants were advised to complete the distance as quickly as possible. Work rate was self-selected and recorded at each change. During DM run trials, the speed from the BD trial was set as the target. Running speed was adjusted to be consistent with the speeds that were self-selected during the BD runs.

During exercise, HR and run speed was recorded continuously on a GPS watch (Garmin, USA) and transcription. Breath by breath gas exchange and ventilation was recorded using a metabolic cart (Oxycon, Jaeger, Germany) at matching timepoints (TPs) to the BD trial (Figure 5.1). Participants were encouraged to consume fluids throughout exercise using their own preferred beverage choice at a rate to maintain euhydration, as during the duathlon trials.

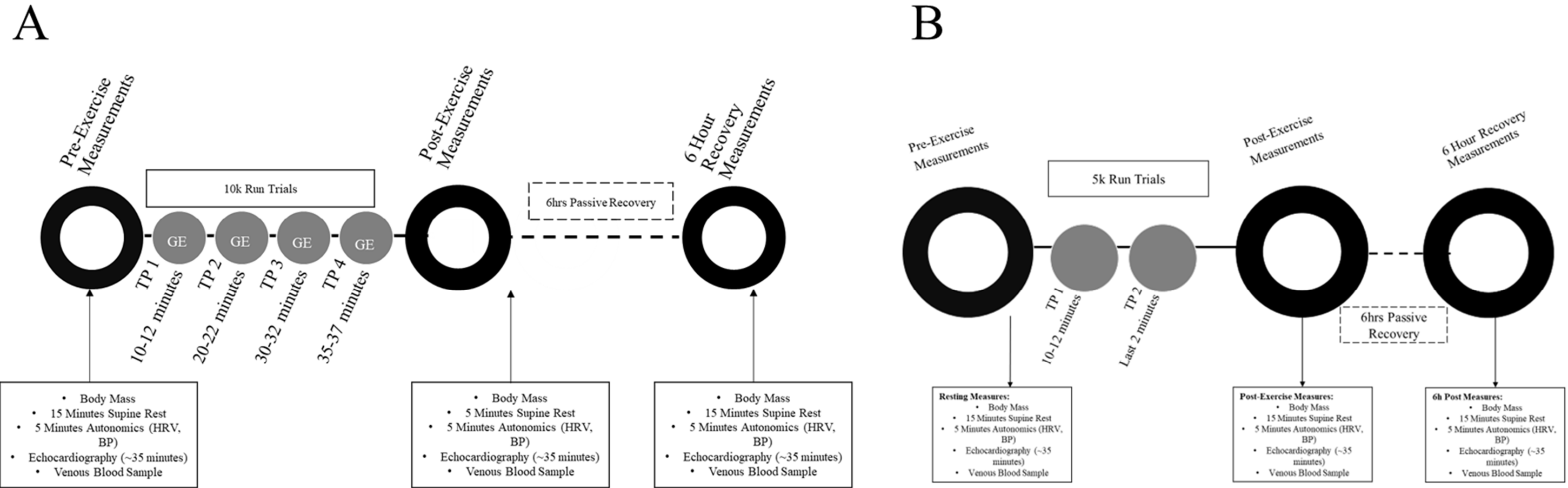


Figure 5.1 Experimental visit protocol for both Max and DM 10k trials (A) and 5k trials (B).

5.3.3 Statistical Analysis

In addition to the statistical analysis described in Chapters 3 and 4, linear regression was performed by pooling the combined data from the DM and Max trials for each run distance (for a total n of 12 per distance) to assess the effects of increased run duration and average heart rate. Pre to immediate post-exercise differences were calculated from echocardiographic and autonomic data to assess the overall impact of each trial and quantify any inter-trial differences in EICF.

5.4 Results

5.4.1 Exercise Results

In both Max trials, there were significant differences in exercise performance, HR, HR Peak, and $\dot{V}O_2$ compared with the DM and duathlon trials over the same distances. In the Max 5k and 10k trials, average speed, heart rate and peak heart rate were significantly higher than both duathlon runs of the same distance (See Tables 5.2 and 5.3). At the last measurement of each run, $\dot{V}O_2$ (absolute and relative) were also significantly higher than in both BD and UD trials.

Table 5.2 Exercise data from the run legs from the BD trial in Chapter 4 are presented for comparison to the standalone trials.

	DM		Max		Broken Duathlon	
	5k DM	10k DM	5k Max	10k Max	10k BD	5k BD
Average HR (beats·min ⁻¹)	156.6 (6.2)*	163.0 (11.7)*	173.8 (4.5)†	172.0 (7.1)†	171.9 (11.7)	172.9 (9.3)
HR _{Peak} (beats·min ⁻¹)	165.6 (7.3)*	175.7 (9.5)	180.0 (8.1)*†	180.3 (6.8)*†	179.3 (8.7)	177.9 (8.7)
Run Speed (km·hour ⁻¹)	14.0 (1.44)	14.6 (0.6)	16.1 (1.5)*†	15.4 (0.9)*†	14.4 (0.6)	14.2 (1.0)
$\dot{V}O_2$ Absolute (L·min ⁻¹)	3.93 (0.4)	4.04 (0.31)	4.60 (0.52)†	4.32 (0.30)	4.07 (0.33)	3.90 (0.30)
$\dot{V}O_2$ Relative (mL·kg·min ⁻¹)	52.2 (5.0)	53.3 (4.4)	58.4 (5.9)*†	56.7 (4.1)*†	53.3 (4.4)	52.1 (5.4)
Fluid (L)	0.0 (0.0)	0.5 (0.1)	0.0 (0.0)	0.3 (0.1)	0.4 (0.1)	0.3 (0.1)
Time (minutes)	21.4 (2.0)	41.1 (1.6)	18.6 (1.6)*†	39.0 (2.2)*†	41.7 (1.7)	21.1 (1.4)

Note: n = 6 participants. Paired samples t-test results * = significantly (P<0.05) different to BD leg of the same distance, † = significantly different to DM trial over the same distance..

Table 5.3 Exercise data at each timepoint during Max and DM trials.

	DM						Max					
	5k DM		10k DM		3	4	5k Max		10k Max		3	4
	1	2	1	2			1	2	1	2		
HR (beats·min ⁻¹)	157.9 (16.1)	161.2 (15.6)	155.4 (13.1)	161.2 (13)	164.2 (9.4)	166.9 (9)	168.7 (14.9)	178.8 (13.4)	165.3 (12.8)	170.3 (13)	174.3 (11.2)	178.2 (10.3)
Run Speed (km·hour ⁻¹)	14 (1.4)	10.1 (0.7)	14.3 (0.7)	14.3 (0.7)	14.4 (0.8)	14.5 (0.9)	15.7 (1.3)*‡†	16.6 (1.6)*‡†	15.1 (0.8)	15.3 (0.9)	15.4 (0.9)	15.9 (0.9)
$\dot{V}O_2$ Relative (mL·kg·min ⁻¹)	51.7 (4.9)	52.6 (5.4)	50.6 (4.5)	51.2 (4.7)	51.5 (4.3)	51.8 (4.7)	56.3 (5.6)	60.5 (6)	54.8 (5.1)	56 (4.6)	57.2 (4.2)	58.8 (3.2)*‡
$\dot{V}O_2$ Absolute (L·min ⁻¹)	3.90 (0.50)	4.00 (0.40)	3.78 (0.3)	3.79 (0.3)	4.04 (0.3)	4.01 (0.4)	5.5 (3.4)	5.8 (3.3) *‡†	4.19 (0.4)	4.27 (0.4)	4.39 (0.3)	4.49 (0.2)*‡

Note: n = 6 participants. Paired samples t-test results * = significantly (P<0.05) different to the same run-leg distance in the UD trial, ‡ = significantly different to the same run-leg distance in the BD run leg, † = significantly different to DM.

5.4.2 Echocardiographic Results

5.4.2.1 Systolic and Diastolic Function

There was a significant effect of exercise on LV systolic and diastolic function within each trial. In the Max 10k trial, EDV ($F=6.34$ and $P=0.02$) and SV ($F=4.68$ and $P=0.04$) were significantly affected by exercise. In the Max 5k trial, EDV ($F=7.27$ and $P=0.01$), SV ($F=6.69$ and $P=0.01$), and \dot{Q} ($F=9.07$ and $P<0.01$), were significantly affected. Findings of the changes in systolic variables at each timepoint are shown below for the 5k trials (Figure 5.2 A) and the 10k trials (Figure 5.2 B) and the diastolic measures are shown for both trials in Table 5.4.

For the TDI and PW measures, there were significant changes from baseline that lasted to the 6h post-exercise measurement. In the Max 10k trial, mitral valve E/A ratio was significantly ($F=8.21$, $P < 0.05$) lower post exercise and at 6h post than baseline. In the Max 5k trial, tricuspid E/A ratio was significantly ($F = 8.23$, $P < 0.01$) lower than baseline at the post-exercise measurement. Aside from mitral E/A ratio in the Max 10k trial, all variables returned to near baseline values within 6h of passive recovery.

Between trials, the post-exercise changes in mitral E/A ratio were significantly ($F = 6.92$, $P < 0.05$) higher in the Max 10k than the DM 5k trial. Additionally, there were significant differences between the Max and DM 5k trials for post-exercise changes in EDV ($F = 5.91$, $P < 0.05$), and SV ($F = 4.75$, $P < 0.05$).

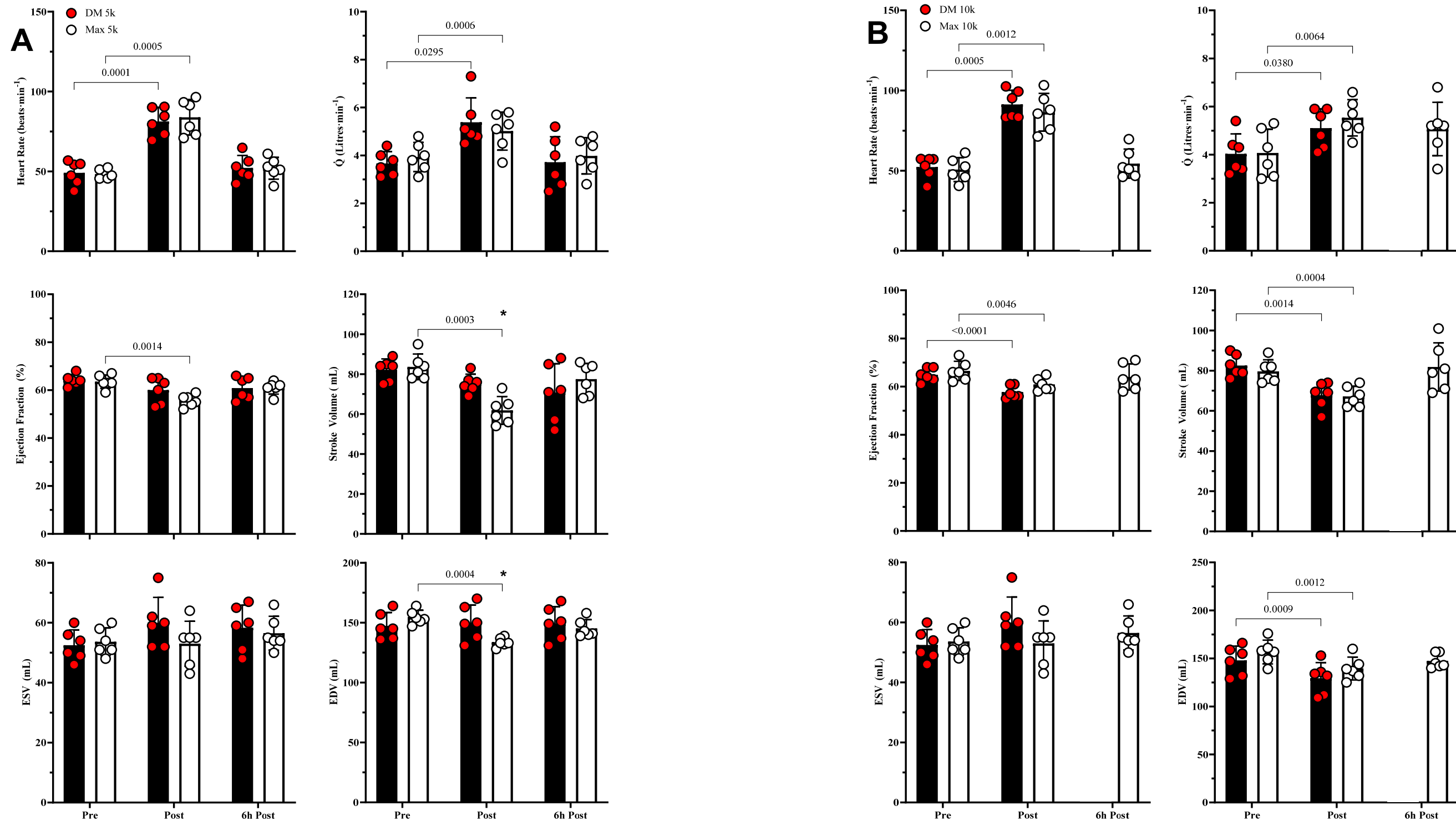


Figure 5.2 Left ventricular systolic functional parameters at each timepoint in the 5k (A) and 10k (B) DM (black bars) and Max (white bars) running trials.

Note: $n = 6$ participants. Results of the paired-samples T-tests results are presented on the figure where significant ($P = 0.05$) differences from the pre-exercise values within each trial occurred. Individual data points are represented by red circles for the DM trials and open circles for the Max trials. * = significantly ($P < 0.05$) different change in post-exercise value than the DM 5k trial.

Table 5.4 Left and right ventricular PW Doppler and TDI measures at each timepoint from each run trial.

	DM 5k			DM 10k			Max 5k			Max 10k		
	Pre	Post	6h Post	Pre	Post	Pre	Post	6h Post	Pre	Post	6h Post	
Left Ventricle Function												
E (m·s ⁻¹)	0.63 (0.03)	0.64 (0.02)	0.64 (0.02)	0.70 (0.04)	0.66 (0.04)	0.64 (0.03)	0.58 (0.07)	0.59 (0.04)	0.67 (0.03)	0.59 (0.04)	0.64 (0.03)	
A (m·s ⁻¹)	0.37 (0.02)	0.40 (0.03)	0.37 (0.03)	0.40 (0.02)	0.44 (0.04)	0.41 (0.03)	0.41 (0.03)	0.38 (0.03)	0.43 (0.04)	0.45 (0.03)	0.48 (0.04)	
E/A Ratio	1.78 (0.20)	1.73 (0.2)	1.83 (0.12)	1.76 (0.09)	1.59 (0.17)	1.59 (0.11)	1.48 (0.24)	1.62 (0.21)	1.60 (0.13)	1.34 (0.09)*	1.36 (0.11)*	
E/E'	3.78 (0.18)	3.97 (0.22)	3.58 (0.19)	4.03 (0.32)	3.71 (0.09)	4.05 (0.36)	3.39 (0.34)	3.44 (0.26)	3.87 (0.23)	3.5 (0.07)	3.6 (0.12)	
Right Ventricle Function												
TAPSE (cm)	2.92 (0.05)	2.77 (0.13)	2.83 (0.07)	2.88 (0.14)	2.65 (0.16)	3.02 (0.07)	2.6 (0.18)	2.92 (0.08)	3.02 (0.12)	2.52 (0.08)	2.97 (0.08)	
E (m·s ⁻¹)	0.51 (0.01)	0.5 (0.03)	0.55 (0.03)	0.55 (0.03)	0.52 (0.03)	0.53 (0.04)	0.47 (0.03)	0.51 (0.04)	0.54 (0.04)	0.53 (0.05)	0.52 (0.03)	
A (m·s ⁻¹)	0.26 (0.01)	0.34 (0.03)	0.29 (0.02)	0.31 (0.01)	0.33 (0.02)	0.29 (0.03)	0.36 (0.04)	0.27 (0.02)	0.32 (0.02)	0.4 (0.05)	0.35 (0.05)	
Tricuspid E/A Ratio	1.93 (0.07)	1.58 (0.2)	1.94 (0.16)	1.74 (0.12)	1.61 (0.12)	1.84 (0.08)	1.40 (0.14)*	1.92 (0.13)	1.71 (0.09)	1.3 (0.12)	1.53 (0.14)	

Note: n = 6 participants. Paired samples t-test results * = significantly (P=0.05) different from baseline within each trial, ‡ = significantly different from post-exercise measurement within each trial, † = DM significantly different than Max trial at same measurement.

5.4.2.2 Ventricular Longitudinal Strain and Strain Rate

The running exercise resulted in significant post-exercise elevations in LV LS over baseline in the Max 10k ($F=6.19$, $P < 0.05$), Max 5k ($F = 6.78$, $P < 0.05$), and DM 10k ($F = 5.12$, $P < 0.05$) trials. Additionally, right ventricular LS was also significantly increased in the Max 10k ($F = 5.60$, $P < 0.05$), Max 5k ($F = 14.1$, $P < 0.01$), DM 5k ($F = 12.68$, $P < 0.01$), and DM 10k ($F = 5.00$, $P < 0.05$) trials. All values had returned to near baseline levels within 6h of passive recovery. There was a significantly increased post-exercise change in both LV LS ($F = 3.58$, $P < 0.05$) and RV LS ($F = 5.68$, $P < 0.05$) in the Max 5k than the DM 5k.

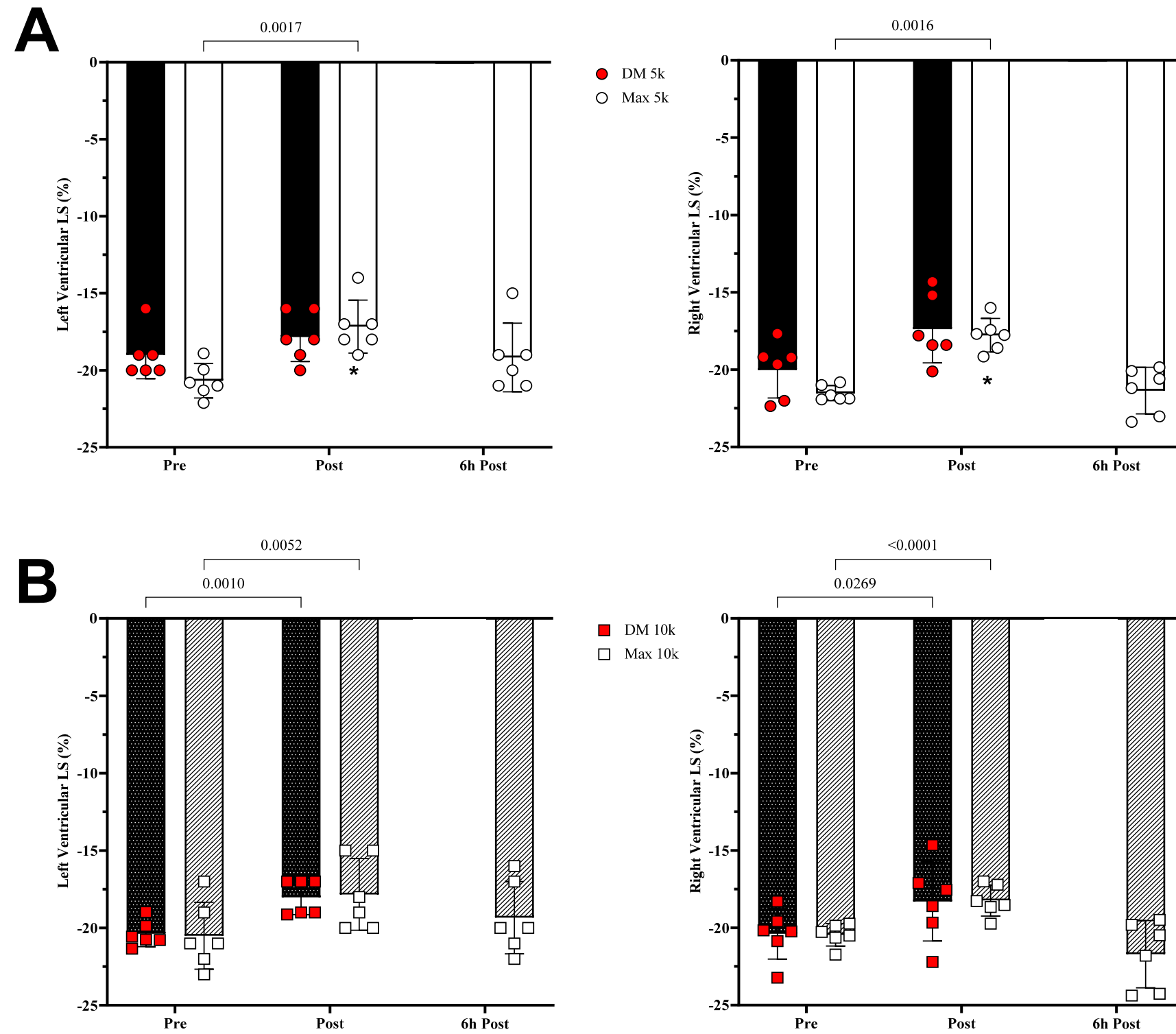


Figure 5.3 Left and right ventricular longitudinal ϵ values at each timepoint across 5k (A) and 10k (B) trials.

Note: $n = 6$ participants. Results of the paired-samples T-tests results are presented on the figure where significant ($P = 0.05$) differences from the pre-exercise values within each trial occurred. Individual data points are represented by red symbols for the DM trials and open symbols for the Max trials. * = significantly ($P < 0.05$) different change in post-exercise value than the DM 5k trial.

	DM 5k			DM 10k		Max 5k			Max 10k		
	Pre	Post	6h Post	Pre	Post	Pre	Post	6h Post	Pre	Post	6h Post
Left Ventricle											
Early Diastolic SR (%/sec)	1.78 (0.18)	1.71 (0.12)	1.71 (0.11)	1.79 (0.26)	1.75 (0.22)	1.72 (0.11)	1.52 (0.21)	1.6 (0.18)	1.69 (0.2)	1.70 (0.33)	1.60 (0.18)
Late Diastolic SR (%/sec)	0.66 (0.17)	0.7 (0.26)	0.69 (0.24)	-	-	0.67 (0.14)	0.78 (0.16)	0.71 (0.14)	0.71 (0.17)	0.85 (0.16)	0.78 (0.17)
Right Ventricle											
Early Diastolic SR (%/sec)	1.32 (0.14)	1.27 (0.14)	1.37 (0.08)‡	1.42 (0.26)	1.2 (0.27)	1.29 (0.18)	1.07 (0.17)	1.27 (0.12)	1.24 (0.34)	1.34 (0.33)	1.38 (0.23)*
Late Diastolic SR (%/sec)	0.71 (0.15)	0.71 (0.23)	0.79 (0.24)	0.87 (0.14)	1.04 (0.34)	0.8 (0.06)	0.98 (0.18)	0.88 (0.12)	0.78 (0.14)	1.14 (0.32)	1.03 (0.2)

Table 5.5 Left and right ventricular LS variables for 5k and 10k, DM and Max run trials.

Note: n = 6 participants. Paired samples t-test results * = significantly (P < 0.05) different from baseline within each trial Pre, ‡ = significantly (P < 0.05) different from post-exercise measurement within each trial.

5.4.2.3 Longitudinal Strain Correlation Analyses

The pooled 10k run trial data demonstrated a moderate, relationship between immediate post-exercise changes in left ventricular LS and 10k run duration (Figure 5.5).

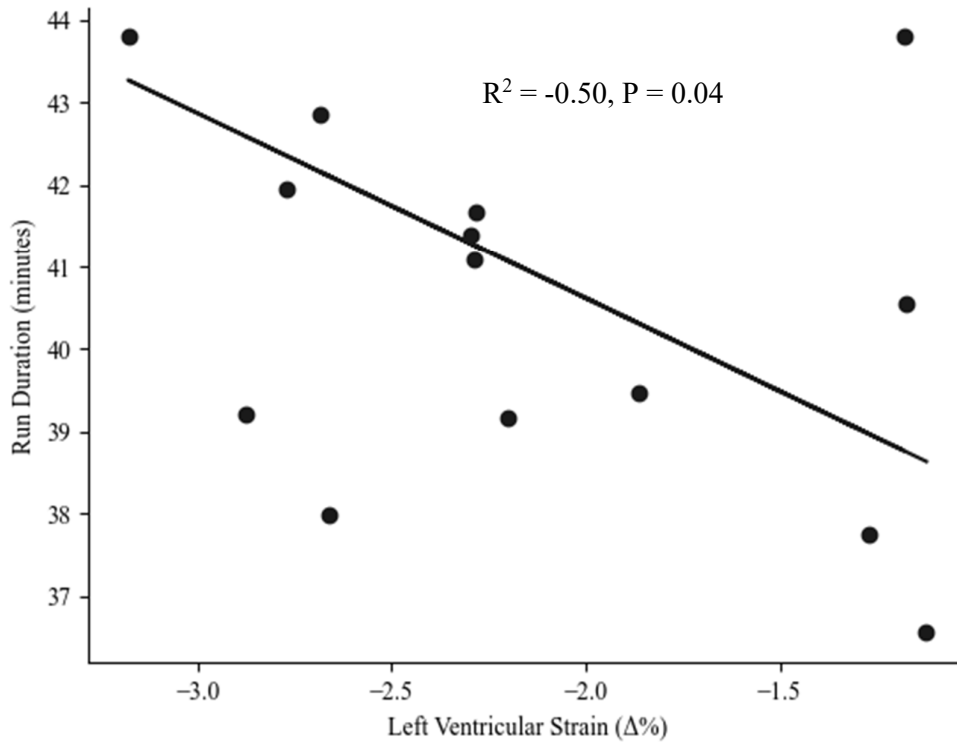


Figure 5.4 Scatter plot of the correlation of 10k run durations and LV LS change. Note: n = 14 observations, 7 participants. The negative relationship corresponds to an improved or maintained post-exercise LV longitudinal contractile function at faster 10k run speeds.

There was also a moderate positive relationship between 5k run time and post-exercise changes in LV LS (Figure 5.6).

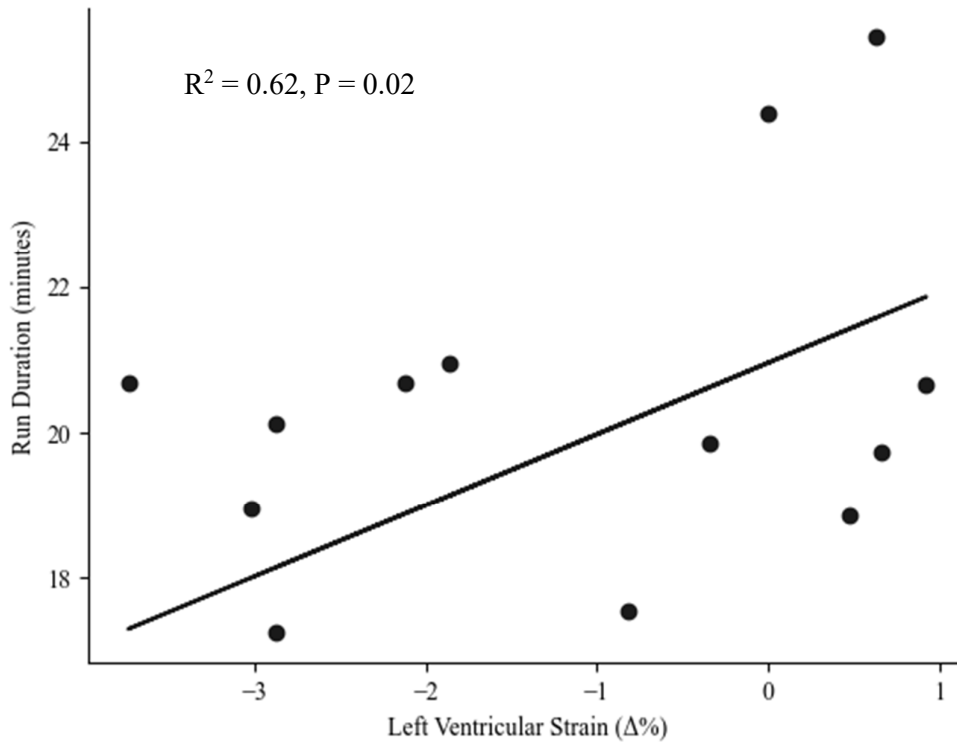


Figure 5.5 Scatter plot of the correlation of 5k run durations and LV LS change. Note: $n = 14$ observations, 7 participants. The positive relationship corresponds to an improved or maintained post-exercise LV longitudinal contractile function at slower 5k run speeds.

5.4.3 Haemodynamics and Heart Rate Variability

There was a significant effect of trial on LF/HF ($F=3.19$ and $P=0.03$), sBP ($F=3.00$ and $P=0.04$) and HR ($F=5.10$ and $P<0.01$). The post-exercise change in LF/HF ratio was significantly higher in the DM 5k trial than both the Max 5k and 10k trials (Table 5.8).

In the DM 5k trial there was a significant effect of exercise on LF/HF ratio ($F = 7.47$, $P < 0.05$), PSD ($F = 5.91$, $P < 0.05$), and RMSSD ($F = 13.50$, $P < 0.01$). The LF/HF ratio was increased over pre-exercise levels, and the PSD and RMSSD were all lower than pre-exercise at the post-exercise measurement. In the Max 5k there was a significant effect of exercise on

RMSSD ($F = 4.80, P < 0.05$), which was reduced from baseline post-run. The Max 10k trial resulted in significant reductions in RMSSD ($F = 22.50, P < 0.01$), PSD ($F = 13.02, P < 0.01$), and increased LF/HF ratio ($F = 4.60, P < 0.05$) at the post-exercise measurement. Similarly, the DM 10k trial resulted in significant post exercise reductions in RMSSD ($F = 3.98, P < 0.05$), PSD ($F=8.98, P < 0.05$) and elevated LF/HF ratio ($F=6.72, P < 0.05$) (Table 5.8).

Table 5.6 HRV and haemodynamic data from all Max and DM run trials.

	DM 5k			DM 10k			Max 10k			Max 5k		
	Pre	Post	6h Post	Pre	Post	Pre	Post	6h Post	Pre	Post	6h Post	
Haemodynamics												
HR (beats·min ⁻¹)	48.45 (7.1)	81.23 (8.68)*	53.03 (8.2)‡	52.08 (6.71)	89.67 (7.86)*	50.71 (7.48)	88.68 (9.21)*	55.75 (9.09)‡	49.47 (5.46)	89.11 (6.39)*	53.33 (7.2)‡	
sBP (mmHg)	108.03 (8.36)	116.97 (8.68)	109.54 (11.82)	117.96 (10.93)	115.78 (9.25)	103.86 (14.21)	121.04 (6.27)	114.11 (11.83)	107.73 (10.12)	116.55 (5.59)	116.45 (6.97)	
mBP (mmHg)	75.82 (32.8)	88.71 (5.33)	81.06 (8.15)	88.9 (9.37)	87.4 (5.93)	75.77 (23.13)	89.67 (8.74)	83.19 (7.52)	79.9 (4.97)	87.5 (3.79)	83.35 (5.11)	
dBp (mmHg)	51.15 (25.55)	70.34 (3.08)	64.52 (6.33)	69.77 (7.32)	68.58 (3.97)	58.43 (17.46)	69.71 (8.25)	66.1 (5.0)	61.98 (3.37)	68.81 (4.54)	64.99 (4.93)	
Autonomics												
RMSSD (ms)	80.0 (29.37)	27.16 (32.74)*	64.88 (32.4)‡	62.72 (16.41)	31.64 (25.19)*	69.99 (12.42)	18.03 (10.43)*	51.09 (17.57)‡	79.32 (24.03)	31.77 (30.21)	66.09 (26.06)	
PSD (ms)	8.84 (0.93)	5.43 (1.72)*	8.09 (1.24)‡	7.96 (0.75)	5.37 (1.97)*	8.75 (0.26)	5.31 (1.43)*	7.56 (0.96)*‡	8.88 (0.85)	6.41 (2.19)	8.4 (0.76)	
LF/HF	1.44 (0.51)	10.32 (7.79)* [#]	1.37 (0.63)‡	1.5 (0.8)	4.3 (3.9)*	1.29 (0.86)	3.62 (1.32)*	1.33 (0.79)*	1.47 (0.63)	2.62 (2.64)	1.44 (0.38)	

Note: n = 6 participants. Paired samples t-test results * = significantly (P < 0.05) different from baseline measurement within each trial, ‡ = significantly (P < 0.05) different from post-exercise measurement within each trial. HRV frequency data are given in log₁₀ units. [#] = significantly different change from baseline compared to the Max 5k and Max 10k trials.

5.4.4 Cardiac Troponin

Results of the tests for hs-cTnT are shown in Figures 5.6 below. All participants were below the LOD for the assay at BL. Exercise resulted in a significant increase in hs-cTnT in all trials, which was still significantly elevated above baseline and the LOD at 6h post exercise in the DM 5k, Max 5k, and Max 10k trials. The post-exercise change in hs-cTnT concentrations were significantly higher than the DM 5k trial in the Max 5k ($F= 5.45$, $P < 0.5$) and Max 10k ($F = 8.34$, $P < 0.05$) trial. Additionally, the change in hs-cTnT release was significantly higher in the Max 10k than the Max 5k trial ($F=7.86$, $P < 0.05$) (Figure 5.7).

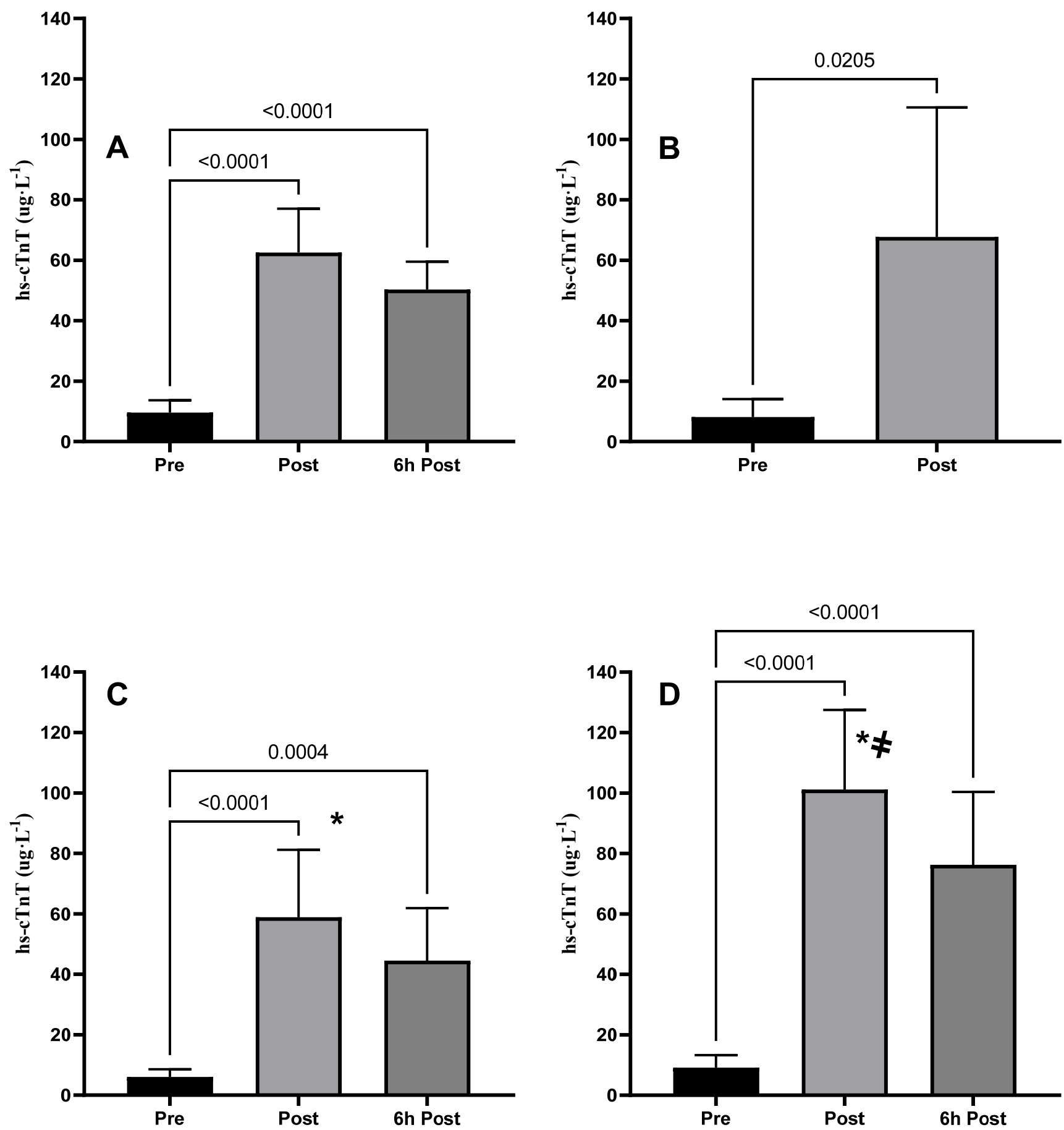


Figure 5.6 Bar plot of hs-cTnT concentrations in the DM 5k (A), DM 10k (B), Max 5k (C) and Max 10k (D) trials.

Note: $n = 6$ participants. P values given are pairwise comparisons from pre-exercise levels and the 2 post-exercise timepoints within each trial. * = significantly ($P < 0.05$) different change in post-exercise value than the DM 5k trial, # = significantly ($P < 0.05$) different change in post-exercise value than the Max 5k trial.

Correlation Analysis

Results of the linear regression for exercise induced hs-cTnT concentration and exercise performance revealed a moderate positive relationship between 5k run completion time and post-exercise cTn release (Figure 5.8), and a moderate negative relationship between 10k completion time and hs-cTnT release (Figure 5.9).

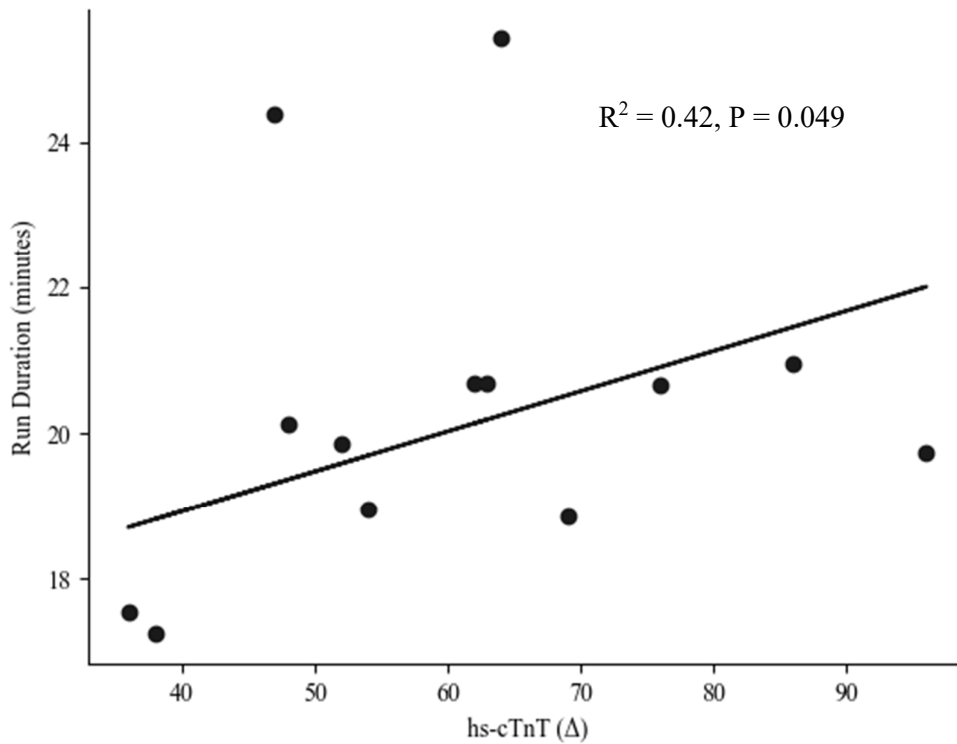


Figure 5.7 Post 5k run troponin release and 5k run duration.

Note: $n = 14$ observations, 7 participants. A positive regression line indicates increased cTn release with slower 5k run time.

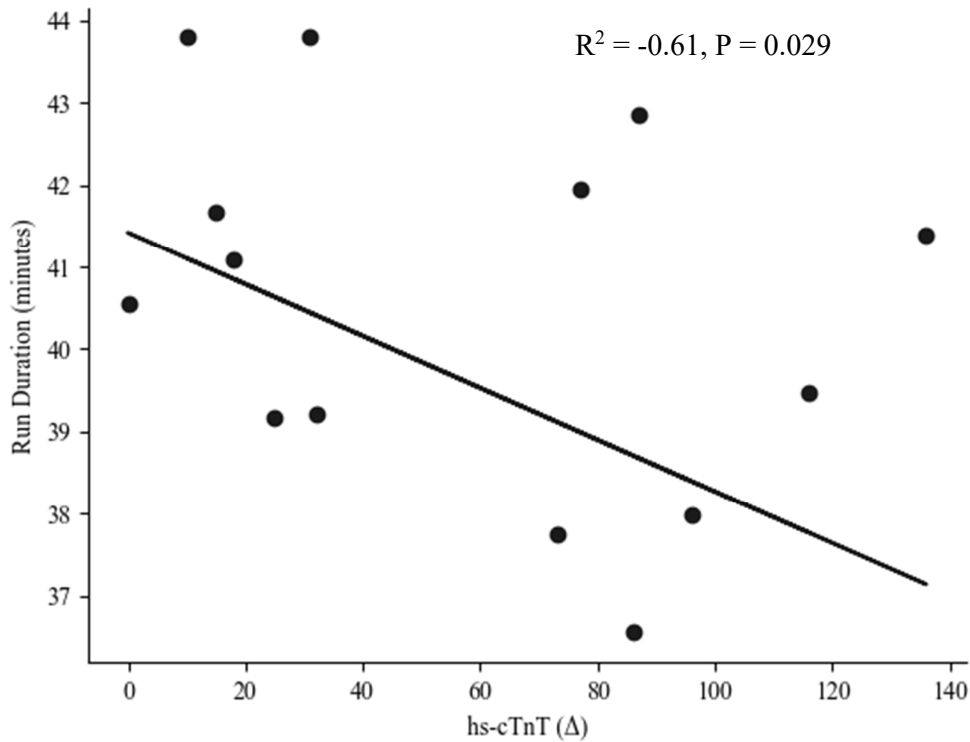


Figure 5.8 Post 10k run troponin release and 10k run duration.

Note: $n = 14$ observations, 7 participants. A negative regression line indicates reduced cTn release with slower 10k run time.

5.5 Discussion

5.5.1 Key Findings

This study is the first to examine the individual contributions to EICF, LV mechanics, and biomarker release of each run leg of an Olympic distance duathlon. Additionally, this study builds upon the findings of previous work investigating the effects of exercise intensity and investigations into the separate effects of exercise mode and intensity. To the authors current knowledge, there has been little investigation into the effects of 5k and 10k standalone running trials on these variables in trained adults, at any intensity. The work in this study adds more evidence to the investigation into the effects of shorter duration, intense prolonged exercise on EICF and troponin release. The key findings were that:

- Average and maximal heart rates achieved during a maximal effort 5 and 10k run were comparable to those during an Olympic distance duathlon.
- Additionally, heart rates were significantly higher during the final 5k run in the duathlon compared to running at the same speeds in a standalone performance. When the runs were performed standalone, HR variables were significantly lower for the same work rates.
- Moderate to high intensity 5k runs cause significant cardiac troponin release and reductions in left and right ventricular longitudinal function, which return to pre-exercise levels within 6h of passive recovery.
- Left ventricular longitudinal function was more affected in faster 10k runners, and they also released more troponin. Whereas faster 5k runners had a greater post-exercise reduction in LV LS and released less troponin.

5.5.2 Exercise Performance

When compared to the paces achieved during the DM trials, the participants in this study were able to increase their work rates by 6.5% on the 10k, and 15% on the 5k runs. This indicates that similarly to the cycling trials, the participants possessed greater functional exercise reserves when not limited by the constraints of pacing requirements during the duathlon. Additionally, it is likely that they were less inhibited by the fatigue induced by previous legs and anticipated during the additional legs. Interestingly, there does not appear to be any prior research to investigate the functional reserves of triathletes over conventional standalone race distances (5k and 10k runs).

5.5.3 Effect of 5k and 10k Running Efforts

The findings from this study demonstrate that both 5 and 10k running results in reduced ventricular longitudinal function, altered autonomic regulation and release of cTn. This study was the first to compare these two popular event distances at maximal intensity and it was initially hypothesised that the Max 10k would be the most strenuous. Unfortunately, the data

from this study was not able to detect inter-trial differences, which may be due to a lack of statistical power or samples. However, the observation of similar transient exercise induced reductions in cardiac function following both distances demonstrate the potential fatigue caused by short-duration running events where the intensity and effort are high. While early research into EICF investigated the effects of increasing exercise duration, from conventional marathons to multi-day events (Middleton et al., 2006, Shave et al., 2007), the influence of intensity has only recently been subjected to closer scrutiny (Shave et al., 2010b, Stewart et al., 2015, Stewart et al., 2016, Kleinnibbelink et al., 2021b). The duration of all exercise bouts in this study ranged from 17 to 44 minutes, which is comparable to recent research (but also novel as there is little prior investigation into short duration, high intensity exercise). Across the separate legs in both conditions, duration increased approximately by 50% for each leg from the 5k run to the 10k run, and intensity measured by average HR ranged from 80 – 94% of HR_{MAX}. Overall, the Max 5k, DM 10k and Max 10k runs elicited a greater magnitude of changes to cardiac functional alterations and troponin release, whereas the DM 5k resulted in fewer changes and no alterations to LV LS or autonomic control. The maximal effort 5 and 10k runs resulted in similar post-exercise reductions in systolic function as the maximal effort cycling and duathlon trials, which was characterised by reductions in EDV and SV, with elevated HR to sustain or increase \dot{Q} . Runners who completed their Max 5k run in the fastest times released lower amounts of troponin and experienced greater reductions in ventricular longitudinal function than slower athletes, and this finding was the opposite for the Max 10k runs. This may be explained by the participants fitness levels and the potential effect of a greater number of training years. It has been suggested that increased levels of myocardial adaptation, as a result of training, result in a lower cTn release (Shave and Oxborough, 2012) and it is possible that this explains the lower cTn release in the faster 5k runners in this study. Alternatively, it may be the case that slower 5k runners were less well-trained and released more cTn. Conversely, the 10k run legs potentially posed a more considerable cardiac challenge to the faster 10k runners, as cTn release was increased with run speed. We also observed increased cTn following the lower-intensity DM 5k runs, with the absence of any cardiac functional changes. This intensity and duration combination may

present the optimal exercise intensity boundary for adaptation and health (Stewart et al., 2016), but evidently also requires the most exertion. Few studies to date have investigated the effects of 10k run racing on cardiac function and these findings demonstrate considerable cardiac functional perturbations and sustained biomarker release with reduced recovery in SAX LV function following just 40 minutes of exercise. Further research into the demands and consequential adaptation following this event are therefore recommended.

The Max 5k runs caused a similar level of cardiac perturbation and troponin release as the Max 40k cycle legs from the previous Chapter, despite the average 5k run duration being ~30% of that of the cycling exercise. This is a particularly relevant finding, since each week in the UK thousands of runners compete in ParkRun, a free timed 5k event (Stevinson et al., 2015, Hindley, 2020). As such the discovery that intense bouts of 5k running exercise can cause similar reductions in cardiac function as ~60 minutes of moderate intensity cycling exercise warrants future investigation. There was evidence of autonomic perturbation following the 5k runs in both conditions, in particular the longer-duration DM trials where LF/HF ratio was significantly altered. Prior research has proposed (O'Keefe et al., 2012, O'Keefe et al., 2020) the potentially harmful effects of diminished LV strain parameters and disrupted autonomic function following exercise may expose participants with known or occult cardiomyopathy to increased risk to development or progression of electrophysiological disorders (Franklin et al., 2020a, Zhang et al., 2020). From the adaptive model perspective, it is interesting to note that there was a more substantial effect of prior training on cardiac biomarker release during the 5k and both cycle legs that was absent in the results of the 10k trials. Participants who took longer to complete these trials released greater amounts of troponin than fitter athletes, indicating a potential relationship between improved aerobic fitness and troponin release. This may be the result of prior training stimulating improved myocardial function (Stohr et al., 2012) or indicative of a naturally stronger myocardium in fitter athletes. Conversely, the ~40-minute efforts of the DM and Max 10k runs resulted in negative relationships between duration and biomarker release, with the fastest athletes releasing the most troponin and also demonstrating lower reductions in resting

post-exercise ventricular longitudinal function. This finding adds more evidence to potentially explain the relationship between exercise intensity and duration with cardiac function and it has emerged from this study that participant training history or fitness level is potentially a mediating factor.

The reduced number of functional changes following the DM 5k trial suggests that the DM 5k runs did not pose as significant of a challenge to the fitter participants. Participants were able to significantly increase their speed during the Max 5k, which suggests the preferred pacing strategy during the duathlons in this study was to aim to maintain the speed set during the initial 10k run over the final 5k. Alternatively, duathlon 5k run speed may have been limited by cumulative fatigue, and therefore these runs were performed at a low relative intensity due to factors such as glycogen depletion, thermal stress, or mental fatigue.

Evidence of a greater degree of myocardial adaptation in the faster athletes is supported by evidence of a moderate, significant negative relationship ($r = 0.50$, $P = 0.045$) between alterations in LVLS and exercise duration in the Max 10k runs. This finding is also indicative of a greater preservation of cardiac contractility in faster athletes in spite of the increased exercise duration of the 10k runs (Crandall and González-Alonso, 2010, Takimoto and Kass, 2012, Hellsten and Nyberg, 2015a). These findings demonstrate the varied response to prolonged strenuous running of durations exceeding 15 minutes (Scharhag et al., 2008, Elliott and La Gerche, 2015, Beaumont et al., 2017, Lord et al., 2018, Donaldson et al., 2019). The potential mechanism for the varied response between the left and right ventricles has been explained previously (Oxborough et al., 2012, La Gerche et al., 2012a, Lord et al., 2018, Lewicka-Potocka et al., 2020, Kleinnibbelink et al., 2021b), however in this study RV and LV longitudinal function were similarly affected by the exercise in all trials. (Lord et al., 2018, Donaldson et al., 2019, Bjorkavoll-Bergseth et al., 2020).

To account for the different cardiac functional alterations between 5k and 10k running in this study, it is proposed that exercise duration alone does not fully account for the varied findings, and that exercise intensity should also be considered. In the present study, it is likely that the fastest athletes were able to considerably improve their speed during the 5k trials

from the duathlon-matched runs in the Max trials, and this resulted in marked perturbation myocardial function (Elliott and La Gerche, 2015). During the 10k runs, the amplified duration at a similar intensity (as indicated by non-significantly different average heart rates) to the 5k runs provoked reduced biomarker release, which is proposed to be due to the improved myocardial adaptation of the more aerobically fit athletes' LV (Stohr et al., 2012), however the greater magnitude on LV longitudinal function warrants further investigation.

It is not possible to directly compare the effects of exercise mode in the results of this thesis, due to substantially greater exercise duration in the bike legs. However, it is interesting to observe that despite working at a higher relative $\dot{V}O_2$ and $\%HR_{MAX}$ during the bike legs, fitter athletes demonstrated lower levels of cardiac perturbations than slower cyclists, and the opposite was true for the 10k run legs. Importantly, absolute running heart rates and $\dot{V}O_2$ were higher than during cycling and the dynamic nature of running may be a contributing factor as the increased physical motion of the heart and reduced efficiency of skeletal muscle aided venous return during running may both contribute to increased intensity placing a greater cardiac vs. locomotor muscle burden than during cycling (Faulkner et al., 1971, Kohrt et al., 1987, Millet et al., 2009, Suriano and Bishop, 2010). Additionally, despite a lower mean exercise duration in the Max 10k trial than the bike legs, the Max 10k elicited the greatest concentration of troponin release, which was sustained at 6h post exercise.

5.5.4 Conclusions

The most notable alteration to LV systolic function following the single sport trials was the prolonged reduction in E/A ratio at 6h post the Max 10k run trials. Additionally, during the immediate recovery from all max trials there were signs of reduced systolic function as indicated by the reductions in EDV, EF and SV. The reduction in post-exercise SV was caused by reduced EDV, in line with previous findings (Watanabe et al., 2020, Crandall and González-Alonso, 2010, Stöhr et al., 2011b, Stöhr et al., 2011a), and the concomitant sympathetic suppression following maximal effort PSE was apparently enough to mitigate any augmentation of cardiac contractility to preserve post-exercise SV (González-Alonso et

al., 1999). In terms of overall impact, the DM 5k elicited the least alterations in LV systolic function, whereas the Max 10k trials elicited the most. Despite higher relative $\dot{V}O_2$ and HRs during the Max 5k there were no prolonged effects of exercise on these variables, and thus the overall impact of an all-out 5k may not be as challenging despite the increased intensity. It is therefore suggested that the 10k distance represented the greatest cardiac challenge to this highly trained cohort due to the sustained reductions in mitral valve E/A ratio after 6h of passive recovery. Additionally, the findings of reduced LV longitudinal function, and significant cTn release immediately following a maximal 5k run are worthy of further investigation. Tens of thousands of runners across the world regularly partake in weekly 5k Park Run events (Hindley, 2020). The data from this thesis demonstrates that such exertions involving a 5k running time trial lead to significant cardiac functional alterations, which may put vulnerable individuals at a greater risk of acute coronary or cardiac incidents (O'Keefe et al., 2012). The present study exclusively examined highly trained participants, and further research is needed to assess EICF following 5k runs in the general, recreationally trained population.

Chapter 6 General Discussion

6.1 Summary

Within this thesis several areas in the literature that were previously insufficiently explored were addressed in the following aims:

1. To assess the effects of Olympic Distance duathlon exercise on cardiac function and to clarify the performance reserves of highly-trained triathletes over the individual duathlon legs.
2. To review and meta-analyse previous research that investigated the influence of prolonged strenuous exercise (PSE) on cardiac function, biomarker release, and autonomic control.
3. To explore the relationship between these variables to determine whether less invasive resting measures could be used to estimate post-exercise cardiac function and biomarker changes.

Chapter 1 identified and meta-analysed the body of literature prior to this thesis that had investigated the 3 cardiac factors (myocardial function, cardiac autonomic modulation, and biomarker release). The primary findings from this meta-analysis and systematic review were that strenuous endurance cycling, running and triathlon exercise causes transient reductions in LV EF and E/A ratio, and clinically relevant elevations in cTn. Exercise intensity measured by average HR explains a large degree of the variance in troponin release and E/A ratio in the data, indicating exercise bouts at a high intensity elicit both cTn release and reduced diastolic function (Donaldson et al, 2019). The recommendations for future research studies from these findings were to:

1. Utilise specific echocardiographic techniques such as myocardial speckle tracking.
2. Ensure participants are euhydrated during post-exercise measurements to prevent accountable alterations in haemodynamics caused by reduced or increased blood volume.

3. Repeat measures in the hours following exercise to assess symptom progression or recovery.
4. Further explore the relationship between training history and exercise intensity on cTn release and functional changes.

Within the methodological scope of this thesis, the aim was to investigate the influence of a prolonged bout of duathlon exercise performed at race intensity to observe whether it would elicit the previously reported cardiac perturbations that occur following ultra-distance triathlon and cycling road races. As highly trained triathletes are able to maintain a high relative percentage of $\dot{V}O_{2\max}$ for an Olympic distance triathlon lasting 1.75-2 hours (Millet and Bentley, 2004, Millet et al., 2009) it was hypothesised that this would present the optimal balance of duration and high intensity exercise. The sport of duathlon has been extensively investigated in the lab (Vallier et al., 2003, Sparks et al., 2005, Alvero-Cruz et al., 2011, Ronconi and Alvero-Cruz, 2011, Berry, 2012, Berry et al., 2016, Tsuzuki et al., 2019), but not in an cardiac function context prior to this thesis. Additionally, the individual legs of the duathlon are also popular standalone competition distance, which warrant further investigation in the context of exercise induced cardiac functional changes. . Therefore, the conclusions of Chapter 1 informed the methodology of the following 3 experimental studies.

The findings of Chapter 3 were the first to investigate the reliability of conducting lab-based Olympic distance (OD) duathlons to investigate cardiac function in well-trained athletes. By performing 2 trials and allowing participants to self-select their own work rates it was possible to demonstrate the reproducibility of pacing and performance among the cohort. Despite the added period of rest in the BD trials, performances were similar between trials and there were no significant differences in any of the variables measured at the post and 24h post TPs. Therefore, Chapter 3 demonstrated the validity of the inclusion of a ‘broken’ format to investigate inter-leg variations in cardiac function during multisport events. The findings of Chapter 3 in brief were that LV and RV LS, and functional parameters were transiently reduced at the end of an OD duathlon, and the results of the BD trial demonstrated the

majority of these alterations occurred during the first 10k run. Participants had fully recovered to baseline status after 24h of passive recovery, indicating that this should be a sufficient period of recovery to prevent cumulative increments in cardiac fatigue that may be a precursor to the development of cardiac disease (O'Keefe et al., 2020, Wilson et al., 2011a).

In Chapters 4 and 5 the legs of each duathlon trial were separated and compared as standalone duathlon-paced legs vs. maximal effort trials over the same distance. It was found that participants were able to increase their work rate by 5-15% across the 3 legs over the equivalent duathlon performances, the first 10k run in the duathlon was performed closest to maximal intensity, which may form part of an optimal pacing strategy, and the reduced intensity of the duathlon 5k run resulted in fewer cardiac function changes when performed standalone. Additionally, the results of this study suggest that during the duathlon, the pacing strategy involves maintaining speed during the final 5k run close to that achieved during the first 10k run. There was accompanying evidence of transient cardiac perturbations of varying degrees on; biomarker release, impaired cardiac function, and disrupted autonomic control, following the exercise bouts at both intensities. The variance of these findings was somewhat explained by modifications in exercise intensity, mode, and duration; however, due to the methodological limitations of this thesis it was not possible to definitively identify the individual influence of each component and it was not directly possible to compare mode. Overall, it appeared that increased effort (Max vs. DM) over any distance elicited the greater amounts of cardiac perturbations compared to duathlon race-pace effort, but no significant differences in the magnitude. Critically, the potentially negative impact of the 5k runs should be investigated further as this is the most widely participated event format across the UK, with hundreds of free-participation ParkRun events taking place every Saturday (Stevinson et al., 2015).

Additionally, the reported changes in cardiac function shown in each experimental chapter in this thesis add to the body of evidence related to constant workload exercise (Travers et al., 2020). One feature that was lacking from the experimental design was the inclusion of continuous measurements of cardiac function during exercise, which has proven to be

methodologically challenging to perform during running exercise (Jouffroy et al., 2015). Like the methodology employed in this thesis, Jouffroy et al. (2015) were able to perform serial, intra-exercise measures of cardiac function during an ultra-distance (80km) trail run (Jouffroy et al., 2015). Of the 28 participants in this study, there were no notable modifications to LV function at the 21km and 53km measurement points. However, upon arrival at the race finish there were reductions in mitral E/A ratio, SV, and LV LS. In comparison to the findings of this thesis, the lack of intra-exercise changes in LV LS and systolic function may potentially be explained by a lower exercise intensity (mean running speed of 8.2 vs. 14.8 km·hour⁻¹), which was associated with alterations in LV LS in Chapters 4 and 5.

Echocardiographic measurements taken during cycling exercise were performed by Travers et al (2020), who demonstrated the continuous rise in \dot{Q} , EDV and SV throughout 180 minutes of cycling exercise at ~65% VO_{2Max}, alongside augmentations in LV twist-untwist (Travers et al., 2020). Of note in this study, was the maintenance of SV and EDV while exercising in the heat when hydration levels were maintained (see Figure 6.1 overleaf).

In this thesis, the most strenuous exercise led to declines in resting longitudinal function, mitral E/A ratio, and LV filling capacity, it should be noted that this is likely not indicative of cardiac fatigue due to the concomitant increase in \dot{Q} in all participants. Due to methodological differences, it is difficult to directly compare the data in this thesis with the previously reported changes in exercise cardiac function for several reasons; (1) the participants in this thesis were highly trained and accustomed to the duration of the exercise trial, which may not be true of participants performing 3 hours of hyperthermic cycling exercise, (2) the participants were highly motivated to complete the set distances in the fastest possible time, similarly to how they would race in competition, (3) the methodologies and procedures used in this thesis were likely less sensitive to the progressive changes in cardiac function during constant workload exercise. However, it must be noted that the outcomes related to the post-exercise restoration of cardiac function is likely of value to coaches and athletes training for similar events.

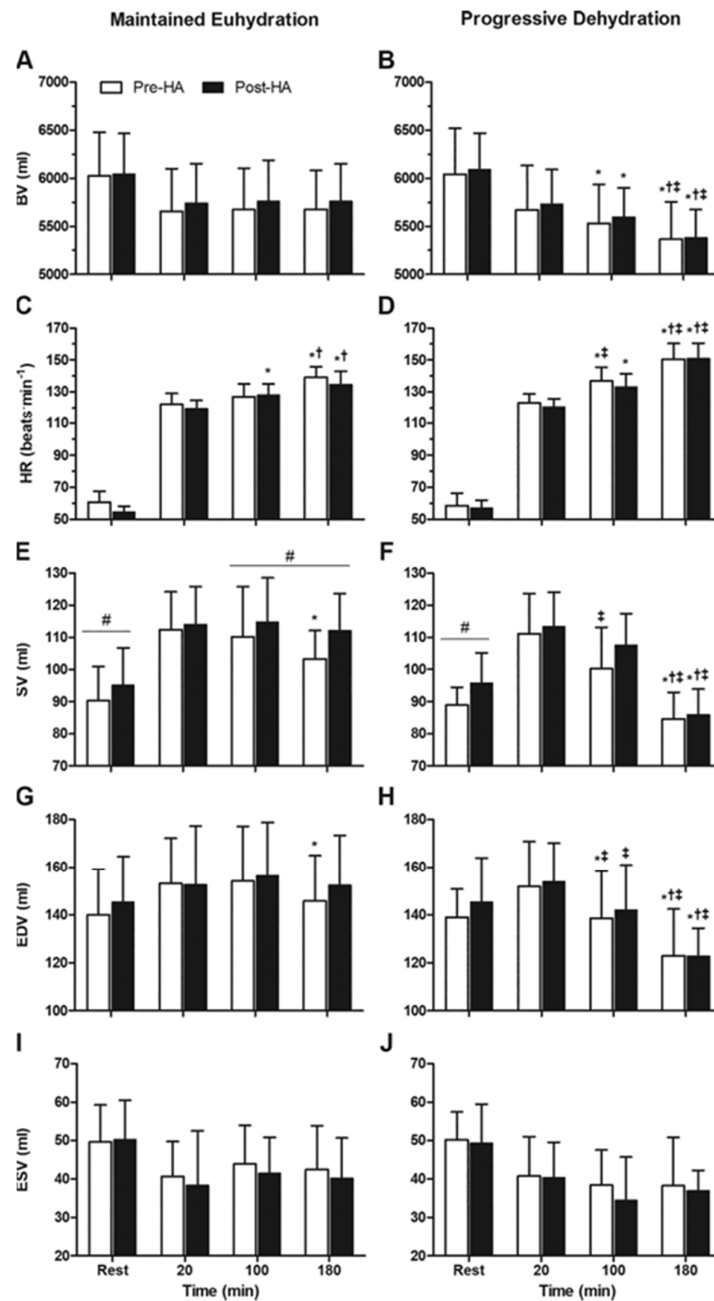


Figure 6.1 Reproduced from Travers et al., (2020). Note: Blood volume (BV;A and B), heart rate (HR; C and D),stroke volume (SV; E and F), end diastolic volume (EDV;G and H), and end-systolic volume (ESV; I and J) at rest and during bouts of semi-recumbent exercise under moderate heat stress pre-and post-heat acclimation (HA). Responses were measured while euhydration was maintained (left) or during progressive dehydration (right) via fluid restriction. Data are means SD for 8 participants. *P < 0.05 vs. 20min; †P < 0.05 vs. 100min; ‡P < 0.05 vs. euhydration; #P 0.05 vs. pre-HA. Key Issues

6.1.1 Comparison of Outcomes Between Cycling and Running Trials

The hypothesis of this thesis was that cycling exercise at duathlon race pace would result in fewer cardiac functional changes, both during the duathlon and standalone. Also, at all intensities running exercise would elicit greater levels of cardiac perturbations than cycling exercise. The overall results of both studies demonstrate that this research hypothesis can be largely accepted, but with several caveats.

When interpreting and comparing the results, it is important to note that the methodological design did not allow for direct comparison of duration or intensity between mode, and that participants achieved different levels of absolute work rate and metabolism during the cycle and run legs. However, it is possible that the reduced intensities achieved during cycling may possibly be a function of exercise mode, as the DM cycling trials were based on the performances during the original duathlon trials the average exercise intensity achieved by this mode may have been limited by fatigue of the preceding 10k runs and not due to modal differences. The participant's pacing strategies may have relied upon a near-maximal effort in the first 10k to take advantage of the non-weightbearing nature of cycling as an opportunity to achieve the maximum possible recovery before the final run leg. These data, alongside previous studies (Sparks et al., 2005, Berry et al., 2016, Tsuzuki et al., 2019) which have demonstrated that the cycling leg is of lower-intensity (as measured by gas exchange and HR) during lab-based duathlons, would tend to support this assumption. Additionally, the contention that running places more physiological demand than cycling has been demonstrated in previous works on runners and cyclists by Nieman and colleagues (2014). The authors of this work demonstrated significantly ($P < 0.05$) greater levels of skeletal muscle damage, inflammation and reported muscle soreness (DOMS) following a 3-day training camp of either running or cycling exercise for $2.5 \text{ hours} \cdot \text{day}^{-1}$ (Nieman et al., 2014). This finding supports the results of Koller and colleagues (1998), who reported more pronounced skeletal and cardiac muscle injury following short bouts of downhill running compared to 230km of alpine cycling (Koller et al., 1998).

In the current thesis, as the cycling legs were of considerably longer duration than the 10k runs, the reduced intensity was potentially a result of the athlete's pacing strategies to ensure optimal completion of the entire event. When compared to the duathlon and DM bike legs, the results of Chapter 5 found that participants were able to improve their work rate and performance by 15% in the max legs, as opposed to 5% and 9% in the 10k and 5k runs in Chapter 6, respectively. The max cycling leg demonstrated marked levels of post-exercise cardiac perturbations that were like the max and duathlon 10k runs, and of a greater magnitude than the DM bike leg and 5k runs. This demonstrates the potential for relatively short-duration, high-intensity cycling exercise to cause cumulative cardiac dysfunction and long-term damage (Stewart et al., 2015, Stewart et al., 2016, Bjorkavoll-Bergseth et al., 2020). The results of Chapter 4 and a consensus amongst many other studies tends to confirm these findings, and it is likely the lower impact of the duathlon cycling leg on cardiac function is probably caused by other factors related to fatigue and thermoregulation rather than intrinsic modal differences between running and cycling exercise (Millet and Bentley, 2004, Millet et al., 2009). Therefore, it is suggested that the differences in cardiac perturbations resulting from the duathlon and DM bike legs were due to the sum of the participant's pacing strategies and prior 10k run fatigue.

6.1.2 The Effects of Duathlon Performance

The approximately two-hour duration of the duathlon trials in Chapter 4 was similar to the duration of previously studied OD triathlons (McGavock et al., 2002, Millet and Bentley, 2004, Park et al., 2014). The current thesis findings were comparable to other studies investigating exercise bouts of this duration in terms of cardiac troponin release and function. Park et al. (2014) investigated the release of cardiac biomarkers following an OD triathlon (123.4 ± 1.6 minutes) in elite and non-elite triathletes and found that cTnT significantly increased at 2hrs post-exercise in the elite group, who shared similar characteristics as the cohort in Chapter 4. Interestingly, Park et al. (2014) found no significant cTn release at the immediate post-exercise timepoint (Park et al., 2014). However, this is likely due to the use

of a non-high sensitivity assay in this study. In the broken duathlon trial, it was demonstrated that hs-cTnT is released following the first 10k run leg and hs-cTnT is continuously released across the duration of the entire duathlon. The findings of McGavock et al. (2002) who investigated intra and post-exercise cardiac function demonstrated how ~2hrs of strenuous exercise can negatively affect cardiac performance. In this study on female triathletes, FAC was significantly reduced after exercise and LV systolic function was enhanced during exercise (McGavock et al., 2003).

While the achieved results were comparable with the previous research into ~2hrs of exercise at equivalent intensities (~90% HR_{MAX}), an important consideration is that to date most of the research of this nature into multisport exercise has been conducted on ‘ultra’ endurance exercise events such as the Ironman triathlon which lasts 8-17hrs (Oxborough et al., 2010). While the majority of research has previously focused on ultra-long distance endurance events, ‘short’ format triathlon racing is the most widely participated in, and most accessible event distance (Franklin et al., 2020b). The most direct investigation of event duration in this thesis was performed in Chapter 6, which compared 5 and 10k running legs at both maximal and duathlon paces. The results of the Max trials demonstrated marked cardiac perturbations following each event, but the magnitude of these was greater following the 10k run. While the Max 5k was performed at a higher average HR and fractional $\dot{V}O_{2max}$, the results demonstrated that exercise duration and distance were the key determinants of cardiac perturbations. A consideration of this finding is that there were no significant differences in average run speed between Max 5k and 10k trials; however, this was largely a factor of inter-individual variation as some athletes greatly increased their speed over the shorter duration runs compared to the 10ks. While the Max 10k was seemingly the most fatiguing distance, this research has proven the impact of a short duration (<20 minutes) maximal run on subsequent cardiac function, regulation and biomarker release. Within the field, there is a paucity of research into events of this duration and future research should be conducted to further investigate the 5k run, particularly in the context of less-trained individuals than the participants in this study. Recent data from the work of Klennibbelink (2021), demonstrated

the marked disruption of normal cardiac function following 45-minutes of high-intensity running (Kleinnibbelink et al., 2021b), and these findings support this alongside demonstrating the relevance of investigating the maximal effort 5k run in an EICF context.

Additionally, the data from Chapter 6 agrees with the findings of Stohr and colleagues (2012), which demonstrated the positive relationship between aerobic fitness and post-exercise cardiac function and therefore, recreationally fit athletes may experience a greater degree of functional changes post-5k running races. Parkrun is a mass participation, grassroots 5k running event that the majority of recreational, and competitive, runners take part in. Often, athletes treat these weekly runs as a competition, and it is likely that among the large population of regular highly trained Parkrun competitors each week there is a degree of cardiac fatigue occurring. The mass-participative nature of Parkrun, and alongside the data presented in Chapter 5 highlights the need for future research to further investigate the 5k run in the context of possible cardiac fatigue.

Regression analysis of the standalone trials data also demonstrated the influence of fitness on cardiac perturbations, with those taking the longest to complete the 5k run trials demonstrating the smallest reductions in longitudinal strain. Conversely, the fastest athletes in the 10k trials demonstrated the ability to maintain LV longitudinal contractile function. This finding would support previous evidence that the LV is adapted to a greater extent in more highly-trained, aerobically-fitter athletes and thus less vulnerable to greater degrees of post-exercise dysfunction (La Gerche et al., 2008, Oxborough et al., 2011, Stewart et al., 2016, Stewart et al., 2015).

The findings of this thesis also demonstrate the influence of duathlon event duration by comparing the 2nd and 3rd legs of the duathlon to the standalone duathlon paced trials, thus factoring the impact of the prior exercise. The main finding from this analysis was the absence of any significant differences in troponin release following the BD bike leg despite the increased duration, which would suggest that the initial release of troponin is of the greatest magnitude and that smaller secretions that do not affect the mean plasma concentration are subsequently released. In the broken duathlon, following the 10k run it is

possible that the troponin released was metabolised throughout the cycling leg and resulted in the reduced levels over the standalone workload matched DM bike legs. It is therefore suggested that further research should investigate the time course and metabolism or excretion mechanisms of troponin during exercise. Additionally, the findings demonstrate that in the highly trained athletes in both studies, there was little difference in the magnitude of change in any cardiac variables that resulted from the preceding exercise during the duathlons.

6.1.3 Increased Standalone-Performance Work Rates

One of the hypotheses outlined in Chapter 1 predicted that the experienced athletes recruited in this thesis would be able to substantially increase their work rates, either cycling power output or running velocity, over the duathlon-matched efforts when performing the individual legs as standalone bouts. This was investigated in Chapters 4 and 5, and while the chosen method did not allow for direct comparison of exercise intensities *per se*, the increased work rates yielded higher exercise HRs and oxygen consumption. Whether this also provoked increased magnitudes of cardiac perturbations is still unclear, however. The overall results demonstrated no significant differences in post exercise changes in cardiac function between each duathlon paced leg and their matched max-effort trial. However, while the magnitude of the changes in the Max trials was not significantly higher there was a trend for increased perturbations over the DM trials. Therefore, there appeared to be a minimal reduction in cardiac function that followed the lower intensity DM trials that was not significantly increased following a similar-length performance at an elevated work rate across the three legs. To thoroughly assess the influence of exercise intensity, the methods chosen by Stewart et al (2016) are recommended, which in brief required participants to perform at cycling power-outputs based on fixed-percentages of their individual gas exchange thresholds (Stewart et al, 2016).

It could be argued that hs-cTnT release was affected by the increase in distance and overall cardiac work in the Max 10k trial, over the DM and Max 5k run legs, as this leg elicited the greatest degree of hs-cTnT release. It is suggested that the sustained elevation in HR during

the Max 10k, for twice the distance of the 5k provoked this release, and therefore a combination of both heavy-domain exercise that is maintained beyond a certain duration (approximately 30 minutes is suggested) may represent a critical boundary of exercise tolerance. Similar findings have been previously demonstrated in meta-analyses (Lord et al., 2018, Donaldson et al., 2019) and lab-based studies in cyclists (Stewart et al., 2015, Stewart et al., 2016). The potential relationship between increased exercise intensity and duration, and hs-cTnT release could be explained by the hypothesised mechanistic basis for troponin release resulting from oxidative damage causing sarcolemma permeability and for cytoplasmic stores of troponin to be released) (Shave et al., 2010a). An additional potential mechanism of release is through persistent mechanical deformation causing stretch responsive integrins to facilitate the release of stored troponins from the myocardial membrane (Baker et al., 2019). Figure 6.2 outlines these mechanisms and how they may occur in an exercise setting.

Therefore, the data from Chapters 4 and 5 would support either of these theories as an increased exercise work rate was positively associated with hs-cTnT release. The concept of a tolerable boundary of exercise, which could provoke EICF has was previously mentioned in this thesis. The data presented supports this hypothesis, and Figure 6.3 outlines a potential model for such a threshold. Importantly, the factors responsible for magnitude and reversibility of cardiac functional changes appear to be related to not one, but a combination of both exercise intensity and duration, as the results showed differing cardiac perturbations following similar intensity runs of vastly different duration in the Max 5k and 10k trials.

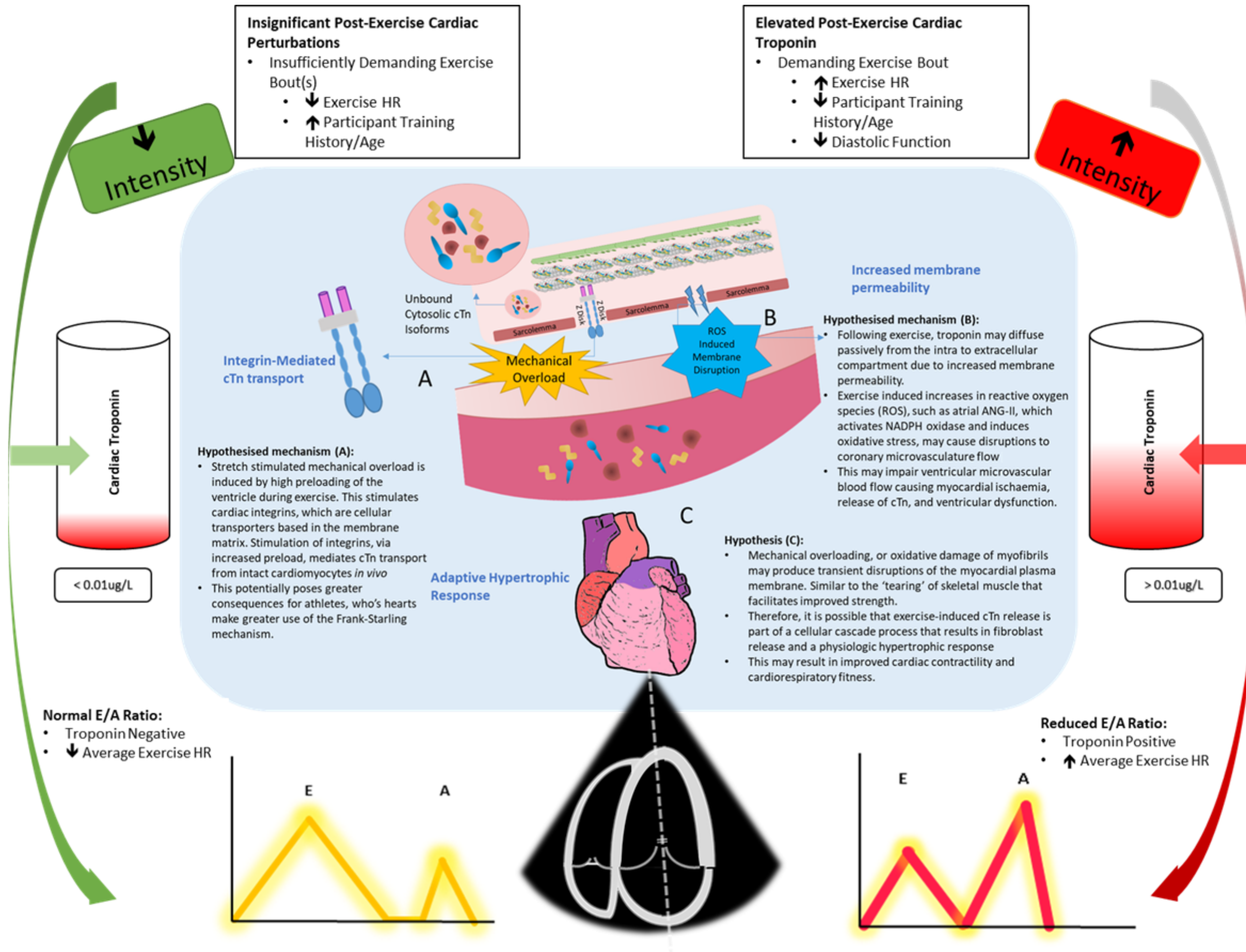


Figure 6.2 Hypothesized mechanistic basis for exercise-induced hs-cTnT release.

6.2 Implications

From a clinical perspective, the results of this thesis apply the current scientific understanding of the potential long-term implications of cardiac fatigue to conventionally performed event distances and durations. It is widely reported that individuals at either end of the exercise participation spectrum are the most susceptible to potential cardiac complications, and the cohort of this study falls towards the extreme high end (O'Keefe et al., 2012, O'Keefe et al., 2020). It is most likely that the findings of the 6h follow up measures in Chapters 4 and 5 bear the most clinical relevance to real life competition training, since this is the period in which elite multisport athletes would typically perform a second daily training session. There was a lingering effect of exercise in resting cardiac function following the Max 10k trial, where hs-cTnT levels were also raised >700% above the clinical threshold of $14 \text{ ng}\cdot\text{L}^{-1}$. Whether or not the repeated cardiac fatigue elicited by such demanding exercise goes on to develop myocardial injury, fibrosis, or some alternative substrate for cardiac arrhythmia is beyond the scope of this thesis. However, a potential long-term cardiac risk of such behaviour cannot be ruled out. The recommendation from this thesis is that sports doctors and coaches should evaluate the potential for cumulative cardiac fatigue within their training prescriptions and ensure sufficient (at least 24h24h) rest following maximal-effort time trials. Additionally, the importance of the potential application of post-exercise RMSSD in providing insights into post-exercise cardiac recovery is highlighted by this work. However, this area warrants further exploration *via* serial follow-up measurements alongside cardiac function measures throughout the 24h recovery period.

6.2.1 Future Research

While this thesis attempted to answer the research questions outlined at the beginning, there are also several areas for improvement within the methodology and use of emerging techniques going forwards.

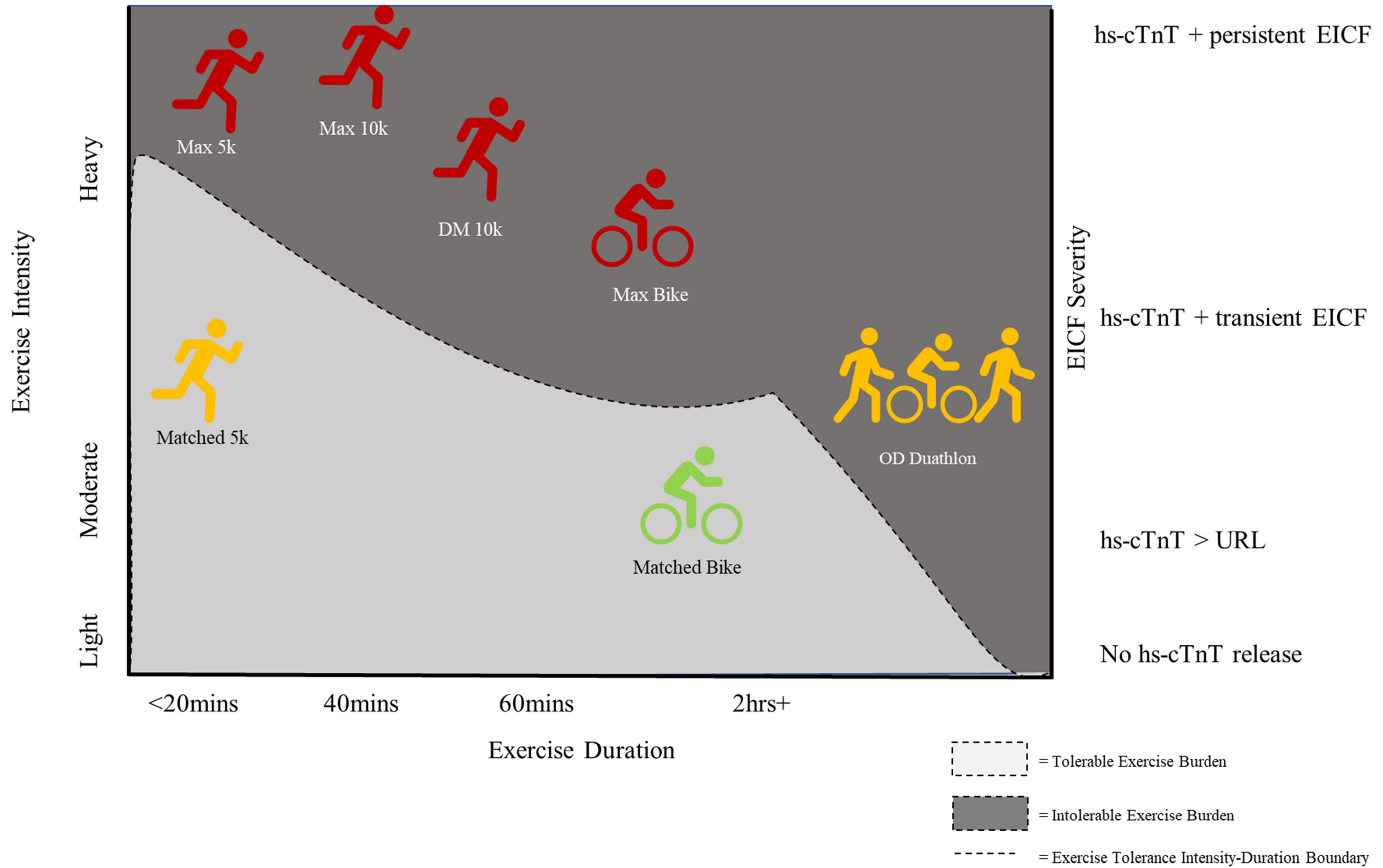


Figure 6.3 Hypothesised exercise boundary to establish a tolerable limit before provoking EICF.

6.2.2 Influence of rate of HR increase

This is a potentially important and relatively new metric that has been used to assess the potential level of over-training related fatigue (Thomson et al., 2016). It should be considered that the rate, or acceleration, of HR increase over time may be related to baseline cardiac parameters and therefore should be investigated alongside a thorough echocardiographic and autonomic examination.

6.2.3 DFA Alpha 1

Additionally, non-linear quantification of HRV metrics have shown a strong link with cardiovascular health (Shaffer and Ginsberg, 2017). In this thesis the Alpha 1 coefficient from detrended fluctuation analysis (DFA) was extracted from the resting HRV signal before and after exercise, which proved to be reliable in differentiating between trials of differing intensity. Based upon this finding and the fact that , the use of DFA Alpha 1 during exercise has demonstrated remarkable prescriptive ability to identify exercise intensity thresholds, it is suggested that it should be more widely implemented to similar studies (Gronwald and Hoos, 2020).

6.3 Conclusion

The primary and novel aim of this thesis was to investigate the effects of duathlon exercise on cardiac function. The use of the broken format demonstrated the reliability of lab-based duathlon performances and for the sequential investigation of how EICF progresses throughout the event. Additionally, it has been demonstrated that running 10k and 5k at varied intensities does produce statistically similar cardiac perturbations to cycling exercise of substantially increased duration at similar average heart rates. This work also highlights that the nature of multi-sport exercise may cause fatigue not related to cardiac function that limits performance, and therefore this should be considered in future investigations. When discussing the contribution of exercise intensity, mode, and duration to cardiac perturbations, the evidence from this thesis suggests exercise intensity is a more reliable predictor of post-

exercise cardiac function in trained individuals, This is supported by the finding that the maximal effort standalone 5k run, 10k run and 40k bike legs provoked the greatest and most sustained reductions in function compared to their lower-intensity, similar duration DM counterparts. Importantly, for many the UK's weekly Parkrun competitors, the Max 5k run effort provoked cardiac functional changes and the lower-intensity DM 5k elicited very few cardiac perturbations. The findings in this thesis agree with the majority of EICF research, which suggests that LV systolic function is transiently impaired due to reduced filling, and that rotational mechanics of the LV are preserved or enhanced while longitudinal function declines during the immediate recovery period. In addition, the return to resting levels is complete in most of the exercise bouts within 6-24h of recovery.

6.3.1 Limitations

Although throughout this thesis statistical corrections for confounding factors were made, some of the presented findings may have been influenced by sympathetic withdrawal following exercise cessation (Gronwald and Hoos, 2020, Kleinnibbelink et al., 2021b). This has been shown to contribute to masking exercise induced cardiac fatigue as BP and HR both decline from exercise levels (Stewart et al., 2017). While the magnitude of functional changes reported during the acute-recovery phase from exercise is typically ameliorated within 24h of recovery in studies reporting follow-up data, the true extent of potential cardiac fatigue may be masked by post-exercise sympathovagal excitation due to post exercise hypotension. To mitigate the influence of post exercise hypotension on echocardiographic indices of cardiac function, recent studies (Stewart et al., 2016, Kleinnibbelink et al., 2021b) have employed low-intensity exercise challenges following bouts of PSE. It is possible to conduct an echocardiographic exam during recumbent cycling exercise at low work rates (50-100W) and studies that have utilised this methodology have revealed a greater magnitude of EICF than during passive recovery. This methodology was not employed due to equipment limitations; however, future investigations into the effect of PSE on cardiac function should aim to do so where possible. If implemented in the current work, this would have permitted assessment of

the effect of altered post-exercise loading conditions on myocardial performance and ventricular augmentation (Stewart et al., 2017).

Similarly, the interpretation of the data in this thesis must be considered alongside the fact that no resting or low-intensity exercise controls were performed in any of the trials. Inclusion of such control trials would have demonstrated the magnitude of potential methodological and biological variabilities inherent within the study procedures. Low-intensity exercise and resting trials would provide potentially important information and a baseline measure for the physiological responses that occur during prolonged strenuous exercise. Without these trials, it is difficult to assess the relative effects of high-intensity exercise on performance and physiological responses in the general population who perform more recreational forms of exercise. Despite this limitation, the results of this study provide valuable insights into the effects of high-intensity exercise on physiological responses and performance outcomes, particularly in populations who engage in high-intensity exercise as part of their training or competition.

Additionally, though the methodology provided separate data for the running and cycling trials the extent of any comparison between these was limited by the variations in intensity and duration performed by the participants used, and there were no control measures in place to standardise the workload performed between modes (Oxborough et al., 2010). Therefore, attempting to standardise exercise intensity and duration across both modes was not possible and would not have been ecologically valid or comparable to previous research. A more targeted approach to compared cycling and running trials matched for overall energy expenditure and myocardial work is a viable means to achieve this.

6.3.2 Guidance for Athletes and Coaches

In triathlon and duathlon, the bike-run transition has been shown to be the biggest determinant of performance and is the transition that multisport athletes typically train the most (Millet et al., 2011). Both the OD duathlon and triathlon involve a 10k run; however, there may be an influence of order-effect that differentiates triathlon from the levels of

cardiac perturbations demonstrated in this study. In duathlon the most strenuous leg is first and is likely performed at a greater intensity than in the 10k run at the end of a triathlon (Nikolaidis et al., 2021), which would suggest the cardiac perturbations are also increased when the second run is also taken into consideration. Furthermore, the substitution of the non-weight bearing swim leg for the additional run may result in the duathlon being a more strenuous off-season/winter month alternative event for triathletes, and more research should be done to investigate the differences between duathlon and triathlon on EICF.

When interpreting the findings of this thesis in the context of performing repeated training sessions it is important to note that this work has exclusively investigated the influence of exercise on cardiac fatigue. The athlete undertaking a heavy training period may experience a reduction in performance in the absence of cardiac fatigue, due to skeletal muscular, neural, and/or emotional fatigue at different periods in the yearly training cycle (Lorenz et al., 2010). In such instances, the rate of HR increase appears to be a reliable and convenient measurement to diagnose training-induced fatigue and future research should endeavour to investigate its relationship to cardiac function prior to a training session (Thomson et al., 2016, Bjorkavoll-Bergseth et al., 2020, Bechke et al., 2020, Bellenger et al., 2018). Recently, there has been a plethora of wearable devices developed to assist with athlete recovery, sleep and training intensity prescription (Düking et al., 2018). There would be great value in determining whether non-invasive autonomic measures correlate well with athlete recovery, to negate the requirement for biomarker analysis. Additionally, the need for further investigation into the potentially negative impact of high-intensity, short duration running bouts on the cardiac system is highlighted.

References

- AAGAARD, P., SAHLEN, A., BERGFELDT, L. & BRAUNSCHEWIG, F. 2014. Heart rate and its variability in response to running—associations with troponin. *Med Sci Sports Exerc*, 46, 1624-30.
- ABHAYARATNA, W. P., SEWARD, J. B., APPLETON, C. P., DOUGLAS, P. S., OH, J. K., TAJIK, A. J. & TSANG, T. S. 2006. Left atrial size: physiologic determinants and clinical applications. *J Am Coll Cardiol*, 47, 2357-63.
- AENGEVAEREN, V., BAGGISH, A., CHUNG, E., GEORGE, K., KLEIVEN, Ø., MINGELS, A., ØRN, S., SHAVE, R., THOMPSON, P., THIJIS & EIJSVOGELS, T. 2021. Exercise-Induced Cardiac Troponin Elevations: From Underlying Mechanisms to Clinical Relevance. *Circulation*, 144, 1955-1972.
- AKSELROD, S., GORDON, D., UBEL, F. A., SHANNON, D. C., BERGER, A. C. & COHEN, R. J. 1981. Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science*, 213, 220-2.
- ALAM, M., HOGLUND, C. & THORSTRAND, C. 1992. Longitudinal systolic shortening of the left ventricle: an echocardiographic study in subjects with and without preserved global function. *Clin Physiol*, 12.
- ALAM, M., WARDELL, J., ANDERSSON, E., SAMAD, B. A. & NORDLANDER, R. 1999. Characteristics of mitral and tricuspid annular velocities determined by pulsed wave Doppler tissue imaging in healthy subjects. *J Am Soc Echocardiogr*, 12, 618-28.
- ALBOUAINI, K., EGRED, M., ALAHMAR, A. & WRIGHT, D. J. 2007. Cardiopulmonary exercise testing and its application. *Postgraduate medical journal*, 83, 675-682.
- ALEXOPOULOS, E. C. 2010. Introduction to multivariate regression analysis. *Hippokratia*, 14, 23-8.
- ALVERO-CRUZ, J. R., RONCONI, M., CARRILLO DE ALBORNOZ, M., GARCÍA ROMERO, J. C., ROSADO VELÁZQUEZ, D. & DIEGO ACOSTA, M. D. 2011. Thyroid hormones response in simulated laboratory sprint duathlon. *Journal of Human Sport and Exercise*, 6, 323-327.
- AMUNDSEN, B. H., HELLE-VALLE, T., EDVARDSSEN, T., TORP, H., CROSBY, J., LYSEGGEN, E., STØYLEN, A., IHLEN, H., LIMA, J. A., SMISETH, O. A. & SLØRDAHL, S. A. 2006. Noninvasive myocardial strain measurement by speckle tracking echocardiography: validation against sonomicrometry and tagged magnetic resonance imaging. *J Am Coll Cardiol*, 47, 789-93.
- ANAWEKAR, N. S., GERSON, D., SKALI, H., KWONG, R. Y., YUCEL, E. K. & SOLOMON, S. D. 2007. Two-dimensional assessment of right ventricular function: an echocardiographic-MRI correlative study. *Echocardiography*, 24, 452-6.
- ANDERSEN, N. H., TERKELSEN, C. J., SLOTH, E. & POULSEN, S. H. 2004. Influence of preload alterations on parameters of systolic left ventricular long-axis function: a Doppler tissue study. *J Am Soc Echocardiogr*, 17, 941-7.
- ASHLEY, E. A., KARDOS, A., JACK, E. S., HABENBACHER, W., WHEELER, M., KIM, Y. M., FRONING, J., MYERS, J., WHYTE, G., FROELICHER, V. & DOUGLAS, P. 2006. Angiotensin-converting enzyme genotype predicts cardiac and autonomic responses to prolonged exercise. *J Am Coll Cardiol*, 48, 523-31.
- BAGGISH, A. L. & LEVINE, B. D. 2017. Coronary Artery Calcification Among Endurance Athletes. *Circulation*, 136, 149-151.
- BAGGISH, A. L., YARED, K., WANG, F., WEINER, R. B., HUTTER, A. M., PICARD, M. H. & WOOD, M. J. 2008. The impact of endurance exercise training on left

- ventricular systolic mechanics. *American Journal of Physiology-Heart and Circulatory Physiology*, 295, H1109-H1116.
- BAILLOT, M. & HUE, O. 2015. Hydration and thermoregulation during a half-ironman performed in tropical climate. *Journal of sports science & medicine*, 14, 263-268.
- BAKER, D. W. 1970. Pulsed Ultrasonic Doppler Blood-Flow Sensing. *IEEE Transactions on Sonics and Ultrasonics*, 17, 170-184.
- BAKER, P., LECKIE, T., HARRINGTON, D. & RICHARDSON, A. 2019. Exercise-induced cardiac troponin elevation: An update on the evidence, mechanism and implications. *Int J Cardiol Heart Vasc*, 22, 181-186.
- BANKS, L., SASSON, Z., BUSATO, M. & GOODMAN, J. M. 2010. Impaired left and right ventricular function following prolonged exercise in young athletes: influence of exercise intensity and responses to dobutamine stress. *J Appl Physiol (1985)*, 108, 112-9.
- BEAUMONT, A., GRACE, F., RICHARDS, J., HOUGH, J., OXBOROUGH, D. & SCULTHORPE, N. 2017. Left Ventricular Speckle Tracking-Derived Cardiac Strain and Cardiac Twist Mechanics in Athletes: A Systematic Review and Meta-Analysis of Controlled Studies. *Sports Med*, 47, 1145-1170.
- BECHKE, E., KLISZCZEWICZ, B., MCLESTER, C., TILLMAN, M., ESCO, M. & LOPEZ, R. 2020. An examination of single day vs. multi-day heart rate variability and its relationship to heart rate recovery following maximal aerobic exercise in females. *Sci Rep*, 10, 14760.
- BELLENGER, C. R., THOMSON, R. L., DAVISON, K., ROBERTSON, E. Y., NELSON, M. J., KARAVIRTA, L. & BUCKLEY, J. D. 2018. Optimization of Maximal Rate of Heart Rate Increase Assessment in Runners. *Research Quarterly for Exercise and Sport*, 89, 322-331.
- BELONJE, A., NANGRAHARY, M., DE SWART, H. & UMANS, V. 2007. Major Adverse Cardiac Events During Endurance Sports. *The American Journal of Cardiology*, 99, 849-851.
- BENDITT, D. G., FERGUSON, D. W., GRUBB, B. P., KAPOOR, W. N., KUGLER, J., LERMAN, B. B., MALONEY, J. D., RAVIELE, A., ROSS, B., SUTTON, R., WOLK, M. J. & WOOD, D. L. 1996. Tilt table testing for assessing syncope. American College of Cardiology. *J Am Coll Cardiol*, 28, 263-75.
- BERGER, R. D., SAUL, J. P. & COHEN, R. J. 1989. Transfer function analysis of autonomic regulation. I. Canine atrial rate response. *Am J Physiol*, 256, H142-52.
- BERRY, N. T. The effects of a 10 km run and 30 km cycling time-trial on ventilatory threshold during the final running leg of an ITU Duathlon in highly-trained multi-sport athletes. 2012.
- BERRY, N. T., WIDEMAN, L., SHIELDS, E. W. & BATTAGLINI, C. L. 2016. The Effects of a Duathlon Simulation on Ventilatory Threshold and Running Economy. *J Sports Sci Med*, 15, 247-53.
- BIANCHI, A. M., MAINARDI, L. T., MELONI, C., CHIERCHIA, S. & CERUTTI, S. 1997. Continuous monitoring of the sympatho-vagal balance through spectral analysis. *IEEE Eng Med Biol Mag*, 16, 64-73.
- BITMEAD, J. & OLIVER, G. 2018. A safe procedure: best practice for intravenous peripheral cannulation. *British Journal of Nursing*, 27, S1-S8.
- BJORKAVOLL-BERGSETH, M., KLEIVEN, O., AUESTAD, B., EFTESTOL, T., OSKAL, K., NYGARD, M., SKADBERG, O., AAKRE, K. M., MELBERG, T., GJESDAL, K. & ORN, S. 2020. Duration of Elevated Heart Rate Is an Important Predictor of Exercise-Induced Troponin Elevation. *J Am Heart Assoc*, 9, e014408.

- BLABER, A. P., YAMAMOTO, Y. & HUGHSON, R. L. 1995. Methodology of spontaneous baroreflex relationship assessed by surrogate data analysis. *Am J Physiol*, 268, H1682-7.
- BOM, N., LANCEE, C. T., ZWIETEN, G. V., KLOSTER, F. E. & ROELANDT, J. 1973. Multiscan Echocardiography. *Circulation*, 48, 1066-1074.
- BONACCI, J., GREEN, D., SAUNDERS, P. U., BLANCH, P., FRANETTOVICH, M., CHAPMAN, A. R. & VICENZINO, B. 2010. Change in running kinematics after cycling are related to alterations in running economy in triathletes. *J Sci Med Sport*, 13, 460-4.
- BONACCI, J., VLECK, V., SAUNDERS, P. U., BLANCH, P. & VICENZINO, B. 2013. Rating of perceived exertion during cycling is associated with subsequent running economy in triathletes. *J Sci Med Sport*, 16, 49-53.
- BORCHINI, R., VERONESI, G., BONZINI, M., GIANFAGNA, F., DASHI, O. & FERRARIO, M. M. 2018. Heart Rate Variability Frequency Domain Alterations among Healthy Nurses Exposed to Prolonged Work Stress. *International journal of environmental research and public health*, 15, 113.
- BOVET, P., HUNGERBUHLER, P., QUILINDO, J., GRETTVE, M. L., WAEBER, B. & BURNAND, B. 1994. Systematic difference between blood pressure readings caused by cuff type. *Hypertension*, 24, 786-792.
- BREUER, H. W., SKYSCHALLY, A., SCHULZ, R., MARTIN, C., WEHR, M. & HEUSCH, G. 1993. Heart rate variability and circulating catecholamine concentrations during steady state exercise in healthy volunteers. *British Heart Journal*, 70, 144-149.
- BURNS, A. T., LA GERCHE, A., PRIOR, D. L. & MACISAAC, A. I. 2009. Left ventricular untwisting is an important determinant of early diastolic function. *JACC Cardiovasc Imaging*, 2, 709-16.
- CALVERT, J. & LEFER, D. 2012. Chapter 6@ Overview of Cardiac Muscle Physiology. *Muscle Fundamental Biology and Mechanisms of Disease*, 57-66.
- CHAN-DEWAR, F., GREGSON, W., WHYTE, G., GAZE, D., WATERHOUSE, J., WEN, J. & GEORGE, K. 2013a. Do the effects of high intensity 40 km cycling upon left ventricular function and cardiac biomarker during recovery vary with time of day? *J Sports Sci*, 31, 414-23.
- CHAN-DEWAR, F., GREGSON, W., WHYTE, G., KING, J., GAZE, D., CARRANZA-GARCIA, L. E., LEGAZ-ARRESE, A. & GEORGE, K. 2013b. Cardiac electromechanical delay is increased during recovery from 40 km cycling but is not mediated by exercise intensity. *Scand J Med Sci Sports*, 23, 224-31.
- CHAPMAN, A. B., ZAMUDIO, S., WOODMANSEE, W., MEROUANI, A., OSORIO, F., JOHNSON, A., MOORE, L. G., DAHMS, T., COFFIN, C., ABRAHAM, W. T. & SCHRIER, R. W. 1997. Systemic and renal hemodynamic changes in the luteal phase of the menstrual cycle mimic early pregnancy. *Am J Physiol*, 273, F777-82.
- CHENG, C. P., IGARASHI, Y. & LITTLE, W. C. 1992. Mechanism of augmented rate of left ventricular filling during exercise. *Circ Res*, 70, 9-19.
- CLARKSON, P. M., NOSAKA, K. & BRAUN, B. 1992. Muscle function after exercise-induced muscle damage and rapid adaptation. *Med Sci Sports Exerc*, 24, 512-20.
- CNSYSTEMS 2014. Vascular unloading technique [Online]. Available: <http://www.cnsystems.com/innovation/cnap-technology/vascular-unloadingtechnique>.
- CNSYSTEMS 2021. Task Force Monitor.
- CRANDALL, C. G. & GONZÁLEZ-ALONSO, J. 2010. Cardiovascular function in the heat-stressed human. *Acta Physiologica*, 199, 407-423.

- CUSHMAN, W. C., COOPER, K. M., HORNE, R. A. & MEYDRECH, E. F. 1990. Effect of back support and stethoscope head on seated blood pressure determinations. *Am J Hypertens*, 3, 240-1.
- D'ANDREA, A., RIEGLER, L., GOLIA, E., COCCHIA, R., SCARAFILE, R., SALERNO, G., PEZZULLO, E., NUNZIATA, L., CITRO, R., CUOMO, S., CASO, P., DI SALVO, G., CITTADINI, A., RUSSO, M. G., CALABRÒ, R. & BOSSONE, E. 2013. Range of right heart measurements in top-level athletes: the training impact. *Int J Cardiol*, 164, 48-57.
- DALLA VECCHIA, L. A., BARBIC, F., DE MARIA, B., COZZOLINO, D., GATTI, R., DIPAOLO, F., BRUNETTA, E., ZAMUNER, A. R., PORTA, A. & FURLAN, R. 2019. Can strenuous exercise harm the heart? Insights from a study of cardiovascular neural regulation in amateur triathletes. *PLoS One*, 14, e0216567.
- DANDEL, M., LEHMKUHL, H., KNOSALLA, C., SURAMELASHVILI, N. & HETZER, R. 2009. Strain and strain rate imaging by echocardiography - basic concepts and clinical applicability. *Curr Cardiol Rev*, 5, 133-48.
- DAWSON, E., GEORGE, K., SHAVE, R., WHYTE, G. & BALL, D. 2003. Does the human heart fatigue subsequent to prolonged exercise? *Sports Med*, 33, 365-80.
- DAWSON, E. A., SHAVE, R., GEORGE, K., WHYTE, G., BALL, D., GAZE, D. & COLLINSON, P. 2005. Cardiac drift during prolonged exercise with echocardiographic evidence of reduced diastolic function of the heart. *Eur J Appl Physiol*, 94, 305-9.
- DELICCE, A. V. & MAKARYUS, A. N. 2021. Physiology, Frank Starling Law. *StatPearls*. Treasure Island (FL): StatPearls Publishing
- Copyright © 2021, StatPearls Publishing LLC.
- DITOR, D. S., KAMATH, M. V., MACDONALD, M. J., BUGARESTI, J., MCCARTNEY, N. & HICKS, A. L. 2005. Effects of body weight-supported treadmill training on heart rate variability and blood pressure variability in individuals with spinal cord injury. *Journal of Applied Physiology*, 98, 1519-1525.
- DONALDSON, J. A., WILES, J. D., COLEMAN, D. A., PAPADAKIS, M., SHARMA, R. & O'DRISCOLL, J. M. 2019. Left Ventricular Function and Cardiac Biomarker Release-The Influence of Exercise Intensity, Duration and Mode: A Systematic Review and Meta-Analysis. *Sports Med*, 49, 1275-1289.
- DOUCENDE, G., SCHUSTER, I., RUPP, T., STARTUN, A., DAUZAT, M., OBERT, P. & NOTTIN, S. 2010. Kinetics of left ventricular strains and torsion during incremental exercise in healthy subjects: the key role of torsional mechanics for systolic-diastolic coupling. *Circ Cardiovasc Imaging*, 3, 586-94.
- DOUGLAS, P. S., O'TOOLE, M. L. & KATZ, S. E. 1998. Prolonged exercise alters cardiac chronotropic responsiveness in endurance athletes. *J Sports Med Phys Fitness*, 38, 158-63.
- DOUGLAS, P. S., O'TOOLE, M. L., MILLER, W. D. B. & REICHEK, N. 1990. Different effects of prolonged exercise on the right and left ventricles. *Journal of the American College of Cardiology*, 15, 64-69.
- DÜKING, P., ACHTZEHN, S., HOLMBERG, H.-C. & SPERLICH, B. 2018. Integrated Framework of Load Monitoring by a Combination of Smartphone Applications, Wearables and Point-of-Care Testing Provides Feedback that Allows Individual Responsive Adjustments to Activities of Daily Living. *Sensors*, 18, 1632.
- EDLER, I. & LINDSTRÖM, K. 2004. The history of echocardiography. *Ultrasound Med Biol*, 30, 1565-644.

- EIJSSVOGELS, T. M., FERNANDEZ, A. B. & THOMPSON, P. D. 2016. Are There Deleterious Cardiac Effects of Acute and Chronic Endurance Exercise? *Physiol Rev*, 96, 99-125.
- ELLIOTT, A. D. & LA GERCHE, A. 2015. The right ventricle following prolonged endurance exercise: are we overlooking the more important side of the heart? A meta-analysis. *British Journal of Sports Medicine*, 49, 724.
- ERBEL, R., SCHWEIZER, P., LAMBERTZ, H., HENN, G., MEYER, J., KREBS, W. & EFFERT, S. 1983. Echoventriculography -- a simultaneous analysis of two-dimensional echocardiography and cineventriculography. *Circulation*, 67, 205-15.
- ESLER, M., JENNINGS, G., LAMBERT, G., MEREDITH, I., HORNE, M. & EISENHOFER, G. 1990. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev*, 70, 963-85.
- EYSMANN, S. B., GERVINO, E., VATNER, D. E., KATZ, S. E., DECKER, L. & DOUGLAS, P. S. 1996. Prolonged exercise alters beta-adrenergic responsiveness in healthy sedentary humans. *J Appl Physiol (1985)*, 80, 616-22.
- FAULKNER, J. A., ROBERTS, D. E., ELK, R. L. & CONWAY, J. 1971. Cardiovascular responses to submaximum and maximum effort cycling and running. *Journal of Applied Physiology*, 30, 457-461.
- FENG, J., SCHAUS, B. J., FALLAVOLLITA, J. A., LEE, T. C. & CANTY, J. M., JR. 2001. Preload induces troponin I degradation independently of myocardial ischemia. *Circulation*, 103, 2035-7.
- FIELD, A. P. 2000. *Discovering Statistics Using SPSS for Windows: Advanced Techniques for the Beginner*, Sage Publications.
- FISHER, J. P., YOUNG, C. N. & FADEL, P. J. 2015. Autonomic adjustments to exercise in humans. *Compr Physiol*, 5, 475-512.
- FLEMING, A. D., MCDICKEN, W. N., SUTHERLAND, G. R. & HOSKINS, P. R. 1994. Assessment of colour Doppler tissue imaging using test-phantoms. *Ultrasound Med Biol*, 20, 937-51.
- FLORAS, J. S. 2009. Sympathetic nervous system activation in human heart failure: clinical implications of an updated model. *J Am Coll Cardiol*, 54, 375-85.
- FOALE, R., NIHOYANNOPOULOS, P., MCKENNA, W., KLEINEBENNE, A., NADAZDIN, A., ROWLAND, E. & SMITH, G. 1986. Echocardiographic measurement of the normal adult right ventricle. *Br Heart J*, 56, 33-44.
- FORTIN, J., HABENBACHER, W., GRUELLENBERGER, R., WACH, P. & SKRABAL, F. Real-time monitor for hemodynamic beat-to-beat parameters and power spectra analysis of the biosignals. Proceedings of the 20th Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Vol.20 Biomedical Engineering Towards the Year 2000 and Beyond (Cat. No.98CH36286), 1-1 Nov. 1998 1998. 360-363 vol.1.
- FORTIN, J., MARTE, W., GRÜLLENBERGER, R., HACKER, A., HABENBACHER, W., HELLER, A., WAGNER, C., WACH, P. & SKRABAL, F. 2006. Continuous non-invasive blood pressure monitoring using concentrically interlocking control loops. *Comput Biol Med*, 36, 941-57.
- FORTIN, J. H., G; BOJIC, A; HABENBACHER, W; GRULLENBERGER, R; HELLER, A; PACHER, R; WACH, P; SKRABAL, F 2001. Validation and Verification of the Task Force Monitor®. *Results of Clinical Studies for F DA 510(k) n°*, K014063, 1-7.
- FRANKLIN, B. A., THOMPSON, P. D., AL-ZAITI, S. S., ALBERT, C. M., HIVERT, M. F., LEVINE, B. D., LOBELO, F., MADAN, K., SHARRIEF, A. Z. & EIJSVOGELS, T. M. H. 2020a. Exercise-Related Acute Cardiovascular Events and Potential Deleterious Adaptations Following Long-Term Exercise Training: Placing the Risks

- Into Perspective-An Update: A Scientific Statement From the American Heart Association. *Circulation*, 141, e705-e736.
- FRANKLIN, B. A., THOMPSON, P. D., AL-ZAITI, S. S., ALBERT, C. M., HIVERT, M. F., LEVINE, B. D., LOBELO, F., MADAN, K., SHARRIEF, A. Z., EIJSVOGELS, T. M. H., AMERICAN HEART ASSOCIATION PHYSICAL ACTIVITY COMMITTEE OF THE COUNCIL ON, L., CARDIOMETABOLIC, H., COUNCIL ON, C., STROKE, N., COUNCIL ON CLINICAL, C. & STROKE, C. 2020b. Exercise-Related Acute Cardiovascular Events and Potential Deleterious Adaptations Following Long-Term Exercise Training: Placing the Risks Into Perspective-An Update: A Scientific Statement From the American Heart Association. *Circulation*, 141, e705-e736.
- FRASER, A., PAYNE, N., GROCCOTTMASON, R., JANEROT-SJÖBERG, B., SANDBLOM, I., LIND, B., BERGER, K., ROSENHEK, R., SUTHERLAND, G. & BRODIN, L. 1999. 294 Could stress echocardiography with off-line tissue doppler quantification of long-axis function be used to diagnose ischaemia? *European Journal of Echocardiography*, 1.
- GARCIA, M. J., THOMAS, J. D. & KLEIN, A. L. 1998. New Doppler echocardiographic applications for the study of diastolic function. *J Am Coll Cardiol*, 32, 865-75.
- GEORGE, K., WHYTE, G., STEPHENSON, C., SHAVE, R., DAWSON, E., EDWARDS, B. E. N., GAZE, D. & COLLINSON, P. 2004. Postexercise Left Ventricular Function and cTnT in Recreational Marathon Runners. *Medicine & Science in Sports & Exercise*, 36, 1709-1715.
- GEORGE, K., WHYTE, G. P., GREEN, D. J., OXBOROUGH, D., SHAVE, R. E., GAZE, D. & SOMAUROO, J. 2012. The endurance athletes heart: acute stress and chronic adaptation. *Br J Sports Med*, 46 Suppl 1, i29-36.
- GERRITSEN, J., DEKKER, J. M., TENVOORDE, B. J., KOSTENSE, P. J., HEINE, R. J., BOUTER, L. M., HEETHAAR, R. M. & STEHOUWER, C. D. 2001. Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. *Diabetes Care*, 24, 1793-8.
- GEYER, H., CARACCILOLO, G., ABE, H., WILANSKY, S., CARERJ, S., GENTILE, F., NESSER, H. J., KHANDHERIA, B., NARULA, J. & SENGUPTA, P. P. 2010. Assessment of myocardial mechanics using speckle tracking echocardiography: fundamentals and clinical applications. *J Am Soc Echocardiogr*, 23, 351-69; quiz 453-5.
- GIAVARINA, D. & LIPPI, G. 2017. Blood venous sample collection: Recommendations overview and a checklist to improve quality. *Clin Biochem*, 50, 568-573.
- GOEDECKE, J. H., GIBSON, A. S. C., GROBLER, L., COLLINS, M., NOAKES, T. D. & LAMBERT, E. V. 2000. Determinants of the variability in respiratory exchange ratio at rest and during exercise in trained athletes. *American Journal of Physiology-Endocrinology and Metabolism*, 279, E1325-E1334.
- GONZÁLEZ-ALONSO, J., CRANDALL, C. G. & JOHNSON, J. M. 2008. The cardiovascular challenge of exercising in the heat. *The Journal of Physiology*, 586, 45-53.
- GONZÁLEZ-ALONSO, J., MORA-RODRÍGUEZ, R. & COYLE, E. F. 1999. Supine exercise restores arterial blood pressure and skin blood flow despite dehydration and hyperthermia. *Am J Physiol*, 277, H576-83.
- GOSWAMI, N., ROESSLER, A., LACKNER, H. K., SCHNEDITZ, D., GRASSER, E. & HINGHOFER-SZALKAY, H. G. 2009. Heart rate and stroke volume response patterns to augmented orthostatic stress. *Clin Auton Res*, 19, 157-65.

- GRABSKAYA, E., SPIRA, C., HOFFMANN, R., ALTIOK, E., OCKLENBURG, C., HOFFMANN, R. & BECKER, M. 2010. Myocardial rotation but not circumferential strain is transducer angle dependent: a speckle tracking echocardiography study. *Echocardiography*, 27, 809-14.
- GRATZE, G., FORTIN, J., HOLLER, A., GRASENICK, K., PFURTSCHELLER, G., WACH, P., SCHÖNEGGER, J., KOTANKO, P. & SKRABAL, F. 1998. A software package for non-invasive, real-time beat-to-beat monitoring of stroke volume, blood pressure, total peripheral resistance and for assessment of autonomic function. *Comput Biol Med*, 28, 121-42.
- GRESSLIEN, T. & AGEWALL, S. 2016. Troponin and exercise. *Int J Cardiol*, 221, 609-21.
- GRONWALD, T. & HOOS, O. 2020. Correlation properties of heart rate variability during endurance exercise: A systematic review. *Ann Noninvasive Electrocardiol*, 25, e12697.
- GUEZENNEC, C. Y., VALLIER, J. M., BIGARD, A. X. & DUREY, A. 1996. Increase in energy cost of running at the end of a triathlon. *Eur J Appl Physiol Occup Physiol*, 73, 440-5.
- GUTIÉRREZ-CHICO, J. L., ZAMORANO, J. L., PÉREZ DE ISLA, L., OREJAS, M., ALMERÍA, C., RODRIGO, J. L., FERREIRÓS, J., SERRA, V. & MACAYA, C. 2005. Comparison of left ventricular volumes and ejection fractions measured by three-dimensional echocardiography versus by two-dimensional echocardiography and cardiac magnetic resonance in patients with various cardiomyopathies. *Am J Cardiol*, 95, 809-13.
- HAAHR, M. 2021. *RANDOM.ORG: True Random Number Service*. [Online]. Available at: <https://www.random.org>. [Accessed 22 November 2021].
- HADDAD, F., HUNT, S. A., ROSENTHAL, D. N. & MURPHY, D. J. 2008. Right Ventricular Function in Cardiovascular Disease, Part I. *Circulation*, 117, 1436-1448.
- HAM, D. J. & KNEZ, W. L. 2009. An Evaluation of 30-km Cycling Time Trial (TT30) Pacing Strategy Through Time-to-Exhaustion at Average TT30 Pace. *The Journal of Strength & Conditioning Research*, 23.
- HARMS, M. P., WESSELING, K. H., POTT, F., JENSTRUP, M., VAN GOUDOEVER, J., SECHER, N. H. & VAN LIESHOUT, J. J. 1999. Continuous stroke volume monitoring by modelling flow from non-invasive measurement of arterial pressure in humans under orthostatic stress. *Clin Sci (Lond)*, 97, 291-301.
- HARRIS, C. R., MILLMAN, K. J., VAN DER WALT, S. J., GOMMERS, R., VIRTANEN, P., COURNAPEAU, D., WIESER, E., TAYLOR, J., BERG, S., SMITH, N. J., KERN, R., PICUS, M., HOYER, S., VAN KERKWIJK, M. H., BRETT, M., HALDANE, A., DEL RÍO, J. F., WIEBE, M., PETERSON, P., GÉRARD-MARCHANT, P., SHEPPARD, K., REDDY, T., WECKESSER, W., ABBASI, H., GOHLKE, C. & OLIPHANT, T. E. 2020. Array programming with NumPy. *Nature*, 585, 357-362.
- HARRIS, K. M., CRESWELL, L. L., HAAS, T. S. & ET AL. 2017. Death and cardiac arrest in u.s. triathlon participants, 1985 to 2016: A case series. *Annals of Internal Medicine*.
- HART, E., DAWSON, E., RASMUSSEN, P., GEORGE, K., SECHER, N. H., WHYTE, G. & SHAVE, R. 2006. Beta-adrenergic receptor desensitization in man: insight into post-exercise attenuation of cardiac function. *J Physiol*, 577, 717-25.
- HART, E., SHAVE, R., MIDDLETON, N., GEORGE, K., WHYTE, G. & OXBOROUGH, D. 2007. Effect of preload augmentation on pulsed wave and tissue Doppler echocardiographic indices of diastolic function after a marathon. *J Am Soc Echocardiogr*, 20, 1393-9.

- HEAPS, C. L., GONZÁLEZ-ALONSO, J. & COYLE, E. F. 1994. Hypohydration causes cardiovascular drift without reducing blood volume. *Int J Sports Med*, 15, 74-9.
- HEATHERS, J. A. J. 2014. Everything Hertz: methodological issues in short-term frequency-domain HRV. *Frontiers in Physiology*, 5.
- HEIN, I. A. & BRIEN, W. D. O. 1993. Current time-domain methods for assessing tissue motion by analysis from reflected ultrasound echoes-a review. *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control*, 40, 84-102.
- HELAK, J. W. & REICHEK, N. 1981. Quantitation of human left ventricular mass and volume by two-dimensional echocardiography: in vitro anatomic validation. *Circulation*, 63, 1398-1407.
- HELBING, W. A., BOSCH, H. G., MALIEPAARD, C., REBERGEN, S. A., VAN DER GEEST, R. J., HANSEN, B., OTTENKAMP, J., REIBER, J. H. & DE ROOS, A. 1995. Comparison of echocardiographic methods with magnetic resonance imaging for assessment of right ventricular function in children. *Am J Cardiol*, 76, 589-94.
- HELLSTEN, Y. & NYBERG, M. 2015a. Cardiovascular Adaptations to Exercise Training. *Comprehensive Physiology* [Online], 6. Available: <http://europepmc.org/abstract/MED/26756625>
- <https://doi.org/10.1002/cphy.c140080> [Accessed 2015/12/].
- HELLSTEN, Y. & NYBERG, M. 2015b. Cardiovascular Adaptations to Exercise Training. *Compr Physiol*, 6, 1-32.
- HENRY, W. L., DEMARIA, A., GRAMIAK, R., KING, D. L., KISSLO, J. A., POPP, R. L., SAHN, D. J., SCHILLER, N. B., TAJIK, A., TEICHHOLZ, L. E. & WEYMAN, A. E. 1980. Report of the American Society of Echocardiography Committee on Nomenclature and Standards in Two-dimensional Echocardiography. *Circulation*, 62, 212-7.
- HERKNER, H., MÜLLNER, M., DOMANOVITS, H., BUR, A., WOISETSCHLÄGER, C., GAMPER, G., LAGGNER, A. N. & HIRSCHL, M. M. 2000. Use of an age-adjusted Doppler E/A ratio in patients with moderate to severe hypertension. *J Hypertens*, 18, 1477-81.
- HERMANSEN, L., EKBLUM, B. & SALTIN, B. 1970. Cardiac output during submaximal and maximal treadmill and bicycle exercise. *J Appl Physiol*, 29, 82-6.
- HERMANSEN, L. & SALTIN, B. 1969. Oxygen uptake during maximal treadmill and bicycle exercise. *Journal of Applied Physiology*, 26, 31-37.
- HERRING, N. 2018. *Levick's introduction to cardiovascular physiology*, Boca Raton : CRC Press.
- HESSEL, M. H. M., AT SMA, D. E., VAN DER VALK, E. J. M., BAX, W. H., SCHALIJ, M. J. & VAN DER LAARSE, A. 2008. Release of cardiac troponin I from viable cardiomyocytes is mediated by integrin stimulation. *Pflugers Archiv*, 455, 979-986.
- HIGGINS, J. P. T. & THOMPSON, S. G. 2002. Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine*, 21, 1539-1558.
- HINDLEY, D. 2020. "More Than Just a Run in the Park": An Exploration of Parkrun as a Shared Leisure Space. *Leisure Sciences*, 42, 85-105.
- HIRSCHL, M. M., BINDER, M., HERKNER, H., BUR, A., BRUNNER, M., SEIDLER, D., STÜHLINGER, H. G. & LAGGNER, A. N. 1996. Accuracy and reliability of noninvasive continuous finger blood pressure measurement in critically ill patients. *Crit Care Med*, 24, 1684-9.
- HUE, O., LE GALLAIS, D., BOUSSANA, A., CHOLLET, D. & PREFAUT, C. 1999. Ventilatory responses during experimental cycle-run transition in triathletes. *Med Sci Sports Exerc*, 31, 1422-8.

- HUE, O., LE GALLAIS, D., BOUSSANA, A., CHOLLET, D. & PREFAUT, C. 2000. Performance level and cardiopulmonary responses during a cycle-run trial. *Int J Sports Med*, 21, 250-5.
- HUE, O., LE GALLAIS, D., CHOLLET, D., BOUSSANA, A. & PRÉFAUT, C. 1998. The influence of prior cycling on biomechanical and cardiorespiratory response profiles during running in triathletes. *Eur J Appl Physiol Occup Physiol*, 77, 98-105.
- HULTEEN, R. M., SMITH, J. J., MORGAN, P. J., BARNETT, L. M., HALLAL, P. C., COLYVAS, K. & LUBANS, D. R. 2017. Global participation in sport and leisure-time physical activities: A systematic review and meta-analysis. *Prev Med*, 95, 14-25.
- ISAAZ, K., THOMPSON, A., ETHEVENOT, G., CLOEZ, J. L., BREMBILLA, B. & PERNOT, C. 1989. Doppler echocardiographic measurement of low velocity motion of the left ventricular posterior wall. *Am J Cardiol*, 64, 66-75.
- JAMES, J. E. 2004. Critical review of dietary caffeine and blood pressure: a relationship that should be taken more seriously. *Psychosom Med*, 66, 63-71.
- JOUFFROY, R., CAILLE, V., PERROT, S., VIEILLARD-BARON, A., DUBOURG, O. & MANSENCAL, N. 2015. Changes of Cardiac Function During Ultradistance Trail Running. *Am J Cardiol*, 116, 1284-9.
- KANG, S. J., SONG, J. K., SONG, J. M., KANG, D. H., LEE, E. Y., KIM, J., NAM, G. B., CHOI, K. J., KIM, J. J. & KIM, Y. H. 2006. Usefulness of ventricular longitudinal contractility assessed by Doppler tissue imaging in the prediction of reverse remodeling in patients with severe left ventricular systolic dysfunction. *J Am Soc Echocardiogr*, 19, 178-84.
- KAWEL-BOEHM, N., MACEIRA, A., VALSANGIACOMO-BUECHEL, E. R., VOGEL-CLAUSSEN, J., TURKBHEY, E. B., WILLIAMS, R., PLEIN, S., TEE, M., ENG, J. & BLUEMKE, D. A. 2015. Normal values for cardiovascular magnetic resonance in adults and children. *J Cardiovasc Magn Reson*, 17, 29.
- KHANKIRAWATANA, B., KHANKIRAWATANA, S. & PORTER, T. 2004. How should left atrial size be reported? Comparative assessment with use of multiple echocardiographic methods. *American Heart Journal*, 147, 369-374.
- KITABATAKE, A., INOUE, M., ASAO, M., TANOUCHE, J., MASUYAMA, T., ABE, H., MORITA, H., SENDA, S. & MATSUO, H. 1982. Transmitral blood flow reflecting diastolic behavior of the left ventricle in health and disease--a study by pulsed Doppler technique. *Jpn Circ J*, 46, 92-102.
- KLEINNIBBELINK, G., HULSHOF, H. G., VAN DIJK, A. P. J., TEN CATE, T., GEORGE, K. P., OXBOROUGH, D. L. & THIJSSSEN, D. H. J. 2021a. Effects of Preload Manipulation on Right Ventricular Contractility: Invasive Pressure-Area Loop versus Non-invasive Strain-Area Loop. *J Am Soc Echocardiogr*, 34, 447-449.
- KLEINNIBBELINK, G., VAN DIJK, A. P. J., FORNASIERO, A., SPERETTA, G. F., JOHNSON, C., HOPMAN, M. T. E., SCULTHORPE, N., GEORGE, K. P., SOMAUROO, J. D., THIJSSSEN, D. H. J. & OXBOROUGH, D. L. 2021b. Exercise-Induced Cardiac Fatigue after a 45-min Bout of High-Intensity Running Exercise Is Not Altered under Hypoxia. *J Am Soc Echocardiogr*.
- KOHRT, W. M., MORGAN, D. W., BATES, B. & SKINNER, J. S. 1987. Physiological responses of triathletes to maximal swimming, cycling, and running. *Med Sci Sports Exerc*, 19, 51-5.
- KOLLER, A., MAIR, J., SCHOBERSBERGER, W., WOHLFARTER, T., HAID, C., MAYR, M., VILLIGER, B., FREY, W. & PUSCHENDORF, B. 1998. Effects of prolonged strenuous endurance exercise on plasma myosin heavy chain fragments and other muscular proteins. Cycling vs running. *J Sports Med Phys Fitness*, 38, 10-7.

- KORINEK, J., WANG, J., SENGUPTA, P. P., MIYAZAKI, C., KJAERGAARD, J., MCMAHON, E., ABRAHAM, T. P. & BELOHLAVEK, M. 2005. Two-dimensional strain--a Doppler-independent ultrasound method for quantitation of regional deformation: validation in vitro and in vivo. *J Am Soc Echocardiogr*, 18, 1247-53.
- KRAUT, J. A. & MADIAS, N. E. 2014. Lactic Acidosis. *New England Journal of Medicine*, 371, 2309-2319.
- KREIDER, R. B., BOONE, T., THOMPSON, W. R., BURKES, S. & CORTES, C. W. 1988. Cardiovascular and thermal responses of triathlon performance. *Med Sci Sports Exerc*, 20, 385-90.
- KUMAR, A. & CANNON, C. P. 2009. Acute Coronary Syndromes: Diagnosis and Management, Part I. *Mayo Clinic Proceedings*, 84, 917-938.
- LA GERCHE, A., BOYLE, A., WILSON, A. M. & PRIOR, D. L. 2004. No evidence of sustained myocardial injury following an Ironman distance triathlon. *Int J Sports Med*, 25, 45-9.
- LA GERCHE, A., BURNS, A. T., MOONEY, D. J., INDER, W. J., TAYLOR, A. J., BOGAERT, J., MACISAAC, A. I., HEIDBÜCHEL, H. & PRIOR, D. L. 2012a. Exercise-induced right ventricular dysfunction and structural remodelling in endurance athletes. *Eur Heart J*, 33, 998-1006.
- LA GERCHE, A., BURNS, A. T., TAYLOR, A. J., MACISAAC, A. I., HEIDBÜCHEL, H. & PRIOR, D. L. 2012b. Maximal oxygen consumption is best predicted by measures of cardiac size rather than function in healthy adults. *Eur J Appl Physiol*, 112, 2139-47.
- LA GERCHE, A., CONNELLY, K. A., MOONEY, D. J., MACISAAC, A. I. & PRIOR, D. L. 2008. Biochemical and functional abnormalities of left and right ventricular function after ultra-endurance exercise. *Heart*, 94, 860-6.
- LA GERCHE, A., INDER, W. J., ROBERTS, T. J., BROSNAN, M. J., HEIDBUCHEL, H. & PRIOR, D. L. 2015. Relationship between Inflammatory Cytokines and Indices of Cardiac Dysfunction following Intense Endurance Exercise. *PLoS One*, 10, e0130031.
- LA GERCHE, A. & PRIOR, D. L. 2007. Exercise--is it possible to have too much of a good thing? *Heart Lung Circ*, 16 Suppl 3, S102-4.
- LAAKSONEN, M. S., KIVELÄ, R., KYRÖLÄINEN, H., SIPILÄ, S., SELÄNNE, H., LAUTAMÄKI, R., NUUTILA, P., KNUUTI, J., KALLIOKOSKI, K. K. & KOMI, P. V. 2006. Effects of exhaustive stretch-shortening cycle exercise on muscle blood flow during exercise. *Acta Physiol (Oxf)*, 186, 261-70.
- LANFRANCHI, P. A. & SOMERS, V. K. 2002. Arterial baroreflex function and cardiovascular variability: interactions and implications. *Am J Physiol Regul Integr Comp Physiol*, 283, R815-26.
- LANG, R. M., BADANO, L. P., MOR-AVI, V., AFILALO, J., ARMSTRONG, A., ERNANDE, L., FLACHSKAMPF, F. A., FOSTER, E., GOLDSTEIN, S. A., KUZNETSOVA, T., LANCELLOTTI, P., MURARU, D., PICARD, M. H., RIETZSCHEL, E. R., RUDSKI, L., SPENCER, K. T., TSANG, W. & VOIGT, J. U. 2015. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*, 28, 1-39.e14.
- LANG, R. M., BIERIG, M., DEVEREUX, R. B., FLACHSKAMPF, F. A., FOSTER, E. & PELLIKKA, P. A. 2005. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*, 18.

- LE GOFF, C., FARRE SEGURA, J., DUFOUR, P., KAUX, J. F. & CAVALIER, E. 2020. Intense sport practices and cardiac biomarkers. *Clin Biochem*, 79, 1-8.
- LEETMAA, T. H., DAM, A., GLINTBORG, D. & MARKENVARD, J. D. 2008. Myocardial response to a triathlon in male athletes evaluated by Doppler tissue imaging and biochemical parameters. *Scand J Med Sci Sports*, 18, 698-705.
- LEGAZ-ARRESE, A., LOPEZ-LAVAL, I., GEORGE, K., JOSE PUENTE-LANZAROTE, J., CASTELLAR-OTIN, C., REVERTER-MASIA, J. & MUNGUIA-IZQUIERDO, D. 2015. Individual variability of high-sensitivity cardiac troponin levels after aerobic exercise is not mediated by exercise mode. *Biomarkers*, 20, 219-24.
- LEWICKA-POTOCKA, Z., DABROWSKA-KUGACKA, A., LEWICKA, E., GALASKA, R., DANILOWICZ-SZYMANOWICZ, L., FARAN, A., NABIALEK-TROJANOWSKA, I., KUBIK, M., KALETA-DUSS, A. M. & RACZAK, G. 2020. Right Ventricular Diastolic Dysfunction after Marathon Run. *Int J Environ Res Public Health*, 17.
- LINDSAY, M. M. & DUNN, F. G. 2007. Biochemical evidence of myocardial fibrosis in veteran endurance athletes. *Br J Sports Med*, 41, 447-52.
- LORD, R., SOMAUROO, J., STEMBRIDGE, M., JAIN, N., HOFFMAN, M. D., GEORGE, K., JONES, H., SHAVE, R., HADDAD, F., ASHLEY, E. & OXBOROUGH, D. 2015. The right ventricle following ultra-endurance exercise: insights from novel echocardiography and 12-lead electrocardiography. *Eur J Appl Physiol*, 115, 71-80.
- LORD, R. N., UTOMI, V., OXBOROUGH, D. L., CURRY, B. A., BROWN, M. & GEORGE, K. P. 2018. Left ventricular function and mechanics following prolonged endurance exercise: an update and meta-analysis with insights from novel techniques. *Eur J Appl Physiol*, 118, 1291-1299.
- LORENZ, D. S., REIMAN, M. P. & WALKER, J. C. 2010. Periodization: current review and suggested implementation for athletic rehabilitation. *Sports Health*, 2, 509-18.
- LOW, P. A. 1996. Assessment: Clinical autonomic testing report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology. *Neurology*, 46, 873-80.
- LUCIA, A., SERRATOSA, L., SABORIDO, A., PARDO, J., BORAITA, A., MORAN, M., BANDRES, F., MEGIAS, A. & CHICHARRO, J. L. 1999. Short-term effects of marathon running: no evidence of cardiac dysfunction. *Med Sci Sports Exerc*, 31, 1414-21.
- MALIK, M. 1996. Heart Rate Variability. *Circulation*, 93, 1043-1065.
- MALLIANI, A. 1999. The Pattern of Sympathovagal Balance Explored in the Frequency Domain. *Physiology*, 14, 111-117.
- MARKS, L. A. & GROCH, A. 2000. Optimizing cuff width for noninvasive measurement of blood pressure. *Blood Press Monit*, 5, 153-8.
- MATSUI, H., KITAMURA, K. & MIYAMURA, M. 1978. Oxygen uptake and blood flow of the lower limb in maximal treadmill and bicycle exercise. *European Journal of Applied Physiology and Occupational Physiology*, 40, 57-62.
- MATSUO, H., KITABATAKE, A., HAYASHI, T., ASAO, M. & TERAU, Y. 1977. Intracardiac flow dynamics with bi-directional ultrasonic pulsed Doppler technique. *Jpn Circ J*, 41, 515-28.
- MCGAVOCK, J., HAYKOWSKY, M., WARBURTON, D., TAYLOR, D., QUINNEY, A. & WELSH, R. 2003. Left ventricular systolic performance during prolonged strenuous exercise in female triathletes. *Dyn Med*, 2, 2.
- MCGAVOCK, J. M., WARBURTON, D. E. R., TAYLOR, D., WELSH, R. C., QUINNEY, H. A. & HAYKOWSKY, M. J. 2002. The effects of prolonged strenuous exercise on

- left ventricular function: A brief review. *Heart & Lung: The Journal of Acute and Critical Care*, 31, 279-294.
- MEHTA, R., GAZE, D., MOHAN, S., WILLIAMS, K. L., SPRUNG, V., GEORGE, K., JEFFRIES, R., HUDSON, Z., PERRY, M. & SHAVE, R. 2012. Post-exercise cardiac troponin release is related to exercise training history. *Int J Sports Med*, 33, 333-7.
- MIDDLETON, N., GEORGE, K., WHYTE, G., GAZE, D., COLLINSON, P. & SHAVE, R. 2008. Cardiac troponin T release is stimulated by endurance exercise in healthy humans. *J Am Coll Cardiol*, 52, 1813-4.
- MIDDLETON, N., SHAVE, R., GEORGE, K., WHYTE, G., HART, E. & ATKINSON, G. 2006. Left ventricular function immediately following prolonged exercise: A meta-analysis. *Med Sci Sports Exerc*, 38, 681-7.
- MIDDLETON, N., SHAVE, R., GEORGE, K., WHYTE, G., SIMPSON, R., FLORIDA-JAMES, G. & GAZE, D. 2007. Impact of repeated prolonged exercise bouts on cardiac function and biomarkers. *Med Sci Sports Exerc*, 39, 83-90.
- MILLARD-STAFFORD, M., SPARLING, P. B., ROSSKOPF, L. B. & DICARLO, L. J. 1991. Differences in Peak Physiological Responses During Running, Cycling and Swimming. *The Journal of Strength & Conditioning Research*, 5, 213-218.
- MILLET, G. P. & BENTLEY, D. J. 2004. The physiological responses to running after cycling in elite junior and senior triathletes. *Int J Sports Med*, 25, 191-7.
- MILLET, G. P. & VLECK, V. E. 2000. Physiological and biomechanical adaptations to the cycle to run transition in Olympic triathlon: review and practical recommendations for training. *British Journal of Sports Medicine*, 34, 384.
- MILLET, G. P., VLECK, V. E. & BENTLEY, D. J. 2009. Physiological differences between cycling and running: lessons from triathletes. *Sports Med*, 39, 179-206.
- MILLET, G. P., VLECK, V. E. & BENTLEY, D. J. 2011. Physiological requirements in triathlon. *Journal of Human Sport and Exercise*, 6, 184-204.
- MIURA, H. K., K.; ISHIKO; T. 1994. Characteristics of VO₂max and ventilatory threshold in triathletes. *Jpn J Exerc Sports Physiol*, 1, 99-106.
- MODESTO, K. M., CAUDURO, S., DISPENZIERI, A., KHANDHERIA, B., BELOHLAVEK, M., LYSYANSKY, P., FRIEDMAN, Z., GERTZ, M. & ABRAHAM, T. P. 2006. Two-dimensional acoustic pattern derived strain parameters closely correlate with one-dimensional tissue Doppler derived strain measurements. *European Journal of Echocardiography*, 7, 315-321.
- MONTAIN, S. J. & COYLE, E. F. 1992. Influence of graded dehydration on hyperthermia and cardiovascular drift during exercise. *J Appl Physiol (1985)*, 73, 1340-50.
- MOR-AVI, V., LANG, R. M., BADANO, L. P., BELOHLAVEK, M., CARDIM, N. M., DERUMEAUX, G., GALDERISI, M., MARWICK, T., NAGUEH, S. F., SENGUPTA, P. P., SICARI, R., SMISETH, O. A., SMULEVITZ, B., TAKEUCHI, M., THOMAS, J. D., VANNAN, M., VOIGT, J. U. & ZAMORANO, J. L. 2011. Current and evolving echocardiographic techniques for the quantitative evaluation of cardiac mechanics: ASE/EAE consensus statement on methodology and indications endorsed by the Japanese Society of Echocardiography. *J Am Soc Echocardiogr*, 24, 277-313.
- MYBURGH, E., KRUGER, M. & SAAYMAN, M. 2018. Aspects influencing the commitment of endurance athletes: a tourism perspective. *Journal of Sport & Tourism*, 22, 275-301.
- NAGUEH, S. F., SMISETH, O. A., APPLETON, C. P., BYRD, B. F., 3RD, DOKAINISH, H., EDVARDBSEN, T., FLACHSKAMPF, F. A., GILLEBERT, T. C., KLEIN, A. L., LANCELLOTTI, P., MARINO, P., OH, J. K., POPESCU, B. A. & WAGGONER, A. D. 2016. Recommendations for the Evaluation of Left Ventricular Diastolic Function

- by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr*, 29, 277-314.
- NEILAN, T. G., JANUZZI, J. L., LEE-LEWANDROWSKI, E., TON-NU, T. T., YOERGER, D. M., JASSAL, D. S., LEWANDROWSKI, K. B., SIEGEL, A. J., MARSHALL, J. E., DOUGLAS, P. S., LAWLOR, D., PICARD, M. H. & WOOD, M. J. 2006. Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. *Circulation*, 114, 2325-33.
- NEUMAYR, G., PFISTER, R., MITTERBAUER, G., MAURER, A., GAENZER, H., STURM, W. & HOERTNAGL, H. 2002. Effect of the "Race Across The Alps" in elite cyclists on plasma cardiac troponins I and T. *Am J Cardiol*, 89, 484-6.
- NIE, J., GEORGE, K. P., TONG, T. K., TIAN, Y. & SHI, Q. 2011. Effect of repeated endurance runs on cardiac biomarkers and function in adolescents. *Med Sci Sports Exerc*, 43, 2081-8.
- NIEMAN, D. C., LUO, B., DRÉAU, D., HENSON, D. A., SHANELY, R. A., DEW, D. & MEANEY, M. P. 2014. Immune and inflammation responses to a 3-day period of intensified running versus cycling. *Brain Behav Immun*, 39, 180-5.
- NIKOLAIDIS, P. T., VILLIGER, E. & KNECHTLE, B. 2021. Participation and Performance Trends in the ITU Duathlon World Championship From 2003 to 2017. *J Strength Cond Res*, 35, 1127-1133.
- NIKOLAIDIS, P. T., VILLIGER, E., VICTOR SOUSA, C., ROSEMANN, T. & KNECHTLE, B. 2019. The Effect of Aging on Pacing Strategies in Short and Long Distance Duathlon. *Exp Aging Res*, 45, 223-233.
- NOTTIN, S., DOUCENDE, G., SCHUSTER, I., TANGUY, S., DAUZAT, M. & OBERT, P. 2009. Alteration in left ventricular strains and torsional mechanics after ultralong duration exercise in athletes. *Circ Cardiovasc Imaging*, 2, 323-30.
- O'KEEFE, E. L., TORRES-ACOSTA, N., O'KEEFE, J. H. & LAVIE, C. J. 2020. Training for Longevity: The Reverse J-Curve for Exercise. *Mo Med*, 117, 355-361.
- O'KEEFE, J. H., PATIL, H. R., LAVIE, C. J., MAGALSKI, A., VOGEL, R. A. & MCCULLOUGH, P. A. 2012. Potential Adverse Cardiovascular Effects From Excessive Endurance Exercise. *Mayo Clinic Proceedings*, 87, 587-595.
- O'CONNOR, K., LANCELLOTTI, P., DONAL, E. & PIÉRARD, L. A. 2010. Exercise echocardiography in severe asymptomatic aortic stenosis. *Archives of Cardiovascular Diseases*, 103, 262-269.
- OLCINA, G., PEREZ-SOUSA, M. Á., ESCOBAR-ALVAREZ, J. A. & TIMÓN, R. 2019. Effects of Cycling on Subsequent Running Performance, Stride Length, and Muscle Oxygen Saturation in Triathletes. *Sports (Basel, Switzerland)*, 7, 115.
- OMMEN, S. R., NISHIMURA, R. A., APPLETON, C. P., MILLER, F. A., OH, J. K., REDFIELD, M. M. & TAJIK, A. J. 2000. Clinical utility of Doppler echocardiography and tissue Doppler imaging in the estimation of left ventricular filling pressures: A comparative simultaneous Doppler-catheterization study. *Circulation*, 102, 1788-94.
- ONIKI, T., HASHIMOTO, Y., SHIMIZU, S., KAKUTA, T., YAJIMA, M. & NUMANO, F. 1992. Effect of increasing heart rate on Doppler indices of left ventricular performance in healthy men. *British Heart Journal*, 68, 425.
- OPIE, L. H. 2004. *Heart physiology : from cell to circulation*, Philadelphia, Pa., London, Philadelphia, Pa., London : Lippincott Williams & Wilkins.
- OXBOROUGH, D., BIRCH, K., SHAVE, R. & GEORGE, K. 2010. "Exercise-induced cardiac fatigue"--a review of the echocardiographic literature. *Echocardiography*, 27, 1130-40.

- OXBOROUGH, D., SHARMA, S., SHAVE, R., WHYTE, G., BIRCH, K., ARTIS, N., BATTERHAM, A. M. & GEORGE, K. 2012. The right ventricle of the endurance athlete: the relationship between morphology and deformation. *J Am Soc Echocardiogr*, 25, 263-71.
- OXBOROUGH, D., SHAVE, R., MIDDLETON, N., WHYTE, G., FORSTER, J. & GEORGE, K. 2006. The impact of marathon running upon ventricular function as assessed by 2D, Doppler, and tissue-Doppler echocardiography. *Echocardiography*, 23, 635-41.
- OXBOROUGH, D., SHAVE, R., WARBURTON, D., WILLIAMS, K., OXBOROUGH, A., CHARLESWORTH, S., FOULDS, H., HOFFMAN, M. D., BIRCH, K. & GEORGE, K. 2011. Dilatation and dysfunction of the right ventricle immediately after ultraendurance exercise: exploratory insights from conventional two-dimensional and speckle tracking echocardiography. *Circ Cardiovasc Imaging*, 4, 253-63.
- PAGANI, M., MONTANO, N., PORTA, A., MALLIANI, A., ABBOUD, F. M., BIRKETT, C. & SOMERS, V. K. 1997. Relationship between spectral components of cardiovascular variabilities and direct measures of muscle sympathetic nerve activity in humans. *Circulation*, 95, 1441-8.
- PARATI, G., CASADEI, R., GROPELLI, A., DI RIENZO, M. & MANCIA, G. 1989. Comparison of finger and intra-arterial blood pressure monitoring at rest and during laboratory testing. *Hypertension*, 13, 647-55.
- PARATI, G., ONGARO, G., BILO, G., GLAVINA, F., CASTIGLIONI, P., DI RIENZO, M. & MANCIA, G. 2003. Non-invasive beat-to-beat blood pressure monitoring: new developments. *Blood pressure monitoring*, 8, 31-36.
- PARK, C. H., KIM, K. B., HAN, J., JI, J. G. & KWAK, Y. S. 2014. Cardiac Damage Biomarkers Following a Triathlon in Elite and Non-elite Triathletes. *Korean J Physiol Pharmacol*, 18, 419-23.
- PASSAGLIA, D. G., EMED, L. G., BARBERATO, S. H., GUERIOS, S. T., MOSER, A. I., SILVA, M. M., ISHIE, E., GUARITA-SOUZA, L. C., COSTANTINI, C. R. & FARIA-NETO, J. R. 2013. Acute effects of prolonged physical exercise: evaluation after a twenty-four-hour ultramarathon. *Arq Bras Cardiol*, 100, 21-8.
- PEELING, P. D., BISHOP, D. J. & LANDERS, G. J. 2005. Effect of swimming intensity on subsequent cycling and overall triathlon performance. *British Journal of Sports Medicine*, 39, 960.
- PETERS, G. L., BINDER, S. K. & CAMPBELL, N. R. 1999. The effect of crossing legs on blood pressure: a randomized single-blind cross-over study. *Blood Press Monit*, 4, 97-101.
- PICKERING, T. G., HALL, J. E., APPEL, L. J., FALKNER, B. E., GRAVES, J., HILL, M. N., JONES, D. W., KURTZ, T., SHEPS, S. G. & ROCCELLA, E. J. 2005. Recommendations for blood pressure measurement in humans and experimental animals: part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. *Circulation*, 111, 697-716.
- PICKERING, T. G., SHIMBO, D. & HAAS, D. 2006. Ambulatory blood-pressure monitoring. *N Engl J Med*, 354, 2368-74.
- POLLOCK, J. D. & MAKARYUS, A. N. 2021. Physiology, Cardiac Cycle. *StatPearls*. Treasure Island (FL): StatPearls Publishing
- Copyright © 2021, StatPearls Publishing LLC.
- POMERANZ, B., MACAULAY, R. J., CAUDILL, M. A., KUTZ, I., ADAM, D., GORDON, D., KILBORN, K. M., BARGER, A. C., SHANNON, D. C., COHEN, R. J. & ET AL.

1985. Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol*, 248, H151-3.
- POTTER, J. F., WATSON, R. D., SKAN, W. & BEEVERS, D. G. 1986. The pressor and metabolic effects of alcohol in normotensive subjects. *Hypertension*, 8, 625-31.
- PRINGLE, J. S., DOUST, J. H., CARTER, H., TOLFREY, K., CAMPBELL, I. T., SAKKAS, G. K. & JONES, A. M. 2003. Oxygen uptake kinetics during moderate, heavy and severe intensity "submaximal" exercise in humans: the influence of muscle fibre type and capillarisation. *Eur J Appl Physiol*, 89, 289-300.
- PRIOR, D. L. & LA GERCHE, A. 2012. The athlete's heart. *Heart*, 98, 947.
- PUJADAS, S., DONATE, M., LI, C. H., MERCHAN, S., CABANILLAS, A., ALOMAR, X., PONS-LLADO, G., SERRA-GRIMA, R. & CARRERAS, F. 2018. Myocardial remodelling and tissue characterisation by cardiovascular magnetic resonance (CMR) in endurance athletes. *BMJ Open Sport Exerc Med*, 4, e000422.
- PUMPRLA, J., HOWORKA, K., GROVES, D., CHESTER, M. & NOLAN, J. 2002. Functional assessment of heart rate variability: physiological basis and practical applications. *Int J Cardiol*, 84, 1-14.
- REHMAN, S. & NELSON, V. L. 2021. Blood Pressure Measurement. *StatPearls*. Treasure Island (FL): StatPearls Publishing
- Copyright © 2021, StatPearls Publishing LLC.
- REYES DEL PASO, G. A., LANGEWITZ, W., MULDER, L. J., VAN ROON, A. & DUSCHEK, S. 2013. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. *Psychophysiology*, 50, 477-87.
- REZK, C. C., MARRACHE, R. C., TINUCCI, T., MION, D., JR. & FORJAZ, C. L. 2006. Post-resistance exercise hypotension, hemodynamics, and heart rate variability: influence of exercise intensity. *Eur J Appl Physiol*, 98, 105-12.
- RIFAI, N., DOUGLAS, P. S., O'TOOLE, M., RIMM, E. & GINSBURG, G. S. 1999. Cardiac troponin T and I, echocardiographic [correction of electrocardiographic] wall motion analyses, and ejection fractions in athletes participating in the Hawaii Ironman Triathlon. *Am J Cardiol*, 83, 1085-9.
- RODEVAN, O., BJORNERHEIM, R., LJOSLAND, M., MAEHLE, J., SMITH, H. J. & IHLEN, H. 1999. Left atrial volumes assessed by three- and two-dimensional echocardiography compared to MRI estimates. *Int J Card Imaging*, 15, 397-410.
- RONCONI, M. & ALVERO-CRUZ, J. R. 2011. Respuesta de la frecuencia cardiaca y consumo de oxígeno de atletas varones en competiciones de duatlón sprint. *Apunts. Medicina de l'Esport*, 46, 183-188.
- ROWELL, L. B. 1974. Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev*, 54, 75-159.
- ROWLAND, T. 2009. Endurance Athletes' Stroke Volume Response to Progressive Exercise. *Sports Medicine*, 39, 687-695.
- RUDSKI, L. G., LAI, W. W., AFILALO, J., HUA, L., HANDSCHUMACHER, M. D., CHANDRASEKARAN, K., SOLOMON, S. D., LOUIE, E. K. & SCHILLER, N. B. 2010. Guidelines for the echocardiographic assessment of the right heart in adults: a report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *J Am Soc Echocardiogr*, 23, 685-713; quiz 786-8.
- RUST, C. A., KNECHTLE, B., KNECHTLE, P., PFEIFER, S., ROSEMANN, T., LEPERS, R. & SENN, O. 2013. Gender difference and age-related changes in performance at the long-distance duathlon. *J Strength Cond Res*, 27, 293-301.

- SAHN, D. J., DEMARIA, A., KISSLO, J. & WEYMAN, A. 1978. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation*, 58, 1072-83.
- SAM, C. & BORDONI, B. 2021. Physiology, Acetylcholine. *StatPearls*. Treasure Island (FL): StatPearls Publishing
- Copyright © 2021, StatPearls Publishing LLC.
- SANDERCOCK, G. R., BROMLEY, P. D. & BRODIE, D. A. 2005. The reliability of short-term measurements of heart rate variability. *Int J Cardiol*, 103, 238-47.
- SARAIVA, R. M., DEMIRKOL, S., BUAKHAMSRI, A., GREENBERG, N., POPOVIĆ, Z. B., THOMAS, J. D. & KLEIN, A. L. 2010. Left atrial strain measured by two-dimensional speckle tracking represents a new tool to evaluate left atrial function. *J Am Soc Echocardiogr*, 23, 172-80.
- SAREBAN, M., WINKERT, K., SPERLICH, B., BERGER, M. M., NIEBAUER, J., STEINACKER, J. M. & TREFF, G. 2018. Speckle tracking-derived bi-atrial strain before and after eleven weeks of training in elite rowers. *Sci Rep*, 8, 14300.
- SATO, N., MIYAKE, S., AKATSU, J. & KUMASHIRO, M. 1995. Power spectral analysis of heart rate variability in healthy young women during the normal menstrual cycle. *Psychosom Med*, 57, 331-5.
- SCHABORT, E. J., KILLIAN, S. C., ST CLAIR GIBSON, A., HAWLEY, J. A. & NOAKES, T. D. 2000. Prediction of triathlon race time from laboratory testing in national triathletes. *Med Sci Sports Exerc*, 32, 844-9.
- SCHARHAG, J., SCHNEIDER, G., URHAUSEN, A., ROCHETTE, V., KRAMANN, B. & KINDERMANN, W. 2002. Athlete's heart. *Journal of the American College of Cardiology*, 40, 1856-1863.
- SCHARHAG, J., SHAVE, R., GEORGE, K., WHYTE, G. & KINDERMANN, W. 2008. "Exercise-induced increases in cardiac troponins in endurance athletes: a matter of exercise duration and intensity?". *Clin Res Cardiol*, 97, 62-3; author reply 61.
- SCHERR, J., BRAUN, S., SCHUSTER, T., HARTMANN, C., MOEHLENKAMP, S., WOLFARTH, B., PRESSLER, A. & HALLE, M. 2011. 72-h kinetics of high-sensitive troponin T and inflammatory markers after marathon. *Med Sci Sports Exerc*, 43, 1819-27.
- SCHMID, E., HILBERATH, J. N., BLUMENSTOCK, G., SHEKAR, P. S., KLING, S., SHERNAN, S. K., ROSENBERGER, P. & NOWAK-MACHEN, M. 2015. Tricuspid annular plane systolic excursion (TAPSE) predicts poor outcome in patients undergoing acute pulmonary embolectomy. *Heart, lung and vessels*, 7, 151-158.
- SCHNEIDER, D. A. & POLLACK, J. 1991. Ventilatory threshold and maximal oxygen uptake during cycling and running in female triathletes. *Int J Sports Med*, 12, 379-83.
- SCHNELL, F., CLAESSEN, G., LA GERCHE, A., BOGAERT, J., LENTZ, P.-A., CLAUS, P., MABO, P., CARRÉ, F. & HEIDBUHEL, H. 2016. Subepicardial delayed gadolinium enhancement in asymptomatic athletes: let sleeping dogs lie? *British Journal of Sports Medicine*, 50, 111.
- SCHROEDER, E. B., LIAO, D., CHAMBLESS, L. E., PRINEAS, R. J., EVANS, G. W. & HEISS, G. 2003. Hypertension, Blood Pressure, and Heart Rate Variability. *Hypertension*, 42, 1106-1111.
- SEILER, K. S. & KJERLAND, G. Ø. 2006. Quantifying training intensity distribution in elite endurance athletes: is there evidence for an "optimal" distribution? *Scandinavian Journal of Medicine & Science in Sports*, 16, 49-56.
- SEILER, S., HAUGEN, O. & KUFFEL, E. 2007. Autonomic recovery after exercise in trained athletes: intensity and duration effects. *Med Sci Sports Exerc*, 39, 1366-73.

- SERRANO-OSTARIZ, E., LEGAZ-ARRESE, A., TERREROS-BLANCO, J. L., LOPEZ-RAMON, M., CREMADES-ARROYOS, D., CARRANZA-GARCIA, L. E., IZQUIERDO-ALVAREZ, S. & BOCOS-TERRAZ, P. 2009. Cardiac biomarkers and exercise duration and intensity during a cycle-touring event. *Clin J Sport Med*, 19, 293-9.
- SERRANO OSTARIZ, E., LÓPEZ RAMÓN, M., CREMADES ARROYOS, D., IZQUIERDO ÁLVAREZ, S., CATALÁN EDO, P., BAQUER SAHÚN, C. & LEGAZ ARRESE, A. 2013. Post-exercise left ventricular dysfunction measured after a long-duration cycling event. *BMC Research Notes*, 6, 211-211.
- SHAFFER, F. & GINSBERG, J. P. 2017. An Overview of Heart Rate Variability Metrics and Norms. *Front Public Health*, 5, 258.
- SHAFFER, F., MCCRATY, R. & ZERR, C. L. 2014a. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Front Psychol*, 5, 1040.
- SHAFFER, F., MCCRATY, R. & ZERR, C. L. 2014b. A healthy heart is not a metronome: an integrative review of the heart's anatomy and heart rate variability. *Frontiers in psychology*, 5, 1040-1040.
- SHAPIRO, D., JAMNER, L. D., LANE, J. D., LIGHT, K. C., MYRTEK, M., SAWADA, Y. & STEPTOE, A. 1996. Blood pressure publication guidelines. *Psychophysiology*, 33, 1-12.
- SHARMA, R., O'DRISCOLL, J. M., SAHA, A., SRITHARAN, M., SUTTON, R. & ROSEN, S. D. 2015. Differing autonomic responses to dobutamine stress in the presence and absence of myocardial ischaemia. *The Journal of physiology*, 593, 2171-2184.
- SHAVE, R., BAGGISH, A., GEORGE, K., WOOD, M., SCHARHAG, J., WHYTE, G., GAZE, D. & THOMPSON, P. D. 2010a. Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. *J Am Coll Cardiol*, 56, 169-76.
- SHAVE, R., DAWSON, E., WHYTE, G., GEORGE, K., GAZE, D. & COLLINSON, P. 2004. Altered cardiac function and minimal cardiac damage during prolonged exercise. *Medicine and science in sports and exercise*, 36, 1098-1103.
- SHAVE, R., GEORGE, K. P., ATKINSON, G., HART, E., MIDDLETON, N., WHYTE, G., GAZE, D. & COLLINSON, P. O. 2007. Exercise-induced cardiac troponin T release: a meta-analysis. *Med Sci Sports Exerc*, 39, 2099-106.
- SHAVE, R. & OXBOROUGH, D. 2012. Exercise-induced cardiac injury: evidence from novel imaging techniques and highly sensitive cardiac troponin assays. *Prog Cardiovasc Dis*, 54, 407-15.
- SHAVE, R., ROSS, P., LOW, D., GEORGE, K. & GAZE, D. 2010b. Cardiac troponin I is released following high-intensity short-duration exercise in healthy humans. *Int J Cardiol*, 145, 337-339.
- SHAVE, R. E., DAWSON, E., WHYTE, G., GEORGE, K., BALL, D., GAZE, D. C. & COLLINSON, P. O. 2002. Evidence of exercise-induced cardiac dysfunction and elevated cTnT in separate cohorts competing in an ultra-endurance mountain marathon race. *Int J Sports Med*, 23, 489-94.
- SHAVE, R. E., LIEBERMAN, D. E., DRANE, A. L., BROWN, M. G., BATTERHAM, A. M., WORTHINGTON, S., ATENCIA, R., FELTRER, Y., NEARY, J., WEINER, R. B., WASFY, M. M. & BAGGISH, A. L. 2019. Selection of endurance capabilities and the trade-off between pressure and volume in the evolution of the human heart. *Proc Natl Acad Sci U S A*, 116, 19905-19910.
- SINGH, S. & GOYAL, A. 2007. The origin of echocardiography: a tribute to Inge Edler. *Texas Heart Institute journal*, 34, 431-438.

- SIVESGAARD, K., CHRISTENSEN, S. D., NYGAARD, H., HASENKAM, J. M. & SLOTH, E. 2009. Speckle tracking ultrasound is independent of insonation angle and gain: an in vitro investigation of agreement with sonomicrometry. *J Am Soc Echocardiogr*, 22, 852-8.
- SOU, S. M., PUELACHER, C., TWERENBOLD, R., WAGENER, M., HONEGGER, U., REICHLIN, T., SCHAERLI, N., PRETRE, G., ABACHERLI, R., JAEGER, C., RUBINI GIMENEZ, M., WILD, D., RENTSCH, K. M., ZELLWEGGER, M. J. & MUELLER, C. 2016. Direct comparison of cardiac troponin I and cardiac troponin T in the detection of exercise-induced myocardial ischemia. *Clin Biochem*, 49, 421-432.
- SPARKS, S. A., CABLE, N. T., DORAN, D. A. & MACLAREN, D. P. 2005. Influence of environmental temperature on duathlon performance. *Ergonomics*, 48, 1558-67.
- STEVENS, P. M. 1966. Cardiovascular dynamics during orthostasis and the influence of intravascular instrumentation. *Am J Cardiol*, 17, 211-8.
- STEVINSON, C., WILTSHIRE, G. & HICKSON, M. 2015. Facilitating participation in health-enhancing physical activity: a qualitative study of parkrun. *Int J Behav Med*, 22, 170-7.
- STEWART, G. M., CHAN, J., YAMADA, A., KAVANAGH, J. J., HASELER, L. J., SHIINO, K. & SABAPATHY, S. 2017. Impact of high-intensity endurance exercise on regional left and right ventricular myocardial mechanics. *European Heart Journal - Cardiovascular Imaging*, 18, 688-696.
- STEWART, G. M., KAVANAGH, J. J., KOERBIN, G., SIMMONDS, M. J. & SABAPATHY, S. 2014. Cardiac electrical conduction, autonomic activity and biomarker release during recovery from prolonged strenuous exercise in trained male cyclists. *Eur J Appl Physiol*, 114, 1-10.
- STEWART, G. M., YAMADA, A., HASELER, L. J., KAVANAGH, J. J., CHAN, J., KOERBIN, G., WOOD, C. & SABAPATHY, S. 2016. Influence of exercise intensity and duration on functional and biochemical perturbations in the human heart. *J Physiol*, 594, 3031-44.
- STEWART, G. M., YAMADA, A., HASELER, L. J., KAVANAGH, J. J., KOERBIN, G., CHAN, J. & SABAPATHY, S. 2015. Altered ventricular mechanics after 60 min of high-intensity endurance exercise: insights from exercise speckle-tracking echocardiography. *Am J Physiol Heart Circ Physiol*, 308, H875-83.
- STÖHR, E. J., GONZÁLEZ-ALONSO, J., PEARSON, J., LOW, D. A., ALI, L., BARKER, H. & SHAVE, R. 2011a. Dehydration reduces left ventricular filling at rest and during exercise independent of twist mechanics. *J Appl Physiol (1985)*, 111, 891-7.
- STÖHR, E. J., GONZÁLEZ-ALONSO, J. & SHAVE, R. 2011b. Left ventricular mechanical limitations to stroke volume in healthy humans during incremental exercise. *American Journal of Physiology-Heart and Circulatory Physiology*, 301, H478-H487.
- STOHR, E. J., MCDONNELL, B., THOMPSON, J., STONE, K., BULL, T., HOUSTON, R., COCKCROFT, J. & SHAVE, R. 2012. Left ventricular mechanics in humans with high aerobic fitness: adaptation independent of structural remodelling, arterial haemodynamics and heart rate. *J Physiol*, 590, 2107-19.
- SURIANO, R. & BISHOP, D. 2010. Physiological attributes of triathletes. *J Sci Med Sport*, 13, 340-7.
- SUTHERLAND, G. R., BIJNENS, B. & MCDICKEN, W. N. 1999. Tissue Doppler Echocardiography: Historical Perspective and Technological Considerations. *Echocardiography*, 16, 445-453.
- SUTHERLAND, G. R., DI SALVO, G., CLAUS, P., D'HOOGHE, J. & BIJNENS, B. 2004. Strain and strain rate imaging: a new clinical approach to quantifying regional myocardial function. *J Am Soc Echocardiogr*, 17, 788-802.

- TAKIMOTO, E. & KASS, D. 2012. Regulation of Cardiac Systolic Function and Contractility.
- TAWFIK, G. M., DILA, K. A. S., MOHAMED, M. Y. F., TAM, D. N. H., KIEN, N. D., AHMED, A. M. & HUY, N. T. 2019. A step by step guide for conducting a systematic review and meta-analysis with simulation data. *Tropical Medicine and Health*, 47, 46.
- TEI, C., NISHIMURA, R. A., SEWARD, J. B. & TAJIK, A. J. 1997. Noninvasive Doppler-derived myocardial performance index: correlation with simultaneous measurements of cardiac catheterization measurements. *J Am Soc Echocardiogr*, 10, 169-78.
- THOMAS, J. D. & POPOVIĆ, Z. B. 2006. Assessment of left ventricular function by cardiac ultrasound. *J Am Coll Cardiol*, 48, 2012-25.
- THOMPSON, S. G. & HIGGINS, J. P. 2002. How should meta-regression analyses be undertaken and interpreted? *Stat Med*, 21, 1559-73.
- THOMSON, R. L., ROGERS, D. K., HOWE, P. R. & BUCKLEY, J. D. 2016. Effect of acute exercise-induced fatigue on maximal rate of heart rate increase during submaximal cycling. *Res Sports Med*, 24, 1-15.
- THORNTON, A. & LEE, P. 2000. Publication bias in meta-analysis: its causes and consequences. *Journal of Clinical Epidemiology*, 53, 207-216.
- TIAN, Y., NIE, J., HUANG, C. & GEORGE, K. P. 2012. The kinetics of highly sensitive cardiac troponin T release after prolonged treadmill exercise in adolescent and adult athletes. *J Appl Physiol (1985)*, 113, 418-25.
- TORTORA, G. J. & DERRICKSON, B. H. 2008. *Principles of Anatomy and Physiology*, John Wiley & Sons.
- TRAMMEL, J. E. & SAPRA, A. 2021. *Physiology, Systemic Vascular Resistance. StatPearls. Treasure Island (FL): StatPearls Publishing*
- Copyright © 2021, StatPearls Publishing LLC.
- TRIVERS, G., GONZÁLEZ-ALONSO, J., RIDING, N., NICHOLS, D., SHAW, A. & PÉRIARD, J. D. 2020. Exercise heat acclimation has minimal effects on left ventricular volumes, function and systemic hemodynamics in euhydrated and dehydrated trained humans. *Am J Physiol Heart Circ Physiol*, 319, H965-h979.
- TSUZUKI, T., TSUKIOKA, K. & NAITO, H. 2019. Changes in the blood redox balance during a simulated duathlon race and its relationship with athletic performance. *Physiol Rep*, 7, e14277.
- TULLOH, L., ROBINSON, D., PATEL, A., WARE, A., PRENDERGAST, C., SULLIVAN, D. & PRESSLEY, L. 2006. Raised troponin T and echocardiographic abnormalities after prolonged strenuous exercise--the Australian Ironman Triathlon. *Br J Sports Med*, 40, 605-9.
- UJINO, K., BARNES, M. E., CHA, S. S., LANGINS, A. P., BAILEY, K. R., SEWARD, J. B. & TSANG, T. S. M. 2006. Two-Dimensional Echocardiographic Methods for Assessment of Left Atrial Volume. *The American Journal of Cardiology*, 98, 1185-1188.
- UTOMI, V., OXBOROUGH, D., WHYTE, G. P., SOMAUROO, J., SHARMA, S., SHAVE, R., ATKINSON, G. & GEORGE, K. 2013. Systematic review and meta-analysis of training mode, imaging modality and body size influences on the morphology and function of the male athlete's heart. *Heart*, 99, 1727-33.
- VALIPOUR, A., SCHNEIDER, F., KÖSSLER, W., SALIBA, S. & BURGHUBER, O. C. 2005. Heart rate variability and spontaneous baroreflex sequences in supine healthy volunteers subjected to nasal positive airway pressure. *J Appl Physiol (1985)*, 99, 2137-43.
- VALLAT, R. 2018. Pingouin: statistics in Python. *J. Open Source Softw.*, 3, 1026.

- VALLIER, J. M., MAZURE, C., HAUSSWIRTH, C., BERNARD, T. & BRISSWALTER, J. 2003. [Energy cost of running during a specific transition in duathlon]. *Can J Appl Physiol*, 28, 673-84.
- VAN DE SCHOOR, F. R., AENGEVAEREN, V. L., HOPMAN, M. T., OXBOROUGH, D. L., GEORGE, K. P., THOMPSON, P. D. & EIJSVOGELS, T. M. 2016. Myocardial Fibrosis in Athletes. *Mayo Clin Proc*, 91, 1617-1631.
- VANDENBERG, B. F., WEISS, R. M., KINZEY, J., ACKER, M., STARK, C. A., STANFORD, W., BURNS, T. L., MARCUS, M. L. & KERBER, R. E. 1995. Comparison of left atrial volume by two-dimensional echocardiography and cine-computed tomography. *Am J Cardiol*, 75, 754-7.
- VELLA, C. A. & ROBERGS, R. A. 2005. A review of the stroke volume response to upright exercise in healthy subjects. *Br J Sports Med*, 39, 190-5.
- VINERANU, D., KHOKHAR, A., TWEDDEL, A. C., CINTEZA, M. & FRASER, A. G. 2003. Estimation of Global Left Ventricular Function from the Velocity of Longitudinal Shortening. *Echocardiography*, 19, 177-185.
- VIZZA, C. D., LYNCH, J. P., OCHOA, L. L., RICHARDSON, G. & TRULOCK, E. P. 1998. Right and left ventricular dysfunction in patients with severe pulmonary disease. *Chest*, 113.
- WAGGONER, A. D. & BIERIG, S. M. 2001. Tissue Doppler imaging: a useful echocardiographic method for the cardiac sonographer to assess systolic and diastolic ventricular function. *J Am Soc Echocardiogr*, 14, 1143-52.
- WAHR, D. W., WANG, Y. S. & SCHILLER, N. B. 1983. Left ventricular volumes determined by two-dimensional echocardiography in a normal adult population. *J Am Coll Cardiol*, 1, 863-8.
- WANNAMETHEE, S. G., SHAPER, A. G. & WALKER, M. 2000. Physical activity and mortality in older men with diagnosed coronary heart disease. *Circulation*, 102, 1358-63.
- WATANABE, K., STOHR, E. J., AKIYAMA, K., WATANABE, S. & GONZALEZ-ALONSO, J. 2020. Dehydration reduces stroke volume and cardiac output during exercise because of impaired cardiac filling and venous return, not left ventricular function. *Physiol Rep*, 8, e14433.
- WELSH, R. C., WARBURTON, D. E. R., HUMEN, D. P., TAYLOR, D. A., MCGAVOCK, J. & HAYKOWSKY, M. J. 2005. Prolonged strenuous exercise alters the cardiovascular response to dobutamine stimulation in male athletes. *The Journal of Physiology*, 569, 325-330.
- WHITE, H. D. 2011. Pathobiology of troponin elevations: do elevations occur with myocardial ischemia as well as necrosis? *J Am Coll Cardiol*, 57, 2406-8.
- WHYTE, G. P., GEORGE, K., SHARMA, S., LUMLEY, S., GATES, P., PRASAD, K. & MCKENNA, W. J. 2000. Cardiac fatigue following prolonged endurance exercise of differing distances. *Med Sci Sports Exerc*, 32, 1067-72.
- WILLIAMS, B., POULTER, N. R., BROWN, M. J., DAVIS, M., MCINNES, G. T., POTTER, J. F., SEVER, P. S. & MC, G. T. S. 2004. Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. *J Hum Hypertens*, 18, 139-85.
- WILLIAMS, K., GREGSON, W., ROBERTSON, C., DATSON, N., WHYTE, G., MURRELL, C., WILSON, L., AINSLIE, P., ROSS, E., SHAVE, R., GAZE, D. & GEORGE, K. 2009. Alterations in left ventricular function and cardiac biomarkers as a consequence of repetitive endurance cycling. *European Journal of Sport Science*, 9, 97-105.

- WILLIAMS, K. R. & CAVANAGH, P. R. 1987. Relationship between distance running mechanics, running economy, and performance. *Journal of Applied Physiology*, 63, 1236-1245.
- WILSON, M., O'HANLON, R., PRASAD, S., DEIGHAN, A., MACMILLAN, P., OXBOROUGH, D., GODFREY, R., SMITH, G., MACEIRA, A., SHARMA, S., GEORGE, K. & WHYTE, G. 2011a. Diverse patterns of myocardial fibrosis in lifelong, veteran endurance athletes. *J Appl Physiol (1985)*, 110, 1622-6.
- WILSON, M., O'HANLON, R., PRASAD, S., OXBOROUGH, D., GODFREY, R., ALPENDURADA, F., SMITH, G., WONG, J., BASAVARAJAIAH, S., SHARMA, S., NEVILL, A., GAZE, D., GEORGE, K. & WHYTE, G. 2011b. Biological markers of cardiac damage are not related to measures of cardiac systolic and diastolic function using cardiovascular magnetic resonance and echocardiography after an acute bout of prolonged endurance exercise. *Br J Sports Med*, 45, 780-4.
- WU, A. H. B. 2017. Release of cardiac troponin from healthy and damaged myocardium. *Frontiers in Laboratory Medicine*, 1, 144-150.
- WYATT, F., PAWAR, G. & KILGORE, L. 2011. Exercise Induced Cardiac Fatigue Following Prolonged Exercise in Road Cyclists. *ICHPER-SD Journal of Research*, 6, 61-66.
- YANKELSON, L., SADEH, B., GERSHOVITZ, L., WERTHEIN, J., HELLER, K., HALPERN, P., HALKIN, A., ADLER, A., STEINVIL, A. & VISKIN, S. 2014. Life-Threatening Events During Endurance Sports. *Journal of the American College of Cardiology*, 64, 463-469.
- YIP, G., ABRAHAM, T., BELOHLAVEK, M. & KHANDHERIA, B. K. 2003. Clinical applications of strain rate imaging. *J Am Soc Echocardiogr*, 16, 1334-42.
- YU, C. M., SANDERSON, J. E., MARWICK, T. H. & OH, J. K. 2007. Tissue Doppler imaging a new prognosticator for cardiovascular diseases. *J Am Coll Cardiol*, 49, 1903-14.
- ZHANG, C.-D., XU, S.-L., WANG, X.-Y., TAO, L.-Y., ZHAO, W. & GAO, W. 2020. Prevalence of Myocardial Fibrosis in Intensive Endurance Training Athletes: A Systematic Review and Meta-Analysis. *Frontiers in cardiovascular medicine*, 7, 585692-585692.

Appendices

Ethics Form



FACULTY OF SOCIAL AND APPLIED SCIENCES

APPLICATION FOR FREC APPROVAL

Please type your application. Completed applications should be emailed to the Faculty REC administrator, copied to red.resgov@canterbury.ac.uk

For Faculty Office use only
FREC Protocol No: <i>/SAS/</i> Date received:

Your application **must** comprise the following documents (please tick the boxes below to indicate that they are attached):

Peer Review Form

Application Form

Declaration Form

Risk Assessment Form

Copies of any documents to be used in the study:

Questionnaire

Introductory letter(s)

Participant Information Sheet(s)

Consent Form(s)

Data Collection Instruments

Focus Group Guidelines



FACULTY OF SOCIAL AND APPLIED SCIENCES

APPLICATION FOR FREC APPROVAL

Please type your application. Completed applications should be emailed to the Faculty REC administrator, copied to red.resgov@canterbury.ac.uk

Please ensure that you have answered all questions.

For Committee use only

FREC Protocol No: **/SAS/**

Date received:

1. RESEARCHER(S)

1a. LEAD RESEARCHER

Name: James Donaldson

Department: Sport and Exercise Sciences

Email: james.donaldson@canterbury.ac.uk

Previous experience of research on human participants:

- **2011-2014 – Undergraduate degree: Sport, exercise, and health sciences (BSc).**
Performed maximal exercise testing, ECG, isokinetic dynamometry, and a range of fitness tests on human participants as part of lab practical sessions. For my dissertation participants completed a sub-maximal run speed protocol and had their running economy assessed using Douglas bags.
- **2014-2015 – master’s degree: Sport, exercise, and health sciences (MSc).**
Experience with capillary blood sampling, lung function testing, EMG. Thesis project required transesophageal temperature probe placement, transthoracic ultrasound practice, exercise in environmental chamber, blood sampling for electrolytes, metabolites, and haemoglobin.

1b. SUPERVISOR

Name: Dr Jamie O’Driscoll

Email address: jamie.odriscoll@canterbury.ac.uk

2. **STUDY TITLE:** Reliability of lab-based duathlon performances in a highly trained cohort, and indices of subsequent cardiac performance.

3. **OTHER RESEARCHERS/COLLABORATORS** (*please note their employer if they are not employees of CCCU*)

4. **Intended start date:** December 2016

5. **Projected date of completion:** December 2019

6. **RESEARCH SPONSOR/OTHER ORGANISATIONS INVOLVED**

If your study involves another organisation, please provide details. Evidence that the relevant authority has given permission will be needed (i.e., a letter).

N/A

7. **OTHER ETHICS COMMITTEE APPROVAL** (*Has the proposed study been submitted to any other reviewing body? If so, please provide details.*)

N/A

8. **LAY SUMMARY** (*NO MORE than 300 words*)

Strenuous, long duration (endurance) exercise, such as running and cycling, can alter heart muscle function and regulation (Exercise induced cardiac fatigue [EICF]). In a small proportion of normal athletes, prior research¹ has demonstrated elevations in clinical biomarkers (cardiac troponin [cTn]) following such events, which has been described as heart muscle fatigue. In addition, reductions in heart muscle contraction and relaxation have been shown post exercise². These post-exercise alterations have been demonstrated in multiple studies^{3,4,5}. Prior research has examined the relationship between biomarker release and heart dysfunction in athletic populations^{4,5}. However, there is currently little consensus on the relationship between exercise induced troponin release and EICF, due to the differing methodologies employed in the studies accounting for the variance in findings.

Furthermore, little research has investigated potential alterations in neural control of the heart muscle (cardiac autonomic regulation) in response to exercise. However, it has been hypothesized that exercise demands, enough to suppress parasympathetic cardiac control following 24 hours of rest, may also elicit cTn release. This is supported by Stewarts and colleague's (2013) finding of a positive relationship between exercising heart rate, decreased heart rate variability, and cardiac troponin release following 60-min of strenuous cycling⁵. Exercise mode may also affect the degree of EICF, as running exercise typically elicits higher exercise heart rates than cycling. However, there is currently no study that has compared intra-individual cardiac response following both running and cycling exercise.

As such, the following remains to be elucidated:

- The exercise intensity-duration that elicits a positive cTn response.
- How is the previous factor affected by exercise modality e.g., cycling vs. running exercise?
- The relationship between autonomic balance and biomarker release following exercise, and how is this relationship altered by exercise intensity, duration, and mode.
- The relationship between autonomic regulation and ventricular function following exercise.

In the first instance it is deemed necessary to conduct a reliability study to help determine sample size estimation in subsequent studies in my PhD. In order to ensure population validity for the later studies making up my PhD., a highly-trained cohort of subjects will be used to produce reliability data for investigating the cardiac response to running and cycling exercise. The research question for this study will be ‘How reliable are lab-based duathlon performances, in a highly-trained cohort?’. This novel investigation will focus upon the effect of cycling and running exercise upon the primary measures to be used in later studies with the PhD., including components of cardiac function (ultrasound scan), selected biomarker release (blood samples), and autonomic cardiac control (7 lead ECG) in the duathlon-trained cohort.

References:

1. Shave, R., George, K. P., Atkinson, G., Hart, E., Middleton, N., Whyte, G., Gaze, D., & Collinson, P. O. (2007). Exercise-induced cardiac troponin T release: a meta-analysis. *Medicine and science in sports and exercise*, *12*, 2099–2106.
2. Middleton, N., Shave, R., George, K., Whyte, G., Hart, E., & Atkinson, G. (2006). Left ventricular function immediately following prolonged exercise: A meta-analysis. *Medicine and science in sports and exercise*, *4*, 681–687.
3. Shave, R., Baggish, A., George, K., Wood, M., Scharhag, J., Whyte, G., Gaze, D., & Thompson, P. D. (2010). Exercise-induced cardiac troponin elevation: evidence, mechanisms, and implications. *Journal of the American College of Cardiology*, *3*, 169–176.
4. Shave, R., & Oxborough, D. (2012). Exercise-induced cardiac injury: evidence from novel imaging techniques and highly sensitive cardiac troponin assays. *Progress in cardiovascular diseases*, *5*, 407–415.
5. Stewart, G. M., Kavanagh, J. J., Koerbin, G., Simmonds, M. J., & Sabapathy, S. (2013). Cardiac electrical conduction, autonomic activity, and biomarker release during recovery from prolonged strenuous exercise in trained male cyclists. *European journal of applied physiology*, *1*, 1–10.

9. PURPOSE OF THE STUDY

The purpose of this study is to determine the effects of two modes of exercise (running and cycling) on cardiac function and control. Additionally, the study aims to examine the relationship between the previously mentioned variables to examine the heart-rate dependent

basis of cardiac troponin release. A tertiary aim of the study is to better inform the training strategies of cycle-run multi-sport athletes (duathletes) by identifying workloads that elicit cardiac dysfunction.

10. STUDY DESIGN, METHODS, AND DATA ANALYSIS (*please outline in brief*)

- **Study Design:** This study will employ a repeated-measures design. Data will be collected following several exercise interventions including stand-alone running and cycling, and multi-sport run-cycle exercise bouts.
- **Methods:** The main data of the study will be measured using the following methodologies:
 - **Echocardiography:** Echocardiographic images will be obtained by an experienced researcher (JD), who has completed an NHS Echocardiography course and trained for 1 year at CCCU on various participants under the supervision of Dr. Jamie O'Driscoll. Images will be obtained using a portable ultrasound system (Vivid Q, GE) in the apical 4-chamber, parasternal short-axis, and parasternal long-axis views. From these views, we will measure LV diastolic and systolic function and RV function using Tissue Doppler Imaging (TDI) and M-Mode echocardiography. For the echocardiographic exam, participants will be required to lie in the left-lateral decubitus position to bring the left ventricle closer to the chest wall, and with the left arm abducted to widen the inter-costal space and improve the imaging window.
 - **Venous blood sampling:** Blood samples will be taken from a cannula inserted to the cephalic vein, or *via* butterfly needle inserted in the median cubital vein. We will collect 3 samples per draw, which will be immediately separated by centrifuge and frozen. Two samples will be sent to an external lab with known reliability and analysed for plasma levels of cardiac troponin isoforms T and I, with 1 sample stored at the University for possible future research.
 - **Electrocardiography (ECG):** ECG and blood-pressure data will be acquired using a non-invasive hemodynamic monitoring system (Taskforce Monitor, APC Cardiovascular). This system measures ECG data via 4 surface electrodes, oscillometric blood pressure using a brachial cuff, and beat-to-beat blood pressure from a plethysmographic finger-cuff. The procedure will require participants to lie in the supine position, with mitigated aural and visual stimulation, for 15 minutes to reduce external stimulation and stabilise sympathetic/parasympathetic balance.
 - **Order:** The measurements will be taken in the following order: ECG, Echocardiography, Blood draws. This is due to the chance of a vasovagal reaction occurring on needle insertion, which can influence autonomic balance. The effects of this will be mitigated by the patient

being seated during the blood draw and assessing their comfort with needles beforehand, making reassurances etc. as necessary.

- **Data analysis:** Data will be analysed using SPSS v23. Statistical tests will include ANOVA, correlation, and multiple regression tests.

11. WHO ARE THE REQUIRED PARTICIPANTS FOR YOUR STUDY?

We require 12 participants for this study. This is based on a Gpower calculation using previously reported effect sizes (η^2) of between 0.45 and 0.80 over 3 repeated measures in a single group. To account for training and age-related differences in cardiac health our participants will be 18–35-year-old duathlon/triathlon-trained males. Due to Neilan et al's (2006) finding of an increased risk of cardiac dysfunction and elevated cardiac troponin levels in low vs. high volume training groups, we will recruit athletes who are currently training at least 7 hours per week for this study.

Neilan, T. G., Januzzi, J. L., Lee-Lewandrowski, E., Ton-Nu, T. T., Yoerger, D. M., Jassal, D. S., Lewandrowski, K. B., Siegel, A. J., Marshall, J. E., Douglas, P. S., Lawlor, D., Picard, M. H., & Wood, M. J. (2006). Myocardial injury and ventricular dysfunction related to training levels among nonelite participants in the Boston marathon. *Circulation*, *22*, 2325–2333.

12. HOW WILL PARTICIPANTS BE RECRUITED?

Participants will be recruited by advertisements posted to social media and emails sent to cycling/running/triathlon club secretaries.

Participants from another CCCU Faculty

Will you be recruiting STAFF or STUDENTS from another Faculty? If so, which Faculty?

No.

IMPORTANT: If you intend recruiting participants from another Faculty, this form must be copied to the Dean of the Faculty concerned, and to the Chair of that Faculty's Research Ethics Committee.

13. SELECTION CRITERIA (Inclusion and exclusion criteria)

- Participants will be screened for adequate cardiac images using ultrasound prior to acceptance to the study.
- They will also be excluded based on current or previous cardiovascular disease.
- They must have been training specifically for duathlon or triathlon events for the previous 2 years.

14. CONSENT

14a. **How will consent be obtained?** (*Attach copies of any information sheet(s) and consent forms that will be used*)

See appendices 2 & 3 for consent forms and information sheets.

14b. Will the participants be from any vulnerable groups? (Tick as appropriate)

Those under 18

Those with any form of learning difficulties

Those who may have a particularly dependent relationship with the researcher (e.g., students)

Any other vulnerable group (please give details)

14c. How will you ensure that vulnerable participants are competent to consent to take part? (Please attach any correspondence to parents, guardians, carers, keyworkers etc.)

This study will only recruit physically able male participants between 18 and 35 years of age, and not include vulnerable participants.

14d. Is there anything that might make it difficult for people to refuse to take part in the study (e.g., the potential participants are students or colleagues of the Researcher)? How will you address this?

No. Participants will be recruited anonymously via the internet/email and not in person.

15. PARTICIPANT'S INVOLVEMENT: RISKS, REQUIREMENTS AND BENEFITS

15a. What potential hazards, risks or adverse effects associated with the study?

The study will use the following invasive or potentially harmful methods:

- **Cannulation of the cephalic vein** - to obtain blood samples from the participants at regular intervals throughout the testing procedure. 5mL of blood will be extracted a maximum of 7 samples per visit, with an average of 4. Even at the maximum volume of blood drawn, this still is far short of the typical amount given during a blood donation (470mL). Potential adverse effects arising from cannulation are haematoma and ecchymosis. The risk of this will be reduced by a trained, experienced researcher conducting all blood sampling and cannulation.
- **Strenuous endurance exercise** – there is the possibility of injury, physiological pain, and 1 in 100,000 cases of reported sudden cardiac death following endurance exercise and/or a maximal exercise test. However, this study will not cause participants to undergo any form, duration or intensity of exercise that will be outside of their typical training regime. Therefore, the amount of additional risk imposed by this study will be minimal, arguably it will be safer than their typical exercise regime as a first-aid and defibrillator trained researcher will be on standby during the entire bout of exercise and for 6 hours after.

15b. Has a full risk assessment been carried out in line with University Health & Safety procedures?

Yes, please see appendix for thorough risk assessments pertaining to each activity in this study.

15c. Will group or individual interviews / questionnaires discuss any topics or issues that might be sensitive, embarrassing or upsetting? If so, please list and explain how you will prevent, or respond to, volunteer discomfort.

This study will not use any interviews or questionnaires.

15d. Is it possible that criminal or other disclosures could be made by participants in the study that require action (e.g., evidence of professional misconduct)? What procedures will be put in place to deal with these issues?

No. Participants will only be required to disclose their medical history.

15e. Please describe any expected benefits to the research participant.

By participating in this study, participants will gain insight into their cardiovascular and neurological reaction to strenuous exercise, which can be used to inform their future training programmes by providing an index of their individual recovery time from strenuous exercise. They will also receive a full report of the graded maximal exercise tests (VO₂max) for both running and cycling, which can be used to prescribe training sessions.

15f. What circumstances might lead to premature termination of the study in part or as a whole? Please include an explanation of how you will deal with the remaining participants in this event.

Severe illness or injury befalling the lead researcher. In this instance, participants will be contacted to inform them of the researcher's inability to continue with the study.

16. FINANCIAL INCENTIVES, EXPENSES AND COMPENSATION

16a. Is any financial or other reward (e.g., travelling expenses) to be given to participants? If yes, please give details and justification.

No.

16b. Will the study result in financial payment or payment in kind to the department? Please specify, including the amounts involved.

No.

17. CONFIDENTIALITY, ANONYMITY AND DATA STORAGE

17a. What steps will be taken to ensure confidentiality? *Give details of the anonymisation procedures to be used, and at what stage they will be introduced.*

- All personal information will be coded and stored on a secure university, password-protected computer for the duration of the study and for 5-years following the conclusion of the PhD. Only those directly involved in the study (researchers and supervisors) will have access to the information.
- During data collection, revealing personal information will be coded to protect identity and anonymity of the participants.
- If any data is published, participants will be referred to by number and no revealing information will be included in the manuscript.

17b. Who will have access to the records and resulting data?

Only those directly involved in the data collection and review process will have access to the data and records obtained from this study.

17c. Where, and, for how long, do you intend to store the consent forms and other records?

Physical copies will be stored in a locked private office within the CCCU Canterbury Campus, digital copies will be stored on a local university hard drive and personal encrypted hard drive for 5 years following the conclusion of the PhD.

17d. How do you propose disposing of the consent forms and other records at the end of the retention period?

The physical copies will be incinerated, and all digital copies will be erased.

18. DISSEMINATION AND OUTPUTS

Please indicate how you will disseminate the findings from your study:

Thesis/dissertatio

X

Journal

article

X

Monograph or chapter in book

--

Conference paper

X

Conference poster

X

Research reports to Funders

--

Other *(please give details)*:



FACULTY OF SOCIAL AND APPLIED SCIENCES

FACULTY RESEARCH ETHICS COMMITTEE

DECLARATION

Project Title: Reliability of lab-based duathlon performances in a highly trained cohort, and indices of subsequent cardiac performance.

Project No: /SAS/

- The information in this form is accurate to the best of my knowledge and belief and I take full responsibility for it.
- I undertake to conduct this research in accordance with University Research Governance procedures.
- If the research is approved, I undertake to adhere to the study protocol without deviation and to comply with any conditions set out in the letter sent by the Faculty REC notifying me of this.
- I undertake to inform the Faculty REC of any changes in the protocol and to seek their agreement and to submit annual progress reports. I am aware of my responsibility to be up to date and comply with the requirements of the law and appropriate guidelines relating to security and confidentiality of participant or other personal data, including the need to register when appropriate with the appropriate Data Protection Officer.
- I understand that research records/data may be subject to inspection for audit purposes if required in future and that research records should be kept securely for five years.
- I understand that personal data about me as a researcher in this application will be held by the REC and that this will be managed according to the principles established in the Data Protection Act.

Signature of Researcher: J Donaldson

Print Name: James A Donaldson

Date: 19/01/2017

FOR STUDENT APPLICATION ONLY

I have read the research proposal and application form, and support this submission to the REC.

Signature of Supervisor: JOD

Date: 19/01/2017

Print Name: Jamie O'Driscoll

Consent forms



[MODEL] CONSENT FORM

Title of Project: Reliability of lab-based duathlon performances in a highly trained cohort, and indices of subsequent cardiac performance.

Name of Researcher: James Donaldson

Contact details:

Address:

AG 50, Anselm, CCCU, 1 North Holmes Road, Canterbury, Kent, CT11QU

Tel:

01227763832

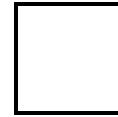
Email:

James.donaldson@canterbury.ac.uk

Please initial box

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.
3. I understand that any personal information that I provide to the researchers will be kept strictly confidential

4. I agree to take part in the above study.



Name of Participant

Date

Signature

Name of Person taking consent
(If different from researcher)

Date

Signature

Researcher

Date

Signature

Copies: 1 for participant
 1 for researcher

Participant Information Sheet

Cardiac Function and Control Following Prolonged Strenuous Cycle/Run Exercise

PARTICIPANT INFORMATION SHEET

A research study is being conducted at Canterbury Christ Church University (CCCU) in the department of sport and exercise sciences by PhD student James Donaldson. The supervisory team includes Dr Jamie O'Driscoll and Dr Jonathan Wiles.

Background

The function of the heart can be impacted following strenuous endurance exercise, such as running and cycling. It is currently not clear if, and at what point, this reduced function occurs during a standard duathlon race. We also wish to explore how this dysfunction is related to changes in autonomic (sympathetic and parasympathetic) regulation of the heart.

What will you be required to do?

Participants in this study will be required to visit the lab 6 times to complete an Olympic distance duathlon at race intensity. We will measure the function of your heart and take a small blood sample at rest, following each bout of exercise and at 6- and 24-hours post exercise. On the first 2 visits you will perform a maximal graded exercise test on a cycle ergometer and a treadmill to determine your maximal aerobic capacity (VO_{2max}) and anaerobic threshold for running and cycling. Each test will last about 30 minutes and be separated by 24 hours of rest. You will not need to return to the lab the following day after these visits, but the remaining visits will require you return 6 and 24 hours later for post-testing. The remaining 4 visits will involve 4 Olympic Distance Duathlons (10km run, 40km cycle, 5km run).

To participate in this research, you must:

Be **male**, aged **18-35 years old**. Have been **training and competing** in duathlon/triathlon races for the previous **2 years**. Free from any known **cardiovascular disease**.

Feedback

By taking part in this study, you will be assisting in research that will aim to explore the role of the autonomic nervous system in cardiac dysfunction following endurance exercise. The data generated will help to inform your future training programmes and potentially inform elite-athlete training regimes. You will receive a full report of your exercise capacity and training zones for both the bike and run, as well as being provided an insight into your ventricular function at rest and following exercise.

Confidentiality

All data and personal information will be stored securely within CCCU premises in accordance with the Data Protection Act 1998 and the University's own data protection requirements. Data can only be accessed by the researchers, James Donaldson, Dr Jamie O'Driscoll, and Dr Jonathan Wiles. After completion of the study, all data will be made anonymous (i.e., all personal information associated with the data will be removed). **Any questions?** Please contact James Donaldson: James.Donaldson@canterbury.ac.uk / (+44) 01227 923832

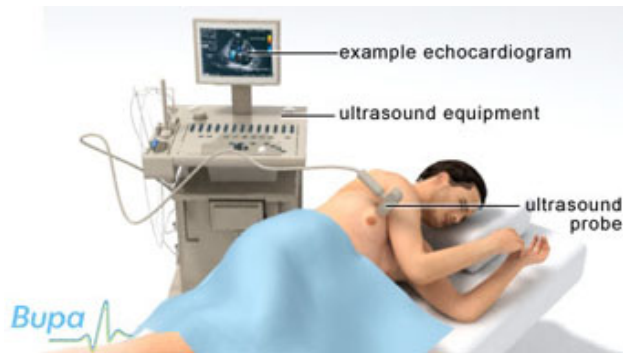
Procedures

The measurements we take will involve non-invasive measurement of cardiac function – ECG and Ultrasound simply require you to lie on a bed with surface electrodes attached around your torso. You may be asked to move into a slightly uncomfortable position during the echocardiographic assessment in order to acquire the best images.



ECG assessment: 4 Electrodes will be attached to your torso. We will also fit brachial and fingertip pressure cuffs. The Taskforce Monitor will measure the variability of your heart rate and blood pressure for 5 minutes. At the start of each visit before exercise, you will need to lie down for 15 minutes in the dark to set a resting

Echocardiography: For this test you will need to lie on your left side with your arm behind your head for 10-15 minutes while we collect images of your heart from different angles. This is a very relaxed procedure, but you may be asked to hold your breath at certain points to stop your lungs from



Blood Samples: We will need to take several small blood samples throughout each visit. A trained researcher will insert a cannula into a vein in your forearm; this will allow us to take multiple blood samples without having to insert a needle each time.

The visits will all be separated by at least 48 hours of rest to ensure you recover and remain injury free.

Risk Assessments - Blood Sampling



DATE of Assessment:	09/09/2016	ASSESSMENT No	1
Assessed by (Name):	James Donaldson	DEPARTMENT name or code:	Section of Sport and Exercise Science
NATURE OF ACTIVITY:	Blood sampling and sterilisation		DATE OF ACTIVITY: Throughout Year
LOCATION:	Sports Science Labs	REVIEW DATE:	09/09/2017

Hazard	Persons at Risk	Current Control Measures	Severity (S)	Likelihood (L)	Risk Rating (S X L)	Additional Control Measures Required	Revised Risk Rating	Action Sign Off Date/ Responsible Person
Venepuncture & cannulation; excessive loss of blood by participant	Participant	Practitioners and support staff are trained first aiders	2	2	4	None	4	James Donaldson

Severity (S):

Likelihood of Harm (L):

Risk Rating:
(S x L)

Risk Evaluation:

Action to follow:

Minor injury or illness	1	Very unlikely	1			
Moderate injury or illness	2	Unlikely	2	1 to 4	Low / Acceptable	No further actions but ensure controls are maintained.
"3-day injury" or illness	3	50 / 50 likelihood	3	5 to 9	Medium / Adequate	Look to improve at next review.
Major injury or illness	4	Likely	4	10 to 16	Medium / Tolerable	Look to improve within specified timescale.
Fatality	5	Very likely / certainty	5	17 to 25	High / Unacceptable	Stop activity immediately and make appropriate improvements.

For Health and Safety advice, go to www.canterbury.ac.uk/support/health.safety or contact health.safety@canterbury.ac.uk

Hazard	Persons at Risk	Current Control Measures	Severity (S)	Likelihood (L)	Risk Rating (S X L)	Additional Control Measures Required	Revised Risk Rating	Action Sign Off Date/ Responsible Person
Syncope	Participant	Participants will be asked how comfortable they are with needles and the sight of blood prior to intervention and will be in a semi-reclined position to reduce risk of injury and promote recovery.	1	1	1	None	1	

Risk of infection from body fluids	All associated staff, students, and visitors	All repeated use equipment exposed to bodily fluids sterilised straight after use in Virkon solution (multipurpose disinfectant cleaner)	2	1	2	None	2	
Needle stick injury to practitioner	Practitioner	<p>Only the member of staff conducting the assessment takes samples, they are fully trained and are required to wear gloves and overalls.</p> <p>Blood loss by practitioner can be controlled by practitioner and first aid trained support staff.</p> <p>Needles put in sharps bin after use. All lancets are single use and disposed of after use</p>	1	1	1	None	1	

Risk of blood borne infection from needle stick injury	Practitioner	<p>Only the member of staff conducting the assessment takes samples, they are fully trained and are required to wear gloves and overalls.</p> <p>Any needle stick injury needs to be reported and that person will require medical support.</p> <p>Needles put in sharps bin after use. All lancets are single use and disposed of after use</p>	2	2	4	None	4	
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Bloods areas: Risk of blood borne infection.	All associated staff, students, and visitors	Yellow biohazard tape clearly highlights where the bloods area is. Area and equipment are thoroughly cleaned after use. All students and visitors are instructed not to touch/use area. Needles put in sharps bin after use. All lancets are single use and disposed of after use	2	1	2	None	2	
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Risk Assessments - Laboratory Exercise

DATE of Assessment:	09/09/2016	ASSESSMENT No	1
Assessed by (Name):	James Donaldson	DEPARTMENT name or code:	Section of Sport and Exercise Science
NATURE OF ACTIVITY:	Exercise in Laboratory Setting		DATE OF ACTIVITY: Throughout Year
LOCATION:	Sports Science Labs	REVIEW DATE:	09/09/2017



Severity (S):	Likelihood of Harm (L):	Risk Rating: (S x L)	Risk Evaluation:	Action to follow:
Minor injury or illness	1 Very unlikely	1		
Moderate injury or illness	2 Unlikely	2	1 to 4 Low / Acceptable	No further actions but ensure controls are maintained.
"3-day injury" or illness	3 50 / 50 likelihood	3	5 to 9 Medium / Adequate	Look to improve at next review.
Major injury or illness	4 Likely	4	10 to 16 Medium / Tolerable	Look to improve within specified timescale.
Fatality	5 Very likely / certainty	5	17 to 25 High / Unacceptable	Stop activity immediately and make appropriate improvements.

For Health and Safety advice, go to www.canterbury.ac.uk/support/health.safety or contact health.safety@canterbury.ac.uk

Hazard	Persons at Risk & Nature of harm	Current Control Measures	Severity (S)	Likelihood (L)	Risk Rating (S x L)	Additional Control Measures Required <i>(Further action required)</i>	Revised Risk Rating	Action Sign Off Date/ Responsible Person
Fire	Visitors, students, and all associated staff.	Fire evacuation procedure explained. Fire routes, extinguishers and signs checked by trained staff. Visitors & students supervised at all times by First Aid trained staff.	4	2	8	None	8	James Donaldson
Accident	Visitors, students, and all associated staff.	List of University First Aid trained staff available on intranet. All staff involved in visits and laboratory activities are First Aid and AED trained. Small First Aid Boxes provided in studio (Ag50), physiology lab (Ag59) & control room (Ag56). AED provided in studio (Ag50). First aid notice displayed with emergency contact numbers. All staff involved in visits with persons under the age of 18 are CRB checked, and visitors are supervised at all times.	4	2	8	None	8	
Electric Shock	Visitors, students, and all associated staff.	All equipment connected to the mains via removable mains plug is Portable Appliance Tested (PAT tested).	1	1	1	None	1	
Exposure to chemicals e.g., cleaning agents	Visitors, students, and all	COSHH sheets available for all chemicals. Visitors & students are supervised at all times. Staff properly trained regarding	1	1	1	None	1	

Hazard	Persons at Risk & Nature of harm	Current Control Measures	Severity (S)	Likelihood (L)	Risk Rating (S x L)	Additional Control Measures Required <i>(Further action required)</i>	Revised Risk Rating	Action Sign Off Date/ Responsible Person
and blood analysis products.	associated staff.	current safety procedures on those products.						
Wet floors from research and or cleaning.	Visitors, students, and all associated staff.	Wet floors due to research equipment being cleaned on completion of testing. Where appropriate wet floor signs displayed and removed when no longer required Visitors supervised at all times.	1	1	1	None	1	
Compressed Gasses, risk of bottles falling and the valves becoming dislodged. Possibility of gasses being dispersed into testing environment.	Visitors, students, and all associated staff.	Hazard warning signs are displayed on the lab door, and walls. Staff trained in moving bottles and replacing regulators. Protective footwear, gloves and glasses provided. All gas cylinders are chained to their associated equipment or a wall. Transportation boxes are used for both delivery and removal of gas cylinders. None of the gasses used are toxic and the environment is not enclosed.	4	1	4	None	4	
Falling objects.	Visitors, students, and all associated staff.	Proper shelving provided and at appropriate height. Shelving and equipment monitored at regular intervals. Visitors and students supervised at all times.	2	1	2	None	2	
Exposure to faulty equipment.	Visitors, students, and all	All departmental and University equipment is checked and maintained at appropriate intervals.	4	1	4	None	4	

Hazard	Persons at Risk & Nature of harm	Current Control Measures	Severity (S)	Likelihood (L)	Risk Rating (S x L)	Additional Control Measures Required <i>(Further action required)</i>	Revised Risk Rating	Action Sign Off Date/ Responsible Person
	associated staff.							
<p>Research equipment demonstration by staff member.</p> <p>Fitness test assessment by visitor/student:</p> <p>Risk of strains, injuries, falls or adverse reaction to exercise whilst using equipment.</p>	Visitors, students, and all associated staff.	<p>Training on the relevant piece of equipment given to staff member concerned.</p> <p>Visitors and students fully informed and supervised. The participant will fill out a health screening questionnaire and read then sign an informed consent.</p> <p>Before exercise visitors/students will complete a warmup. Suitable clothing and footwear to be worn. Equipment setup to suit each participant.</p> <p>Participant told to cease activity if causing distress.</p> <p>Asthma sufferers to keep pump with them at all times.</p> <p>Visitors and students will be supervised at all times.</p>	4	1	4	None	4	
Capillary blood sample taking for assessment. Exposure to human bloods (possibly containing	Visitor, students, or staff participating , staff conducting	Visitors and students fully informed and supervised. The participant will fill out a health screening questionnaire and read and sign an informed consent. Only the member of staff conducting the assessment takes samples, they are fully	4	1	4	None	4	

Hazard	Persons at Risk & Nature of harm	Current Control Measures	Severity (S)	Likelihood (L)	Risk Rating (S x L)	Additional Control Measures Required <i>(Further action required)</i>	Revised Risk Rating	Action Sign Off Date/ Responsible Person
blood-borne diseases).	the demonstrati on and visitor or students watching.	trained and are required to wear gloves and overalls. All work surfaces are kept clean with the appropriate cleaning products and labelled with biohazard tape. All students and visitors are instructed not to touch/use area. Needles put in sharps bin after use.						

PARTICIPANTS NEEDED FOR TRI/DUATHLON PhD STUDY

WHO?

- 2 years of triathlon or duathlon racing and training.
- Male.
- Age 18-35.
- Free from known cardiovascular disease & hypertension.
- Currently exercising a minimum of 7 hours a week.



WHAT?

- **6 Visits** to SportsLab at CCCU.
 - **2 VO₂max and Lactate Threshold** tests for both running and cycling.
 - **6 Experimental visits.**
- Blood samples, Cardiac Ultrasound, ECG monitoring before and after exercise.
- Recovery measurements @ 6 & 24h24h post-exercise.

WHY?

- **Full report** of physiological testing including your **FTP**, **VO₂max** for cycling and running, **running economy**, and **heart rate training zones** for running and cycling.
- Excellent opportunity for lab-controlled training.
- Assist in new research exploring the role of the nervous system in cardiac fatigue following endurance exercise.

CONTACT

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