The effect of prolonged exercise and environmental temperature upon left ventricular function and cardiac biomarker release

JOANNE HANKEY

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Supervisory Team

Director of Studies

Professor Keith George

Associate Dean for Research (Faculty of Science)

Research Institute for Sport and Exercise Sciences

Liverpool John Moores University

Second Supervisor

Professor Greg Whyte

Professor of Applied Sport Sciences

Research Institute for Sport and Exercise Sciences

Liverpool John Moores University

Third Supervisor

Professor Warren Gregson

Professor of Applied Exercise Physiology

Research Institute for Sport and Exercise Sciences

Liverpool John Moores University

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Abstract

This thesis examined the effect of single and repeat bouts of prolonged exercise as well as an environmental temperature challenge upon left ventricular (LV) function and cardiac biomarker release. The primary aims were; (1) does repeated exercise bouts mediate a cumulative decrease in LV function and/or increase in cardiac biomarker concentrations; and (2) to examine the impact of prolonged exercise in a hyperthermic environment upon cardiac function and cardiac biomarker levels.

Study 1 demonstrated evidence of LV systolic and diastolic dysfunction after exercise that persisted 22 hours into recovery following 10 days of cycling in amateur cyclists. There was however, limited support for a cumulative change in function across days. A highly individual, but not cumulative, pattern of cardiac biomarker appearance was observed with rapid clearance. This data suggests that the cardiovascular system of the amateur cyclists coped well with the accumulated exercise stress imposed by the repeated cycling over 10 days. Study 2 revealed that a single bout of prolonged exercise (37 km), in a hyperthermic environment, resulted in a decrease in LV systolic and diastolic function and an elevation in cardiac biomarkers. There was no evidence of cumulative changes in function or biomarker appearance over a further 5 days of exercise in a hyperthermic environment. Significant individual variation between participant's responses were again noted.

Study 3 employed a controlled exercise stimulus in a laboratory setting and revealed both LV systolic and diastolic function were not significantly altered following 60 minutes of running in either a normothermic (13^{o}C) or hyperthermic (30^{o}C) environment. The release of cardiac biomarkers was limited, with a tendency for markers to be higher in the hyperthemic condition. Participants coped well with the exercise stress, however, the

"low" exercise dose in this study likely negates any meaningful impact upon cardiac function and biomarker release.

The final study manipulated core temperature (T_c), through pre-cooling, prior to exercise in a hyperthermic environment (32.4 ± 0.9ºC and 46.8 ± 6.4% RH). Diastolic, but not systolic, function was reduced following 90 minutes of running, with no difference apparent between pre-cooling and control conditions. cTnT was evident in all participants following both trials, with a limited release of NT-proBNP that was not mediated by pre-cooling. Pre-cooling appeared to have no beneficial or adverse effect on the cardiovascular function and biomarkers that again displayed high inter-individual variability.

In conclusion, we observed evidence that acute exercise can result in changes in both cardiac function and biomarkers. There was, however; (1) no evidence of an accumulation of cardiac function or biomarker data across multiple bouts of exercise across a number of days, and (2) limited evidence that either a hyperthermic environment or a pre-cooling intervention altered cardiac function and biomarker data after exercise in a controlled laboratory design. Changes in cardiac function and biomarkers were transient in nature, of relatively small magnitude and subject to high individual variability. It would seem that these changes represent an acute physiologic perturbation as opposed to pathology.

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CHAPTER 1

Introduction

Participation in regular moderate exercise has beneficial effects on the risk factor of cardiovascular disease, as well as a variety of other chronic diseases which include diabetes mellitus, cancer, obesity, hypertension and depression (Warburton *et al.*, 2006). Whilst regular exercise-training can reduce the incidence of coronary heart disease (CHD) events, each exercise bout is associated with a transient increase in the risk of sudden cardiac death (SCD) and acute myocardial infarction (AMI) in susceptible individuals (Thompson *et al.*, 2007). The substantial benefits of exercise greatly outweigh the increased risk of a cardiac event, with evidence suggesting the greatest benefit in those who exercised in excess of 42 metabolic equivalents (METs) per week (Tanasescu *et al*., 2002). Whilst a number of elite athletes are reported to exercise in excess of 20 hours or 300 METs per week (La Gerche *et* al., 2008), little is known regarding the upper limit of exercise volume for cardiac health (Whyte, 2008). La Gerche and Prior (2007) proposed that at extreme levels of endurance training, there may be a decline in the overall benefits of exercise when compared to an increased risk of a cardiac event.

Taking part in prolonged exercise, such as training for and participating in endurance and ultra-endurance events is becoming increasingly popular. In this thesis, prolonged exercise is defined as endurance exercise of 60 minutes or greater. According to Shave *et al.* (2007b), participation in the London marathon has risen from ~8,000 participants in 1981 to over 30,000 participants in 2006, with many more applying for places. This is despite the fact that there is some controversy over the beneficial effect of prolonged exercise (Michielsen *et al.*, 2008). This controversy is not limited to scientific debate in journal studies but has crossed over to the mass media about the safety of exercise, thus having a greater reach and impact (Burfoot, 2008; Parker-Pope, 2009; Yared and Wood, 2009). Such speculation within the media can act as a deterrent to the physically inactive and raises concern with those taking part in recreational endurance events (Shave *et al.*,

2007a; Scott *et al*., 2009). Endurance athletes are now seeking advice from scientists, coaches and clinicians over safety concerns of the exercise they partake (George *et al.*, 2008).

Moderate steady-state exercise of as little as 10 minutes creates an increased hemodynamic and metabolic load, requiring an athlete to maintain a high cardiac workload to ensure adequate muscle perfusion (Dawson *et al*., 2003). As the exercise duration becomes prolonged (greater than 60 minutes) the cardiac workload is further exuberated due to increased thermal load and blood volume redistribution/reduction, placing a substantial strain upon the cardiovascular system (Coyle and González-Alonso, 2001). A growing evidence-base suggests that the physiological demands of maintaining a high cardiac workload for a prolonged period of 60 minutes or greater (Middleton *et al*., 2006a) may result in a transient impairment in cardiac function, evidenced by a reduction in indices of global LV function such as filling pressures, filling velocities, ejection fraction (EF), stroke volume (SV) and systolic and diastolic tissue velocities (e.g. Douglas *et al.*, 1990b; George *et al.*, 2004, 2005; Middleton *et al.*, 2007a; Shave *et al.*, 2004a, 2004c; Vanoverschelde *et al.*, 1991). The release of cardiac biomarkers into the systemic circulation such as cardiac troponin (cTn), a biomarker of cardiomyocyte cell insult, immediately following acute bouts of prolonged exercise in apparently healthy individuals has been documented (Shave *et al*., 2007a). In addition, there is no consensus regarding the prevalence, mechanisms and clinical management of exercise-induced cTn release (Shave *et al*., 2010).

A combination of factors such as exercise duration, intensity and volume, participants training experience, as well as environmental factors have been suggested to precipitate changes in LV function and biomarker appearance (Gresslien and Agewall, 2016;

Oxborough *et al.*, 2010; Sedaghat-Hamedani *et al.*, 2015). However, data interpretation is often confounded due to the field-based competitive nature of endurance events, particularly in relation to the inability to manipulate environmental conditions. Furthermore, whilst single bouts of prolonged exercise provide a substantial cardiovascular challenge, these events are typically followed by periods of rest or reduced activity allowing the athlete to recover. Many athletes however, engage in repeated bouts of exercise either during competition, through the repetitive element of training, or a combination of the two. Therefore, the evaluation of single bouts of prolonged exercise provides limited information into the practical understanding of cardiac dysfunction and biomarker release in different exercise settings. Participation in multi-day endurance events, with repeated endurance exercise exposure and limited recovery time, have also proved increasingly popular (Knoth *et al*., 2012), with such events likely representing the upper limit of physiological stress placed upon the human heart (George *et al.*, 2011). Despite this, there is a lack of evidence examining the cardiovascular consequences of these types of events.

To further the current understanding of the impact of single or repeated bouts of prolonged exercise, as well as a mediating role for environmental temperature, upon LV function and markers of cardiac damage, the aims of this thesis were as follows; to examine LV function and markers of cardiac damage in healthy individuals in response to (1) 10 days of road cycling, (2) single and multi-day exercise (running) in a field-based hyperthermic environment, (3) prolonged running in a lab-based hyperthemic and normothermic environment and (4) prolonged running in a lab-based hyperthermic environment with and without pre-cooling.

The following Chapter of this thesis provides an overview of the existing literature in relation to; cardiovascular responses to acute aerobic exercise, the impact of single and repeated bouts of endurance exercise upon cardiac function and biomarker release and the effect of exercise, thermoregulation and environmental temperature upon cardiovascular function. The specific study designs and results from each study are presented in Chapters three, four, five and six, respectively. Finally, the overall findings are discussed in Chapter seven and the conclusions from the four studies are presented.

CHAPTER 2

Literature Review

2.1 Introduction

The benefits of physical activity are undisputed, with many studies demonstrating that physical activity is associated with a reduced risk of cardiovascular disease, cancer, diabetes and dementia (Shiroma and Lee, 2010; Warburton *et al.*, 2006). Several epidemiological studies have reported an inverse relationship between physical activity and cardiovascular disease (Paffenbarger *et al.*, 1978, 1986), with regular exercise improving the cardiovascular disease risk profile by lowering blood pressure (Whelton *et al.* 2002), improving endothelial function (Joyner and Green, 2009) and enhancing vagal tone (Beere *et al.*, 1992), amongst other adaptations. A dose-response relationship between physical activity and cardiovascular outcomes is also well described (Eijsvogels and Thompson, 2015), with as little as 15 mins per day of moderate-intensity exercise significantly lowering the risk of all-cause mortality and increasing life expectancy (Wen *et al*., 2011). The World Health Organisation (2011) recommends 150 mins of moderate-intensity or 75 mins of vigorous-intensity aerobic exercise per week, for adults aged 18-64 years. However, whilst these exercise volumes yield a significant health improvement (Powell *et al.*, 2011), with further increasing exercise volumes producing further smaller risk reductions (Eijsvogels *et al.*, 2016), a large population do not meet these exercise guidelines. In stark contrast, participation in prolonged endurance training and events has grown in popularity, particularly in marathons, triathlons and cycling events (Knechtle *et al.*, 2011; Shave *et al.*, 2007b; Shoak *et al.*, 2013), whereby participants are frequently participating in more than double the recommended daily/weekly exercise guidelines. Whilst greater volumes have been found to yield a greater cardiovascular benefit (Sarna *et al.*, 1993), the health consequences of participating in extreme volumes of exercise is under debate (Eijsvogels *et al.*, 2016; Eijsvogels and Thompson, 2017). Little is known regarding the upper limit of exercise volume for cardiovascular health benefits, with some authors suggesting no upper limit (Mandsanger *et al*., 2018), whilst others suggest a

plateau or possible increased risk with very high exercise volumes (La Gerche and Prior 2007; Whyte, 2008). A number of studies have also reported potentially adverse cardiovascular responses to prolonged exercise, including post-exercise cardiac dysfunction (Oxborough *et al*., 2010) and exercise-induced elevations in cardiac biomarkers (Shave *et al.,* 2007a). Whilst a growing evidence-base suggests that the physiological demands of maintaining a high cardiac workload for a prolonged period of 60 mins or greater (Middleton *et al*., 2006a) may result in a transient impairment in cardiac function, the release of cardiac biomarkers into the systemic circulation may occur following as little as 30 mins exercise (Shave *et al*., 2010). It is also suggested that a combination of factors such as exercise duration, intensity and volume, participants training experience, as well as environmental factors that precipitate changes in ventricular function and biomarker appearance (Gresslien and Agewall, 2016; Oxborough *et al.,* 2010; Sedaghat-Hamedani *et al.,* 2015), although more research is required in this controversial area (Dawson *et al.,* 2003). Participation in multi-day prolonged endurance events, with repeated endurance exercise exposure more than double the World Health Organisation (2011) guidelines with limited recovery time is also becoming increasingly popular (Knoth *et al*., 2012). However, limited attention has been given to the effect of multi-day exercise upon cardiac function and biomarkers of cardiac damage or stress.

The focus of this literature review, and the empirical studies within this thesis is to develop our knowledge of acute cardiovascular consequences of undertaking single and repeated bouts of prolonged exercise. Therefore, the following literature review initially describes the cardiovascular response to acute aerobic exercise, followed by a review of the existing findings in relation to the impact of single and repeated bouts of endurance exercise upon cardiac function and biomarker release. Finally, the literature pertinent to cardiovascular function during heat stress and strategies to reduce the physiological strain

of exercising in thermally stressful environments is evaluated before the aims and hypotheses of this thesis are presented.

2.2 Cardiovascular Responses to Acute Aerobic Exercise

Prior to reviewing the literature relating to the cardiovascular response to endurance exercise, an understanding of the cardiac cycle is important to comprehend before a discussion of how the cardiovascular system responds to an acute bout of sub-maximal aerobic exercise. The normal cardiac cycle consists of a phase of myocardial contraction (systole) and a period of myocardial relaxation (diastole), which results in the ejection and filling of blood of the LV. A well-coordinated sequence of pressure and volume shifts must occur to maintain the continuous change between filling and ejection from the LV (Figure 2.1).

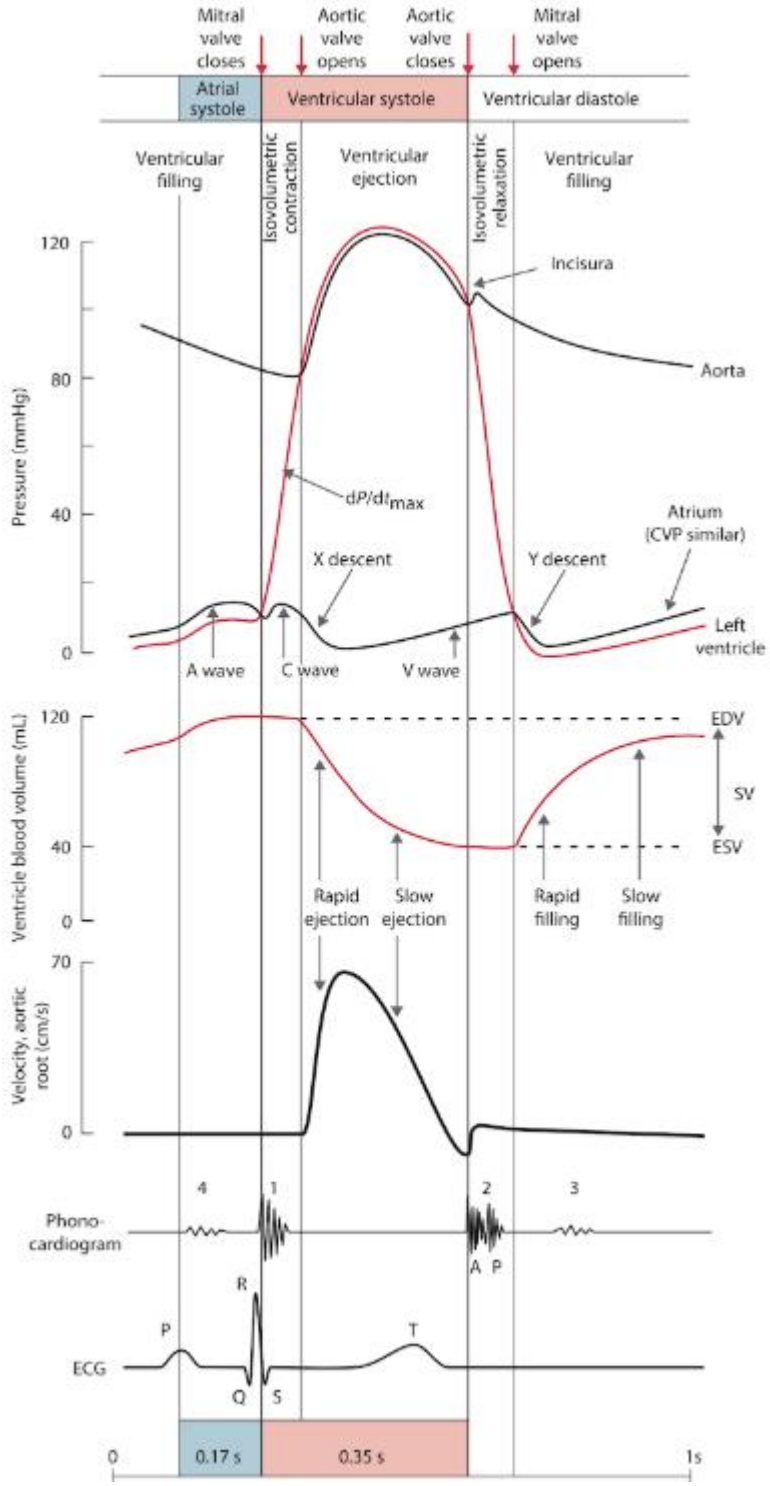


Figure 2.1 The normal left ventricular (LV) cardiac cycle. Interaction of the changes in pressure, volume and flow in the aorta, LV and left atrium. EDV: end diastolic volume; ESV: end systolic volume; SV: stroke volume (From Herring and Paterson, 2018; Reprinted with permission from Publisher).

LV systole is initiated by a brief period of isovolumetric contraction, whereby a shortening of endocardial myofibres and stretch of the epicardial fibres result in an increase in intra-ventricular pressure, without a change in LV volume (Herring and Paterson, 2018). As the ventricles contract, LV pressure rapidly exceeds left atrial pressure creating a pressure gradient, pushing back the leaflets of the mitral valves, forcing them to close (Calvert and Lefer, 2012). Further contraction of the LV increases LV pressure above that of the diastolic pressure in the aorta. As a result, the aortic valve is forced open, ejecting blood into the circulation, reducing the LV volume. The continued contraction of the LV increases the pressure in the aorta which peaks approximately half way through the systolic ejection period, after which the intra-ventricular pressure starts to decline below that in the aorta. This allows for the blood in the aorta to push back on the aortic valve, forcing it to close (Calvert and Lefer, 2012). The reduction in LV volume at the end of this phase represents the LV end systolic volume (LVESV). The period of systole is closely followed by a period of diastole. LV diastole is defined as the period from the end of aortic ejection until the onset of ventricular tension of the succeeding beat (Gibson and Francis, 2003) and is determined by ventricular relaxation and effective chamber compliance (Nishimura and Tajik, 1997). It initiates at the terminal phase of systolic activity through the decline in myocardial tension caused by the uptake of calcium into the sarcoplasmic reticulum (Gibson and Francis, 2003). The rapid fall in intra-ventricular pressure at the end of systole (~80 mmHg to 0-5 mmHg), known as isovolumetric relaxation time, allows for the myofibres to relax prior to any change in LV volume and begins at the onset on the closure of the aortic valve. The reduction in pressure creates a pressure gradient between the left atrium and LV that forces open the mitral valve, initiating early LV filling (E). Initially, ventricular pressure falls despite an increase in blood volume due to the relaxing ventricle recoiling elastically from its deformed end systolic shape. As a result, blood is sucked into the ventricle in early diastole (Herring

and Paterson, 2018). Peak E velocity can be measured by pulsed Doppler, with values usually ranging 1.0-1.2 $m.s^{-1}$ in young healthy adults. The rate of filling slows down as the ventricle reaches its inherent relaxed volume (diastasis). Venous pressure drives further filling, distending the ventricle, causing a gradual increase in ventricular diastolic pressure (Calvert and Lefer, 2012). Contraction of the atria propels further blood into the ventricle, accounting for 10-20% of ventricular filling at rest (Herring and Paterson, 2018). This phase of atrial contraction is known as late diastolic filling (A) and produces a further increase in left atrial and LV pressures. By the end of the filling phase, the volume has increased to represent the LV end diastolic volume (LVEDV), following the closure of the mitral valves. The difference between LVEDV and LVESV is known as SV and therefore represents the volume of blood ejected from the ventricle with each contraction of the heart. During increased cardiovascular demand i.e. during exercise, the volumes and durations within the cardiac cycle may all be influenced by three factors: 1) preload, 2) afterload and 3) contractility.

A major determinant of preload is venous return, quantified as the amount of blood returning to the right atrium each minute (Calvert and Lefer, 2012). Factors that lead to an increased venous return/preload include: an increased cardiac output i.e. through exercise, increased central blood volume, increased venous tone and rapid ventricular relaxation. An enhanced preload leads to further distention of the ventricle during diastole, this increased tension of the cardiac myofibres causes a reflex increase in myofibre contractility and a greater ejection of blood into the aorta, contributing to an increased SV (Calvert and Lefer, 2012). The intrinsic ability of the heart to adapt to changes in preload is known as the Frank-Starling mechanism. Preload, therefore, reflects the end-point of venous return, LV filling, pressure and stretch on the LV wall, known as

LVEDV. It is also important to note that the LV becomes less distensible with greater filling (larger fiber lengths) at extreme LVEDV's.

Afterload (i.e. aortic pressure) reflects the resistance that the LV must overcome to eject blood (Calvert and Lefer, 2012). Therefore, an increase in afterload requires a higher peak systolic pressure during systole, impacting upon SV. An increase in afterload reduces the force production of the LV myofibres due to more energy being used to overcome the heightened aortic pressure before blood ejection is possible during isovolumetric contraction (Herring and Paterson, 2018). Consequently, a progressive increase in afterload can inhibit myofibre shortening during the ejection phase, attenuating a reduction in LVESV and subsequently SV. Afterload can be estimated by measuring the pressure that the ventricle produces during systolic ejection (systolic blood pressure; SBP).

Neural input from the sympathetic and parasympathetic nervous systems influence the rate (chronotropic state), force of LV contraction (inotropic effect) and relaxation (lusitropic effect), independent of preload (fiber 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. As a consequence, 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). This process of myocyte contraction and relaxation occurs on an average rate of 60 times a minute, otherwise known as heart rate (HR). At rest, HR reflects the predominant activity of the

parasympathetic nervous system, releasing acetylcholine which slows the intrinsic activity of the heart. 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-andrenergic receptors in the myocardium, resulting in an increase in chronotropic, inotropic and lusitropic effects (Opie, 2004) from a greater release and uptake of calcium into the sarcoplasmic reticulum. An increase in contractility through sympathetic stimulation produces a significant change in SV, reflected by an increase in EF, calculated as SV expressed as a fraction of LVEDV (Konstam and Abboud, 2017). It is important to note that EF is influenced by both preload (diastolic) and afterload (systolic) and consequently should not be interpreted as a measure of contractility without knowledge of LV loading (Konstam and Abboud, 2017).

The cardiovascular adaptations that occur in response to an acute bout of submaximal exercise are well regulated and reflect a rapid adjustment to the increased demands placed upon the body. A 30 min bout of submaximal running is tolerated well by most individuals, with a rapid recovery post-exercise i.e. rapid decrease in HR when exercise ceases. When exercise exposure is significantly increased i.e. increased duration, the cardiovascular workload is also elevated. Whether the cardiovascular system copes with this higher dose of exercise is under debate and is the focus of this thesis.

2.3 The Impact of Single Bouts of Endurance Exercise on Cardiac Function and Biomarker Release

During prolonged exercise, particularly ultra-endurance exercise, there is a considerable amount of cardiac work. Concurrently the heart must also deal with an elevation in T_c, increased mechanical work, increased levels of catecholamines, altered pH and an

exposure to reactive oxygen species (Dawson *et al*., 2003; George *et al.*, 2012). Moderate exercise (e.g. 50-75% VO_2max) of greater than 10 mins is typically associated with a progressive rise in HR, despite a maintained workload, commonly known as cardiovascular drift. Cardiovascular drift is due to a progressive increase in cutaneous blood flow as body temperature rises, increasing cutaneous venous volume. As a result, a progressive reduction in ventricular filling pressure, EDV and SV are apparent, with a parallel increase in HR observed in order to maintain cardiac output (Coyle and González-Alonso, 2001). Though the benefits of participating in physical activity are apparent, whether the heart can maintain performance alongside such challenges is less clear and will be described in the next section.

2.3.1 Alterations in Cardiac Function

A growing evidence-base suggests that the physiological demands of maintaining a high cardiac workload for a prolonged period may result in an immediate depression in systolic and/or diastolic function (Douglas *et al.*, 1990b; George *et al.*, 2004, 2005; Middleton *et al.*, 2007a; Shave *et al.*, 2004a, 2004c; Vanoverschelde *et al.*, 1991). The transient impairment in cardiac function immediately following prolonged exercise in the absence of underlying cardiovascular diseases has been termed as exercise-induced cardiac fatigue (EICF; Banks *et al.*, 2010; Shave *et al*., 2004c; Whyte *et al.*, 2000). This area has been given a vast amount of attention in recent years, with reviews (Dawson *et al.*, 2003; Oxborough *et al*., 2010; Shave *et al.*, 2008) and a meta-analysis (Middleton *et al.*, 2006a) providing more support for the existence of EICF. The majority of previous studies have focused upon post-exercise changes in LV function (Shave *et al.*, 2008), therefore, EICF has been mainly characterised by an acute post-exercise reduction in LV systolic and/or diastolic function (Dawson *et al.*, 2003; Shave *et al*., 2004c). The mechanisms involved in the process are unclear and the exercise parameters that augment cardiac fatigue are

poorly described (Oxborough *et al.*, 2010). A wide range of exercise modes, durations and intensities within differing environmental conditions have been studied to elucidate the causes of EICF, however, there are many discrepancies within the findings (Table 2.1).

Table 2.1 provides an over-arching summary of the literature which includes past studies which assessed LV systolic and diastolic function before and after exercise using standard 2D echocardiography, flow-Doppler and tissue-Doppler (TDI) indices. The table details the studies by mode (running, cycling, multimode), exercise distance (duration), the intensity of the exercise and environmental conditions, alongside the study's findings.

Table 2.1 Studies utilising standard 2D, flow-Doppler and tissue-Doppler echocardiography following single bouts of prolonged exercise

Author	Exercise distance (duration)	Intensity of Exercise	Systolic			Diastolic			Environmental Conditions
			↑	=	↓	↑	=	↓	
RUNNING									
Shave *et al*., 2002c	Maximal ramping treadmill test	Maximal		EF, SV, FS			E, A, E:A		NR
	30 min downhill running	70% maximal running velocity		EF, SV, FS			E, A, E:A		NR
Weippert *et al*., 2016	Trial 1: 60 min	Two blocks separated by 3 mins (12 x 90% HRpeak (30 sec sprint with 15 sec recovery))		EF, FS			E, E’		NR
	Trial 1: 60 min	70% HRpeak continuous		EF, FS			E, E’		NR
Alshaher *et al*., 2007	Test to exhaustion (84 ± 39 min)	70% VO_2max		EF	SV			E:A	NR
Tian *et al.*, 2014	Trial 1: 90 min	95% VT		EF, SV		A		E, E:A	20ºC, 50% RH
	Trial 2: 90 min	95% VT		EF, SV		A		E, E:A	20ºC, 50% RH
Vanoverschelde *et al.*, 1991	20 km (95 ± 15 min)	Self-paced			EF, SV, FS	A		E, E:A	NR
Seals *et al*., 1988	Exercise to exhaustion (170 ± 10 min)	Alternate high and low intensities			FS				NR

Fragasso *et al*., 2004	High Altitude race: 30 km (4 h 27 min) High Altitude race: 25 km (2 h 59 min)	Self-paced Self-paced					A (A, E, E:A 24 h post) A (A, E, E:A 24 h post)	E, E:A E, E:A	1191 – 4559 m 2100 – 4165 m
Sahlén *et al*., 2009a	30 km cross-country race (119 ± 28 min)	Self-paced	S’	EF	SV	A, A’		E, E:A, E’	NR
Sahlén *et al*., 2010	30 km cross-country race	Self-paced		EF					NR
Banks *et al*., 2010	150 min 150 min	60% VO_2max 80% VO_2max		EF EF			E:A	E’A’ E:A, E’:A’	NR NR
Banks *et al*., 2011	150 min Younger cohort 150 min Older cohort	80% VO_2max 80% VO_2max		EF, SV EF	 SV	A, A’ A, A’	E	E:A, E’, E’:A’ E, E:A, E’, E’:A’	NR
Dalla Vecchia *et al*., 2014	Half-marathon (96 min 30 s)	Self-paced		EF		A		E, E:A, E’	15ºC
Chan-Dewar *et al*., 2010b	Marathon (229 ± 38 min)	Self-paced		EF				E:A	Midday 21ºC
Dawson *et al*., 2008	Marathon (229 ± 38 min)	Self-paced		EF, S’				E:A, E’:A’	Midday 21ºC
George *et al*., 2004	Marathon (256 ± 46 min)	Self-paced		EF, FS, SBP/LVESV	SV	A	E	E:A	11ºC, 40% RH
George *et al*., 2005	Marathon (256 ± 46 min)	Self-paced				A, A’		E, E:A, E’, E’:A’	14ºC, 50% RH, light winds
George *et al*., 2006	Marathon (256 ± 46 min)	Self-paced		EF, FS	SV, S’				14ºC, 50% RH
Hart *et al*., 2007	Marathon (216 ± 40 min)	Self-paced		EF		A, A’		E, E:A, E’, E’:A’	12.5ºC

Hewing *et al*., 2015	Marathon (263 ± 37 min)	Self-paced	FS, S’			A, A’		E, E:A, E’	NR
Kasikcioglu *et al*., 2006	Marathon	Self-paced		(FS 1 d post)			(E, A, E:A 1 d post)		NR
Knebel *et al*., 2009	Marathon (256 ± 37 min)	Self-paced	FS				A’	E:A, E’	23.5ºC, 46% RH at midday
Knebel *et al*., 2014	Marathon: Premenopausal Marathon: Postmenopausal	Self-paced Self-paced		FS FS		A’ A’	E’ (E:A, E’, A 2 w post in both groups)	E:A E:A, E’	12ºC, 75% RH at midday
Lucía *et al*., 1999a	Marathon	Self-paced	EF, FS			A		E, E:A	11-22ºC, 60-65% RH
Middleton *et al*., 2006b	Marathon (212 ± 36 min)	Self-paced	S’	EF, SBP/LVESV		A, A’		E, E:A, E’, E’:A’	Max 11ºC, frequent rain showers
Middleton *et al*., 2007a	Marathon: 2004 (201 ± 29 min) Marathon: 2005 (212 ± 50 min)	Self-paced		EF, SBP/LVESV EF, SBP/LVESV				E:A, E’:A’ E:A, E’:A’	NR NR
Mousavi, *et al*., 2009	Marathon (245 ± 68 min)	Self-paced		EF, S’		A, A’ (A’ 1 wk post)		E, E:A, E’ (E’ 1 wk post)	NR
Neilan *et al*., 2006a	Marathon (4 h 5 min)	Self-paced		EF		A, A’		E, E:A, E’	NR
Neilan *et al*., 2006b	Marathon (3.54 ± 0.56 h)	Self-paced		EF		A, A’ (A 3-4 wk post)		E, E:A, E’ (E:A, E’ 3-4 wk post)	NR

Oxborough *et al*. 2006	Marathon (230 ± 42 min)	Self-paced				A		E, E:A	NR
Perrault *et al*., 1986	Marathon (2 h 46 min)	Self-paced		EF, FS					27.2ºC, 94% RH
Shave *et al*., 2009	Marathon (213 ± 41 min)	Self-paced HRmean 161 ± 12 beats.min^{-1}		EF, FAC, S’		A, A’		E, E:A, E’, E’:A’	NR
Sierra *et al*., 2016	Marathon	Self-paced		EF	S’		A, E’, A’	E, E:A	Race start: 17.8ºC, 55% RH, 1 m.s^{-1} wind velocity Race end: 22.8ºC, 59% RH, 2 m.s^{-1} wind velocity
Wilson *et al*., 2011	Marathon (209 ± 19 min)	Self-paced				A (A 6 h post)	E (E 6 h post)	E:A (E:A 6 h post)	Max 10ºC, Approx. 40% RH
Whyte *et al*., 2005	Marathon (245.3 ± 46.0 min)	Self-paced		EF	SV	A, A’		E, E:A, E’, E’:A’	Max 14ºC, Approx. 50% RH
Carrió *et al*., 1990	6 hour race (mean distance 54.7 km)	Self-paced	EF						13 – 15ºC, 84% mean RH
Jouffroy *et al*., 2015	80 km trail run (9 h 55 min ± 1 h 19 min)	Self-paced			EF, FS		A, A’	E, E:A, E’, E’:A’	NR
Chan-Dewar *et al*., 2010a	The Comrades Marathon: 89 km (586 ± 80 min)	Self-paced			EF, S’			E:A, E’, A’	Midday 20ºC

George *et al*., 2009	The Comrades Marathon: 89 km (586 ± 80 min)	Self-paced			EF, FAC, SBP/LVES V		A	E, E:A	4 - 24ºC
Cote *et al*., 2015	100 km or 160 km mountain marathon Sex differences	Male: 4.7 ± 1.1 $km.h^{-1}$ Female: 4.8 ± 0.7 $km.h^{-1}$		SBP/LVES V SBP/LVES V	EF, SV EF, SV		E, A E, A	E:A E:A	6 – 30ºC, ascent 600 – 2300 m
Niemela *et al*., 1984	24 h run (114 – 227 km)	Self-paced		SV, FS, SBP/LVES V					7 – 15ºC, 50 – 60% RH, 1 - 7 $m.s^{-1}$ wind velocity
Passaglia *et al*., 2013	24 h ultramarathon (140.32 km)	Self-paced		EF		A, A’	E, E’:A’	E:A, E’	9 - 21ºC
Dávila-Román *et al*., 1997	163 km mountain ultramarathon	Self-paced		EF					2 – 24ºC, 10 – 60% RH, 2350 – 4300 m altitude
Scott *et al*., 2009	100 mile trial race (25.5 ± 3.2 h)	Self-paced	SBP/LVES V	EF, SV, FAC			A	E, E:A	NR
Maufrais *et al*., 2016	300 km mountain ultramarathon (126 h 2 min)	Self-paced	SV	EF		E	A, E:A		Elevation gain +24000 m
CYCLING									
Crawford *et al*., 1979	Exercise to exhaustion (9 – 18 mins)	Incremental			FS				NR

Ketelhut *et al*., 1994	5 min 30 min	130-140 beats.min^{-1} 130-140 beats.min^{-1}	EF, SV, FS		EF, SV, FS				NR
Eysmann *et al*., 1996	Exercise to exhaustion (95 min)	Self-paced			EF			E:A	NR
Stewart *et al*., 2015	60 min	Self-paced		EF, SV				E:A, E’	NR
Chan-Dewar *et al*., 2013	8.00am: 40 km (76 ± 6 min) 6:00pm: 40 km (76 ± 7 min)	90-100% LT 90-100% LT		EF, FS EF, FS	SV, S’ SV, S’	A’ A’	E, A, E:A E, A, E:A	E’, E’:A’ E’, E’:A’	20ºC, 40% RH 20ºC, 40% RH
Stewart *et al*., 2016	90 min 120 min	110% GET 80% GET		 SV	SV				22ºC, ~55% RH
Goodman *et al*., 2009	120 mins	~75% HRmax or 60-65% VO_2max		EF, SV, SBP/LVESV					NR
Stickland *et al*. 2004	Exercise to exhaustion: (2.51 ± 0.86 h)	25 watts below lactate threshold			SV				21ºC
Vitello *et al*., 2013	3 x 6 min incremental workloads preceded 180 min	20, 30 and 40% max aerobic power > 130 beats.min^{-1}		S’	EF, SV, FS		A, A’	E, E:A, E’	NR
Dawson *et al*., 2005	4 h	5% below LT		SBP/LVESV (SBP/LVESV 24 h post)		A	E (E, A, E:A 24 h post)	E:A	NR

Shave *et al.* 2004b	50 mile (126 ± 7 min) 50 mile (125 ± 6 min)	LT LT		SV, FS SV, FS			E, A, E:A E, A, E:A		19ºC, normobaric normoxia 19ºC, normobaric hypoxia
Shave *et al.* 2004c	100 mile (256 ± 15 min) 100 mile (254 ± 13 min)	5% below LT 5% below LT		EF, SV, SBP/ESV EF, SV, SBP/ESV		A	 E, A, E:A	E, E:A	0 ± 0.1ºC 19 ± 0.4ºC
Serrano-Ostariz *et al.*, 2013	206 km (accumulated slope 3800 m)	Self-paced			EF			E:A	16.0 - 32.5ºC
COMBINATION									
Scharhag *et al.*, 2006	1 h running or cycling 3 h running or cycling	95-100% AT 75% AT		EF, FS EF, FS		A A, A’		E, E:A, E’, E’:A’ E, E:A, E’, E’:A’	NR NR
McGavock *et al.*, 2003	Olympic Triathlon: 40 km cycle, 10 km run (121 ± 8 min)	90 ± 3% HRmax		FAC					NR
Haykowsky *et al.*, 1991	Half-ironman triathlon	Self-paced			FAC, SBP/LVESV				NR
Shave *et al.*, 2004a	Half-ironman triathlon: 1.9 km swim, 90 km cycle, 21.1 km run (5 h 01 min ± 25 min)	Self-paced		(SBP/LVESV 48 h post)	SBP/LVESV	A	(E, A, E:A 48 h post)	E, E:A	19ºC, 50% RH

Welsh *et al*., 2005	Half-ironman triathlon: 2 km swim, 90 km cycle, 21 km run (5 h 01 min ± 25 min)	Self-paced			SBP/ESCA				18ºC, precipitation 0.8 cm, wind speed 7 $km.h^{-1}$
Whyte *et al*., 2000	Half-ironman triathlon: 1.9 km swim, 90 km cycle, 21 km run (5 h 29 min ± 20 min)	Self-paced		EF, FS (EF, SV, FS 48 h post)	SV	A		E, E:A (E, A, E:A 48 h post)	NR
Leetmaa *et al*., 2008	Triathlon: 4 km swim, 120 km cycle, 30 km run (7 h 21 min)	Self-paced		EF, S’			A	E, E:A	NR
Whyte *et al*., 2000	Ironman triathlon: 3.8 km swim, 180 km cycle, 42.2 km run (10 h 40 min)	Self-paced		(EF, SV, FS 48 h post)	EF, SV, FS	A		E, E:A (E, A, E:A 48 h post)	NR
Douglas *et al*., 1987	Ironman triathlon: 2.4 mile swim, 112 mile cycle, 26.2 mile run (12 h 30 min)	Self-paced		(FS 1 d post)	FS	A (E, A, E:A 1 d post)	E, E:A		Air: 24 - 42ºC, Water: 26ºC, 40-85% RH

Douglas *et al*., 1990a	Ironman triathlon: 3.9 km swim, 180.2 km cycle, 42.2 km run	Self-paced		FAC		E, A (E 28 h post)	E:A (A, E:A 28 h post)		Air: 24 - 42ºC, Water: 26ºC, 40 - 85% RH
Douglas *et al*., 1990b	Ironman triathlon: 3.9 km swim, 180.2 km cycle, 42.2 km run	Self-paced		(EF 1 d post)	EF				Air: 24 - 42ºC, Water: 26ºC, 40 - 85% RH
Douglas *et al*., 1998	Ironman triathlon (3.9 km swim, 180.2 km cycle, 42.2 km run)	Self-paced			EF				NR
Hassan *et al*., 2006	Ironman triathlon: 3.8 km swim, 180 km cycle, 42.2 km run (712 ± 96 min)	Self-paced		EF, SV			A	E, E:A	Air: 15.6 – 20.9ºC, Water: 15ºC, 48 - 79% RH
La Gerche *et al.*, 2004	Ironman triathlon: 3.8 km swim, 180 km cycle, 42.2 km run (592 ± 100 min)	Self-paced		EF			E, A, E:A		16 - 27ºC

La Gerche *et al.*, 2008	Ironman triathlon: 3.8 km swim, 180 km cycle, 42.2 km run (10 h 50 min ± 1 h 15 min)	Self-paced		EF			E:A, E’		NR
Tulloh *et al*., 2006	Ironman triathlon (694 min)	Self-paced			EF, SV				NR
Rifia *et al.* 1999	Ironman triathlon (3.9 km swim, 180.2 km cycle, 42.2 km run)	Self-paced			EF				NR
Ashley *et al*., 2006	300 miles Adventure race	Self-paced			EF, FS		E, A		NR
OTHER									
Poh *et al*., 2008	3000 m speed skating	Self-paced	EF, S’			E, A’	A, E’		NR
Planer *et al*., 2012	Field training exercise (85 – 103 h)	Self-paced	EF, S’				A, E:A, E’, A’	E	Discomfort index: >28 day, 25.2 ± 2.4 night

EF: ejection fraction, SV: stroke volume, FS: fractional shortening, SBP/LVESV: systolic blood pressure/left ventricular end systolic volume, S’: peak systolic myocardial tissue velocity, FAC: fractional area change, SBP/ESCA: systolic blood pressure/end systolic cavity area, E: peak early trans-mitral flow velocity, A: peak atrial filling velocity, E:A: early to late diastolic filling ratio, E’: peak early diastolic myocardial velocity, A’: peak late diastolic myocardial velocity, E’/A’: peak early to late myocardial tissue velocity ratio, VT: ventilatory threshold, AT: anaerobic threshold, GET: gas exchange threshold, LT: lactate threshold, NR: not reported.

The increased sympathetic activity, increased preload, and/or alteration in blood flow in response to prolonged exercise, has in many cases led to an unchanged or improved systolic function, as measured by EF, SV, fractional shortening (FS), systolic blood pressure/left ventricular end systolic volume (SBP/LVESV) or peak systolic myocardial tissue velocity (S'; Table 2.1). No alterations in EF (Mousavi *et al.,* 2009; Neilan *et al*., 2006b) or S' (Shave *et al*., 2009) were reported following a marathon run, whilst 1 h/3 h of running or cycling did not provoke any changes in FS (Scharhag *et al.*, 2006). A lack of change in SV and SBP/LVESV has also been demonstrated following 100 miles of cycling (Shave *et al.* 2004c). Overall, short duration exercise appears to have little impact upon systolic function (Table 2.1). Conversely, Vanoverschelde *et al.* (1991) reported a reduction in EF following 20 km running, whilst Seals *et al.* (1988) reported a reduced FS following an exercise run to exhaustion. Whilst these changes may be mediated by lower levels of fitness in the participants, older imaging techniques provide a lower sensitivity and resolution. Decreases in LV systolic function following prolonged exercise has been reported in several studies (Table 2.1), more commonly following ultraendurance exercise. For example, Rifai *et al*., (1999) demonstrated a 24% reduction in EF following an ironman triathlon, which is greater than what would be expected from measurement variability. EF was also below the lower limit for normal LV systolic function, yet the clinical significance is yet to be elucidated. Following a single bout of exercise, systolic function has however, typically returned to normal after 24-48 hours of recovery (Shave *et al.,* 2004a; Whyte *et al.,* 2000). A meta-analysis has suggested that shorter duration exercise of < 6 hours exerts minimal impact upon LV systolic function (Middleton *et al.*, 2006a). However, more recently it is suggested to be a combination of factors such as exercise duration, intensity and volume, participants training experience, as well as environmental factors that precipitate changes in ventricular function

(Oxborough *et al.*, 2010) and will reviewed in Section 2.3.3. More research is required in this controversial area (Dawson *et al.*, 2003) and will be a focus of this thesis.

It is important to note however, that changes in EF indices should be interpreted with care as these are dependent upon changes in preload and afterload, especially when taking into consideration the effect of prolonged exercise on dehydration, hypovolemia and BM (Dawson *et al*., 2003). Therefore, it is possible that reported changes in LV function may represent an alteration in hemodynamics rather than a depression in inotropy or intrinsic relaxation properties (Scott and Warburton, 2008). For example, Goodman *et al*. (2009) found a depression in LV systolic performance following exercise, however, no change was observed during exercise. Possible explanations for this were attributed to an abrupt reduction in venous return and sympathetic withdrawal following exercise.

Evidence for changes in LV diastolic function after prolonged exercise has been reported within numerous studies (Table 2.1), with changes in diastolic function reported to precede changes in systolic function (Shave *et al*., 2004c). The most common finding was a reduction in the early to late atrial (E:A) peak flow velocity filling ratio, as a consequence of a reduction in E and an increase in A (Neilan *et al*., 2006b; Shave *et al*., 2009). The E:A ratio has previously been used to represent a global index of diastolic function, with a reduction often associated with LV diastolic stiffness in pathological conditions. A meta-analysis by Middleton *et al*. (2006a), reported an overall immediate post-exercise reduction in E:A ratio in all subgroups studied, reflecting altered diastolic filling dynamics. The change in E:A ratio was not correlated with changes in the loading variables of left ventricular internal dimension–diastole (LVIDd), SBP and HR, suggesting changes in LV diastolic function are not fully explained by changes in loading post-exercise. This is also supported by Whyte *et al*. (2005) and Dawson *et al*. (2008)

who both reported depressions in diastolic function did not correlate with changes in HR and loading, suggesting alterations in the intrinsic relaxation properties of the LV. Not all studies however, have found a decrease in diastolic function following prolonged exercise (Table 2.1). La Gerche *et al.* (2008) and Kasikcioglu *et al*. (2006) found insignificant changes in conventional measures of diastolic function (E:A) following an Ironman triathlon and marathon, respectively. The study by Kasikcioglu *et al*. (2006) should, however, be interpreted with caution due to a lack of statistical power by only recruiting 6 participants, as well as post-exercise data collection being conducted 1 day following exercise cessation. Many studies have shown that upon follow-up, any alterations in diastolic function have returned to baseline levels. However, Mousavi *et al*. (2009) showed LV diastolic dysfunction remained 1 week following a marathon, whilst Neilan *et al.* (2006b) found that during a follow-up measure 3-4 weeks post-marathon, A and E:A remained abnormal.

It is suggested that myocardial tissue velocities, measured through TDI are less load dependant compared to standard pulsed-wave Doppler imaging as a measure of diastolic function (Pela *et al*., 2002; Yalcin *et al*., 2002), though this remains controversial (Burns *et al*., 2007; Firstenberg *et al*., 2001). A number of studies (Table 2.1), including Whyte *et al.* (2005), demonstrated a reduction in peak early diastolic myocardial velocity (E'), an increase in peak late diastolic myocardial velocity (A'), which resulted in a reduced E':A'. Whyte *et al*. (2005) also found a significant correlation between TDI and standard Doppler measures of diastolic function, suggesting either methods are appropriate tools in investigating diastolic functional changes. An alteration in E could originate from a change in left atrial pressure immediately prior to the opening of the mitral valve and/or a disturbance in the pressure decay in the LV during the diastolic period (Hees *et al.*, 2004). The ratio of E to E' (E:E') can be calculated as a non-invasive estimate of left

atrial pressure (Naugueh *et al.,* 1998), and in some studies has been found to be maintained despite reductions in diastolic function, suggesting a decay in LV pressure (George *et al.*, 2005). However, other authors have criticised this method as being too insensitive for detecting pressure changes (Neilan *et al.*, 2006b).

Whilst there are a number of studies which evidence a reduction in cardiac function following prolonged exercise, the mechanisms responsible for these exercise induced changes are not fully understood. Changes in cardiac loading i.e. LVEDV and SBP have accounted for some, but not all of the apparent functional reductions, with a multifactorial model of cardiac fatigue being proposed by Shave and Oxborough (2012; Figure 2.2).

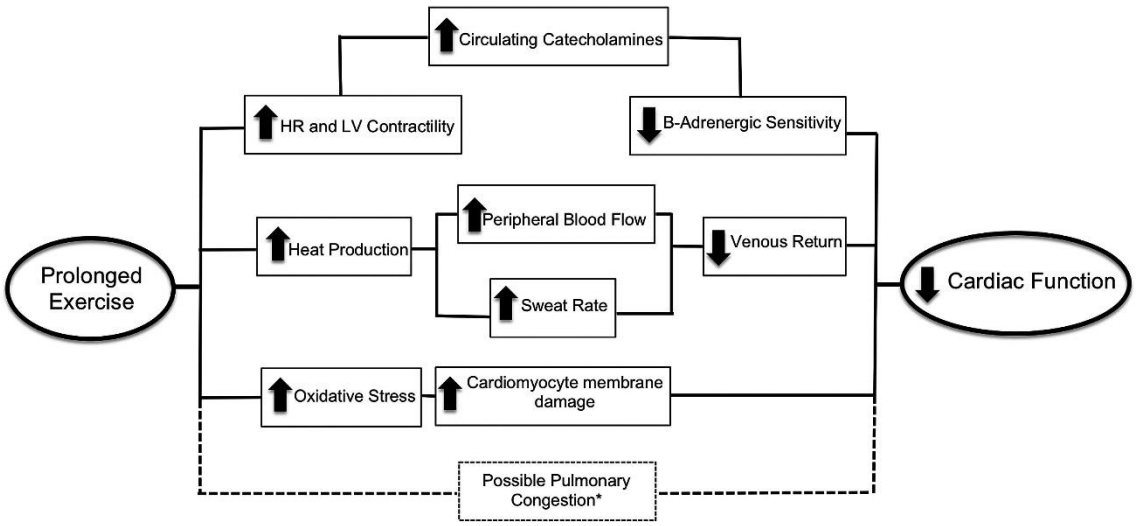


Figure 2.2 Potential mechanisms responsible for impaired cardiac function after prolonged exercise (From Shave and Oxborough, 2012; Reprinted with permission from Publisher).

Two potential mechanisms have been proposed as intrinsic, non-load related mechanisms behind changes in cardiac function: 1) β-andrenergic receptor downregulation (Hart *et al.,* 2006) and 2) Myocyte damage (Neilan *et al.,* 2006b). The sustained exposure to catecholamine's during prolonged exercise, has been found to result in a desensitization

or down regulation of cardiac β-andrenergic receptors (Banks *et al.,* 2010; Oxborough *et al*., 2010) and autonomic responsiveness (Seiler *et al.,* 2007), leading to a reduced force of contraction and impaired rate of LV relaxation. Previous single day studies have also suggested an association between cTn release and cardiac function (Neilan *et al.,* 2006b; Rifai *et al.,* 1999), whilst others have refuted this finding (Shave *et al.,* 2004c). The following section of the literature review discusses the extant evidence of cardiac damage associated with single bouts of endurance exercise.

2.3.2 The Appearance of Tissue-Specific Markers of Cardiac Damage

The appearance of cardiac biomarkers, normally indicative of cell damage and myocardial infarction, have been observed in numerous individuals without coronary artery disease such as myocarditis, supra-ventricular tachycardia, pericarditis, as well as non-cardiac conditions such as high dose chemotherapy, renal failure, primary pulmonary hypertension, cerebrovascular accident and, more recently, endurance exercise such as marathons (Amman *et al*., 2004; Bakshi *et al*., 2002; Kelley *et al.,* 2009). Participants in endurance exercise maintain an elevated cardiac output with a normal or elevated SBP for a prolonged period of time. This sustained elevation of cardiac work results in a considerable stress on the cardiovascular system, which may result in myocardial injury (Shave *et al.,* 2007b). The appearance of tissue-specific markers of cardiac damage following prolonged exercise has been termed exercise-induced cardiac damage (EICD; Shave *et al*., 2004c; Whyte *et al.,* 2005).

Troponin is a protein component of the contractile apparatus within skeletal and cardiac myocytes, which regulate and facilitate the interaction between actin and myosin filaments during a muscle contraction (Garg *et al.,* 2017). cTn is a complex comprising of three subunits: 1) troponin T attaches the troponin complex to the actin filament, 2)

troponin C which binds to calcium, and 3) troponin I which inhibits the ATPase activity of actomyosin complex (Katrukha, 2013). Whilst cTn C is synthesised in skeletal and cardiac muscle, cTn I (cTnI) and T (cTnT) isoforms are highly sensitive and specific markers to cardiac myocytes (Garg *et al.*, 2017), with detection in the bloodstream indicative of myocardial injury (Amman *et al*., 2004). The use of cTn remains important in the diagnosis of AMI, or now commonly known as acute coronary syndrome (ACS; Ammann *et al*., 2004; Shave *et al.*, 2007b). Following the onset of AMI, cTn's appear in the serum 4-10 h (Jaffe *et al*., 1996), peaking following 12-48 h and remaining elevated for 4-10 days (Garg *et al.*, 2017). A growing amount of research suggests an elevation in cTn, immediately following acute bouts of prolonged exercise (Shave *et al*., 2007a; Table 2.2). As the number of individuals participating in endurance exercise continues to increase, medical personnel will more frequently have to assess patients presenting elevated cardiac biomarkers following prolonged exercise (Fortescue *et al*., 2007; Knebel *at al*., 2009) and should check for other symptoms before assuming that an athlete has suffered ischemic myocardial injury (Thompson *et al.*, 2006). Misdiagnosis of myocardial injury following prolonged exercise can result in unnecessary invasive interventions and costs, delays in management decisions, as well as being a psychologically damaging process for the athlete (Bakshi *et al*., 2002; Kelley *et al.*, 2009; Whyte, 2008).

Table 2.2 provides an overview of the literature which assessed cardiac biomarkers before and after exercise. The table details the studies by mode (running, cycling, multimode), exercise distance (duration), the intensity of the exercise and environmental conditions, alongside the study's findings. Details of the type of biomarkers used are also given, which include cTnT (1s/2nd/3rd generation), cTnI, highly sensitive assays (hs-cTnT and hs-cTnI) as well as markers of cardiac stress.

Table 2.2 Studies utilising cTnT, cTnI and other cardiac biomarkers during/following single bouts of prolonged exercise

Author	Exercise distance (duration)	Intensity of Exercise	Blood Measurement	Elevated (Number > URL)	Peak	Environmental Conditions
RUNNING						
Mingels *et al*., 2010	5.4 km fun run	Walk/run	hs-cTnT (Roche)	Increase in mean value (13/122) post	NR	20ºC
			NT-proBNP (Elecsys/Roche)	Increase in mean value (9/122) post	NR	
	5 km (28 ± 4 min)	10.9 ± 1.3 km.h^{-1}	hs-cTnT (Roche)	No increase in mean value (0/43) post	NR	23ºC
			NT-proBNP (Elecsys/Roche)	Increase in mean value (1/43) post	NR	
	15 km (78 ± 9 min)	11.6 ± 1.3 km.h^{-1}	hs-cTnT (Roche)	Increase in mean value (5/38) post	NR	23ºC
			NT-proBNP (Elecsys/Roche)	Increase in mean value (0/38) post	NR	
	21 km (118 ± 14 min)	10.9 ± 1.1 km.h^{-1}	hs-cTnT (Roche)	Increase in mean value (4/10) post	NR	23ºC
			NT-proBNP (Elecsys/Roche)	No increase in mean value (0/10) post	NR	
	42 km (228 ± 30 min)	11.3 ± 1.5 km.h^{-1}	hs-cTnT (Roche)	Increase in mean value (73/85) post	NR	23ºC
			NT-proBNP (Elecsys/Roche)	Increase in mean value (4/85) post	NR	
Shave *et al*., 2002c	Maximal ramping treadmill test	Maximal	cTnT (3rd Gen. Elecsys)	0/8 post, 0/8 48 h post	NR	NR
			cTnI (Immulite)	0/8 post, 0/8 48 h post	NR	
	30 min downhill running	70% maximal running velocity	cTnT (3rd Gen. Elecsys)	0/8 post, 0/8 48 h post	NR	NR
			cTnI (Immulite)	0/8 post, 0/8 48 h post	NR	

Shave *et al*., 2010	30 min treadmill	85-90% VO_2max (HRmean 171 ± 6 beats.min^{-1})	cTnI (TnI-Ultra)	3/8 post, 2/8 1 h, 4/8 2 h, 4/8 3 h, 4/8 4 h, 2/8 5 h, 2/8 24 h	NR	NR
Ranjbar *et al*., 2017	Trial 1: 40 min Trial 2: 40 min	50% HRreserve (2 min)/80% HRreserve (1 min) 60% HRreserve continuous	hs-cTnT (Roche) hs-cTnT (Roche)	(0) post, (0) by 1 h post (1) post, (1) by 1 h post	13.4 ng.L^{-1}, 12.7 ng.L^{-1} 31.0 ng.L^{-1}, 33.0 ng.L^{-1}	24ºC, 50% RH 24ºC, 50% RH
Nie *et al*., 2011	Run 1: 45 min 255 min recovery Run 2: 45 min	VT	cTnT (3rd Gen. Elecsys) NT-proBNP (ECLIA, Roche)	0/12 post run 1, 8/12 (3) pre run 2, 7/12 (2) post run 2, 4/12 (2) 4 h post Increase post run 1, Decrease pre run 2, Increase post run 2, Decrease 4 h post	ND, 0.375 ng.mL-1, 0.303 ng.mL^{-1}, 0.110 ng.mL^{-1} 178.4 pg.mL^{-1}, 121.8 pg.mL^{-1}, 150.8 pg.mL^{-1}, 125.2 pg.mL^{-1}	20ºC, 50% RH
Weippert *et al*., 2016	Trial 1: 60 min Two blocks separated by 3 mins (12 x (30 sec sprint with 15 sec recovery)) Trial 2: 60 min	90% HRpeak 70% HRpeak continuous	hs-cTnT (5th gen. Roche) hs-cTnT (5th gen. Roche)	(5) by 4 h post (0) by 4 h post	NR NR	NR NR
Fu *et al*., 2009	45 min 45 min 90 min 90 min	80% VT 100% VT 80% VT 100% VT	cTnT (3rd Gen. Elecsys)	0/13 post, 0/13 post 2 0/13 post, 8/13 (3) post 2 0/13 post, 2/13 (1) post 2 7/13 post, 12/13 (8) post 2	ND, ND ND, 0.375 ng.mL^{-1} ND, 0.133 ng.mL^{-1} 0.071 ng.mL^{-1}, 0.417 ng.mL^{-1}	NR

Serrano-Ostáriz *et al.*, 2011	45 min 45 min 90 min 90 min 180 min 180 min	85% AT 95% AT 85% AT 95% AT 85% AT 95% AT	cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	Significant effect for longer duration and higher intensity (0-9% across trials) Increase in mean value 30 min and 3 h post (5-14% across all trials)	NR	16.8 ± 2.0ºC, 67.7 ± 10.0% RH
Tian *et al.*, 2014	Trial 1: 90 min Trial 2: 90 min	95% VT 95% VT	hs-cTnT (Roche) NT-proBNP (ECLIA, Roche) hs-cTnT (Roche) NT-proBNP (ECLIA, Roche)	(10) by 3 h post (0) post (9) by 3 h post (0) post	NR NR NR NR	20ºC, 50% RH 20ºC, 50% RH
Tian *et al.*, 2012	90 min Adult 90 min Adolescent	95% VT 95% VT	hs-cTnT (Roche) NT-proBNP (Elecsys/Roche) hs-cTnT (Roche) NT-proBNP (Elecsys/Roche)	1/13 post, 5/13 1 h post, 8/13 2 h post, 11/13 3 h post, 11/13 4 h post, 10/13 5 h post, 9/13 6 h post, 2/13 24 h post Increase in mean value post to 24 h post 8/13 post, 11/13 1 h post, 12/13 2 h post, 12/13 3 h post, 12/13 4 h post, 12/13 5 h post, 12/13 6 h post, 9/13 24 h post Increase in mean value post to 24 h post	15.6 $ng.L^{-1}$, 54.9 $ng.L^{-1}$, 171.0 $ng.L^{-1}$, 305.6 $ng.L^{-1}$, 291.0 $ng.L^{-1}$, 255.4 $ng.L^{-1}$, 208.3 $ng.L^{-1}$, 24.7 $ng.L^{-1}$ NR 69.9 $ng.L^{-1}$, 280.1 $ng.L^{-1}$, 591.9 $ng.L^{-1}$, 794.5 $ng.L^{-1}$, 659.3 $ng.L^{-1}$, 559.6 $ng.L^{-1}$, 437.4 $ng.L^{-1}$, 80.6 $ng.L^{-1}$ NR	NR NR
Nie *et al.*, 2011	21 km (80.8 ± 0.8 min) Adolescents	Self-paced (HRmean 178 ± 12 $beats.min^{-1}$)	cTnT (Elecsys) cTnI (AccuTnI)	8/12 (7) 2 h post, 8/12 (8) 4 h post, 1/12 24 h post 8/12 (2) 2 h post, 11/12 (3) 4 h post, 5/12 24 h post	0.08 $ng.mL^{-1}$, 1.22 $ng.mL^{-1}$, 0.05 $ng.mL^{-1}$ 1.13 $ng.mL^{-1}$, 2.21 $ng.mL^{-1}$, 0.17 $ng.mL^{-1}$	5 - 8ºC, 30 - 40% RH

Tian *et al*., 2006	21 km (80.7 ± 0.6 min) Adolescents	Self-paced	cTnT (3rd Gen. Elecsys) cTnI (AccuTnI)	6/10 (3) 2 h post, 6/10 (4) 4 h post, 1/10 (0) 24 h post 10/10 (6) 2 h post, 10/10 (6) 4 h post, 10/10 (1) 24 h post	NR, 0.29 ng.mL^{-1}, NR NR, 0.51 ng.mL^{-1}, NR	5ºC, ~35% RH
Dalla Vecchia *et al*., 2014	Half-marathon (96 min 30 s)	Self-paced	cTnI (ELFA)	1/35 (1) post	0.45 µg.L^{-1}	15ºC
Kong *et al*., 2017	Half-marathon (adolescents)	Self-paced	cTnT (3rd Gen. Elecsys)	All: 37/38 (30) 4 h post Male: 19/19 (18) 4 h post Female: 18/19 (12) 4 h post	1360 ng.L^{-1} 1360 ng.L^{-1} 550 ng.L^{-1}	5-10ºC, 30-50% RH
Lippi *et al*., 2008a	Half-marathon	Self-paced	cTnT (3rd Gen. Elecsys)	0/15 post, 3 h post, 6 h post, 24 h post	NR	22-25ºC, 55-60% RH
Lippi *et al*., 2008b	Half-marathon	Self-paced	cTnT (3rd Gen. Elecsys) NT-proBNP	(0/17 post, 3 h post, 6 h post, 24 h post) Elevation post, 3 h post, 6 h post, 24 h post (0/17 post, 3 h post, 6 h post, 24 h post)	NR NR	NR
Lippi *et al*., 2008c	Half-marathon	Self-paced	cTnI (Randox)	0/10 post, 3 h, 6 h and 24 h post	NR	22-25ºC, 55-60% RH
Vassalle *et al*., 2018	Half-marathon	Self-paced	hs-cTnT (Roche) hs-cTnI (STAT) NT-proBNP (Roche)	(7/18) 20 min post, (0/18) 24 h post, (0/7) 48 h post (2/18) 20 min post, (3/18) 24 h post, (2/18) 48 h post (3/18) 20 min post, (1/18) 24 h post, (0/7) 48 h post	26.0 ng.L$^{-1,}$ 12.0 ng.L^{-1}, 9.0 ng.L^{-1} 57.0 ng.L^{-1}, 58.0 ng.L^{-1}, 39.0 ng.L^{-1} 179.0 ng.L^{-1}, 140.0 ng.L^{-1}, 93.0 ng.L^{-1}	NR

Vidotto *et al.*, 2005	Half-marathon (121 min females, 104 min males)	Self-paced	cTnT (Dimension/Dade) NT-proBNP (Elecsys/Roche)	(3) 20 min post, (8) 2 h post 25/25 (8) 20 min post, 25/25 (4) 2 h post	0.12 ng.mL^{-1}, 0.16 ng.mL^{-1} 319 pg.mL^{-1}, 262 pg.mL^{-1}	17.1-18.8ºC, 52-62% RH, 217-221 m altitude
Niemelä *et al.*, 2016	Half-marathon (132 ± 5 min) Marathon (199 ± 9 min)	Self-paced 9.6 ± 0.3 km.h^{-1} Self-paced 12.7 ± 0.6 km.h^{-1}	cTnT (Roche) NT-proBNP	Increased above baseline 3 h and 48 h post Increased above baseline 3 h and 48 h post	NR	18-21ºC, 40-50% RH, 15-22 km.h^{-1} wind speed
Sahlén *et al.*, 2008	30 km cross-country race (203 ± 32 min)	Self-paced	cTnT (Roche) NT-proBNP (Roche)	23/43 (19) (21/43)	0.09 µg.L^{-1} NR	Race 1 + 2: 13.4 – 22.6ºC, 50 - 90% RH
Sahlén *et al.*, 2009a	30 km cross-country race (119 ± 28 min)	Self-paced	cTnT (Roche) NT-proBNP (Roche)	(9/15) post, (0/15) 1 d post (7/15) post, elevated above baseline 1 d post, Returned to baseline 6 d post	NR NR	NR
Sahlén *et al.*, 2009b	30 km cross-country race (210.3 ± 33.7 min)	Self-paced	cTnT (Roche) NT-proBNP (Roche)	(75/185) post (79/185) post	NR NR	NR
Sahlén *et al.*, 2010	30 km cross-country race	Self-paced	cTnT (Roche)	(14/43) post	NR	NR
Jassal *et al.*, 2009	Half-marathon (150 ± 20 min) Marathon (310 ± 30 min)	Self-paced	cTnT (3rd Gen. Elecsys, Roche)	19/61 post, 28/61 1 h post Increase in mean value post and 1 h post	NR NR	NR NR
Apple *et al.*, 2002	Marathon (4 h 49 min)	Self-paced	cTnT (Elecsys, Roche) cTnI (Dimension RxL)	(10/19) post, (1/19) 24 – 48 h post (6/19) post, (1/19) 24 – 48 h post	NR, 0.04 µg.L^{-1} NR, 0.5 µg.L^{-1}	NR

Baker *et al*., 2014	Marathon	Self-paced	cTnI (Alere Ltd) cTnT (Roche) hs-cTnT (Roche) BNP (Alere Ltd)	5/48 post 31/45 post 45/45 post 25/48 post	NR NR NR NR	NR
Banfi *et al.*, 2010	Mountain marathon (164 – 262 min)	Self-paced	cTnI (Vitros) NT-proBNP (Vitros)	(4) post (2) post	0.262 ng.mL^{-1} 190 pg.mL^{-1}	16-24ºC, 6000 m ascent/decent
Cummins *et al*., 1987	Marathon (3.25 h)	Self-paced	cTnI (radioimmunoassay)	(2/11) post	NR	NR
Da Ponte *et al*., 2017	Uphill marathon (5 h 50 min ± 54 min)	Self-paced	cTnI (AccuTnI)	22/22 (11) post	NR	Start: 28ºC, 33% RH End: 2ºC, 65% RH, 0 – 2850 m
Dawson *et al*., 2008	Marathon (229 ± 38 min)	Self-paced	cTnI (Ultra)	12/13 (7) post	NR	Midday 21ºC
Eijsvogels *et al.*, 2014a	Marathon	Self-paced	cTnI (Ultra)	(19/23)	NR	NR
Eijsvogels *et al.*, 2014b	Marathon (4 h 4 min ± 41 mins) Control Statin use	Self-paced	cTnI hs-cTnI (Siemens) NT-proBNP (Siemens) cTnI hs-cTnI (Siemens) NT-proBNP (Siemens)	29/41 (19) post, 23/41 (12) 24 h post 41/41 (21) post, 41/41 (10) 24 h post Increase in mean value post, 24 h post 26/30 (14) post 16/30 (6) 24 h post 30/30 (17) post 30/30 (9) 24 h post Increase in mean value post, 24 h post	NR NR NR NR NR NR	NR

Eijsvogels *et al.*, 2015	Marathon (227 ± 28 mins)	Self-paced 11.3 ± 1.4 km.h^{-1} HRmean 161 ± 9 beats.min^{-1}	hs-cTnI (Centaur TnI-Ultra)	77/80 (55/80) post	530 ng.L^{-1}	Wet globe 18.8ºC, 52% RH
Fortescue *et al.*, 2007	Marathon	Self-paced	cTnT (3rd Gen. Elecsys) cTnI (Centaur)	328/482 overall (54) (18)	 0.74 ng.mL^{-1} 4.17 ng.mL^{-1}	NR
Gaudreault *et al.*, 2013	Marathon (232 ± 40 min)	Self-paced	cTnT NT-proBNP	13/20 (3) post	NR	35ºC, 46% RH
George *et al.*, 2004	Marathon (256 ± 46 min)	Self-paced	cTnT (3rd Gen. Elecsys)	26/33 (6) post	0.080 µg.L^{-1}	11ºC, 40% RH
George *et al.*, 2005	Marathon (256 ± 46 min)	Self-paced	cTnT (3rd Gen. Elecsys)	21/28 post	0.733 µg.L^{-1}	14ºC, 50% RH, light winds and scattered cloud cover
Herrmann *et al.*, 2003	Marathon	Self-paced	cTnT (2nd Gen. Elecsys) cTnI (AccuTnI) NT-proBNP (Roche Elecsys 2010)	(23/45) 15 min post, 18/45) 3 h post, (0/9) 24 h post (27/45) 15 min post, (33/45) 3 h post, (1/9) 24 h post (38/45) 15 min post, (30/45) 3 h post, NR 24 h post	0.103µg.L^{-1}, 0.174 µg.L^{-1}, ND 0.360µg.L^{-1}, 0.930 µg.L^{-1}, NR 953 ng.L^{-1}, 550 ng.L^{-1}, 194 ng.L^{-1}	NR
Hewing *et al.*, 2015	Marathon (263 ± 37 min)	Self-paced	cTnT (4th Gen. Roche) NT-proBNP (Roche Elecsys 2010)	62/167 post, 0/167 14 d post 57/167 post	NR NR	NR

Hottenrott *et al.*, 2016	Marathon: run/walk strategy (254 ± 19 min) Marathon: Run (247 ± 6 min)	60 sec walking every 2.5 km (HRmean 158 ± 7 beats.min^{-1}) Self-paced (HRmean 154 ± 6 beats.min^{-1})	CTnI BNP cTnI BNP	1/21 (1) post, 0/21 (0) 4 d post Increase in mean value post, return to baseline 4 d post 1/19 (0) post, 0/19 (0) 4 d post Increase in mean value post and 4 d post	0.28 ng.mL^{-1} NR 0.15 ng.mL^{-1} NR	180 m difference in altitude
Hubble *et al*., 2009	Marathon (4 hr 5 min)	Self-paced	cTnI (Ultra)	28/88	1.4 µg.L^{-1}	NR
Kim *et al.*, 2015	Marathon	Self-paced	cTnI (ADVIA centaur) NT-proBNP (Modular Analytics E170)	Increase in mean value Increase in mean value	NR NR	15ºC, 35% RH pre-race (9am)
Knebel *et al*., 2009	Marathon (256 ± 37 min)	Self-paced	cTnT (4th Gen. Roche) NT-proBNP (Roche Elecsys 2010)	30/78 (5/78) post, 0/78 2 wk post 24/78 post, 2/78 2 wk post	NR NR	23.5ºC, 46% RH at midday
Knebel *et al*., 2014	Marathon Premenopausal Postmenopausal	Self-paced	cTnT (4th Gen. Roche) NT-proBNP (Roche Elecsys 2010) cTnT (4th Gen. Roche) NT-proBNP (Roche Elecsys 2010)	19/54 post, ND 2 w post 24/54, no increase 2 w post 13/35, ND 2 w post 8/35, no increase 2 w post	NR NR NR NR	12ºC, 75% RH at midday
Koller *et al*., 1995	Marathon	Self-paced	cTnT (1st Gen. Boehringer) cTnI (ERIA)	(1/19) (1/19)	1.0 µg.L^{-1} 0.7 µg.L^{-1}	NR

Koller *et al*., 2008	Downhill marathon	Self-paced	cTnT (3rd Gen. ECLIA, Roche) cTnI (MEIA) NT-proBNP (ECLIA, Roche)	(5/13) post, (1/13) ~ 12 h post (7/13) post, (9/13) ~ 12 h post Increase in mean value post and ~ 12 h post	NR NR NR	795 vertical difference
Kratz *et al*., 2002	Marathon (4 h ± 30 min)	Self-paced	cTnI (Triage)	Increase in mean value 4 h post, 0/11 24 h post	NR	11.7 – 13.3ºC, wind speed 1 – 5 mph
Leers *et al*., 2006	Marathon	Self-paced	cTnT (Elecsys) BNP (Advia) NT-proBNP (Elecsys/Roche)	9/27 post, 0/27 24 h post No increase in mean value post and 24 h post Increase in mean value post, no increase in mean value (1) 24 h post	NR NR NR	NR
Legaz-Arrese *et al*., 2011	Marathon (202 ± 14 min)		cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	Increase in mean value 30 min and 3 h post (6/14) Increase in mean value 30 min and 3 h post (0/14)	NR NR	NR
	Duration matched trial	85% AT	cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	Increase in mean value 30 min and 3 h post Increase in mean value 30 min and 3 h post (0/14)	NR NR	
	Duration matched trial	95% AT	cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	Increase in mean value 30 min and 3 h post Increase in mean value 30 min and 3 h post (0/14)	NR NR	
Lucía *et al*., 1999a	Marathon	Self-paced	cTnT (Elecsys) cTnI (ERIA)	1/22 post, 1/22 6 h post, 0/22 24 h post, 1/22 48 h post 0/22 post, 6 h post and 24 h post, 1/22 48 h post	NR NR	11 - 22ºC, 60 - 65% RH

Lucía *et al*., 1999b	Marathon	Self-paced	cTnI (ERIA)	(0/10)	NR	11 - 22ºC, 60 - 65% RH
Mair *et al*., 1992	Marathon	Self-paced	cTnT (1st Gen. Boehringer)	(1/20) 2 d post	1.0 µg.L^{-1}	NR
Mehta *et al.*, 2012	Marathon: highly trained (224 ± 38 min) Marathon: less trained (285 ± 43 min) Marathon	Self-paced Self-paced Self-paced	cTnI (Ultra assay)	22/27 (9) post 24/25 (15) post 93/104 (51) post	NR NR NR	NR
Melanson *et al.*, 2006	Marathon (250 ± 33 min)	Self-paced	cTnT (3rd Gen. Roche) NT-proBNP (Roche Elecsys 1010)	17/22 (11) post 22/22 (2) post	0.072 ng.mL^{-1} 397 pg.mL^{-1}	69-70ºF, wind speed 3mph
Middleton *et al*., 2006b	Marathon (212 ± 36 min)	Self-paced	cTnT (3rd Gen. Roche) NT-proBNP (Roche Elecsys 1010)	9/13 post, 9/13 1 h post, 1/9 24 h post Increase in mean value post and 1 h post, recovery 24 h	0.37 µg.L^{-1}, 0.459 µg.L^{-1}, NR NR	Max 11ºC, frequent rain showers
Middleton *et al*., 2007a	Marathon: 2004 (201 ± 29 min) 2005 (212 ± 50 min)	Self-paced	cTnT (3rd Gen. Roche)	4/8 post 3/8 post	0.128 µg.L^{-1} 0.033 µg.L^{-1}	NR
Middleton *et al*., 2008	Treadmill Marathon	NR	cTnT (3rd Gen. Roche)	During: 0/9 30 min, 5/9 60 min, 5/9 90 min, 9/9 120 min, 4/7 180 min Post: 1/9 Post, 0/9 1 h, 0/9 3 h, 3/9 6 h, 7/9 12 h, 5/9 24 h	0.03 µg.L^{-1} 0.04 µg.L^{-1}	NR

Mingels *et al*., 2009	Marathon	Self-paced	hs-cTnT (Roche) cTnT (4th Gen. Roche) cTnI (Architect)	73/85 38/85 69/85	NR NR NR	Max 23.4ºC, south wind < 14 $m.s^{-1}$
Mousavi, *et al*., 2009	Marathon (245 ± 68 min)	Self-paced	cTnT (3rd Gen. Elecsys)	14/14 (14) post, 0/14 (0) 1 wk post	NR	NR
Neilan *et al*., 2006a	Marathon (4 h 5 min)	Self-paced	cTnT (3rd Gen. Elecsys) NT-proBNP (Elecsys/Roche)	38/60 (28) post (32) post	0.82 $ng.mL^{-1}$ 506 $pg.mL^{-1}$	NR
O'Hanlon *et al*., 2010	Marathon (209 ± 19 min)	Self-paced	cTnI (ADVIA centaur) NT-proBNP (Roche Elecsys 2010)	(8/17) post, (11/17) 6 h post Increase in mean value post and 6 h post	0.43 $\mu g.L^{-1}$, NR NR	NR
Richardson *et al*. 2018	Marathon (265 ± 52 min)	Self-paced 10.1 ± 2.4 $km.h^{-1}$ HRmean 157 ± 14 $beats.min^{-1}$	hs-cTnT (5th Gen Roche)	52/52 (52) post	NR	18-23ºC, 40-50% RH
Roca *et al*., 2017	Marathon	Self-paced	hs-cTnT NT-proBNP	(8/79) post and 24 h post Increase in median value post, no increase in median value 24 h post	NR NR	NR
Saenz *et al*., 2006	Marathon	Self-paced	cTnT (3rd Gen. Roche)	21/30 (7) post	0.098 $ng.mL^{-1}$	NR
Scarhag *et al*., 2005	Marathon	Self-paced	cTnT (3rd Gen. Roche) cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	(24/46) (34/46) Increase in mean value post	NR NR NR	NR

Scherr *et al.*, 2011	Marathon (3 h 47 min ± 26 min)	Self-paced	hs-cTnT (Roche) NT-proBNP (Roche)	(91/102) post, (27/102) 24 h post, 43/102 (4) 72 h post Increase in mean value post, 24 h and 72 h post	631 ng.L^{-1}, NR, NR NR	NR
Shave *et al.*, 2005	Marathon (253 ± 48 min)	Self-paced	cTnT (3rd Gen. Roche)	56/72 (26)	0.733 µg.L^{-1}	NR
Siegel *et al.*, 1997	Marathon	Self-paced	cTnT (Rapid assay) cTnT (1st Gen. ELISA, Boehringer) cTnT (2nd Gen. Cardiac Enzymun, Boehringer) cTnI (Behring) cTnI (Cardiac STATus)	6/43 4 h post 1/43 4 h post, 1/43 24 h post 0/43 4 h post 1/43 post 1/43 post	NR NR ND NR NR	NR
Siegel *et al.*, 2001	Marathon (1997) Marathon (1998-2000) Marathon (2001)	Self-paced	cTnI (Cardiac STATus) cTnI (ACS) cTnI (Triage cardiac panel) cTnT (Elecsys 1010) BNP (Triage BNP)	2/41 4 h post, 1/13 24 h post Increase in mean value 4 h and 24 h post No significant increase No significant increase No significant increase 4 h post, significant increase 24 h post	NR NR NR NR NR	NR NR NR
Siegel *et al.*, 2008	Marathon	Self-paced	cTnT (4h Gen. Elecsys, Roche) NT-proBNP (Roche Elecsys 2010)	18/99 (8) post (5/99) post	0.172 µg.L^{-1} 331 µg.L^{-1}	NR
Smith *et al.*, 2004	Marathon	Self-paced	cTnI (Triage)	(0/34)	NR	NR

Traiperm *et al*., 2012	Marathon Adolescent (4 h 53 min)	Self-paced	cTnT (Roche) cTnI (Beckman)	(30/37 cTnT and/or cTnI) post, 24 h post	NR	16.6 – 24.5ºC, 45 – 82% RH
Trivax *et al*., 2010	Marathon (256.2 ± 43.5 min)	Self-paced	cTnI (chemiluminescence) BNP	Increase in mean value Increase in mean value	NR NR	Start 33ºF (1ºC)
Wilson *et al*., 2011	Marathon (209 ± 19 min)	Self-paced	cTnI (ADVIA centaur) NT-proBNP (Elecsys/Roche)	17/17 (8) post, 17/17 (13) 6 h post Increase in mean value post and 6 h post	0.13 $\mu g.L^{-1}$, 0.43 $\mu g.L^{-1}$ 136 $ng.L^{-1}$, 159 $ng.L^{-1}$	Max 10ºC, Approx. 40% RH
Whyte *et al*., 2005	Marathon (245.3 ± 46.0 min)	Self-paced	cTnT (3rd Gen. Elecsys)	32/39 (20) post	0.73 $\mu g.L^{-1}$	Max 14ºC, Approx. 50% RH
Lippi *et al*., 2012	60 km	Self-paced (82 ± 4% VO_2max)	hs-cTnI (hs-AccuTnI) cTnI (AccuTnI)	15/15 (12) post 12/15 (3) post	NR NR	6 – 8ºC, 54 - 87% RH
Salvagno *et al*., 2014	60 km	Self-paced (84 ± 4% VO_2max)	hs-cTnI (hs-AccuTnI) NT-proBNP (ECLIA, Roche)	Increase in mean value post (4/18) Increase in mean value post (5/18)	NR NR	6 – 8ºC, 54 - 87% RH
George *et al*., 2009	The Comrades Marathon: 89 km (586 ± 80 min)	Self-paced	cTnT (3rd Gen. Roche)	5/12 post	0.272 $\mu g.L^{-1}$	4 - 24ºC
Kim *et al*., 2012	100 km (819.6 ± 54.6 min)	Self-paced	cTnI (ADVIA centaur) NT-proBNP (Modular Analytics E170)	Increase in mean value Increase in mean value	NR NR	25ºC, 65% RH pre-race (10pm)
Musha *et al*., 1997	100 km (10 h 43 min)	Self-paced	cTnT (1st Gen. ELISA, Boehringer)	10/13 (7) post, 7/13 (1) 16 – 18 h post	2.38 $ng.mL^{-1}$, 0.36 $ng.mL^{-1}$	NR

Scarhag *et al*., 2005	100 km	Self-paced	cTnT (3rd Gen. Roche) cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	(23/45) (39/45) Increase in mean value post	NR NR NR	NR
Ohba *et al*., 2001	100 km (661 ± 52 min)	Self-paced	cTnT (1st Gen. Enzymu-test) BNP (Siono-RIA)	10/10 (9) post 10/10 post	3.36 $ng.mL^{-1}$ 47.85 $fmol.mL^{-1}$	9.7 – 12.6ºC, 82 - 99% RH
Dávila-Román *et al*., 1997	163 km mountain ultramarathon	Self-paced	cTnI (Baxer)	1/14 post, 1/14 18 h post	5.0 $ng.mL^{-1}$, 1.8 $ng.mL^{-1}$	2 – 24ºC, 10 – 60% RH, 2350 – 4300 m altitude
Laslett *et al*., 1996	100 mile	Self-paced	cTnT (ELISA)	5/5 (5) post	8.55 $ng.mL^{-1}$	NR
Laslett and Eisenbud	100 mile (23.5 h)	Self-paced	cTnT (1st Gen. ELISA) cTnT (2nd Gen. Enzymun-Test)	21/23 post 0/23 post	9.23 $ng.mL^{-1}$ 0.16 $ng.mL^{-1}$	NR
Scott *et al*., 2009	100 mile trial race (25.5 ± 3.2 h)	Self-paced	cTnT (3rd Gen. ECLIA, Roche) NT-proBNP (ECLIA, Roche)	5/25 post 24/24 (24) post	0.05 $ng.L^{-1}$ 3427 $ng.L^{-1}$	NR
Passaglia *et al*., 2013	24 h ultramarathon (140.32 km)	Self-paced	cTnT	2/12 (0) post	0.015 $ng.mL^{-1}$	9 - 21ºC
Roth *et al*., 2007	216 km ultra-endurance marathon	Self-paced	cTnT (3rd Gen. Roche)	0/10 post	NR	Max 54ºC, 3962 m ascent, 1433 m descent
Bartzeliotou *et al*., 2007	246 km footrace	Self-paced	cTnT (ECLIA, Roche) NT-proBNP (ECLIA, Roche)	0/20 post and 48 h post Increase in mean value post and 48 h post	NR NR	5 - 36ºC, 60 – 85% RH

Klapcinska *et al*., 2013	48 h ultra-marathon (183 – 320 km)	Self-paced	hs-cTnT (Roche) NT-proBNP (Elecsys/Roche)	Peak in mean value 12 h during exercise (3), detectable values 48 h post Peak in mean value 24 h during exercise (7), detectable values 48 h post	NR NR	Start: 22ºC, 53% RH End: 12ºC, 94% RH
CYCLING						
Stewart *et al*., 2015	60 min	Self-paced	hs-cTnT (Roche) hs-cTnI (Abbott)	Increase in mean value Increase in mean value	NR NR	NR
Chan-Dewar *et al*., 2013	8.00am: 40 km (76 ± 6 min) 6:00pm: 40 km (76 ± 7 min)	90-100% lactate threshold 90-100% lactate threshold	cTnI (cTnI Ultra, ADVIA centaur) NT-proBNP (ECLIA, Roche) cTnI (cTnI Ultra, ADVIA centaur) NT-proBNP (ECLIA, Roche)	1/12 (0) 10 min post, 1/12 (0) 60 min post 10/12 (0) 10 min post, 8/12 (0) 60 min post 1/12 (0) 10 min post, 1/12 (0) 60 min post 10/12 (0) 10 min post, 10/12 (0) 60 min post	0.04 µg.L-1, 0.04 µg.L^{-1} NR, NR 0.02 µg.L-1, 0.03 µg.L^{-1} NR, NR	20ºC, 40% RH 20ºC, 40% RH
Stewart *et al*., 2014	2 h	GET	hs-cTnT (Roche)	Increase in mean value 60 min post, no increase in mean value 24 h post	NR	20ºC, ~55% RH
Stewart *et al*., 2016	90 min 120 min	110% GET 80% GET	hs-cTnI (Abbott)	Increase in mean value above URL Increase in mean value	NR	22ºC, ~55% RH
Shave *et al.* 2004b	50 mile (126 ± 7 min) 50 mile (125 ± 6 min)	Lactate threshold Lactate threshold	cTnT (Elecsys 1010) cTnT (Elecsys 1010)	0/8 post and 24 h post 1/8 post, 0/8 24 h post	ND 0.016 µg.L^{-1}	19 ºC, normobaric normoxia 19 ºC, normobaric hypoxia

Stickland *et al*. 2004	Exercise to exhaustion: (2.51 ± 0.86 h)	25 watts below LT	cTnI (ADVIA centaur)	1/11 20-24 h post	0.2 µg.L^{-1}	21ºC
Dawson *et al*., 2005	4 h	5% below LT (151 ± 39 W)	cTnT (3rd Gen. Elecsys)	2/16 post, 0/16 24 h post	0.021 µg.L^{-1}, ND	NR
Skadberg *et al.* 2017	91 km mountain bike race (4.2 h)	Self-paced HRmean 158 beats.min^{-1}	hs-cTnI (STAT) BNP	97/97 (68) 15 min post, (80) 3 h post, (20) 24 h post Elevated highest 24 h post	NR	NR
Skadberg *et al.* 2018	91 km mountain bike race (4.2 h)	Self-paced HRmean 158 beats.min^{-1}	hs-cTnT (Roche) hs-cTnI (STAT)	90/94 15 min post, 89/94 3 h post, 30/94 24 h post 71/94 15 min post, 82/94 3 h post, 22/94 24 h post	NR	NR
Ortega *et al*., 2006	95 km mountain bike challenge (332 min)	Self-paced	cTnI (Beckman)	Increase in mean value (0/11)	0.39 µg.L^{-1}	Altitude difference 2430 m
Scarhag *et al*., 2005	Mountain bike marathon (110 km)	Self-paced	cTnT (3rd Gen. Roche) cTnI (AccuTnI) NT-proBNP (Elecsys/Roche)	(3/14) (8/14) Increase in mean value post	NR NR NR	Altitude difference 2800 m
Shave *et al.* 2004c	100 mile (256 ± 15 min) 100 mile (254 ± 13 min)	5% below lactate threshold 5% below lactate threshold	cTnT (Elecsys 1010) cTnT (Elecsys 1010)	0/8 (0) post, 0/8 (0) 24 h post 2/8 (0) post, 0/8 (0) 24 h post	ND 0.034 µg.L^{-1}, ND	0 ± 0.1ºC 19 ± 0.4ºC
Serrano-Ostariz *et al.*, 2013	206 km (accumulated slope 3800 m)	Self-paced	cTnI (AccuTnI) NT-proBNP (Elecsys proBNP)	(39/91) (59/91)	NR NR	16.0 - 32.5ºC
Mair *et al*., 1992	230 km	Self-paced	cTnT 1st Gen. Boehringer)	(0/8) 2 d post	NR	Altitude difference 5000 m

Neumayr *et al*., 2001	230 km	Self-paced	cTnI (AxSYM)	13/38 post, 1/38 1 d post	4.9 µg.L^{-1}, 4.0 µg.L^{-1}	Altitude difference 5500 m
Neumayr *et al*., 2005	230 km	Self-paced	cTnT (3rd Gen. Troponin T Stat) NT-proBNP (Elecsys proBNP)	13/29 (8) post, 2/29 (0) 1 d post Increase in mean value post, no increase in mean value 1 d post	0.224 µg.L^{-1}, NR NR	Altitude difference 5500 m
Kim *et al*., 2012	308 km ultra-marathon	Self-paced Mean speed 5 km.h^{-1}	cTnI (Dimension Xpand) NT-proBNP (Dimension Xpand)	No difference in mean value Increase in mean value	NR NR	13.0 – 31.9ºC
Neumayr *et al*., 2002	509 km	Self-paced	cTnT (3rd Gen. Troponin T Stat) cTnI (AxSYM)	1/16 (1) post, 0/16 24 h post 6/16 (2) post, 0/16 24 h post	0.11 µg.L^{-1}, ND 5.1 µg.L^{-1}, ND	Cumulative altitude difference 12,200 m
COMBINATION						
Scharhag *et al*., 2006	Marathon	Self-paced	cTnT (3rd Gen. Elecsys) cTnI (AccuTnI) NT-proBNP (Elecsys proBNP)	18/20 (18) post 20/20 (20) post (12) post	0.56 µg.L^{-1} 1.93 µg.L^{-1} 523 ng.L^{-1}	NR
	1 h running or cycling	95-100% AT	cTnT (3rd Gen. Elecsys) cTnI (AccuTnI) NT-proBNP (Elecsys proBNP)	3/20 (3) post 20/20 (5) post (4) post	0.02 µg.L^{-1} 0.06 µg.L^{-1} 267 ng.L^{-1}	
	3 h running or cycling	75% AT	cTnT (3rd Gen. Elecsys) cTnI (AccuTnI) NT-proBNP (Elecsys proBNP)	4/20 (4) post 20/20 (5) post (3) post	0.06 µg.L^{-1} 0.14 µg.L^{-1} 231 ng.L^{-1}	

Koller *et al.*, 1998	67km running 230 km cycling	Self-paced Self-paced	cTnT (2nd Gen. Boehringer) cTnI (enzyme immunoassay)	(0/28) post (0/12) post	NR NR	NR NR
Park *et al*., 2014	Olympic triathlon: 1.5 km swim, 40 km cycle, 10 km run Elite (103.4 ± 1.6 min) Amateur (164.1 ± 14.7 min)	Self-paced	cTnT	No increase in mean value post, increase in mean value 2 h post, no increase in mean value of group 7 d post No increase in mean value of group post, 2 h post and 7 d post	NR NR	NR
Shave *et al*., 2004a	Simulated half-ironman triathlon: 1.9 km swim, 90 km cycle, 21.1 km run (5 h 01 min ± 25 min)	Self-paced	cTnT (Elecsys)	Swim: 0/9 post Cycle: 2/9 post Run: 4/9 post 1/9 24 h post	ND, 0.016 $\mu g.L^{-1}$ 0.021 $\mu g.L^{-1}$ 0.010 $\mu g.L^{-1}$	19ºC, 50% RH
Welsh *et al*., 2005	Half-ironman triathlon: 2 km swim, 90 km cycle, 21 km run (5 h 01 min ± 25 min)	Self-paced	cTnI (ELISA)	(1/9) post (0/9) 24 h post	9.0 $\mu g.L^{-1}$, NR	18ºC, precipitation 0.8 cm, wind speed 7 $km.h^{-1}$
Whyte *et al*., 2000	Half-ironman triathlon: 1.9 km swim, 90 km cycle, 21 km run (5 h 29 min ± 20 min)	Self-paced	cTnT (ELISA)	Significantly increased post, 0/14 48 h post	NR	NR

Leetmaa *et al*., 2008	Triathlon: 4 km swim, 120 km cycle and 30 km run (7 h 21 min)	Self-paced	cTnT (Roche) NT-proBNP (Elecsys proBNP)	(8/13) post, (1/13) 12 – 24 h post Increase in mean value post (13/13), No increase in mean value 12 – 24 h post (4/13)	0.14 µg.L^{-1}, NR NR	NR
La Gerche *et al.*, 2004	Ironman triathlon: 3.8 km swim, 180 km cycle and 42.2 km run (592 ± 100 min)	Self-paced	cTnI (Abbott AxSYM)	1/15 post	0.9 µg.L^{-1}	16 - 27^{o}C
La Gerche *et al.*, 2008	Ironman triathlon: 3.8 km swim, 180 km cycle and 42.2 km run (10 h 50 min ± 1 h 15 min)	Self-paced	cTnI (Abbott AxSYM) BNP (Biosite)	15/26 post, 0/26 1 wk post 26/26 post, 0/26 1 wk post	NR NR	NR
Rifia *et al.* 1999	Ironman triathlon: 3.9 km swim, 180.2 km cycle and 42.2 km run 1994 1995	Self-paced 661 ± 89 min 699 ± 95 min	cTnT (1st Gen. ELISA) cTnT (2nd Gen. Enzymun) cTnI (Opus) cTnT (2nd Gen. Enzymun)	11/23 post 6/23 (2) post 2/23 post 6/19 (2) post	0.37 µg.L^{-1} NR NR 0.19 µg.L^{-1}	NR NR
Tulloh *et al*., 2006	Ironman triathlon (694 min)	Self-paced	cTnT (Roche)	32/36 (6) post, 9/36 (2) 24 h post	1.130 ng.mL^{-1}, 0.221 ng.mL^{-1}	NR
Whyte *et al*., 2000	Ironman triathlon: 3.8 km swim, 180 km cycle and 42.2 km run (10 h 40 min)	Self-paced	cTnT (ELISA)	Significantly increased post, 0/14 48 h post	NR	NR

Denvir *et al.* 1999	45 km run/ 155 km cycle/ 100 km canoe	Self-paced	cTnI (Beckman)	(6/31 overall)	NR	NR
Ashley *et al*., 2006	300 miles: trekking, mountain biking, kayaking, rope climbing and swimming	Self-paced	cTnI (AccuTnI) BNP (Bayer)	22/54 48/54	0.36 µg.L^{-1} 20.4 pmol. L^{-1}	NR
OTHER						
Legaz-Arrese *et al*., 2015	30 min rowing test Elite	All out	cTnI (AccuTnI) NT-proBNP (Elecsys proBNP)	(1/18) 5 min post, (3/18) 1 h post, (8/18) 3 h post, (9/18) 6 h post, (5/18) 12 h post, (1/18) 24 h post Increase in mean value (0)	NR NR	18 – 21ºC, 50 – 60%
	Amateur		cTnI (AccuTnI) NT-proBNP (Elecsys proBNP)	(1/14) 5 min post, (1/14) 1 h post, (2/14) 3 h post, (3/14) 6 h post, (3/14) 12 h post, (1/14) 24 h post Increase in mean value (0)	NR NR	
Legaz-Arrese *et al*., 2017	60 min maximal swimming test Adolescents and adults	All out	hs-cTnT (Roche) NT-proBNP (Elecsys proBNP)	Elevated 5 min, 1 h, 3 h, 6 h and 12 h post (41/66) Elevated 5 min, 1 h, 3 h, 6 h, 12 h and 24 h post (1/66)	335.0 ng.L^{-1} 144.3 ng.L^{-1}	Water: 26ºC, air: 29ºC, 75% RH
Lippi *et al*., 2010	40 min, walking downhill gradient - 25%	6.4 km.h^{-1}	hs-cTnT (Roche) hs-cTnI (ADVIA Centaur)	Increase in mean value 30 min post (0), recovery 24 h, 48 h, 72 h and 96 h post No increase in mean value 30 min, 24 h, 48 h, 72 h and 96 h post	NR NR	NR

Eijsvogels *et al.*, 2012	Walking 30-50km Lean, Overweight and Obese	Self-paced	hs-cTnI (Centaur)	Increase in mean value of all groups: 86/97 (11)	NR	NR
Eijsvogels *et al.*, 2014a	40.6 km walking	Self-paced	cTnI (Ultra)	(4/23)	NR	NR
Bauer *et al*., 2016	Dragon boat training	Intermittent	hs-cTnT (Elecsys, Roche) NT-proBNP (ECLIA, Roche)	0/65 Increase in mean value	NR	15 – 26°C, 42% RH, wind speed 3 $km.h^{-1}$
Planer *et al*., 2012	Field training exercise (85 – 103 h)	Self-paced	cTnT (3rd Gen. Troponin T Stat) NT-proBNP (ADVIA-Centaur)	0/39 No increase in mean value	NR	Discomfort index: >28 day, 25.2 ± 2.4 night

1st gen.: first-generation assay, 2nd gen.: second-generation assay, 3rd gen.: third-generation assay, BNP: brain natriuretic peptide, NT-proBNP: N-terminal proB-type natriuretic peptide, cTnI: cardiac troponin I, cTnT: cardiac troponin T, HRmean: mean heart rate, VT: ventilatory threshold, AT: anaerobic threshold, GET: gas exchange threshold, LT: lactate threshold, NR: not reported, ND: not detectable.

Like EICF, studies of EICD have produced contradictory findings partially due to methodological variation among the studies, with a 0-100% incidence of elevated cTn's following competitive endurance events (Table 2.2). Some studies, such as that by Koller *et al*. (1998), have shown no evidence for EICD, whilst more recently Mousavi *et al*. (2009) and Dawson *et al*. (2008) found a high incidence of biomarker appearance in participants (100% cTnT and 92% cTnI, respectively) following a marathon run, not only above the detection limit but also above the clinical cut-off criteria for AMI. Therefore laboratory staff and clinicians must have a knowledge of clinical entities other than ACS and heart failure in which increases in cTn's may be displayed (Kelley *et al.*, 2009). This is supported by Middleton *et al*. (2008) who presented elevated cTnT concentrations in some participants 24 hours post-exercise, therefore, 24 hour blood draws cannot be used to differentiate between an early onset of ACS or exercise induced cTn release.

The interpretation of exercise related changes in cardiac specific biomarkers is problematic due to the high inter-individual variation in the field-based data available (Shave *et al*., 2007a). This has been attributed in some part, to the highly variable nature of exercise duration and intensity, whereby field-based competitive studies are based on set distance courses (Serrano-Ostáriz *et al.*, 2009), with a lack of control over environmental conditions, which will be a focus of Section 2.3.3. It has been suggested that changes in cTn are related to exercise-induced changes in plasma volume (PV), however, Eijsvogels *et al.* (2010) reported no changes or increases in PV with elevations in cTnI, which suggests this is unlikely.

The generation of assay used has also contributed to the discrepancy within findings in Table 2.2. The first-generation cTnT assay (ELISA) has demonstrated a 12% cross-reactivity with skeletal troponin T (Wu *et al*., 1994) in the presence of higher levels of

creatine kinase. This is supported by Rifai *et al*. (1999), whom revealed detectable cTnT levels in participants who demonstrated higher levels of creatine kinase. Therefore, it is likely that a false positive identification of cardiac damage has occurred in these studies. A second-generation cTnT assay (ELISA) was developed and subsequently used within a number of studies (Rifai *et al*., 1999; Siegal *et al.*, 1997). Siegal *et al.* (1997) compared the use of different assays following marathon running and reported a rise in cTnT of 20% when using the first-generation assay, conversely, the second-generation assay showed no detectable values. The specificity of the second-generation assay in human populations has also been questioned as it employed a bovine troponin T calibrant. A third-generation assay which utilizes recombinant human cTnT as the calibration material was also developed, which is reported to be cardiac specific and thus a more reliable method for detecting cardiac damage (Dawson *et al*., 2003; Shave *et al*., 2002c). A number of studies have utilised this newer assay, with a range of findings apparent (Table 2.2). The high incidence of cTn release demonstrated by Mousavi *et al.* (2009) and Dawson *et al.* (2008) was attributed to an increased sensitivity in the assay employed. This is supported by a descriptive review by Michielsen *et al.* (2008), which reported elevations of 59% of participants utilising the Roche third-generation cTnT assay. In comparison, only 44% and 13% of participants studied showed elevation using the first and second-generation assays and was attributed to the 10-fold lower detection limit of the newer assay.

It is suggested that any elevation in cTn brought about by exertion can generate clinical concern (Shave *et al*., 2010), and whilst some researchers have reported all elevations (Chan-Dewar *et al.*, 2013; Melanson *et al.*, 2006), others have reported participants above the upper reference limit (URL; Tian *et al.*, 2014), AMI criteria or changes in group mean values (Kim *et al.* 2012), confounding data interpretation (Table 2.2). Further to this,

many authors have used the AMI criteria of the publication year (e.g. Fortescue *et al.*, 2007), making comparisons between elevations above AMI cut-off limits difficult (Shave *et al*., 2005). Inter and intra-individual variability was apparent within a small number of studies whom reported individual findings (Middleton *et al*. 2008), highlighting the need for further investigation into the individual response to prolonged exercise.

The vast majority of studies have arisen from individuals participating in organized field-based events, whereby measures of cTn are taken before and immediately after exercise cessation. A controlled lab-based study by Middleton *et al*. (2008) was one of the first studies to show all participants displaying elevations in cTnT during a marathon run, with all but one participant showing further elevations post-exercise. The samples showed a biphasic release of cTnT, which along with repeated post-exercise blood samples, can also partially explain the discrepancy between previous findings due to pre-post exercise design.

As previously mentioned, it has been advocated that EICD underpins EICF (Denvir *et al.*, 1999, Whyte *et al*., 2000), however, due to the lack of specificity of previous cTnT markers, these findings must be viewed cautiously (Shave *et al*., 2002c). More recently, Tulloh *et al.* (2006) found a moderate correlation between change in EF immediately post-race and peak cTnT level. Closer analysis revealed a trend of a greater reduction in EF and SV amongst those athletes that displayed cTnT values above 0.10 $ng.mL^{-1}$. However, when individuals were split into groups according to troponin elevation (<0.10 $ng.mL^{-1}$ and > 0.10 $ng.mL^{-1}$), no significant differences between groups in LV function were observed. This is also supported by George *et al.* (2004, 2005) who's evidence of minor cardiac damage (cTnT) was not associated with changes in LV diastolic filling, suggesting a depression in cardiac function and EICD represent two unrelated

phenomena. It has been proposed there are 6 potentially interlinked mechanisms to explain elevated cTn levels: 1) an increased membrane permeability allowing unbound cTn in the cytosolic pool to enter the circulation; 2) cellular release of proteolytic troponin degradation products whilst membrane integrity remains; 3) formation and release of membranous blebs during temporary ischaemia of cardiac cells; 4) stimulation of myocyte turnover; 5) myocyte necrosis; and 6) increased rate of apoptosis (Eijsvogels *et al*., 2015). The exercise related release of cTn is characterised by a biphasic pattern with an initial rapid increase and decrease during exercise, with a secondary cTn increase post-exercise (Middleton *et al.*, 2008). Within the cytosol, 6-8% cTnT and 3.5% cTnI exists in an unbound form (Korff *et al.*, 2006), however, assays are incapable of distinguishing between cytosolic and complex-bound troponin, complicating interpretation of cTn release post-exercise (Skadberg *et al.*, 2018). George *et al.* (2004) suggested that along with subclinical threshold values and in most cases values rapidly returning to baseline, a transient cytosolic leakage may be occurring as opposed to cardiomyocyte necrosis, supported more recently by Vassalle *et al*. (2018). This theory is disputed by Whyte *et al.* (2005), who suggested if the rise in cTnT observed after a marathon was due to the release of the cytosolic component of cTnT, there would be no alteration in contractile function. Further research is therefore needed to determine the link between cTn release and cardiac function, in an attempt to understand the mechanisms behind both phenomena.

Whilst markers of cardiac injury have been commonly measured following extreme exercise, markers of cardiac stress have less often been used (Table 2.2). B-type natriuretic peptide (BNP) is a cardiac natriuretic peptide hormone secreted from the ventricles in response to volume expansion and pressure overload (Hall, 2004). Within the circulation the BNP hormone is separated from the n-terminal part of the prohormone, known as N-terminal proB-type natriuretic peptide (NT-proBNP). NT-proBNP is a 76

amino acid and is a proposed marker of evaluating and monitoring cardiac abnormalities which are characterised by myocardial wall stress (Mottram *et al*., 2004). NT-proBNP is also reportedly elevated following exercise in response to altered hemodynamics, regional wall-motion abnormalities and exercise induced transitory myocardial wall ischemia (Scharhag *et al.,* 2006). NT-proBNP has been found to be elevated following half-marathon running (Vassalle *et al*., 2018), marathon running (Gaudreault *et al*., 2013; Kim *et al.*, 2015; Melanson *et al.*, 2006), with fewer studies evaluating the effect of cycling (Chan-Dewar *et al.*, 2013) or combined events (Table 2.2). Similarly to cTn, some researchers have reported all elevations (Chan-Dewar *et al.,* 2013; Vidotto *et al.,* 2005), whilst others have reported elevations above the URL (Banfi *et al.*, 2010; Vassalle *et al*., 2018; Vidotto *et al.,* 2005) or changes in group mean values (Kim *et al*. 2012, 2015), confounding data interpretation. Whilst the mechanisms underlying its release are not fully understood, its release may be stimulated by systemic inflammation (McLachlan and Mossop, 2006) resulting in natriuesis, vasodilation and sympathetic nerve blockade. Its release is also hypothesised to be cytoprotective and exert growth-regulating effects (Hamasaki, 2016; Scharhag *et al.*, 2006) by inhibiting the induction of inflammatory mediators such as interleukin-1 and reducing oxidative stress (Hamasaki, 2016).

It has been suggested that a combination of many different factors predispose EICF and EICD and explain the differences/controversial results discussed previously. Factors such as exercise duration, intensity and volume, subjects training status and environmental conditions are amongst those that have been suggested (Dawson *et al*., 2003; George *et al*., 2008; Ortega *et al.*, 2006). It is, therefore, pertinent to investigate the effect of such factors on EICF and EICD, as discussed in the following section.

2.3.3 Exercise Related Factors that may predispose to Exercise-Related Cardiac Fatigue and Damage

As noted in the previous sections, there is a growing evidence base describing an immediate depression in LV systolic and/or diastolic function and an elevation in cardiac biomarkers immediately following a bout of prolonged exercise. Inconsistencies within the findings have been attributed to variations in the methods employed, research designs applied, exercise duration/intensity, participant training status (Middleton *et al.*, 2006a; Shave *et al.*, 2008) and the environment (Neilan and Wood, 2009).

Changes in diastolic function are reported to precede changes in systolic function (Shave *et al.*, 2004c), with diastolic dysfunction appearing to be present after exercise durations of greater than one hour (Shave *et al*., 2008). Speculation from a meta-analysis suggested that a transient depression of LV systolic function may be mediated by exercise durations of greater than 640 minutes (Middleton *et al*., 2006a). A significant reduction in EF within the ultra-duration group compared to the other groups studied immediately post-exercise, support the theory that the onset of LV systolic dysfunction is duration dependant. This is supported by Whyte *et al.* (2000), who compared the effects of exercise duration (half-Ironman compared to an Ironman triathlon competition) upon LV function. The results showed a significant reduction in SV, EF and FS following the Ironman competition, whilst the half-Ironman competition showed a significant reduction in SV only. The authors concluded that the reduction in SV occurred as a result of a minor change in LVIDd, whilst the reductions in EF and FS were attributed to a reduced inotropic state as afterload (SBP) was significantly reduced and preload was unaltered. Further to this, the meta-analysis by Middleton *et al*. (2006a) reported that whilst diastolic filling is compromised following exercise durations greater than 60 minutes, analysis across subgroups indicated that alterations in diastolic filling are unrelated to exercise duration

or training status. Exercise duration is also found to impact cTn release, with a number of studies failing to show any evidence of elevated cTn levels following shorter durations of steady-state exercise. Lippi et al. (2008a, 2008b) demonstrated no elevation in cTnT following a 21km, half marathon run. In contrast, Shave *et al*. (2010) reported elevations in cTn following just 30 min exercise. A meta-analysis by Shave *et al.* (2007) demonstrated lower levels of cTn in longer duration events, advocating the importance of exercise intensity in endurance events. Studies by Serrano-Ostáriz *et al*. (2011) and Legaz-Arrese *et al*. (2011) have shown elevated cTnI with higher intensity work set at a relative or competition intensity in comparison to fixed durations. Legaz-Arrese *et al*. (2011) also suggested there may be a critical exercise intensity or threshold that must be exceeded in order for a cTn elevation to occur. More recently, Richardson *et al*. (2018) found the magnitude of hs-cTnT rise is related to a greater relative exercise intensity but not duration. The relationship between exercise intensity and duration is difficult to derive and may more accurately be viewed as total load, otherwise it is advocated that study design should fix exercise intensity or duration (Eijsvogels *et al*., 2014a).

Whilst the duration of exercise is often noted in studies involving competitive events, information on the intensity of exercise is not always available due to the competitive nature, whereby exercise intensity is self-selected (Table 2.1 and 2.2). Field studies include participants who complete distance events under widely contrasting finish times, corresponding to a wide range of exercising HR's, and therefore exercise intensities (Banks *et al*., 2010). For this reason it makes it difficult to draw conclusions from currently available data collected within a based field study. The competitive stress may also add to the work imposed on the LV if HR's are increased (Dawson *et al*., 2003).

Fortescue *et al*. (2007) found that out of 482 marathon runners, 55 had elevations in cTnT, cTnI or both. The results suggested cTn increases in runners were more likely to be women, younger aged, whilst runners with no marathon experience were almost 3 times likely to have cTn increases compared to those with experience. Neilan *et al.* (2006) suggested amateur athletes were more likely to experience elevated cardiac biomarkers in comparison to individuals with more extensive training backgrounds, however, the meta-analysis by Shave *et al.* (2010) and more recently Richardson *et al.* (2018) did not support this finding. An earlier study by George *et al.* (2004) found that age and finishing time had little impact upon LV function or cTnT.

The majority of studies have been conducted at a competitive event whereby details on the ambient conditions are often not clearly reported or are subject to significant change due to the duration of such events (Table 2.1 and 2.2; Dawson *et al.,* 2003). Lucía *et al.* (1999a) proposed that the effects of environmental strain may be more important than exercise duration in the development of functional changes post-exercise and indeed many of the field-based studies in this area have been carried out within hot, cold, humid and high altitude environments (Bonetti *et al*., 1996; Dávila-Román *et al.*, 1997; Douglas *et al.*, 1987; Gaudreault *et* al., 2013; Neumayr *et al.*, 2001, 2002; Rifai *et al*., 1999). The nature of these events have not allowed for a systematically controlled approach, therefore making it impossible to eliminate the effects of the environment which have been implicated in the aetiology of EICF and EICD (Shave *et al.,* 2004c) and consequently complicate data interpretation. Currently limited lab-based, and thus controlled data has been collected, investigating the impact of environmental temperature on LV function and cardiac biomarker release. Shave *et al*. (2004c) observed the effect of prolonged endurance exercise (100 mile cycle trial) within controlled ambient temperatures of 0^{o}C and 19^{o}C, reporting a significant decrease in diastolic function after exercise in 19^{o}C but

not in 0ºC, with no evidence of systolic dysfunction displayed in either trial. Detectable cTnT was only observed in two cyclists after exercise in the 19ºC trial. This preliminary evidence suggests that ambient temperatures may play some part in exacerbating the post-exercise response of LV function and cardiac biomarker release, but did not extend to warmer environmental conditions. The evidence presented from studies evaluating cardiac fatigue and biomarker release from single bouts of prolonged exercise reveal gaps in the knowledge, particularly within a controlled environment or with elevated environmental temperatures. Therefore a focus of this thesis will consider the effect of the environment and prolonged exercise upon cardiac fatigue and biomarker release.

2.4 The Impact of Repeated Bouts of Endurance Exercise on Cardiac Function and Biomarker Release

Whilst single bouts of prolonged exercise provide a substantial cardiovascular challenge, these events are typically followed by periods of rest or reduced activity allowing the athlete to recover. Participation in multi-day endurance events, with repeated endurance exercise exposure and limited recovery time, have proved increasingly popular (Knoth *et al*., 2012). It is also common for endurance athletes to undertake high volumes of exercise during training and competition with inadequate recovery between sessions. This likely represents the upper limit of physiological stress placed upon the human heart (George *et al.*, 2011). The following section of the literature review discusses the extant evidence of cardiac fatigue and damage associated with repeated bouts of endurance exercise.

2.4.1 Alterations in Cardiac Function

It has been previously speculated by La Gerche *et al.* (2004) that repeated bouts of ultra-endurance exercise, each producing sub-clinical effects, may have a cumulative effect upon cardiac function. There are a small number of studies that have focused upon the

cardiovascular consequences of engaging in repeated bouts of endurance exercise (Table 2.3) which have employed a combination of exercise modes, participants and research designs.

Table 2.3 provides an overview of the previous literature which have assessed LV systolic and diastolic function before and after repeated bouts of exercise using standard 2D echocardiography, flow-Doppler and TDI indices. The table details the studies by mode, exercise duration, the intensity of the exercise and environmental conditions, alongside the study's findings.

Table 2.3 Studies utilising standard 2D, flow-Doppler and tissue-Doppler echocardiography following repeated bouts of prolonged exercise

Author **Mode of Exercise**	**Duration**	**Intensity of Exercise**	**Systolic**			**Diastolic**			**Environmental Conditions**
			↑	=	↓	↑	=	↓	
WALKING/RUNNING									
Benda *et al.*, 2016 3 days walking (30 or 40 km)	Day 1: 424 ± 91 min Day 2: 470 ± 114 min Day 3: 416 ± 111 min	HRmean 109 ± 23 beats.min^{-1} HRmean 108 ± 28 beats.min^{-1} HRmean 109 ± 27 beats.min^{-1}	S’	EF	**SBP/ESV**		E, A, E:A, E’, A’		NR
Middleton *et al*., 2007b Mountain run 3-4 days 15.3 miles per day	Day 1: 138.8 ± 13.5 mins Day 2: 138.6 ± 13.9 mins Day 3: 143.0 ± 23.4 mins Day 4: 139.3 ± 8.2 mins	HRmean 163 ± 11 beats.min^{-1} HRmean 157 ± 18 beats.min^{-1} HRmean 153 ± 17 beats.min^{-1} HRmean 145 ± 14 beats.min^{-1}		EF, SBP/ESV EF, SBP/ESV EF, SBP/ESV EF, SBP/ESV	**EF** **EF**		E:A	E:A E:A E:A	13.0 ± 2.7ºC, 63 ± 11% RH, 3717 ft total elevation per day
Shave *et al*., 2002b 2 day mountain marathon	Day 1: 513 ± 121 mins Day 2: 348 ± 59 mins	Self-paced			EF, SV, FS	A		E, E:A	NR

CYCLING									
Oosthuyse *et al.*, 2012 Simulated 4-day cycling challenge	3 h per day Day 1: Day 2: Day 3: Day 4:	HRmean 154 ± 12 beats.min^{-1} HRmean 152 ± 12 beats.min^{-1} HRmean 142 ± 20 beats.min^{-1} HRmean 147 ± 11 beats.min^{-1}		SBP/ESV, SV	EF, S’	A’	A	E, E’, E:A, E’:A’	17.7 ± 1.4ºC
Williams *et al.*, 2011 Race Across America (team)	6 days, 10 h, 51 min (Approximately 75 h individual riding time)	NR		S’			E’, A’, E’:A’		NR
Appelman *et al.*, 2015 1580 km cycle tour in 8 days	Average cycling time 10 h per day	Average speed 27.9 km.hr^{-1}		FS			E:A		Mean: 23-25ºC
Williams *et al.*, 2009 Replicated Tour de France (20 days cycling in 22 day period)	Range 200 to 677 min	Range HRmean 55.0 ± 6.2 to 67.5 ± 6.9 beats.min^{-1}			EF, SV, S’	A, A’	E	E’, E:A, E’:A’	NR

EF: ejection fraction, SV: stroke volume, FS: fractional shortening, SBP/LVESV: systolic blood pressure/left ventricular end systolic volume, S’: peak systolic myocardial tissue velocity, E: peak early trans-mitral flow velocity, A: peak atrial filling velocity, E:A: early to late diastolic filling ratio, E’: peak early diastolic myocardial velocity, A’: peak late diastolic myocardial velocity, E’/A’: peak early to late myocardial tissue velocity ratio, NR: not reported. **Variables in bold highlight evidence of cumulative effect**.

Shave *et al*. (2002b) studied athletes at a 2-day mountain marathon and observed cardiac dysfunction in 91% of participants following day 2, but no measures were taken at the end of the first day, confounding data interpretation. Middleton *et al.* (2007b) demonstrated a progressive decline in EF over 3 or 4 days of cross-country running in comparison to baseline levels. An increase in post-exercise HR after day 3 could only account for 48% of the change in EF, which was still depressed in a subset of athletes 20 hours post day 4. Diastolic function was reduced pre-post exercise daily, but demonstrated no evidence for a cumulative effect. Benda *et al*. (2016) reported a cumulative decrease in SBP/LVESV over 3 days walking suggesting a decrease in contractility, however, no change in EF and an increase in S' was observed. An unaltered diastolic function was also seen, with the lack of change in LV function being attributed to the low intensity of walking exercise. Oosthuyse *et al*. (2012) confirmed a consistent pattern of reduction in diastolic function each day following 3 hr-sessions of race-simulated cycling for four days in well trained competitive cyclists but did not observe a cumulative decline in EF. Williams *et al*. (2009) also noted a consistent pattern of change in diastolic function with no accumulation effect on EF in a 22 day cycle study, although echocardiograms were only conducted following 4 of the 20 stages. In-event data by Williams *et al*. (2011) demonstrated no changes in septal systolic or diastolic tissue velocities during and after the Race Across America in well trained individuals. More recently, Appleman *et al.* (2015) reported no changes in systolic or diastolic function after 7 days of an 8 day cycle tour, however, no measures were taken during the tour. Further research is warranted to better understand the extent of short term recovery on cardiac function in multi-stage exercise, particularly in less trained individuals, as well as to investigate individual differences in the response to endurance exercise and will therefore be a focus of this thesis.

2.4.2 The Appearance of Tissue-Specific Markers of Cardiac Damage

Limited attention has also been given to the effect of multi-day exercise upon biomarkers of cardiac damage or stress (Table 2.4). Table 2.4 provides an overview of the literature which assessed cardiac biomarkers before and after repeated bouts of exercise. The table details the studies by mode (walking/running, cycling), exercise duration, the intensity of the exercise and environmental conditions, alongside the study's findings. Details of the type of biomarkers used are also given, which include cTnT (1s/2nd/3rd generation), cTnI, highly sensitive assays (hs-cTnT and hs-cTnI) as well as markers of cardiac stress.

Bonetti *et al*. (1996) reported the presence of cTnT (2nd generation) in 5 of the 28 cyclists, assessed prior to, after 1 week, 2 weeks and at the end of the Giro d'Italia cycle race. Limited blood draws and the fact that cTnT was present in the circulation of 3 of the 5 cyclists before the cycle race started confounds data interpretation. Shave *et al.* (2002b) demonstrated a cTnT elevation above the detection limit of the assay in 13/26 participants after day 1 of a mountain marathon, with values returning to undetectable levels after day 2. Conversely, Denissen *et al*. (2012) reported no increases in cTnT before and after a 3 day trail run, which was also supported by Corsetti *et al.* (2012) who reported few changes in the high sensitivity cTnT assay over the course of the Giro D'Italia. Middleton *et al.* (2007b) reported a limited number of cTnT elevations with most occurring following the first day of exercise. Collectively these studies suggest no accumulation effect of repeated endurance exercise bouts on cTnT.

Eijsvogels *et al.* (2010) reported no cumulative effect in cTnI or hs-cTnI release when exposed to the same exercise volume (prolonged walking) on 4 consecutive days, with only a few cases of elevated cTn levels. More recently, Benda *et al.* (2016) reported a significant increase in hs-cTnI across 3 days walking, however, only mean and SD values

were reported confounding data interpretation. Appleman *et al.* (2015) reported a cTnI elevation above the assay detection limit, but below the upper normal limit in 16/20 participants after an 8 day cycling tour. Interestingly in one of the few papers to report case data, Williams *et al*. (2011) observed a high degree of individual variability in the cTnI response to the Race Across America. Sporadic increases in cTnI were also observed after a number of stages during a 20 day cycle study (Williams *et al.*, 2009), with all participants demonstrating elevated values across the tour.

Fewer data are available for NT-proBNP changes with repeated exercise bouts. Corsetti *et al*. (2012) described a progressive increase in NT-proBNP from baseline levels to the 12th day and final day of the Giro d'Italia cycle race, which was also observed by Middleton *et al*. (2007b) over 3-4 days of cross-country running. After 8 days of cycling, Appleman *et al.* (2015) reported BNP levels within the normal limits for all participants, a finding also observed by Benda *et al*. (2016) following 3 days of walking. This finding was also similar to Corsetti *et al*. (2012), who attributed lower NT-proBNP values to the high training status of the participants involved. In a 20 day cycle study Williams *et al.* (2009) observed an early peak NT-proBNP at stage 6 with values dropping close to baseline by stage 20. Inter and intra-individual variability was apparent within the data, as well as that by Williams *et al.* (2011), highlighting the need for further research to expose the response to multi-day exercise.

Table 2.4 Studies utilising cTnT, cTnI and other cardiac biomarkers during/following single bouts of prolonged exercise

Author **Mode of Exercise**	**Duration**	**Intensity of Exercise**	**Blood Measurement**	**Elevated (Number > URL)**	**Peak**	**Environmental Conditions**
WALKING/RUNNING						
Benda *et al.*, 2016 3 days walking (30 or 40 km)	Day 1: 424 ± 91 min Day 2: 470 ± 114 min Day 3: 416 ± 111 min	HRmean 109 ± 23 beats.min^{-1} HRmean 108 ± 28 beats.min^{-1} HRmean 109 ± 27 beats.min^{-1}	hs-cTnI (ADVIA Centaur) BNP (ADVIA Centaur)	Significant increase No change	NR NR	NR
Eijsvogels *et al.*, 2010 4 day walking event (30, 40 or 50 km per day)	Day 1: 8 hrs 37 mins ± 1 hr 38 mins Day 2: 8 hrs 57 mins ± 1 hour 33 minutes Day 3: 8 hrs 36 mins ± 1 hr 47 mins Day 4: 9 hrs 8 mins ± 2 hrs 9 mins	Day 1: 4.6 ± 0.6 km.hr^{-1} Day 2: 4.4 ± 0.6 km.hr^{-1} Day 3: 4.6 ± 0.6 km.hr^{-1} Day 4: 4.4 ± 0.6 km.hr^{-1} (Variation of 72-65% of predicted VO_2max)	cTnI (STAT) hs-cTnI (Centaur TnI-Ultra) cTnI (STAT) hs-cTnI (Centaur TnI-Ultra) cTnI (STAT) hs-cTnI (Centaur TnI-Ultra) cTnI (STAT) hs-cTnI (Centaur TnI-Ultra)	4/103 (4) post 4/103 (4) post 1/103 (1) post 1/103 (1) post 2/103 (2) post 2/103 (2) post 2/103 (2) post 2/103 (2) post	NR 1.17 µg.L^{-1} NR 0.74 µg.L^{-1} NR 0.79 µg.L^{-1} NR 0.44 µg.L^{-1}	Day 1: Min: 13.2ºC Max: 20.6ºC Day 2: Min: 14.2ºC Max: 20.4ºC Day 3: Min:12.3ºC Max: 19.8ºC Day 4: Min:12.5ºC Max: 19.4ºC
Shave *et al*., 2002b 2 day mountain marathon	Day 1: 506 ± 123 mins Day 2: 344 ± 54 mins	Self-paced	cTnT (3rd Gen. Roche)	Day 1: 13/26 Day 2: 1/26	0.035 µg.L^{-1} 0.017 µg.L^{-1}	NR

Middleton *et al.*, 2007b Mountain run 3-4 days 15.3 miles per day	Day 1: 138.8 ± 13.5 mins	HRmean 163 ± 11 beats.min^{-1}	cTnT (3rd Gen. Roche) NT-proBNP (Roche Elecsys 1010)	1/10 post, 4/10 1 h post, 0/10 20 h post Elevated	0.079 µg.L^{-1}, 0.125 µg.L^{-1}, ND NR	13.0 ± 2.7°C, 63 ± 11% RH, 3717 ft total elevation per day
	Day 2: 138.6 ± 13.9 mins	HRmean 157 ± 18 beats.min^{-1}	cTnT (3rd Gen. Roche) NT-proBNP (Roche Elecsys 1010)	1/10 post, 1/10 1 h post, 1/10 20 h post Elevated	NR NR	
	Day 3: 143.0 ± 23.4 mins	HRmean 153 ± 17 beats.min^{-1}	cTnT (3rd Gen. Roche) NT-proBNP (Roche Elecsys 1010)	1/10 post, 1/10 1 h post, 1/10 20 h post Elevated	NR NR	
	Day 4: 139.3 ± 8.2 mins	HRmean 145 ± 14 beats.min^{-1}	cTnT (3rd Gen. Roche) NT-proBNP (Roche Elecsys 1010)	1/10 post Elevated	0.013 µg.L^{-1} NR	
Denissen *et al.*, 2012 3 day trial run	12 h 57 min ± 2 h 51 min		cTnT (NR)		NR	11.5 to 22.8°C, 54 to 22.8% RH, max wind speed 2.8 m.s^{-1}
Day 1: 29.3 km		HRmean 150.8 ± 21.3 beats.min^{-1}		0/19 post		
Day 2: 37.9 km		HRmean 140.7 ± 22.5 beats.min^{-1}		0/19 post		
Day 3: 27.8 km		HRmean 138.5 ± 23.3 beats.min^{-1}		0/19 post, 0/19 24 h post, 0/19 72 h post		

CYCLING						
Williams *et al.*, 2011 Race Across America (team)	6 days, 10 h, 51 min (Approximately 75 h individual riding time)	NR	cTnI (TnI-Ultra ADVIA Centaur) NT-proBNP (ECLIA, Roche)	Day 1: 4/4 (0), Day 2: 1/4 (0), Day 3: 2/4 (0), Day 4: 0/4 (0), Day 5: 1/4 (1), 4 h post: 1/4 (1) Day 1: 4/4 (0), Day 2: 4/4 (0), Day 3: 4/4 (0), Day 4: 4/4 (1), Day 5: 3/4 (1), 4 h post: 1/4 (1)	0.03 $ng.mL^{-1}$, NR. NR, NR, 0.08 $ng.mL^{-1}$, NR NR, NR, NR, 150 $ng.L^{-1}$, NR, 310 $ng.L^{-1}$	NR
Appelman *et al.*, 2015 1580 km cycle tour in 8 days	Average cycling time 10 hours per day	Average speed 27.9 $km.hr^{-1}$	cTnI (NR) BNP (NR)	16/20 (0) post Within normal limits	NR NR	Mean: 23-25ºC
Bonetti *et al*., 1996 Giro d'Italia (22 days)	NR	Self-paced Average speed 37.2 $km.h^{-1}$	cTnT (2^{nd} Gen. Boehringer)	2/25 post week 1, 3/25 post week 2, 2/25 post event	0.05 $\mu g.L^{-1}$, 0.07 $\mu g.L^{-1}$, 0.10 $\mu g.L^{-1}$	Altitude difference 25,985 m
Corsetti *et al*., 2012 Giro d'Italia (22 days)	86 h 47 min	Average speed 35.7 $km.hr^{-1}$	hs-cTnT (Roche) NT-proBNP (Elecsys proBNP)	NR	Day 12: 9.33 $ng.L^{-1}$, Day 22: 21.90 $ng.L^{-1}$ Day 12: 93.31 $ng.L^{-1}$, Day 22: 129.78 $ng.L^{-1}$	NR

Williams *et al.*, 2009 Replicated Tour de France (20 days cycling in 22 day period)	Range 200 to 677 min	Range HRmean 55.0 ± 6.2 to 67.5 ± 6.9 beats.min^{-1}	cTnI (TnI-Ultra ADVIA Centaur)	Stage 1: 3/10, Stage 2: 1/10, Stage 3: 2/10, Stage 6: 1/10, Stage 7: 2/10, Stage 9: 3/10, Stage 12: 5/10, Stage 15: 6/10, Stage 17: 3/10, Stage 18: 3/10, Stage 20: 1/10	NR	NR
			NT-proBNP (ECLIA, Roche)	Stage 1: Elevated, Stage 2: Elevated, Stage 3: Elevated above URL, Stage 6: Elevated above URL, Stage 7: Elevated above URL, Stage 9: Elevated, Stage 12: Elevated, Stage 15: Elevated, Stage 17: Elevated above URL, Stage 18: Elevated above URL, Stage 20: Elevated	NR	

1st gen.: first-generation assay, 2nd gen.: second-generation assay, 3rd gen.: third-generation assay, BNP: brain natriuretic peptide, NT-proBNP: N-terminal proB-type natriuretic peptide, cTnI: cardiac troponin I, cTnT: cardiac troponin T, HRmean: mean heart rate, NR: not reported, ND: not detectable.

2.5 Exercise, Thermoregulation and the Impact of Environmental Heat Stress on Cardiovascular Function

The maintenance of a normal T_c (~37^{o}c) is essential, as deviations as little as 3^{o}c can severely strain physiological systems and lead to death (Crandall and González-Alonso, 2010). Regulatory mechanisms within the human body maintain a homeostasis in internal temperature within a narrow range, with as little as 0.3^{o}c increase initiating critical heat loss responses of cutaneous vasodilation and sweating (Crandall and Wilson, 2015). An increase in skin blood flow enhances convective heat dissipation through sweating, whilst a reduction in skin perfusion impedes heat loss (Rowell, 1974). These heat dissipating responses, along with autonomic and cardiovascular adjustments assist in regulating thermoregulation, blood pressure control and organ perfusion. These adjustments include an elevated cardiac output through increases in HR and cardiac contractility to account for the higher skin perfusion (Rowell, 1974), and an elevated sympathetic activity to reduce blood flow and blood volume to noncutaneous regions (Crandall and Wilson, 2015). Exercise in the heat presents a greater challenge to the cardiovascular system, with an increased need for heat dissipation competing with a greater need for blood flow to the active muscle tissue (González-Alonso, 2007; González-Alonso *et al.,* 2008). Furthermore, a number of studies have demonstrated an impaired exercise capacity (Ely *et al.*, 2010; González-Alonso and Calbet, 2003; González-Alonso *et al*., 1999) when performing exercise in a thermally stressful environment, leading to a number of studies researching into the effect of pre-cooling immediately prior to exercising in thermally stressful environments. The following section summarises the cardiovascular adjustments to heat stress at rest and during exercise prior to a review of the literature of pre-cooling methods prior to prolonged exercise in hyperthermic environments.

2.5.1 Cardiovascular Adjustments to Heat Stress at Rest

During heat stress at rest, skin blood flow is estimated to increase from ~300 ml.min^{-1} towards 7500 ml.min^{-1} (Rowell *et al*., 1969). In order to maintain arterial blood pressure, cardiac output must increase alongside decreases in vascular resistance of non-cutaneous beds to counteract the effect of increases in total vascular conductance associated with cutaneous vasodilation (Crandall and González-Alonso, 2010). As a result, arterial blood pressure is maintained or minimally reduced. The elevation in cardiac output is driven primarily through increases in HR, as SV is either maintained or marginally increased (Rowell *et al*., 1969). Heat stress is reported to increase HR and ventricular contraction through a direct effect on cardiac nodal cells (sinoatrial and atrioventricular) and conduction velocity (positive dromotropic effect), accounting for 40% of the elevation in HR. Sympathetic activation and parasympathetic withdrawal account for the remaining effects on cardiac nodal cells (Crandall and Wilson, 2015).

Several studies have demonstrated a reduction in central blood volume, central venous pressure and LV filling pressure during heat stress at rest, indicating a reduced venous return (Rowell *et al*., 1969; Wilson *et al.*, 2007, 2009). Passive heat stress can induce reductions in central venous pressure, which can approach 0 mmHg depending on the magnitude of the heat stress (Rowell *et al*., 1969; Wilson *et al.*, 2007), potentially as a result of a redistribution of blood from the central circulation to cutaneous vascular beds. Passive heat stress results in a parallel decrease in LV filling pressure (Wilson *et al.*, 2007, 2009), which alongside a reduced central blood volume and a preserved or increased SV, suggests an increase in inotropic state of the heart (enhanced LV function). Therefore, despite a reduced venous return, a maintained SV must be facilitated by an enhanced LV systolic and/or diastolic function. In an attempt to identify the cause of a maintained SV during passive heat stress, Brothers *et al.* (2009) measured LV systolic and diastolic

function using echocardiography. The authors demonstrated that despite a reduction in venous return, E and E' remained unchanged with heat stress at rest, with improved LV systolic (S') and late diastolic function (A). It was concluded that the maintenance of SV was caused by an increased LV systolic and 'atrial systolic function', however, the maintenance of E despite a reduced LV filling pressure also demonstrated an enhanced early diastolic function (Brothers *et al.*, 2009). Whilst the cardiovascular response of passive heat stress has been demonstrated with relatively consistent findings, the cardiovascular response to combined exercise and heat stress have produced conflicting results and will be discussed in the next section.

2.5.2 Cardiovascular Adjustments to Heat Stress during Exercise

The combination of exercise and heat stress provides one of the most severe challenges to the regulation of the cardiovascular system (Crandall and González-Alonso, 2010). Compared to exercise in normothermic conditions, cardiac output during exercise in the heat has shown to be the same (Lafrenz *et al.*, 2008), higher or lower (Rowell *et al.*, 1966). Whilst these discrepancies are likely a consequence of differing exercise intensities, durations and protocols, an increased HR and reduced SV has been consistently observed in hot environments compared to normothermic conditions (Rowell *et al.*, 1966; Lafrenz *et al.*, 2008), suggestive of a decrease in LV function when the need for thermoregulation is elevated. The mechanisms behind the drop in SV is widely debated. A maintained or decreased mean arterial pressure (Rowell *et al.*, 1969; Lafrenz *et al.*, 2008), suggests an enhanced afterload cannot account for the lower SV during exercise in the heat. A reduction in venous return, as a result of a reduced central blood volume (Rowell *et al.*, 1966, 1969) caused by the combined demand for blood flow to the active skeletal muscles and to the skin has also been proposed. Alternatively, an increased HR leading to a

reduced filling time has also been suggested to contribute to the reduction in SV during exercise (Fritzsche *et al.,* 1999).

When performing dynamic exercise in the heat, blood flow to the active muscle tissue is required to meet the energetic demands of the activity, whilst blood flow to the skin is required to aid with temperature regulation (González-Alonso, 2007; González-Alonso *et al.*, 2008). As a consequence, the combined demands for blood flow can result in a competition for the available cardiac output (Rowell, 1974). The increased demands on muscle and skin blood flow can be limited by the cardiac pumping capacity and the limited redistribution of blood flow. As a possible consequence, either: a) the mismatch between cardiac output and total vascular conductance leads to a loss in blood pressure, b) a reduction in muscle VO_2 due to a mismatch between muscle blood flow and metabolism, or c) a compromised skin blood flow leading to a higher T_c (González-Alonso *et al.*, 2008). It is suggested that there is little or no role for a reflex thermoregulatory reduction in active muscle blood flow, but it is a passive event as a result of dehydration enhanced in a hot environment. Whilst blood pressure is generally well maintained during exercise in the heat (Rowell, 1974), a compromised skin blood flow is likely to be enhanced during exercise in the heat. An acute reduction in skin blood flow at the onset of exercise is observed, brought about by increased vasoconstrictor system activity. As exercise duration increases, the vasodilator system is activated and governed by two thresholds (González-Alonso *et al.*, 2008). The first is the internal temperature at which vasodilation begins, which is delayed to a higher temperature during exercise compared to rest (Kellogg *et al*., 1991). As the internal temperature continues to rise (~38^{o}C), a second threshold is met whereby skin blood flow reaches an upper limit despite further increases in internal temperature (González-Alonso *et al.*, 1999), reducing the ability to dissipate heat. This reflects the inability of the cardiovascular system to

maintain arterial blood pressure and cutaneous vasodilation during prolonged exercise. Whilst the effect of heat during prolonged exercise on the cardiovascular system is well reported, the cardiovascular effects of a combination of prolonged exercise and heat exposure post-exercise is less clear and will be a focus of this thesis.

2.5.3 Strategies to enhance exercise performance in the heat

When exercising in the heat, the metabolic heat produced, coupled with the high ambient temperature and/or high humidity can result in an increased T_c (Ross *et al.* 2013), increase in heat related illnesses (Casa *et al.*, 2015), along with an impaired exercise capacity (Ely *et al.*, 2010; González-Alonso and Calbet, 2003; González-Alonso *et al.*, 1999). The main limiting factor inhibiting exercise performance is suggested to be the attainment of a critically high body temperature (González-Alonso *et al.*, 1999), with other adverse effects including cardiovascular strain and metabolic disturbances (Marino, 2002). The increased cardiovascular strain from the enhanced cardiovascular drift in a hyperthermic environment is in part attributed to a peripheral displacement of blood volume and a decreased ventricular filling time due to a hyperthermia-induced tachycardia (González-Alonso *et al.*, 2008).

Heat transfer between the body and the environment occurs via heat flow down temperature or humidity gradients through thermal radiation, convection and evaporation (Brotherhood, 2008). Convective heat transfer is influenced by the thermal properties of the air and skin, as well as the air velocity over the body surface. Whilst evaporative cooling is dependent upon sufficient physiological sweat production, as well as the environments evaporative capacity i.e. air flow over the skin and absolute humidity (Brotherhood, 2008). Sweating can be initiated by central stimulation prior to a rise in body temperature, but also influenced by multiple factors including the amount of

mechanical work being performed, humoral factors i.e. dehydration, and environmental conditions i.e. temperature, humidity and wind flow (Ogawa and Sugenoya, 1993; Saunders *et al*., 2005; Shibasaki and Crandall, 2010). Thermoregulatory mechanisms can also be stimulated by an increase in central brain temperature (Holdcroft, 1980).

Hyperthermia is a major influence upon the impaired exercise capacity (Ely *et al.*, 2010; González-Alonso and Calbet, 2003; González-Alonso *et al*., 1999) observed when performing exercise in a thermally stressful environment. A number of strategies to attenuate the rise in T_c by increasing the body's capacity to store metabolic and environmental heat have been investigated as a means to enhance exercise performance (Booth *et al*., 1997; Kay *et al*., 1999; Ross *et al*., 2013; Wegmann *et al*., 2012), including cooling, acclimation and fluid ingestion. Cooling interventions may increase the heat storage capacity (pre-cooling), attenuate the exercise-induced increase in T_c (per-cooling) and accelerate recovery following intense exercise (post-cooling; Bongers *et al*., 2017). Pre-cooling is described as the rapid removal of heat from the body prior to exercise, and is a popular method used to combat the effects of heat-stress induced fatigue/performance decrements (Périard *et al*., 2017; Ross *et al*., 2013). Pre-cooling utilises techniques including whole body pre-cooling such as cold water immersion and cold air exposure, local cooling such as cooling vests/packs or internal cooling strategies such as ingesting cold water/ice slurry (Bongers *et al*., 2017). Gradual cooling via whole-body water immersion is reported to offer four times greater heat loss to water that air at the same temperature (~4.2 kJ/kg ^{o}C versus ~1.0 kJ/kg ^{o}C, respectively; Ross *et al*., 2013). A recent meta-analysis has reported cold water immersion to be the most effective method of enhancing exercise performance over internal cooling or local cooling (Bongers *et al.*, 2015), suggesting cooling of a large surface area is more effective than local cooling methods. Additionally, the increase in core-to-skin thermal gradient is associated with the

'after-drop' effect, allowing for the continuation of cooling even upon the commencement of exercise (Kay *et al.*, 1999). Furthermore, research into the effect of pre-cooling on the cardiovascular system during exercise has established that reducing the rate of rise in T_c coupled with the hydrostatic effects following immersion, displaces fluids from the extremities towards the thoracic cavity (Farhi and Linnarsson 1977), leading to a maintenance or increase of central blood volume and hence cardiac output as the need for skin blood flow is reduced (Stanley *et* al., 2014; Wegmann *et al*., 2012). A maintained SV and attenuated HR during steady state exercise after pre-cooling has been previously observed (Hessemer *et al.*, 1984; Lee and Haymes, 1995; Schmidt and Brück, 1981), suggesting a reduced cardiovascular strain following pre-cooling. The pre-cooling effect of reducing cardiovascular strain during exercise in the heat upon post-exercise cardiac function has yet to be investigated and warrants further investigation.

2.6 Summary and Link to Empirical Studies

An acute bout of exercise can place a significant amount of stress upon the heart, potentially resulting in a transient reduction in LV systolic and/or diastolic function as well as the release of cardiac-specific biomarkers. Whilst a number of single day field based studies exist, little attention has been given to repeated bouts of exercise. Furthermore, the effect of prolonged exercise within a controlled or hyperthermic environment is unknown. Consequently, more work is required to fully understand these phenomena.

2.7 Thesis Aims and Hypotheses

With the presented literature in mind, the overall aim of this thesis was to examine the impact of prolonged exercise and environmental temperature upon LV function and markers of cardiac damage in healthy individuals. Four empirical studies were completed based on the following aims and hypotheses.

Study 1

Study aim: To assess the cardiac function and cardiac biomarkers of recreational distance cyclists before, during and after a 10 day cycle challenge.

Research hypothesis: A cumulative decrease in LV systolic (EF) and diastolic function (E:A) as well as a cumulative increase in cardiac biomarker (cTnI and NT-proBNP) concentrations would be evident across the 10 day cycle challenge.

Study 2

Study aim(s): To assess whether (1) a single bout of prolonged strenuous running in a hot ambient environment resulted in a reduction in cardiac function and increased levels of biomarkers of cardiac damage, (2) whether five consecutive days of prolonged strenuous running in a hot ambient environment resulted in cumulative decrements in cardiac function and increments in levels of biomarkers of cardiac damage, and (3) what degree of day-to-day recovery in cardiac function and biomarkers will occur.

Research hypothesis 1: An increase in cardiac biomarkers (cTnI and NT-proBNP) and a decrease in cardiac function (EF and E:A) would be apparent after a single bout of exercise in the heat.

Research hypothesis 2: Multi-day exercise in the heat would lead to an accumulation of changes in cardiac function and markers of cardiac damage.

Research hypothesis 3: There would be an insufficient recovery of cardiac function and markers of damage between days.

Study 3

Study aim: To examine the impact of prolonged exercise in a hyperthermic environment upon cardiac function (EF and E:A) and levels of biomarkers of cardiac damage (cTnI and NT-proBNP).

Research hypothesis: Exercise of the same duration (60 min) and the same running speed performed in a hyperthermic environment (30^{o}C) will result in greater changes in LV function and more frequent appearance of cardiac biomarkers than exercise in a normothermic environment (13^{o}C).

Study 4

Study aim: To examine the effect of pre-cooling, prior to exercise in a hyperthermic environment, upon cardiac function (EF and E:A) and markers of cardiac damage (cTnI and NT-proBNP).

Research hypothesis: Performing prolonged exercise in a hot environment, preceded by pre-cooling would result in a maintained LV function and a reduced appearance of cardiac biomarkers in comparison to a control trial.

CHAPTER 3

A unique case series of alterations in left ventricular function and biomarkers of cardiac damage in recreational distance cyclists completing the 1666 km “John O’Groats to Lands End Cycle Challenge”

3.1 Introduction

Clear evidence of a decrease in LV systolic and diastolic function and/or an elevation in cardiac biomarkers following single, prolonged bouts of physical activity is apparent (Chapter 2; Middleton *et al*., 2006a; Shave *et al*., 2007a), with functional and biomarker variables typically returning to baseline values after 24-48 hr of recovery (Middleton *et al.*, 2006b; Neumayr *et al*., 2005; Shave *et al*., 2004a; Stewart *et al.*, 2016). Whilst single bouts of prolonged exercise provide a substantial cardiovascular challenge, many athletes however, engage in repeated bouts of exercise either during competition, through the repetitive element of training, or a combination of the two.

Participation in multi-day endurance events, with repeated endurance exercise exposure and limited recovery time, have proved increasingly popular (Knoth *et al*., 2012) and likely represent the upper limit of physiological stress placed upon the human heart (George *et al.*, 2011). A small number of studies have focused upon the cardiovascular consequences of engaging in repeated bouts of endurance exercise with inconsistent outcomes (Appleman *et al.*, 2015; Bonetti *et al*., 1996; Corsetti *et al*., 2012; Denissen *et al*., 2012; Middleton *et al*., 2007b; Oosthuyse *et al*., 2012; Shave *et al*., 2002b; Williams *et al*., 2009, 2011), whilst also employing a variety of exercise modes, participants and research designs. Most notably was the lack of data collection points, with a number of studies reporting either pre-post event data (Appleman *et al.*, 2015; Denissen *et al*., 2012; Shave *et al*., 2002b) or minimal time points across the event (Bonetti *et al*., 1996; Williams *et al*., 2009) and thus provides part of the rationale for the initial study.

Collectively, Chapter 2 highlighted limited evidence for an accumulation effect of repeated endurance exercise bouts on cardiac function or cardiac biomarkers. Interestingly in one of the few papers to report case data, Williams *et al*. (2011) observed

a high degree of individual variability in response to the Race Across America, however, these participants were highly trained individuals. Whilst the cardiac response to multi-day exercise in professional (Bonetti *et al.*, 1996; Corsetti *et al.*, 2012) and highly trained athletes (Middleton *et al.*, 2007b; Oosthuyse *et al.*, 2012) has been considered, the cardiac consequences of repeated bouts of cycling in amateur or less trained individuals is unknown. Previous research has suggested that training status may mediate both cardiac functional and biomarker responses to acute prolonged exercise (Middleton *et al.*, 2006a), which combined with limited recovery time, may facilitate an accumulation effect. Therefore, a case series approach to better understand the extent of short term recovery during multi-stage exercise on cardiac function and biomarker appearance as well as to investigate individual differences in the response to endurance exercise was adopted. Whilst a laboratory-based study usually allows for the tighter control of extraneous variables, it may also impact on the external validity of the results, which a field-based study can offer. Specifically, a field-based approach also allows for a greater duration of exercise to be performed compared to a laboratory-based study. Consequently, the aim of this study was to assess the cardiac function and cardiac biomarkers of recreational distance cyclists before, during and after a 10 day cycle challenge. It was hypothesised that a cumulative decrease in LV systolic (EF) and diastolic (E:A) function as well as a cumulative increase in cardiac biomarker concentrations (cTnI and NT-proBNP) would be evident in amateur cyclists completing a 10-day cycle challenge.

3.2 Method

3.2.1 Participants and Study Design

Three Caucasian male recreational cyclists (age 25, 37 and 35 years, height 1.87, 1.85 and 1.82 m, body mass 83, 73 and 80 kg, respectively) volunteered to take part in the study. Ethical approval was obtained from the University Ethics Committee prior to data

collection. Before the study commenced, all participants provided written informed consent and self-reported no personal or early family history of cardiovascular disease. No cyclist was ill, injured or taking any medication prior to the start of the study. All cyclists were recreationally-active completing a minimum of 3 prolonged endurance training sessions per week.

All cyclists completed a 1666 km cycle challenge, over a 10 day period, covering between 112 and 198 km per day (Table 3.1) over a range of terrains. The study protocol employed a repeated measures design. Identical echocardiographic and venous blood-sampling procedures were conducted 12 hours prior to day 1 (PRE), post-exercise for all cycling day(s) and 22 hours post-completion of the challenge (POST). Pre and post-exercise body mass (BM) and BP were assessed every day, with BP also being measured POST-challenge. Post-exercise assessments were conducted within 30 min of exercise completion. Total duration (time from exercise initiation to exercise cessation), exercise duration and HR during the exercise period were assessed daily. Cyclists were allowed to consume fluids and foodstuffs *ad-libitum* throughout the study.

Table 3.1 Details of each days distance, elevation, average temperature, average humidity, exercise duration, total duration and % HRmax.

Day	Distance (km)	Elevation (m)	Average Temperature (ºC)	Average Humidity (%)	Exercise Duration (min)	Total Duration (min)	% HRmax
1	126	+1373/ -1398	11.6	66	430	550	72 ± 6
2	183	+1987/ -1982	9.8	66	572	726	65 ± 5
3	137	+1536/ -1526	9.6	68	417	522	62 ± 5
4	177	+1581/ -1581	13.2	71	471	654	59 ± 4
5	189	+2170/ -2076	8.2	73	571	748	61 ± 1
6	183	+1303/ -1313	10.3	71	512	652	60 ± 4
7	112	+1108/ -1031	13.2	72	354	470	60 ± 3
8	198	+2316/ -2389	12.7	82	524	773	60 ± 4
9	183	+2175/ -2066	18.8	39	513	699	62 ± 4
10	179	+2587/ -2769	15.6	60	539	674	61 ± 3

3.2.2 Protocols

Each cyclist underwent resting echocardiographic examination in the left lateral decubitus position. A single sonographer was employed for both imaging and assessment to eliminate inter-observer reliability concerns (Kuecherer *et al*., 1991). All measurements were performed using a commercially available ultrasound system (MyLab CV 30, Esaote, Italy), using a 3.5-MHz transducer. Simultaneous recordings to assess HR were conducted via an integrated ECG. An apical four-chamber view allowed the digital recording of 3-5 complete cardiac cycles from which LVEDV (a measure of preload), LVESV, SV and EF (a measure of LV contractility) were calculated via the area-length method as per the American Society of Echocardiography guidelines (Lang *et al.*, 2015). Variations in the acquisition of LV volumes may result in a technical variability of up to 7% in EF. In addition, participant variability (i.e. autonomic tone, body position) of up to 8% in healthy populations, may result in a total variability in EF typically of up to 13% (Kuecherer *et al*., 1991). Therefore, minimal clinical significant difference may be observed with changes up to 13% and will be considered alongside the clinical cut-off criteria. Diastolic filling was examined using pulsed-wave Doppler interrogation of mitral valve inflow velocities from a LV apical 4 chamber view. Peak E and A velocities were measured, allowing the calculation of E:A. Variability in the measurement of E and A may result in a consequent technical variability up to 0.45, with participant variability contributing to a 0.28 variation (Kuecherer *et al*., 1991). Overall, a typical variation of up to 0.9 in E:A ratio will therefore be considered as clinically insignificant. In addition, tissue-Doppler imaging of the septal wall at the mitral annulus allowed the recording of peak S', E' and A' myocardial tissue velocities. This allowed the calculation of the E':A' ratio and the E:E' ratio which is an index of left atrial pressure (Naugueh *et al.*, 1998). The SBP: LVESV ratio was calculated as a preload-independent measure of LV contractility (Sunagawa *et al*., 1983, 1985).

Whole blood samples were collected by venepuncture without venostasis from an antecubital vein using a 21G butterfly syringe into one plain vacutainer tube (8.5 ml, Becton Dickinson, Oxford, UK). The samples were left to clot prior to centrifuging at 1500 *g* for 10 minutes at 4˚C. Serum was aliquoted into eppendorfs and stored immediately (-20°C) for subsequent analysis of cTnI and NT-proBNP. cTnI was analysed using a cTnI-Ultra ADVIA Centaur CP System assay (Siemens Medical Solutions Diagnostics, Surrey, UK), which is a three-site sandwich immunoassay using direct chemiluminometric technology. The assay range was 0.006-50 $\mu g.L^{-1}$, whilst the coefficient of variation was 20% (functional sensitivity) at 0.017 $\mu g.L^{-1}$ and 10% at 0.03 $\mu g.L^{-1}$. Sample values which did not reach detectable levels for the assay were presented as zero. The upper reference limit (99^{th} percentile) of an apparently healthy population is 0.04 $\mu g.L^{-1}$ (Uettwiller-Geiger *et al.*, 2002). NT-proBNP was analysed using an IMMULITE 2500 NT-proBNP assay (Siemens Medical Solutions Diagnostics, Surrey, UK), which is a solid-phase, two-site chemiluminescent immunometric assay. The assay range was 20-35000 $ng.L^{-1}$, with an analytical sensitivity of 10 $ng.L^{-1}$. The upper reference limit of an apparently healthy population is 125 $ng.L^{-1}$ (Silver *et al.*, 2004).

BM was measured using calibrated digital floor scales on a hard surface to the nearest 0.1 kg (model 875, Seca, Hamburg, Germany). SBP (a measure of afterload) and DBP was measured after 5 minutes rest in a supine position using a Dinamap automated BP monitor (Critikon Corporation, Tampa, USA). Stage/exercise duration, stage distance and ascent/decent and exercising HR were recorded and downloaded daily using a GPS-enabled HR monitor (Garmin Forerunner 305, Garmin Ltd, USA). Analysis of HR data against GPS data was conducted to determine an average daily exercising HR for each cyclist. Maximal HR (HRmax) was determined using the age-predicted equation (HRmax

= 220-age). Each cyclist's daily average HR was then converted to a percentage of HRmax (% HRmax).

3.2.3 Data Analysis

Data are presented descriptively in each cyclist for all variables due to the study sample size. Where possible, comparisons to normal reference values have been made for echocardiographic data, according to Flachskampf *et al.* (2015), Lang *et al.* (2015), Nagueh *et al*. (2016) and Schneiderman *et al.* (2013). Echocardiographic data is expressed as raw data and a percentage change (Equation 3.1). Whilst cardiac biomarkers have been compared to the upper reference limits of an apparently healthy population.

Equation 3.1 Percentage change.

$$\text{Percentage Change} = \frac{\text{Stage Value} - \text{Baseline}}{\text{Baseline}} \times 100$$

3.3 Results

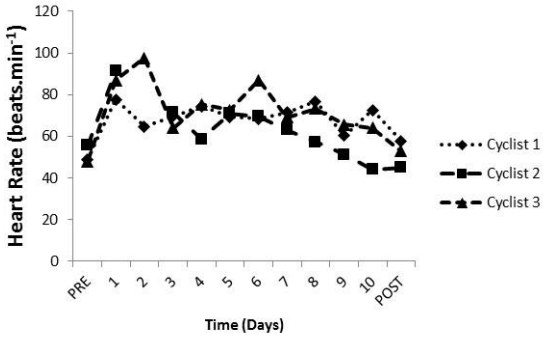
All cyclists completed the 10 day challenge in a total duration of 6468 minutes, of which 4903 minutes was exercise time with the remaining time being brief breaks (e.g. for food; Table 3.1). Daily exercise duration and distance covered was partially mediated by the terrain (Table 3.1). Mean % HRmax during cycling was higher (72%) at the beginning of the challenge with a small decline over the 10 day period. Average BM change ranged between -0.8 and +2.1% across days. All data collection procedures were completed with the exception of the echocardiogram (cyclist 2; day 2) and blood sample (cyclist 2; day 8 and cyclist 3; day 2) due to technical issues.

3.3.1 Echocardiographic Data

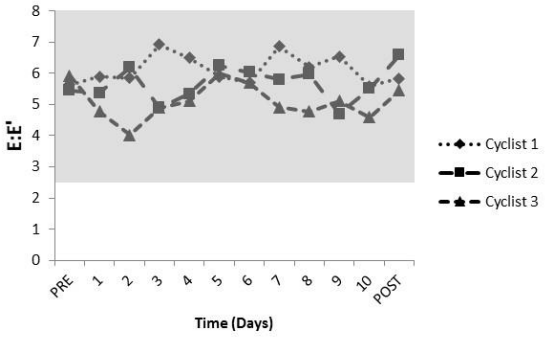
3.3.1.1 Loading

HR PRE-challenge ranged 48-56 beats.min^{-1} and was elevated in all cyclists for the majority of daily post-exercise measures (Figure 3.1a). BM change was also variable day-to-day and between cyclists, with no cyclists showing a percentage daily BM loss of > 1%. Values returned to near baseline measures POST-challenge. PRE-challenge, LVEDV was above normal reference values in all cyclists (Figure 3.1b), with a marked decrease following day 1 in all cyclists (13-18% change). Cyclists 1 and 2 demonstrated a sustained decrease in LVEDV across the challenge, which did not return to baseline levels POST-challenge, whilst LVEDV returned to a PRE-challenge value similar across the remaining days in cyclist 3. Data for E:E', were variable day-to-day and cyclist to cyclist (Figure 3.1c), remaining within normal reference values across the entire challenge. As a general trend SBP and DBP were reduced at the end of each day compared to PRE-challenge data although this was again variable within and between cyclists (Figure 3.2).

a)



c)



b)

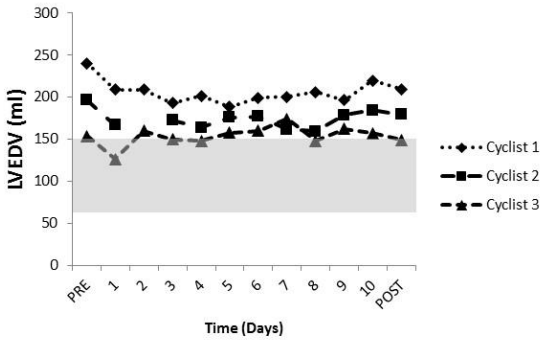


Figure 3.1 Individual data for LV loading over the duration of the cycle challenge where a) post exercise HR, b) LVEDV and c) E:E'. The shaded area represents the normal reference values of an apparently healthy population (Flachskampf *et al.* 2015, Lang *et al.* 2015).

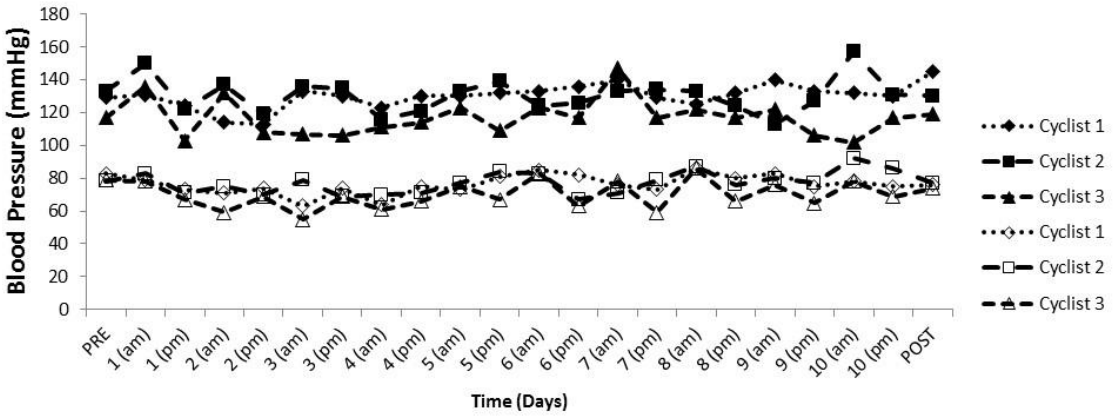


Figure 3.2 Individual data for SBP (filled markers) and DBP (empty markers) over the duration of the cycle challenge. Daily measures denoted by pre exercise (am) and post exercise (pm).

3.3.1.2 LV Systolic Function

EF was within normal reference values in all cyclists PRE-challenge. As a consequence of a drop in LVEDV, and a smaller drop in LVESV, both EF and SV were decreased for the majority of cycling days in cyclists 1 and 2 (+3 to -16 and -4 to -34% change in EF and SV respectively; Figures 3.3a and b). In cyclist 3 LVEDV was well maintained with a slow increase in LVESV across the challenge that resulted in a drop in EF and SV for the majority of stages (+3 to -19 and +6 to -29% change in EF and SV respectively; Figures 3.3a and b). Values for EF and SV remained below PRE-challenge for all cyclists 22 hours POST. The pressure/volume ratio was below the normal reference values in cyclists 1 and 2 PRE-challenge, and showed a varied response across cyclists during the challenge (Figure 3.3c). Cyclist 1 displayed a sustained increase (1-19% change) in SBP/LVESV with little variability across days. Whilst cyclist 2 demonstrated changes -3 to +18% and cyclist 3 decreases of up to 36%, both of which were highly variable. Values did not return to PRE-challenge levels by 22 hours POST. Data for S' was within the normal reference values in all cyclists PRE-challenge and remained within the normal

reference limits for all cyclists until the final day. An accumulated decrease in S' in cyclist 2 up until day 4 was evident (17% change), after which an increase in values were seen to be highly variable (Figure 3.3d). Substantial day-to-day variability was apparent in cyclists 1 and 3 (changes +4 to +35% and -7 to +29%, respectively), who's values remained elevated 22 hours POST.

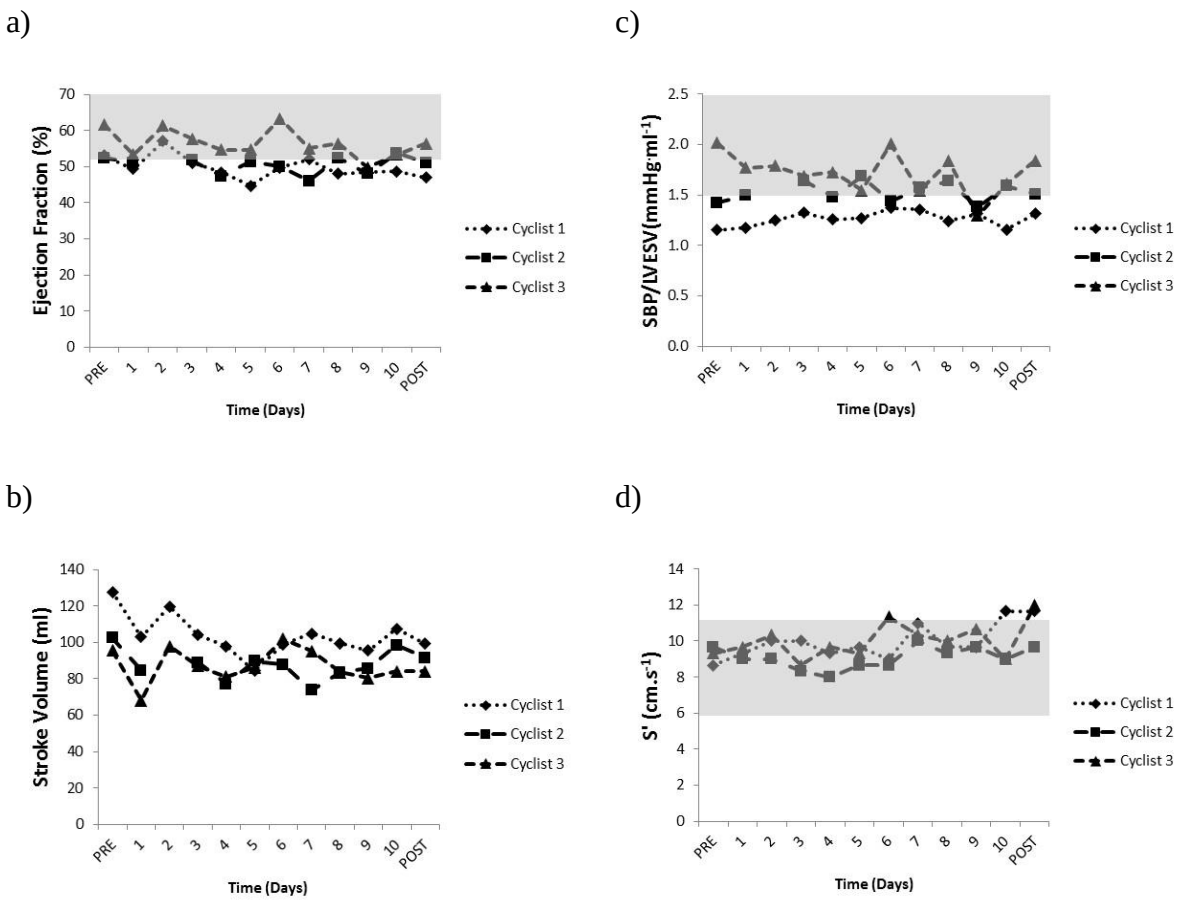


Figure 3.3 Individual data for LV systolic function over the duration of the cycle challenge where a) EF, b) SV, c) SBP/LVESV and d) S'. The shaded area represents the normal reference values of an apparently healthy population (Dalen *et al.* 2010, Lang *et al.* 2015, Schneiderman *et al.*, 2013).

3.3.1.3 LV Diastolic Function

Data for E and A was within normal reference values in all cyclists PRE-challenge (Figure 3.4a and b), whilst E:A marginally exceeded the upper reference limit in cyclist 3 (Figure 3.4c). E:A was consistently reduced in cyclists 1 and 3 across all cycle days and POST-challenge, with values ranging -11 to -47% change (Figure 3.4c), as a consequence of a tendency for E to drop and A to increase. Cyclist 2 presented with changes between -30 up to +53%, with values exceeding the reference values of an apparently healthy population on two occasions due to a maintained E and reduced A. All cyclists demonstrated similar PRE-challenge values for E', A' and E':A', all within normal reference values (Figures 3.4d, e and f). There was substantial within and between cyclist variations in E' (Figure 3.4d). Cyclists 1 and 3 demonstrated a comparable % change ranging -9 to +14. Cyclist 2 had a consistent reduction in E' until day 9 (10 – 26% change), at which point E' increased above PRE-challenge values. Data for A' was also variable within and between cyclists (Figure 3.4e) with a % change ranging -17 to +55. E':A' was consistently reduced in cyclists 1 and 2 with values ranging 2-48% change (Figure 3.4f) with cyclist 1 demonstrating a somewhat cumulative decrease in values up to day 6, after which values became more variable towards PRE-challenge values. Cyclist 3 presented changes between -18 to +28%, with values exceeding the upper reference limit of a healthy population on three occasions due to a maintained E' and reduced A'.

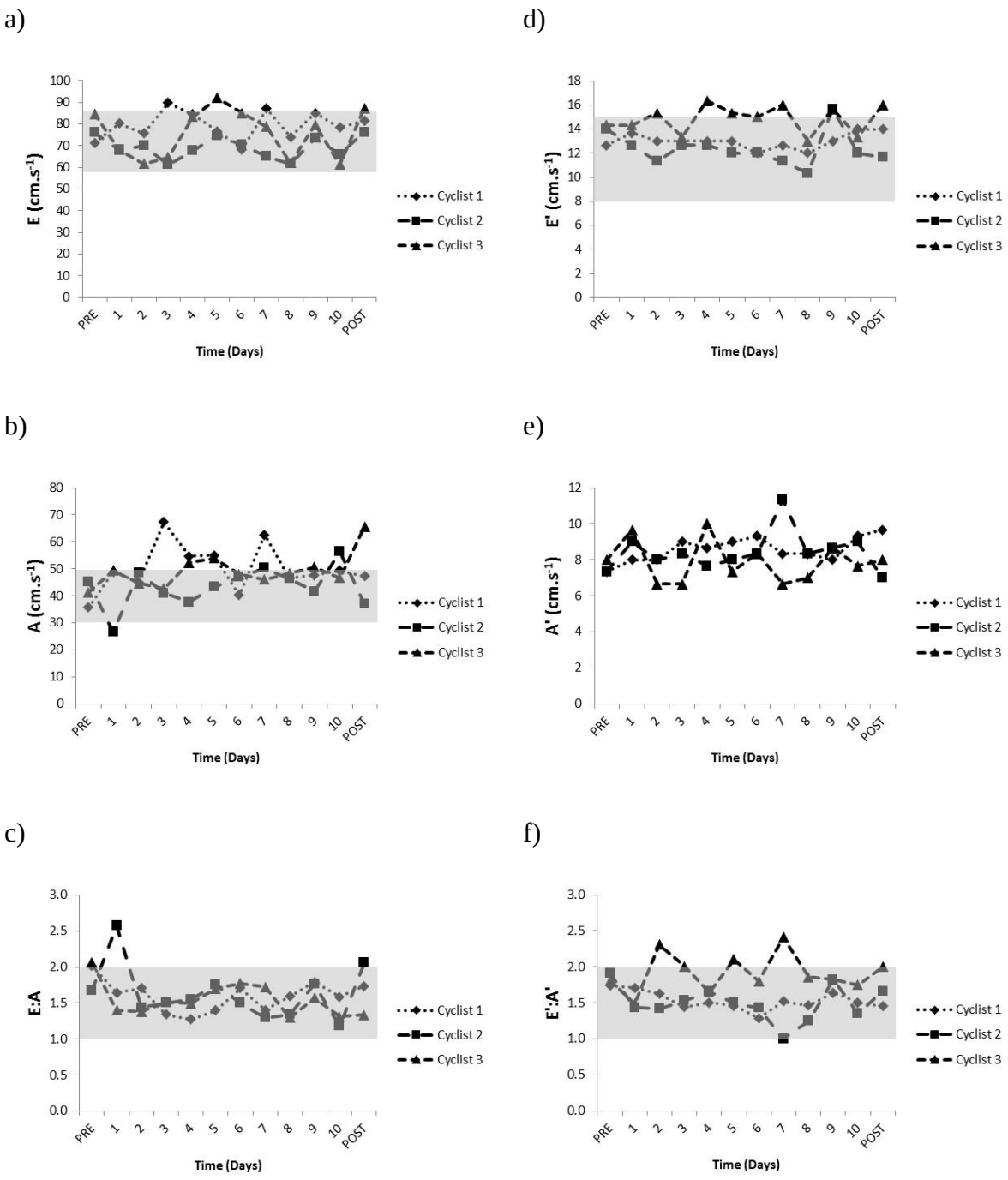


Figure 3.4 Individual data for LV diastolic function over the duration of the cycle challenge where a) E, b) A, c) E:A, d) E', e) A' and f) E':A'. The shaded area represents the normal reference values of an apparently healthy population (Lang *et al.* 2015, Nagueh *et al*. 2016).

3.3.2 Cardiac Biomarkers

No detectable cTnI was present in serum samples taken PRE-challenge, but cTnI was elevated above the detection limit in two cyclists following day 1 (Figure 3.5). Cyclist 1 displayed a double response with further detectable values on day 3 and a peak on day 4 (0.04 $\mu g.L^{-1}$). No detectable cTnI was reported on day 2 and day 6 to post, returning to undetectable values by day 6. Cyclist 2 had a peak cTnI on day 1 (0.027 $\mu g.L^{-1}$) which decreased to undetectable levels from day 3 onwards. Cyclist 3 had no detectable values across all time points.

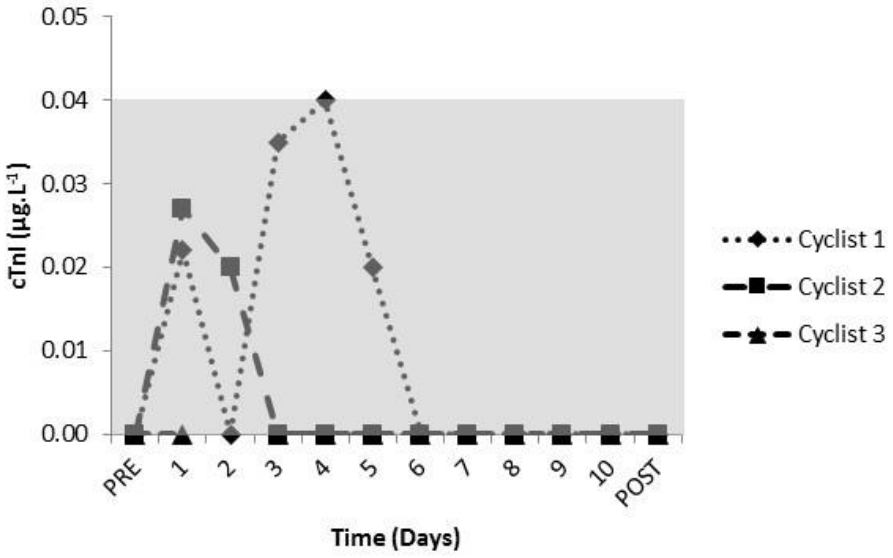


Figure 3.5 Individual data for cTnI over the duration of the cycle challenge (zero values denote that sample values did not reach detectable levels for the assay). The shaded area represents the 99th percentile of an apparently healthy population (≤ 0.04 $\mu g.L^{-1}$; Uettwiller-Geiger *et al*., 2002).

Data for NT-proBNP were below the upper reference limit for a healthy population for all cyclists PRE-challenge. An increase in NT-proBNP to a peak value, above the upper reference limit over the first few days was followed by a decline over the course of the challenge in all cyclists (Figure 3.6). Some between and within cyclist variation was

present, with peak values occurring on different days. All values returned to near baseline levels, and below the upper reference limit 22 hours POST-challenge.

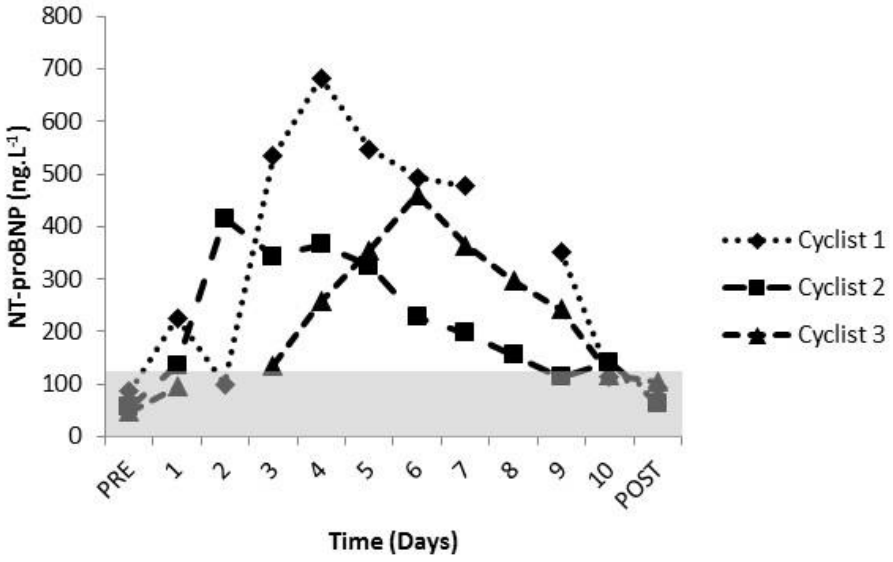


Figure 3.6 Individual data for NT-proBNP over the duration of the cycle challenge. The shaded area represents the upper reference limit of an apparently healthy population (125 ng.L-1; Silver *et al*., 2004).

3.4 Discussion

The results of this study extend the understanding of the consequences of prolonged and cumulative exercise by presenting the effects of 10 days cycling, in less trained individuals, on cardiac function and cardiac biomarker release. Our findings indicate that LV systolic and diastolic functions were depressed in some cyclists across the cycle challenge, although this was highly variable both between and within cyclists, with a general lack of a cumulative effect. Some aspects of systolic and diastolic function had not returned to PRE-challenge levels after 22 hours of rest POST-challenge. Cardiac biomarkers were elevated above detection levels and in some cases above the upper reference limits, showing a marked heterogeneity between cyclists and cycle days. There

was no consistent evidence of cTnI accumulation for any cyclist, whilst NT-proBNP displayed evidence of an accumulation in the earlier days of the challenge.

The exercise duration and % HRmax represent a significant physiological stress placed upon the human heart. Despite this, all cyclists completed the challenge, and with a BM loss of less than 2% prior to each days exercise, it is suggested that cyclists remained relatively well hydrated throughout the challenge. The % HRmax during each stage is of a similar intensity observed in other multi-day cycling activities (Williams *et al.*, 2009). The slight drop in % HRmax during multi-day endurance activities has been reported by other authors (Lucas *et al.*, 2008; Williams *et al.*, 2009) and is suggestive of a likely cumulative musculoskeletal and/or central fatigue (Halson *et al.*, 2002).

3.4.1 LV Systolic Function

Changes in systolic function were apparent across cyclists, most notably by a reduction in EF. An absolute decrease in EF of up to 12% compared to PRE-challenge levels is marginally below what would be expected as a clinically significant change. The current study, alongside those by Shave *et al.* (2002b) and Williams *et al.* (2009), have demonstrated much larger changes in EF (9-12%), following less intense (55-72% HRmax) but longer duration exercise (> 7 hours) thus presenting a greater volume of stress on the heart. This may offer some support that the duration-dependent threshold for single bouts of prolonged exercise (Shave *et al.*, 2004; Whyte *et al.*, 2000) may also exist for exercise induced changes in systolic function during multi-day exercise. Furthermore, a meta-analysis by Middleton *et al.* (2006a) suggested training status as an important variable related to changes in systolic function, which may also be reflected in the large change in EF in the current study. Although the findings focused on moderate duration exercise, a training-induced cardioprotective adaptation was suggested.

The lack of evidence for a cumulative decrease in systolic function of both global (EF) and segmental (S') measures is in agreement with Williams *et al.* (2009) and contrasts work by Middleton *et al.* (2007b). These differences are suggested to be attributed to several reasons, including: a) a recovery of LV systolic function in the short time period between consecutive days cycling; b) exercise duration and total cardiac work preclude an accumulation of cardiac fatigue; or, c) a combination of these factors (Williams *et al.*, 2009). Whilst the lack of accumulation suggests LV systolic function recovers, if only partially, very quickly between exercise bouts, we do not know the exact recovery time course of systolic function. In the present study, some aspects of systolic function were depressed 22 hours after exercise cessation, however, due to time constraints; it was not possible to determine when systolic function returned to PRE-challenge levels. The clinical significance of a reduction in systolic function is yet to be elucidated; however, it is important to note that the EF of all cyclists dropped below the lower limit for normal LV systolic function (52%; Lang *et al.,* 2015) on at least one occasion. Similar exercise HR's across each day, suggest alterations in EF did not seem to impact upon exercise performance, however, further investigation is warranted.

The causes of the reduction in EF, an often widely used measure of LV contractility (Konstam and Abboud, 2017), are not fully understood, however, individual responses displayed two clear patterns. A drop in preload (LVEDV) and small increase in HR resulted in an increase in contractility (SBP/LVESV) in an attempt to maintain EF and SV in cyclists 1 and 2. Changes in EF were not always mimicked by changes in S' (Dawson *et al.*, 2008; Neilan *et al*., 2006b), likely due to the localised nature of this measure. Despite loading values returning towards PRE-challenge values 22 hours post exercise, values for EF and SV remained below PRE-challenge for these cyclists. Therefore, changes in loading may not fully explain the changes observed in global

measures of systolic function. Cyclist 3 demonstrated a maintained LVEDV despite an elevated HR, with a slow increase in LVESV without any large changes in afterload (BP), suggest loading time and volume had minimal impact upon this cyclist. These changes are associated with quite sporadic changes in EF compared to baseline levels. A reduction in LV contractility (SBP/LVESV) may largely explain changes in EF, with some increases in S' coinciding with an increase in EF towards PRE-challenge values. Myocyte damage (Neilan *et al.,* 2006b) and β-andrenergic receptor downregulation (Hart *et al.,* 2006) have been previously proposed as intrinsic, non-load related mechanisms behind changes in systolic function. Within the present study, the cyclist-to-cyclist and day-to-day variability in biomarkers of cardiomyocyte damage (cTnI) and cardiac stress reduces the probability of a link between the two factors. An accumulation of circulating catecholamines during prolonged exercise (Seals *et al.,* 1988) may result in a downregulation of β-andrenergic receptors but we cannot confirm that in the present study.

3.4.2 LV Diastolic Function

Highly variable post-exercise changes in E, E', A and A' resulted in a consistent reduction in E:A and E':A' for all cyclists at the majority of time points. For the majority of parameters and cyclists, the change in LV diastolic function were similar to LV systolic function in that progressive changes were not apparent although in cyclist 1, E':A' displayed a somewhat cumulative decrease until day 6. Similar to systolic function, diastolic filling parameters can be affected by changes in preload and HR (Prasad *et al.,* 2007). Whilst some changes in HR and LVEDV were observed alongside minimal changes in BM, it is unlikely that alterations in preload and HR can explain all of the differences in diastolic parameters. Typically LV diastolic dysfunction displays three clear progressive patterns of abnormal LV filling; 1) impaired relaxation: whereby a

reduced early diastolic filling and increased late diastolic filling results in a reduced E:A; 2) pseudonormalization: an increased left atrial pressure augments the early diastolic filling phase; and 3) restrictive filling patterns: further increases in left atrial pressure restrict almost all LV filling to the early phase of diastole (Dokainish 2015; Nair *et al.,* 2000). Whilst cyclists 2 and 3 display changes that are likely to represent an intrinsic impairment in LV relaxation and/or compliance, cyclist 1 presented a somewhat different response. An increase in left atrial pressure (E:E'), coupled with an increased E and A, is suggestive of pseudo-normalization, a more progressive stage of diastolic dysfunction (Nair *et al.,* 2000). Middleton *et al.* (2007b) reported a return to normal diastolic function between bouts of running. It may, therefore, be suggested that the severity of diastolic dysfunction could well influence the recovery pattern and thus explain the cumulative nature of diastolic dysfunction in cyclist 3. In addition, both E:A and E':A' did not fully recover 22 hours after exercise cessation. Whilst E:A was reduced at the majority of time points across cyclists, to a greater degree of what would be considered technical or participant variability, it did not decrease below the clinical cut-off for diastolic dysfunction (less than 1.0; Lang *et al.,* 2015). Alterations in diastolic parameters did not seem to impact upon exercise performance, suggestive of limited clinical significance.

3.4.3 Cardiac Biomarkers

No detectable cTnI was present in serum samples taken PRE-challenge and is likely explained by the training status and smaller training load of the participants. Increases in cTnI displayed a clear heterogeneity in the individual response, given that cyclist 1 displayed a biphasic response, whilst cyclist 3 demonstrated no elevations across the challenge. It is unclear why cyclist 1 displayed the most detectable samples of cTnI across the challenge; however, this cyclist also demonstrated a clear diastolic dysfunction. The relatively consistent changes in systolic and diastolic function alongside the marked

individual variability in cTnI release that is seen in other multi-day studies (Middleton *et al.,* 2007b; Williams *et al.,* 2009) supports two separate phenomenon with potential different mechanisms. The mechanism(s) behind exercise-induced cTn release remain unclear, however, a release of unbound cTn from the cytosol during periods of membrane stress or inflammation may occur (Scherr *et al.,* 2011). Compared to rest, the increased contraction rate of the myocytes and afterload pressures would result in a greater myocardial wall stress and inflammation, provoking a release of cTn from the myocyte (Serrano Ostariz *et al., 2009*). Whilst not measured in this study, a positive relationship between pro-inflammatory cytokines and increases in cardiac biomarkers has been previously reported (La Gerche *et al*., 2015; Stewart *et al.,* 2016) highlighting the link between inflammation and cTn release post-exercise. The lower concentration of cTnI release within the present study is likely representative of the low exercise intensity (59-72% HRmax), which has previously been linked to lower concentrations of cTn release (Stewart *et al.,* 2016). All cTnI values remained below the upper reference limit of a healthy population, which combined with the absence of any clinical signs and symptoms of an adverse cardiovascular event, the elevations in cTnI are more likely to represent an acute physiologic perturbation, as opposed to underlying cardiac damage or pathology (Shave *et al.,* 2010).

In contrast to cTnI, NT-proBNP demonstrated a progressive increase during each successive exercise bout at the start of the challenge, followed by a decline over the remaining days of the challenge in all cyclists. Cyclist to cyclist variation was present, with peak values occurring on different days. NT-proBNP levels above the 125 $ng.L^{-1}$ upper reference limit of an apparently healthy population were apparent, which in a clinical setting would be representative of LV dysfunction (Shave *et al*., 2007). The changes in the current study more likely represent an increased cardiovascular work,

supported by the fact NT-proBNP concentrations were reduced below the clinical cut-off value following 22 hours of recovery, a finding supported by other multi-day studies (Williams *et al.*, 2009).

3.4.4 Limitations

Some research design and technical limitations in the present study are acknowledged. Firstly, it was not possible to collect echocardiograms and venous blood samples prior to each cycling day, nor were any samples taken following 30 minutes of exercise cessation each day. This was due to a reluctance of participants and time constraints. Whilst the limited blood samples may have missed transient elevations in biomarkers that could have been seen with more frequent sampling post-exercise, it is not believed this has impacted upon the main findings within the present data. It must also be acknowledged that cardiac biomarker concentrations were not corrected for plasma volume changes.

3.5 Conclusion

Ten days of repetitive prolonged cycling in less trained individuals produced evidence of LV systolic and diastolic dysfunction that could be mediated by changes in loading and/or intrinsic factors. There was limited support for a cumulative change in both systolic and diastolic function, however, LV function was still reduced 22 hours into recovery from the event. Any clinical and performance implications related to these changes are, at present, unclear.

A variable release in cTnI and NT-proBNP was observed between individuals and across days. The rapid clearance in these biomarkers after recovery suggest limited clinical relevance in this data. Whilst the present findings add to the limited number of studies focusing upon the cardiovascular consequences of multi-day exercise, the exercise factors

that may contribute to these phenomenon are less clear. Environmental conditions varied across the 10 days and limited attention has been given to factors such as temperature or thermal load on the athletes, which may augment the cardiovascular load and stress response to exercise. This in turn may mediate the impact of prolonged exercise upon cardiac function and biomarker appearance. Therefore, the next Chapter will investigate the effect of single and multi-day prolonged running upon cardiac function and markers of cardiac damage when performed in a high temperature environment.

CHAPTER 4

Alterations in left ventricular function and cardiac biomarkers as a consequence of single and repetitive endurance running in the heat

This data was presented as abstracts at the following conferences:

Hankey, J., Gill, S., Costa, R. J. S., Gregson, W., Whyte, G. and George, K. (2014) The impact of a 37 km foot race in hot ambient conditions upon cardiac function. European College of Sports Science; July, Amsterdam.

Hankey, J., Gill, S., Costa, R. J. S., Gregson, W., Whyte, G. and George, K. (2013) The impact of a 230 km multi-stage ultra-marathon in hot ambient conditions upon cardiac function. British Association of Sport and Exercise Sciences Annual Conference; September, University of Central Lancashire, Preston.

4.1 Introduction

An increasing amount of evidence suggests that single or multi-day bouts of prolonged exercise results in alterations in LV systolic and diastolic function (Chapter 3; Middleton *et al*., 2006a; Oxborough *et al*., 2010), as well as the appearance of cardiac biomarkers during recovery (Chapter 3; Shave *et al*., 2007a; 2010). In the previous Chapter, the impact of 10 consecutive days cycling in recreational cyclists was studied. The results demonstrated alterations in LV function and evidence of biomarker release, with limited evidence for a cumulative effect in post exercise measures. Whilst we have added to the limited number of multi-day exercise studies, data interpretation was confounded due to data collection points being restricted to daily post-exercise measures, providing a partial rationale behind the research design for the subsequent study. Inter and intra-individual variability was apparent, highlighting the need for further research to describe and explain personal or exercise factors that may contribute to these phenomenon.

Others have highlighted that environmental factors associated with any exercise bout may mediate the cardiovascular response to exercise (Dawson *et al*., 2003) and this could, in theory, be extended to cardiac function during recovery and the appearance of biomarkers. When exercise is performed under heat stress, there are competing demands between blood flow to the skin to aid temperature regulation and blood flow to the active muscle to meet the metabolic demands of muscular activity (González-Alonso *et al.*, 2008). This will increase the overall demand on the cardiovascular system during exercise and may mediate greater changes in LV function or preclude an accumulation of cardiac fatigue/markers of cardiac damage, particularly with a short time period between consecutive days exercise. Limited attention has been given to single or multi-stage bouts of prolonged exercise in a hyperthermic environment (Chapter 2) and will be the focus of this Chapter. Observing the progression of changes in cardiac function and markers of cardiac damage pre and post exercise each day of a multi-stage running event will significantly add to our knowledge of the cardiovascular consequences of prolonged exercise in the heat.

The aims of this study were to assess; (1) whether a single bout of prolonged strenuous running in a hot ambient environment resulted in a reduction in cardiac function and increased levels of biomarkers of cardiac damage, (2) whether five consecutive days of prolonged strenuous running in a hot ambient environment resulted in cumulative decrements in cardiac function and increments in levels of biomarkers of cardiac damage, and finally (3) what degree of day-to-day recovery in cardiac function and biomarkers will occur. It was hypothesised that changes in cardiac biomarkers (cTnI and NT-proBNP) and function (EF and E:A) would be apparent after a single bout of exercise in the heat and that this phenomenon would present with evidence of accumulation of

changes with multi-day exercise in the heat, largely due to insufficient recovery between days.

4.2 Method

4.2.1 Participants and Study Design

Thirty trained long distance runners (male n = 20, female n = 10) volunteered to take part in the study. All participants were healthy and free of known or early family history of cardiovascular disease, injury or illness and were not taking medications. Ethical approval was obtained from the University Ethics Committee before data collection. Before the study commenced, all participants provided written informed consent. The study was conducted during the 2011 Al Andalus Ultra Trail Race, during the second week in July, in the region of Loja, Spain. The multi-stage ultramarathon (MSUM) was conducted over 5 stages (5 consecutive days), totalling a distance of 230 km (Table 4.1). Running stages commenced at 09.00 h and consisted of a variety of terrains; predominantly off-road trails and paths, but also including steep and narrow mountain passes, and occasional road section (Table 4.1). Weekly training mileage prior to the MSUM was self-reported to range from 20 to 200 km per week. The MSUM runners were "semi" self-sufficient, whereby participants planned and provided their own foods and beverages (except plain water). Participants were allowed to drink *ad libitum* during the run, with aid stations situated every 10 km along the route providing plain water, fruit (oranges and watermelon) and electrolyte supplementation.

To address the aims of the study, two distinct data collection phases are reported. Phase 1 examined cardiac function and markers of cardiac damage pre- and post-stage 1 of the MSUM. Phase 2 examined cardiac function and markers of cardiac damage, pre- and post each of the 5 stages of the MSUM.

Table 4.1 Details of each stages distance, altitude of ascent-descent range, maximum temperature and maximum relative humidity.

Stage	Distance (km)	Altitude of ascent-descent range (m)	Ambient temperature (^{o}C)	Ambient relative humidity (%)	Predominant course terrain
1	37	503 to 1443	30 to 32	31 to 32	Off-road trails and paths, steep and narrow mountain passes.
2	45	830 to 1338	30 to 34	32 to 33	Off-road trails and paths, steep and narrow mountain passes, and occasional road.
3	40	689 to 1302	32 to 38	35 to 37	Off-road trails and occasional road.
4	65	671 to 1152	32 to 40	31 to 33	Off-road trails and road.
5	38	473 to 1065	37 to 40	37 to 40	Off-road trails and road.

Phase 1

Thirty trained long distance runners (male n = 20, female n = 10) were assessed (mean ± SD; age 41 ± 9 years, height 1.74 ± 0.08 m, BM 71.4 ± 9.6 kg). This phase employed a repeated measures design, with testing procedures taking place immediately prior to stage 1 (PRE) and within 30 minutes of finishing stage 1 (POST). Identical echocardiographic examinations followed by venous blood-sampling procedures were conducted at each assessment point, along with BM. Due to time constraints, 15 participants completed PRE and POST-exercise echocardiographic examination, whilst blood-sampling procedures were carried out in 24 participants.

Phase 2

Fourteen trained long distance runners (male n = 9, female n = 5, mean ± SD; age 42 ± 9 years, height 1.75 ± 0.06 m, BM 70.8 ± 8.3 kg) were assessed in this phase which employed a repeated measures design, with testing procedures taking place immediately prior to (PRE) and immediately post (POST) each stage of the MSUM. Identical echocardiographic and venous blood-sampling procedures were conducted at each assessment point, along with BM. Due to time logistical constraints, 9 participants completed echocardiographic examinations, whilst blood-sampling procedures were carried out in 9 participants.

4.2.2 Protocols

Participants underwent a resting echocardiographic examination, as described in Section 3.2.2, with corresponding measurement variability and critical cut-off limits.

Whole blood samples were collected by venepuncture without venostasis from an antecubital vein using a 21G butterfly syringe into one K_3EDTA vacutainer tube (6 ml, 1.6 mg·ml^{-1}of ethylenediaminetetraacetic acid; Becton Dickinson, Oxford, UK), and one plain vacutainer tube (4 ml, Becton Dickinson, Oxford, UK). Plasma volume (PV) changes were estimated from changes in haemoglobin and haematocrit (Dill & Costill, 1974; Maughan *et al.*, 2001) content of K_3EDTA whole blood samples (100 µl) immediately after sample collection, by coulter counter method (Coulter Ac T diff, Beckman Coulter, Carnaxide, Portugal) as previously reported (Garrett *et al.*, 2009, Garrett *et al.*, 2011, Nielsen *et al.*, 1993; Costa *et al.*, 2012). Whole blood samples collected into plain vacutainer tubes were left to clot prior to centrifuging at 1500 *g* for 10 minutes at 4˚C. Serum was aliquoted into eppendorfs and stored frozen, initially at -20°C during the MSUM competition before being transferred to -80°C storage after

completion of the experimental procedure. Subsequent analysis of cTnI and NT-proBNP was completed (See Section 3.2.1). Results of cTnI and NT-proBNP were corrected for percentage change in PV.

BM was measured using calibrated digital floor scales (BF510, Omron Healthcare, Ukyo-ku, Kyoto, Japan) on a hard surface. For practical reasons, throughout the entire study, participants were weighed with their race clothing and shoes on. Height was recorded PRE-stage 1 only by a wall mounted stadiometer. Stage duration was recorded daily by the race organisation team.

4.2.3 Data Analysis

Descriptive data are presented as mean ± SD and were supported by assessing the mean difference (mean diff) and 95% confidence interval [95% CI]. The Shapiro-Wilk test was applied to all data in order to assess for normal distribution. All statistical analyses were conducted using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA). An alpha level of $p < 0.05$ was considered statistically significant. Furthermore, effect size using Cohen's *d*: defined as trivial, small (> 0.2), moderate (> 0.5) and large (> 0.8; Cohen, 1992) and partial eta squared (η_p^2): defined as small (> 0.01), moderate (> 0.06) and large (> 0.14), were calculated. Cardiac biomarkers were also compared to the upper reference limits of an apparently healthy population. cTnI were analysed descriptively because of the likelihood of undetectable PRE-exercise values.

Phase 1

All PRE-POST exercise variables, excluding cTnI, were analysed using Student's paired *t*-tests. The delta change in cardiac biomarkers were correlated to all variables.

Phase 2

All PRE-stage and POST-stage exercise variables, excluding cTnI, were analysed using a Two-way (Time (PRE-POST) x Stage) repeated-measures ANOVA. Sphericity was analysed by Mauchly's test of sphericity followed by Greenhouse-Geisser adjustment where appropriate. Where any differences were identified, pairwise post hoc comparisons with a Bonferroni correction were used in order to show where they lay. The change in PRE to POST PV was analysed using a One-way repeated-measures ANOVA. Due to only 4 participants completing both echocardiographic and blood sample evaluation, markers of cardiac damage were not correlated with cardiac function.

4.3 Results

4.3.1 Phase 1

All 30 participants completed the stage (completion time 292 ± 39 min, average running speed 7.54 ± 1.12 $km.hr^{-1}$). BM was significantly reduced (mean diff = -1.9; $t_{(14)} = 8.148$, $p < 0.001$; 95% CI -2.4, -1.5]; $d = 0.28$) from 72.0 ± 9.6 kg to 70.1 ± 9.1 kg POST-exercise, a 2.6 ± 1.7% change. A variable reduction in PV of -4 ± 11% [95% CI -26, 19] was also observed.

4.3.1.1 Echocardiographic Data

4.3.1.1.1 Loading

LVEDV was unchanged POST exercise, whilst HR demonstrated a large increase (mean diff = 23; $t_{(14)} = -7.357$, $p < 0.001$; 95% CI [16, 30]; $d = 2.70$) in all participants (Table 4.2). BP was reduced in all participants from PRE to POST exercise for both SBP (mean diff = -26; $t_{(14)} = 6.684$, $p < 0.001$; 95% CI [-34, -18]; $d = 2.71$) and DBP (mean diff = -9; $t_{(14)} = 5.771$, $p < 0.001$; 95% CI [-13, -6]; $d = 1.99$; Table 4.2). The E:E' ratio was reduced post-exercise (mean diff = -1; $t_{(14)} = 2.443$, $p = 0.028$; 95% CI [0, 2]; $d = 0.87$), however, there was some variation in the individual responses (Table 4.2).

Table 4.2 LV loading before (PRE) and after (POST) exercise (n = 15, mean ± SD).

	PRE	POST	p-value
LVEDV (ml)	149 ± 23	139 ± 21	0.100
HR (beats.min^{-1})	60 ± 11	83 ± 12	< 0.001
SBP (mmHg)	141 ± 13	115 ± 12	< 0.001
DBP (mmHg)	81 ± 7	71 ± 6	< 0.001
E/E'	7 ± 2	6 ± 1	0.028

LVEDV: left ventricular end diastolic volume, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, E/E': peak early trans-mitral flow/peak early myocardial tissue velocity

4.3.1.1.2 LV Systolic Function

A large significant reduction in EF (mean diff = -9; $t_{(14)} = 4.617$, $p < 0.001$; 95% CI [-11, -4]; $d = 1.53$) and SV (mean diff = -17; $t_{(14)} = 5.360$, $p < 0.001$; 95% CI [-21, -9]; $d = 1.26$) was observed from PRE-POST exercise (Figure 4.1 a and b, respectively). SBP/LVESV was decreased to a large extent POST compared to PRE (mean diff = -0.63; $t_{(14)} = 4.641$, $p < 0.001$; 95% CI [-0.85, -0.31]; $d = 1.55$; Figure 4.1c), whilst S' was unchanged post-exercise (Figure 4.1d).

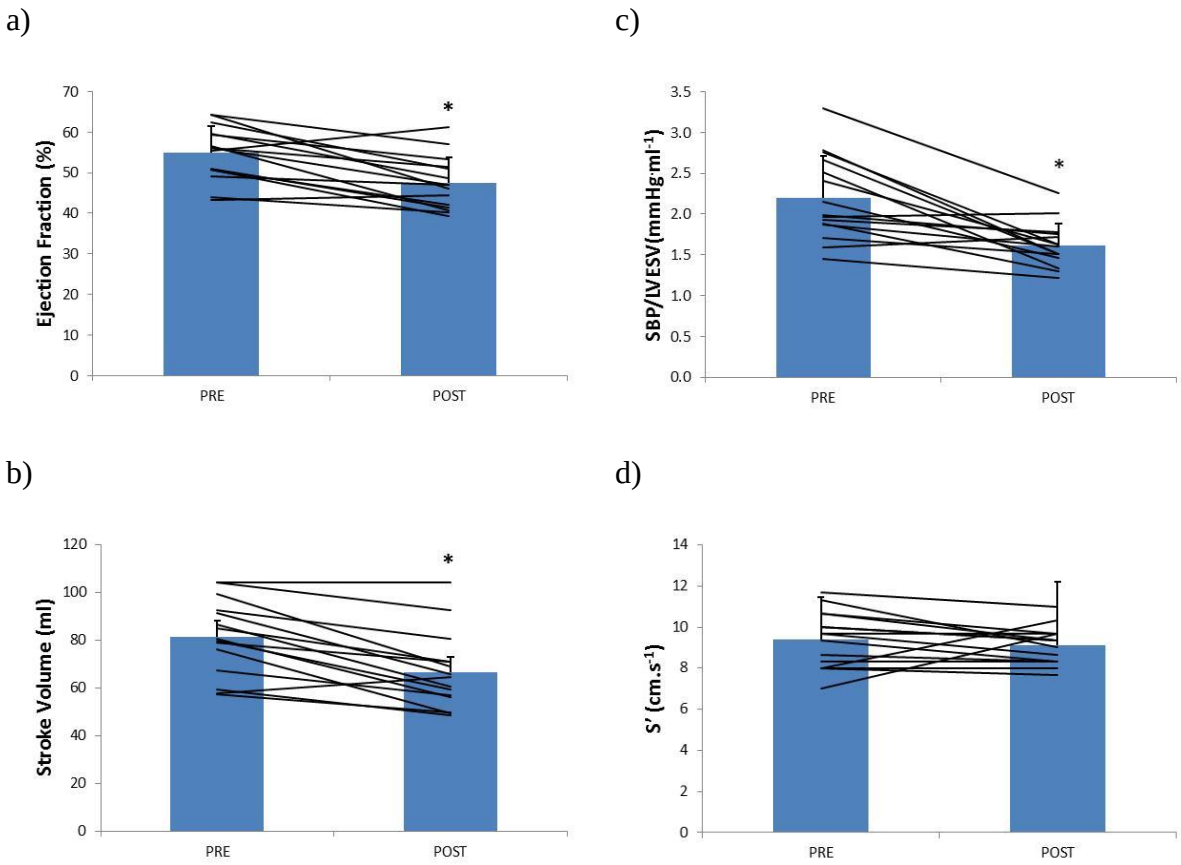


Figure 4.1 A comparison of LV systolic function showing PRE and POST-exercise values (individual and mean ± SD) where, a) EF, b) SV, c) SBP/LVESV and d) S'. Significant change from PRE to POST-exercise, * $p < 0.001$ (n = 15).

4.3.1.1.3 LV Diastolic Function

Peak E was largely reduced from PRE-POST exercise (mean diff = -20; $t_{(14)} = 11.759$, $p < 0.001$; 95% CI [-23, -16]; $d = 2.45$: Figure 4.2a). There was no change in peak A which resulted in a large drop in E:A (mean diff = -0.42; $t_{(14)} = 6.827$, $p < 0.001$; 95% CI [-0.55,-0.29]; $d = 1.84$; Figures 4.2b and c, respectively). Peak E' demonstrated a large reduction PRE-POST exercise (mean diff = 2; $t_{(14)} = 2.188$, $p = 0.046$; 95% CI [0, 3]; $d = 0.84$; Figure 4.2d). Peak A' was unchanged contributing to no change in E':A' (Figures 4.2e and f, respectively).

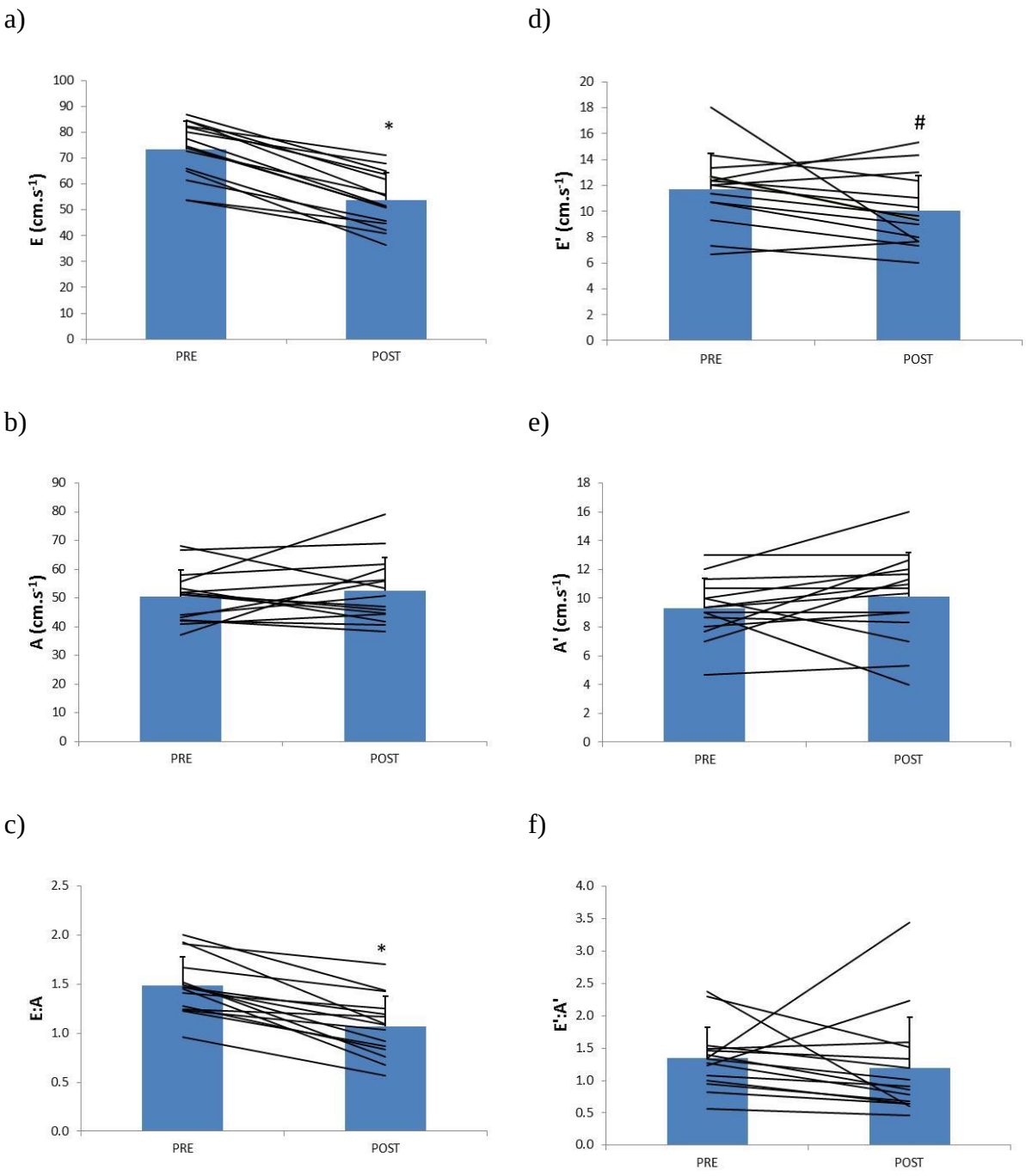


Figure 4.2 A comparison of LV diastolic function showing PRE and POST-exercise values (individual and mean ± SD) where, a) E, b) A, c) E:A, d) E', e) A' and f) E':A'. Significant increase from PRE to POST-exercise, * $p < 0.001$; # $p < 0.05$ ($n = 15$).

4.3.1.2 Cardiac Biomarkers

PRE-exercise, 10 participants demonstrated cTnI values above the detection limit, with 1 participant above the upper reference limit of a healthy population (Figure 4.4). Following exercise, 13 participants demonstrated an elevation in cTnI, 3 of which were above the upper reference limit. Data for NT-proBNP were above the upper reference limit for a healthy population in 19 participants PRE-exercise (Figure 4.5). Although moderately changed, there was no statistically significant change POST-exercise. 19 participants demonstrated an increase in NT-proBNP concentration. Two participants remained below the upper reference limit, whilst 3 participants showed a reduction in concentration below this threshold.

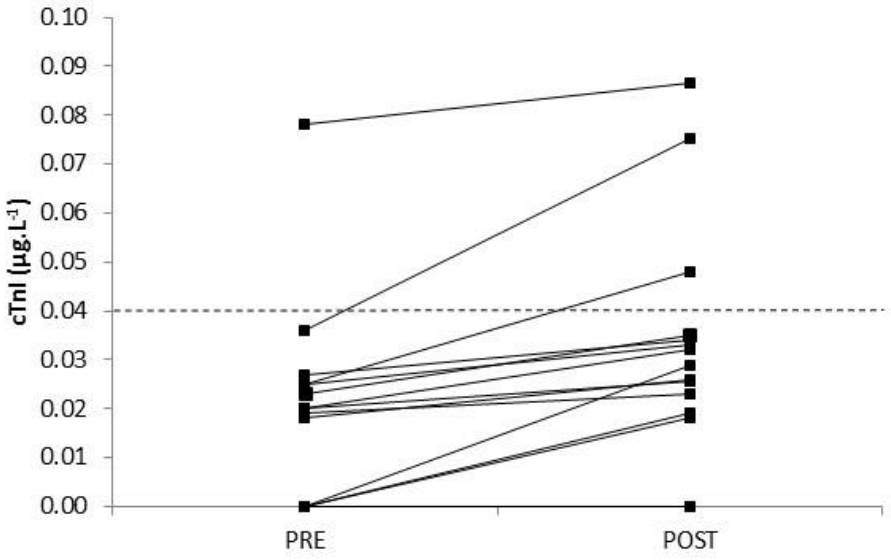


Figure 4.4 Individual participant data for cTnI PRE and POST exercise (zero values denote that sample values did not reach detectable levels for the assay). The dotted line represents the 99th percentile of an apparently healthy population (≤ 0.04 µg.L^{-1}; Uettwiller-Geiger *et al*., 2002; n = 24).

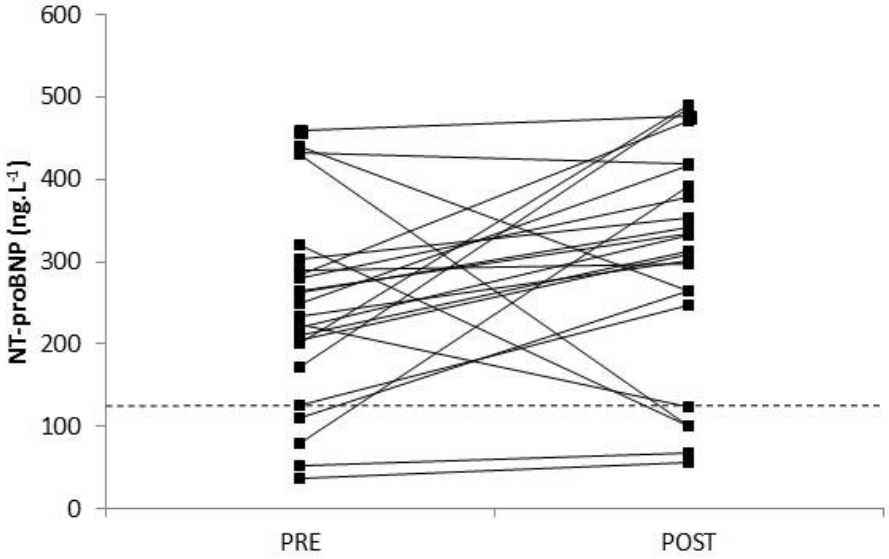


Figure 4.5 Individual participant data for NT-proBNP PRE and POST exercise. The dotted line represents the upper reference limit of an apparently healthy population (125 $ng.L^{-1}$; Silver *et al.*, 2004; n = 24).

4.3.2 Phase 2

All 14 participants completed the entire MSUM in a total time of 1672 ± 238 min and overall average running speed of 8.3 ± 1.2 $km.hr^{-1}$ (Table 4.4 for exercise duration and average running speed for each stage). BM was not changed across stages but demonstrated a large effect over time (mean diff = -1.5; $F_{(1,13)} = 27.960$, $p < 0.001$; 95% CI [-2.1, -0.9]; $\eta_p^2 = 0.68$), whereby BM decreased POST-exercise (Figure 4.6). % change in BM exceeded 2% at each stage (Table 4.4). Changes in PV PRE to POST were small with mean PV changes across stages ranging -6.2 ± 7.0% to 3.0 ± 6.2% (Table 4.4).

Table 4.4 Details of each stages exercise duration, average running speed, POST stage % BM loss and % PV change compared to PRE-stage one

Stage	Exercise Duration (mins)	Average running speed ($km.hr^{-1}$)	POST stage % BM loss compared to PRE-stage one	POST stage % PV change compared to PRE-stage one
1	267 ± 35	8.4 ± 1.1	2.6 ± 1.9	-4.9 ± 14.3
2	422 ± 71	6.6 ± 1.1	2.2 ± 4.8	-4.3 ± 6.9
3	291 ± 47	8.5 ± 1.4	2.9 ± 2.1	-1.7 ± 9.5
4	418 ± 61	9.5 ± 1.4	3.7 ± 2.0	3.0 ± 6.2
5	275 ± 37	8.4 ± 1.2	3.4 ± 2.0	-6.2 ± 7.0

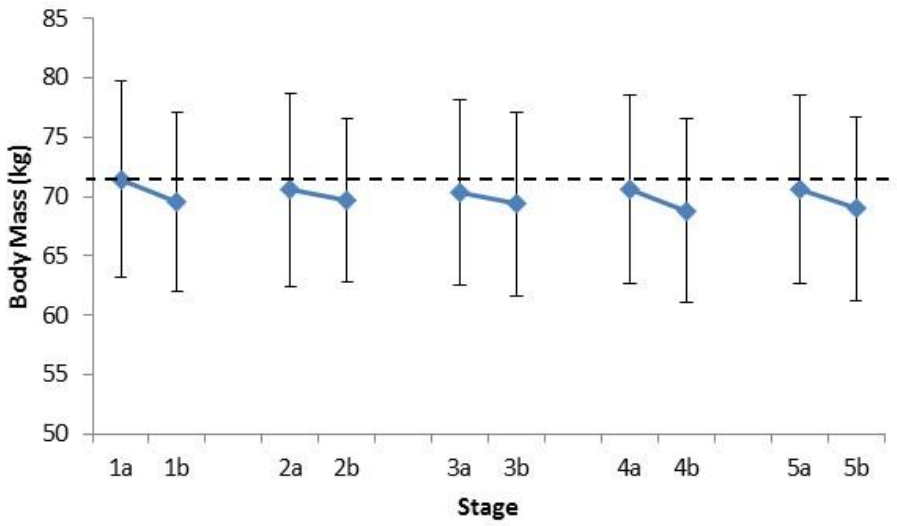


Figure 4.6 A comparison of measures of BM (kg) showing PRE and POST-exercise values on 5 consecutive stages (mean ± SD; The dotted line represents the mean BM PRE stage 1; n = 14).

4.3.2.1 Echocardiographic Data

4.3.2.1.1 Loading

LVEDV demonstrated a small insignificant interaction between stage and time, with no effect noted for time. A large significant effect was apparent across stages ($F_{(4,32)} = 4.564$,

p = 0.005; η_p^2 = 0.36), with post-hoc analysis revealing stage 3 to be lower than stage 5 (mean diff = -15; p = 0.005; 95% CI [-26, -5]; Table 4.5). HR showed an insignificant large interaction between condition and time, as well as in insignificant moderate effect between stages. However, HR was increased POST compared to PRE (mean diff = 19; $F_{(1,8)}$ = 83.654, p < 0.001; 95% CI [14, 23]; η_p^2 = 0.91; Table 4.5). A large significant interaction between stage and time was displayed for SBP ($F_{(4,32)}$ = 6.519, p = 0.001; η_p^2 = 0.45; Table 4.5), with significant reductions POST following stages 1 (mean diff = -27; p = 0.001; 95% CI [-40, -15]), 4 (mean diff = -18; p = 0.001; 95% CI [-27, -10]) and 5 (mean diff = -9; p = 0.001; 95% CI [-14, -5]). DBP demonstrated a large insignificant interaction between stage and time, whilst there was a large but insignificant effect for stage, a large significant decrease POST compared to PRE occurred (mean diff = -6; $F_{(1,8)}$ = 8.780, p = 0.018; 95% CI [-10, -1]; η_p^2 = 0.52; Table 4.5). Similarly, a moderate insignificant interaction was observed between stage and time for E:E', with an insignificant large effect across stages and a large significant reduction POST compared to PRE (mean diff = -1; $F_{(1,8)}$ = 22.775, p = 0.001; 95% CI [-1, 0]; η_p^2 = 0.74; Table 4.5).

Table 4.5 LV loading before (PRE) and after (POST) exercise for each stage of the MSUM (n = 9, mean ± SD).

	Stage 1		Stage 2		Stage 3		Stage 4		Stage 5	
Variable	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
LVEDV (ml)	144±25	137±23$	143±13	133±21	135±17*	130±12*	146±12	141±18$	152±14	144±20$
HR (beats.min^{-1})	58±8	83±13	67±14	89±17	70±24	82±8	73±31	87±31	63±6	82±10
SBP (mmHg)	141±14	114±13	125±14	119±12	127±11	124±12	137±13	119±10	128±11	119±9
DBP (mmHg)	79±7	70±4	75±9	71±5	73±7	72±7	82±7	73±5	75±8	70±6
E/E’	6±2	6±1	6±1	6±2	7±1	6±1	7±1	6±1	7±1	6±2

LVEDV: left ventricular end diastolic volume, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, E/E’: peak early trans-mitral flow/peak early myocardial tissue velocity. * Significantly different compared to stage 5, $p < 0.05$. $ Significantly different POST compared to PRE.

4.3.2.1.2 LV Systolic Function

A large significant interaction between stages and time was displayed for EF ($F_{(4,32)}$ = 2.688, p = 0.049; η_p^2 = 0.25), with a significant reduction POST stage 1 (mean diff = -10; p = 0.005; 95% CI [-16, -4] and a post-hoc power analysis of 0.7. A large effect was observed across stages ($F_{(4,32)}$ = 3.609, p = 0.015; η_p^2 = 0.31), with post-hoc analysis revealing stage 3 to be higher than stage 5 (p = 0.016; 95% CI [0.87, 8.94]). A large effect of time was also noted with a decrease POST compared to PRE ($F_{(1,8)}$ = 9.265, p = 0.016; 95% CI [1.36, 9.83]; η_p^2 = 0.54; Figure 4.7a). SV showed a large significant interaction between stage and time ($F_{(4,32)}$ = 5.601, p = 0.002; η_p^2 = 0.41; Figure 4.7b) with a significant reduction POST stage 1 (mean diff = -17; p = 0.002; 95% CI [-26, -8]), stage 2 (mean diff = -8; p = 0.019; 95% CI [-14, -2]), stage 3 (mean diff = -8; p = 0.017; 95% CI [-15, -2]) and stage 5 (mean diff = -20; p < 0.001; 95% CI [-28, -12]). An insignificant large interaction between stage and time was observed for SBP/LVESV. A large significant effect of stage was noted ($F_{(1.865,\ 14.916)}$ = 4.859, p = 0.026; η_p^2 = 0.38), with post-hoc analysis revealing stage 5 to be lower than stage 3 (mean diff = -0.38; p = 0.009; 95% CI [-0.67, -0.10]; Figure 4.7c). A large drop was noted POST-exercise (mean diff = -0.29; $F_{(1,8)}$ = 15.126, p = 0.005; 95% CI [-0.46, -0.12]; η_p^2 = 0.65). A large significant interaction between stages and time for S' ($F_{(4,32)}$ = 3.380, p = 0.020; η_p^2 = 0.30; Figure 4.7d) was noted with a significant reduction POST stage 2 (mean diff = -1; p = 0.025; 95% CI [0, 2].

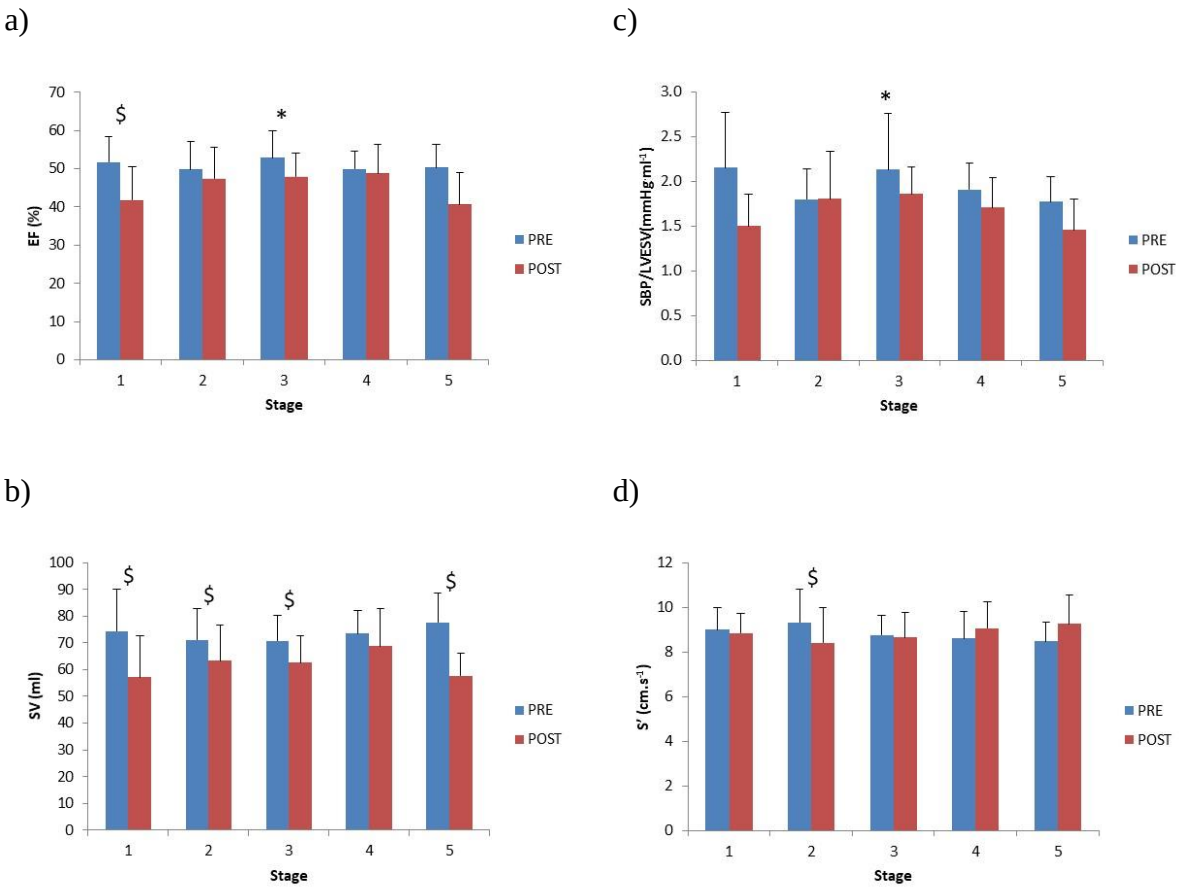


Figure 4.7 A comparison of LV systolic function showing PRE and POST-exercise values on 5 consecutive stages (mean ± SD) where, a) EF, b) SV, c) SBP/LVESV and d) S'. * Significantly different compared to day 5, $p < 0.05$. $ Significantly different POST compared to PRE. (n = 9).

4.3.2.1.3 LV Diastolic Function

E demonstrated an insignificant trivial interaction between stage and time. A large significant effect for stage was observed ($F_{(4,32)} = 5.328$, $p = 0.002$; $\eta_p^2 = 0.40$), with post-hoc analysis revealing stage 2 to be lower than stage 3 (mean diff = -8; $p = 0.025$; 95% CI [-14, -1] and stage 2 lower than stage 4 (mean diff = -11; $p = 0.05$; 95% CI [-23, 0]; Figure 4.8a). A significant large effect of time was also noted (mean diff = -18; $F_{(1,8)} = 104.46$, $p < 0.001$; 95% CI [-22, -14]; $\eta_p^2 = 0.93$), whereby E decreased POST-exercise.

An insignificant large interaction was between stage and time was found for A, which was also not altered by stage or time (Figure 4.8b). As a result, E:A showed an insignificant large interaction between stage and time. There was a large insignificant effect across stages but a large decline across time for E:A (mean diff = -0.50; $F_{(1,8)}$ = 25.412, p = 0.001; 95% CI [-0.73, -0.27]; η_p^2 = 0.76; Figure 4.8c), with a post-hoc power analysis of 0.7. E' demonstrated an insignificant small interaction between stage and time. No impact of stages was noted for E', although E' decreased to a large extent across time (mean diff = -2; $F_{(1,8)}$ = 17.157, p = 0.003; 95% CI [-3, -1]; η_p^2 = 0.68; Figure 4.8d). An insignificant moderate interaction between stage and time was apparent for A', with a moderate effect for stage but a large significant increase POST compared to PRE (mean diff = 1; $F_{(1,8)}$ = 9.380, p = 0.016; 95% CI [0, 3]; η_p^2 = 0.54; Figure 4.8e). As a result, E':A' showed an insignificant moderate interaction between stage and time, with an insignificant moderate effect for stage but a large decrease POST-exercise (mean diff = -0.36; $F_{(1,8)}$ = 12.106, p = 0.008; 95% CI [-0.60, -0.12]; η_p^2 = 0.60; Figure 4.8f).

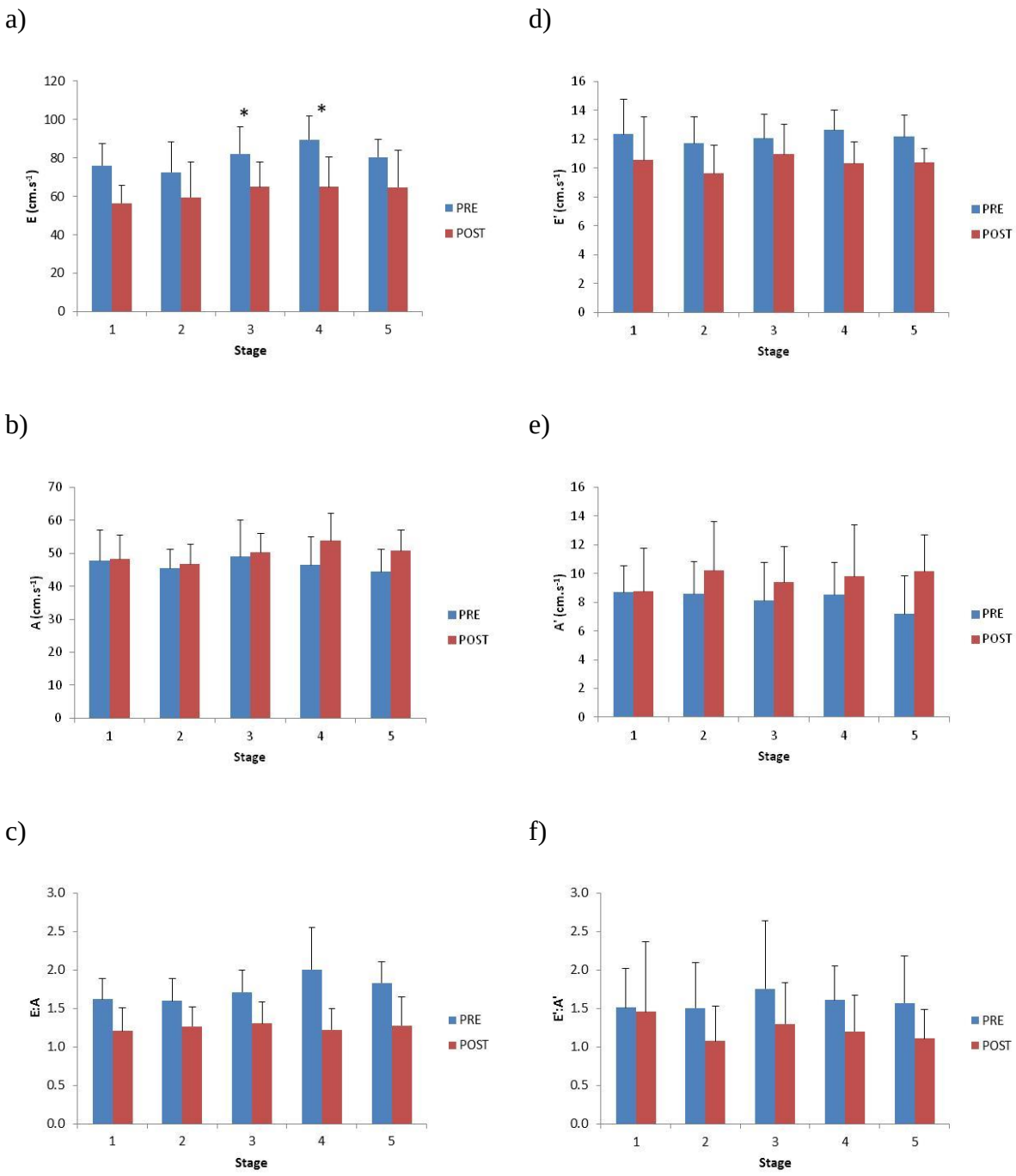


Figure 4.8 A comparison of LV diastolic function showing PRE and POST-exercise values on 5 consecutive stages (mean ± SD) where, a) E, b) A, c) E:A, d) E', e) A' and f) E':A'. * Significantly different compared to day 2, $p < 0.05$ (n = 9).

4.3.2.2 Cardiac Biomarkers

Two participants had detectable cTnI concentrations in serum samples taken PRE stage 1, however, both of these were below the upper reference limit at this time point (Figure 4.9). Three participants had no detectable concentrations across all time points, whilst two participants had detectable elevations POST stage 4, which also corresponds with the longest stage. Participant-to-participant variability was marked with peak values in participants R1, R3, R5, R6 and R9 all occurring at different time points. Data for NT-proBNP was elevated above the upper reference limit for a healthy population in 6 participants PRE stage 1. There was an insignificant moderate interaction between stage and time for NT-proBNP, with an insignificant large effect of stage and small effect of time (Figure 4.10).

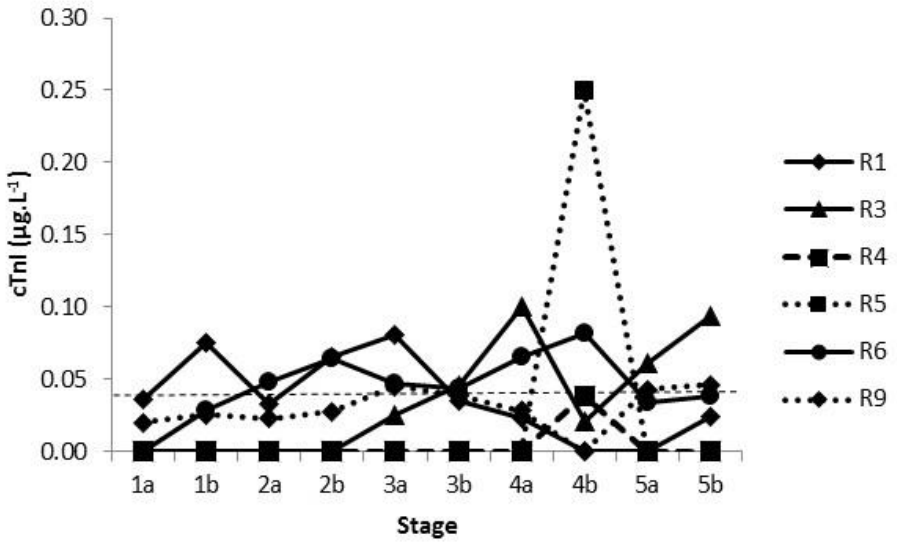


Figure 4.10 Individual participant data for cTnI on 5 consecutive stages (zero values denote that sample values did not reach detectable levels). Daily measures denoted by PRE (a) and POST-exercise (b). The dotted line represents the 99th percentile of an apparently healthy population (≤ 0.04 µg.L^{-1}; Uettwiller-Geiger *et al*., 2002; n = 9).

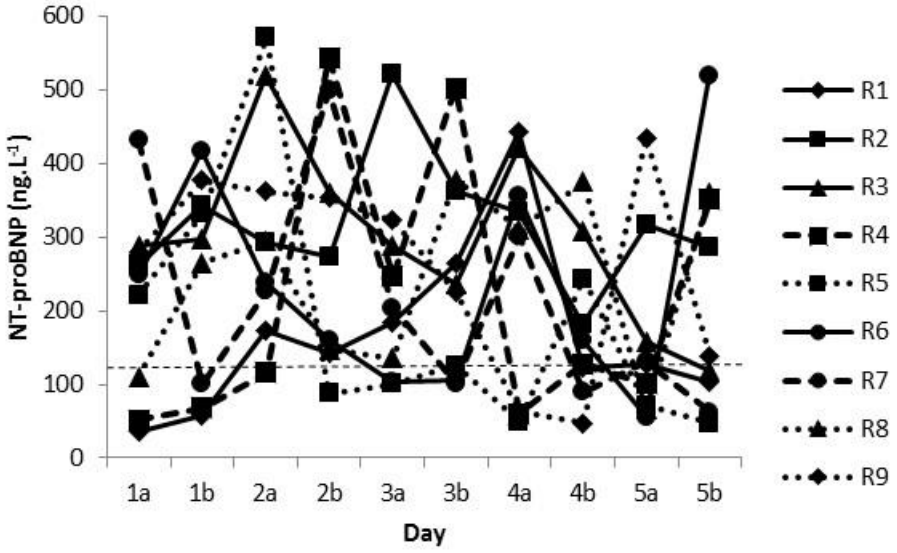


Figure 4.11 A comparison of NT-proBNP showing PRE (a) and POST-exercise (b) values on 5 consecutive stages. The dotted line represents the upper reference limit of an apparently healthy population (125 ng.L^{-1}; Silver *et al.*, 2004; n = 9).

4.4 Discussion

The present study adds to the limited research related to the impact of a single bout of exercise in a hot environment as well as being the first research to examine the effect of multiple prolonged bouts of exercise in a hot environment upon cardiac function and markers of cardiac damage. Phase 1 of the present study indicated evidence of both systolic and diastolic functional change after 37 km running in hot ambient conditions. We also observed detectable or elevated levels of cardiac biomarkers PRE and/or POST exercise in all runners. Both data sets present with notable heterogeneity between participants. The findings of phase 2 indicate that systolic and diastolic functions were depressed POST exercise at different points across the MSUM, with a general lack of a cumulative effect of repeated bouts. Cardiac biomarkers were elevated above detection levels and in some cases above the upper reference limit but with a heterogeneity between runners. Again there was no evidence of an accumulative effect of exercise upon these biomarkers.

4.4.1 Phase 1

The exercise duration, combined with the environmental stress, represents a significant challenge to the cardiovascular system. The decrease in BM was unrelated to all variables including the reduction in PV, with the change in BM likely due to metabolic factors such as substrate loss and muscle breakdown (Hoffman *et al*., 2018; Maughan *et al.,* 2007) rather than intravascular fluid loss alone.

To date this is the first study to report a statistical reduction in global measures of systolic function (EF and SV) following exercise of a near marathon distance. The maintenance of preload (LVEDV), combined with a reduced afterload (BP) suggests impairment of LV intrinsic contractility. This is also supported by a decrease in SBP/LVESV, commonly used as a load-independent marker of LV contractility. The changes in EF were not mimicked by a significant reduction in S', likely due to the localised nature of S'. It is likely that the combination of exercise and thermal stress has placed the heart under greater workload stress that may have mediated a decline in systolic function POST-exercise.

We can only speculate how changes POST-exercise reflect the cardiovascular hemodynamics, and potential for "cardiac fatigue", during exercise. During prolonged exercise, an enhanced cardiovascular drift (increased HR and reduced SV) has been consistently observed in hot environments compared to normothermic conditions (Rowell *et al.,* 1966; Lafrenz *et al.,* 2008), suggestive of a decrease in LV function when the need for thermoregulation is elevated. A tolerable sympathetic load/threshold has been suggested (Stewart *et al.,* 2016), which if exceeded for prolonged periods of time could induce a desensitization or down regulation of cardiac β-andrenergic receptors (Banks *et al.,* 2010; Oxborough *et al*., 2010) and autonomic responsiveness (Seiler *et al.,* 2007)

leading to a reduced force of contraction. Whilst the exercise distance of the current study is shorter than a marathon distance, the duration of the exercise was much greater, which has previously been implicated in the aetiology of systolic dysfunction (Middleton *et al*., 2006a; Oxborough *et al*., 2010). The added demands of the course alongside the increased environmental heat stress may also provide sufficient cardiac load to depress LV contractile function. Individual variation was also apparent with a maintenance or reduction in systolic function POST-exercise observed.

The changes in diastolic function observed are consistent with previous studies of single day exercise of a similar distance or duration (George *et al*., 2004, 2005; Middleton *et al*., 2006b; Mousavi *et al*., 2009; Oxborough *et al*., 2006; Whyte *et al*., 2005), but may in some part be impacted upon by the thermoregulatory effect on pre-load and HR, indicated by the reduction in left atrial driving pressure (E:E'). The decrease in early filling, with little or no compensation in late filling reflect an intrinsic alteration in LV pressure decay due to impaired relaxation/myocardial stiffening during diastole, compliance and atrial contractility (Middleton *et al*., 2006b).

PRE-exercise, cTnI and NT-proBNP were elevated in some runners above the upper reference limit of a healthy population, which may partially be explained by the nature of the runners in the current field-based study with a large training load prior to the event and no control over athlete behaviour. POST-exercise, 13 participants demonstrated an elevation in cTnI, 3 of which were above the upper reference limit. The concentrations of cTnI were lower than those reported in some previous studies (Fortescue *et al*., 2007; George *et al*., 2005; Whyte *et al*., 2005), likely due to the longer exercise duration, and therefore, probable lower intensity/HR (Eijsvogels *et al*., 2015), due to cTn being released from the cytosol during periods of membrane stress or inflammation (Scherr *et al.*, 2011).

POST-exercise, a highly variable change in NT-proBNP concentration was apparent. This may be due to the fact NT-proBNP is released in response to a hemodynamically stressed heart during prolonged exercise (Corsetti *et al*., 2012). It has been previously suggested the increase in NT-proBNP is associated with exercise duration (Serrano-Ostáriz *et al.*, 2011). Legaz-Arrese *et al.* (2011) further suggested the NT-proBNP release post-exercise is independent to exercise intensity once HR exceeds 100-120 beats.min^{-1}, after which exercise duration exerts a bigger influence on NT-proBNP. Therefore, the higher HR induced by exercising in a hot environment (Rowell *et al.*, 1966; Lafrenz *et al.*, 2008) within the current study may not explain the changes in NT-proBNP observed. Baseline values for NT-proBNP can be influenced by age, gender, malnutrition, obesity, HR, volume overload, BP (Chen *et al.*, 2008) and have been implicated in explaining the complex variations in exercise response (Legaz-Arrese *et al.*, 2011).

4.4.2 Phase 2

The multiple stage race in the heat with prolonged stage duration represents a significant cardiovascular challenge. All runners completed the MSUM, with BM losses of greater than 2% following each stage compared to PRE stage 1. An increase in PV across the MSUM compared to PRE stage 1 suggests runners remained relatively well hydrated, therefore, the change in BM again is likely indicative of metabolic factors (Hoffman *et* al., 2018; Maughan *et al.*, 2007) rather than fluid loss alone.

Global measures of systolic function (EF and SV) were reduced POST-exercise compared to PRE across some but not all stages, with no support for a cumulative effect as partial recovery between exercise bouts was apparent. A tendency for LVEDV to decrease by stage 3 then increase above PRE stage 1 values by the final stage of the MSUM is difficult to explain but previous authors have demonstrated PV expansion following 4 or more

days of multistage exercise (Fellmann *et al*., 1999; Wirnitzer and Faulhaber, 2007). Alternatively, transient chamber dilation as a result of excessive prolonged ventricular overload in order to maintain cardiac output has previously been observed following marathon running (Trivax *et al*., 2010). Within the current study, if the observed increase in LVEDV was due to transient chamber dilation, a reduction in EF, SV and SBP:LVESV would follow a similar pattern to LVEVD. PRE-exercise levels of EF and SBP:LVESV at stage 5 were similar to PRE stage 1. Therefore, suggesting any changes in LVEDV are the cause of changes in PV and did not limit changes in systolic function, which are due to a reduced LV contractility, with no systolic accumulative effect being observed. The changes in EF and SV were not mimicked by changes in S' data, a common finding due to its localised nature.

A depression in E:A and E':A' POST compared to PRE-exercise across all stages was apparent, with no strong evidence of a cumulative effect. The changes observed at each POST stage assessment were similar to those observed in *Phase 1*. The significant drop in early diastolic filling and the E:A ratio POST-exercise each day suggests an increase in LV pressure during diastole and/or a drop in left atrial pressure (Nishimura and Tajik, 1997). A lack of change in LVEDV PRE to POST-exercise along with increased LV pressure may result from a reduced compliance or impaired rate of LV relaxation (Scott and Warburton, 2008). In the earlier stages PRE-exercise, an increase in PV and E:E', and concomitant increase in E with a decrease in LVEDV, may actually represent an impaired relaxation/myocardial stiffening of the LV during diastole as opposed to an increase in function. The changes in diastolic filling were not reflected by any changes across stages in myocardial tissue velocities, which are known to be less load-dependant than filling parameters (Gianakki et al., 2008). As previously noted, a decline in β-andrenergic receptor sensitivity during prolonged exercise may exist (Banks *et al*., 2010;

Stewart *et al.*, 2016), which may impair the rate of LV relaxation. Whilst there is evidence for a decrease in LV diastolic function during the MSUM, there is little support for a progressive decline in function as the MSUM progressed.

Cardiac troponin I and NT-proBNP was elevated in some runners PRE stage 1, likely explained by the nature of the athletes in the current study with a large training load prior to the MSUM. Increases in cTnI across the stages were variable across runners and stages. In the present study, each stage varied in terrain, with some stages including steep and narrow mountain passes where running was not possible. Some runners also typically walk on uphill sections and run on downhill sections. Therefore, the walk/run nature of the event may have placed variable hemodynamic loading and stress on the cardiac muscle at irregular intervals, permitting periods of recovery in some runners (Denissen *et al*., 2012). As in-exercise HR data was not collected, this may only be speculated. This may also partially explain the varied response between runners, however, previous multi-stage event have also shown to evidence a heterogeneous response between participants (Chapter 3; Williams *et al*., 2011). Transient elevations in cTnI were evident with elevated samples after one stage followed by an undetectable level at the next sample point. Therefore, it is more likely that elevated cTnI levels are representative of a physiological rather than a pathological response (Eijsvogels *et al*., 2011, 2015). However, we cannot speculate as to which mechanisms may explain elevated cTn levels in the current study.

Variable NT-proBNP concentrations were observed whereby there were no significant changes PRE to POST-exercise or across stages. This may be explained by the high concentration within samples PRE stage 1, which may be a result of prior exercise or heat exposure. A high degree of inter- and intra-individual variation in NT-proBNP was

apparent, which are suggested to occur in response to an exercise-induced increase in end-diastolic ventricular pressure (Urhausen *et al.,* 2004). Previous research has suggested those participants who covered less training distance per week demonstrated a greater elevation in NT-proBNP (Neilan *et al.,* 2006a). A large difference in participant weekly training distance in the present study was apparent and could partially explain the inter-individual variation. A complex interaction of baseline (age, gender, volume overload, HR, BP; Chen *et al.,* 2008), prior exercise (training history; Neilan *et al.,* 2006a) and exercise-related factors (exercise duration; Serrano-Ostáriz *et al.,* 2011) are suggested to explain the variability in detection levels (Legaz-Arrese *et al.,* 2011).

4.4.3 Implications

In a few cases EF dropped below the lower limit for normal LV systolic function (52%; Lang *et al.,* 2015) in phases 1 and 2, however, the change appeared to be transient as EF typically recovered partially, if not fully by the PRE-exercise measurement point each day. The E:A ratio was significantly reduced in phases 1 and 2, in some runners below the clinical cut-off for diastolic dysfunction (less than 1.0), but appearing to be transient in nature. Whilst both variables showed a significant physiological change, neither reached a level which would be classified as a clinical significant change (See Section 3.2.2). Furthermore, neither were associated with any clinical signs and symptoms and did not appear to impact upon exercise performance.

Within phases 1 and 2, runners displayed concentrations of cTn above the clinical cut-off value. As no runners reported any clinical signs and symptoms, again it is likely that elevated cTn levels represent a physiological rather than a pathological response (Eijsvogels *et al*., 2011, 2015). It is suggested an increased NT-proBNP during and after exercise is related to a growth-regulating property of BNP, leading to myocardial

adaptation in healthy athletes (Scharhag *et al*., 2006), rather than a sign of heart damage. Concentrations of NT-proBNP were increased above clinical cut-off values in phases 1 and 2 of the present study, which would be suggestive of LV dysfunction in a clinical setting (Shave *et al*., 2007). The changes in NT-proBNP are more likely to represent the elevated cardiovascular work within each phase of this study.

4.4.4 Limitations

It is possible that some form of heat acclimatization may have occurred before or within the course of the MSUM, as it has previously been reported that 75-80% of the acclimation process occurs within the first 4-7 days of heat exposure (Périard *et al*., 2015; Shapiro *et al*., 1998). Heat acclimatization has been proven to increase submaximal exercise performance (Lorenzo *et al*., 2010) and enhance thermal comfort in the heat (Gonzalez and Gagge, 1976). Adaptations to the heat exposure include PV expansion, increased maintenance of fluid balance, enhanced sweating and cutaneous blood flow, thermal tolerance and a lower exercise metabolic rate (Horowitz 2014; Périard *et al*., 2015). Acute PV expansion occurs following 3-4 days of heat exposure (Sawka and Coyle 1999), with values reaching 3-27%, typically peaking around the fifth day of exercise-heat acclimation (Périard *et al*., 2016). An increase in PV serves to support cardiovascular stability by enhancing vascular filling pressure and therefore increasing SV and BP (Périard *et al*., 2016). Whilst the increase in PV observed may have reduced the cardiovascular demand of the MSUM, it could be also be speculated that the enhanced PV may well mask the degree of cardiac dysfunction in the latter stages of the MSUM. Therefore, further research within this area is warranted. The reluctance of some runners to participate in the study resulted in phase 2 of the present study being slightly underpowered.

Time constraints led to a single POST-exercise assessment of function and cardiac biomarkers in both phases. It was, therefore, not possible to determine the time point in which LV systolic and diastolic function peaked or indeed returned to PRE stage 1 levels. It has also been previously reported by Middleton *et al*. (2008) that a time-dependant change in cTn exists, therefore, peak elevations in cTn may have also been missed. Due to the reluctance of participants, no in-exercise data relating to exercise intensity was obtained, which did not allow for a direct comparison to other studies in relation to the acute cardiovascular stress of the exercise bouts. Whilst collecting data in a field setting presents a real life stimulus, a lack of control over the exercise intensity (i.e. some runners using different pacing strategies than others) and environmental conditions made it more difficult to draw comparisons between stages and also other studies.

4.5 Conclusion

In summary, a single bout of prolonged exercise in a hot environment led to a decrease in LV systolic and diastolic function and an elevation in cardiac biomarkers. Whilst 5 days of consecutive prolonged running produced evidence of a depression in LV systolic and diastolic function, there was limited support for any progressive accumulation in dysfunction.

Across the 5 days individual changes in cTnI and NT-proBNP were sporadic, with limited support for an accumulation across the exercise exposure. The results also presented a high degree of individual variation between participant's responses to the exercise stimulus in both phases of the study.

Whilst the present findings add to the limited number of studies focusing upon the cardiovascular consequences of single and multi-day exercise with the additional stress

of a hyperthemic environment, the field-based setting did not allow for the control of other exercise-related parameters (e.g. pace etc.) or the adoption of a control trial. Consequently the real impact of the environmental factors upon the cardiovascular response to exercise require further study. Therefore, the next Chapter will investigate the effect of controlled, laboratory-based bouts of steady state exercise undertaken at different but controlled environmental temperatures upon cardiac function and markers of cardiac damage.

CHAPTER 5

Left ventricular function and cardiac biomarker release after prolonged steady state exercise in laboratory controlled hyperthermic and normothermic environments.

5.1 Introduction

In Chapter 4, a reduction in cardiac function was observed alongside elevations in cardiac biomarkers following single and multi-day exercise in the heat. As highlighted in Chapter 4, this was the first study to report a reduction in global measures of systolic function (EF and SV) following exercise of a near marathon distance which may be attributed to an increased cardiovascular load compared to a normothermic environment.

The nature of the study in Chapter 4 and previous field-based research has not allowed for a controlled approach to the determination of the impact of environmental strain on cardiac function. To the best of our knowledge only one lab-based, and thus controlled study, has investigated the impact of environmental temperature upon LV function and cardiac biomarker release. Shave *et al*. (2004c) provided preliminary evidence to suggest that ambient temperatures may play some part in exacerbating the post-exercise response of LV function and cardiac biomarker release but did not extend to warmer environmental conditions. To date, the effect of prolonged exercise, within a controlled normothermic and hyperthermic environment, upon LV function and cardiac biomarker release has not been studied and will be the focus of this study. Furthermore, the majority of the field-based research has only looked at pre and post-event measurements of cardiac function and biomarkers. A controlled laboratory-based study allows for repeat assessments of LV function during recovery as well as multiple blood draws during exercise and again into recovery, a limitation of the previous two chapters.

Whilst moderate intensity steady-state exercise of as little as 10 minutes requires an athlete to maintain a high cardiac workload to ensure adequate muscle perfusion (Dawson *et al*., 2003), exercise durations of 60 mins or greater places a substantial strain upon the cardiovascular system due to increased thermal load and blood volume

redistribution/reduction (Coyle and González-Alonso, 2001) and are common exercise durations in healthy individuals. Additionally, Lucía *et al.* (1999a) proposed that the effects of environmental strain may be more important than exercise duration in the development of functional changes post-exercise, consequently, a shorter duration of prolonged exercise will be considered within this study in comparison to previous Chapters. Therefore, the aim of this study was to examine the impact of prolonged exercise in a hyperthermic environment upon cardiac function (EF and E:A) and levels of biomarkers of cardiac damage (cTnI and NT-proBNP). It was hypothesized that exercise of the same duration (60 min) and the same running speed performed in a hyperthermic environment (30^{o}C) will result in greater changes in LV function and more frequent appearance of cardiac biomarkers than exercise in a normothermic environment (13^{o}C).

5.2 Method

5.2.1 Participants and Study Design

In line with Shave *et* al. (2004b), eight male recreational athletes initially volunteered to take part in the study. All participants were healthy and free of known or early family history of cardiovascular disease, injury or illness and were not taking medications. Ethical approval was obtained from the Human Ethics Committee of Liverpool John Moores University prior to the start of data collection. Before the study commenced, all participants provided written informed consent.

A preliminary laboratory session was used to determine the VO_{2PEAK} of the athletes and to allow familiarization with the treadmill and all other equipment. In two subsequent laboratory sessions, participants completed identical 60 min runs (at 65% VO_{2PEAK}) at two different ambient temperatures (conditions; 13^{o}C [COOL] and 30^{o}C [HEAT]) with

constant relative humidity (50%) in a randomised, counterbalanced crossover design. Trial 1 and 2 were separated by 3-7 days.

Baseline assessment occurred 1 h prior to each trial (PRE). Post-trial assessments were then carried out immediately after run completion (POST) and then 1 (1 h POST) and 2 h (2 h POST) into recovery. During PRE and post-trial testing measurements included; BM, HR, BP, echocardiographic imaging and venous blood sampling. A venous blood sample was also obtained after 30 min of each exercise trial. T_c, skin temperature (T_{sk}), HR, and rating of perceived exertion (RPE) were assessed every 10 min during the 60 min trials. Fingertip blood samples for blood lactate (BLa) were obtained at rest and after 30 and 60 min of each trial.

5.2.2 Protocols

VO_{2PEAK} protocol and measurements:

The VO_{2PEAK} test and both 60 min trials were conducted upon a HP-Cosmos treadmill (Nussdorf-Traunstein, Germany). Each participant completed a 5 minute warm up at 8 $km.hr^{-1}$ before the online gas analysis equipment (Metamax, Cortex Biophysic GMbH, Leipzig, Germany) was then fitted, which had been calibrated before each participants test, using gases of known concentrations and volume by a 3 litre syringe. Each test was started at 9 $km.hr^{-1}$ at a 0% gradient. In 2 min incremental stages, the speed was increased by 2 $km.hr^{-1}$ until voluntary exhaustion. In the present study, VO_{2PEAK} was recorded as the highest 30 second average during the test, and the corresponding treadmill running speed identified.

60 min run protocol and measurements:

On the day of the each trial, participants arrived at the laboratory at least 3 h postprandial having refrained from alcohol, tobacco and caffeine consumption as well as exercise during the previous 24 h. All participants recorded dietary intake for 24 h prior to the initial experimental trial. This diet was then replicated before the subsequent experimental trial. A volume of 5 ml of water per kg BM (386 ± 29 ml) was consumed 2 h before arriving at the laboratory to achieve euhydration (Montain and Coyle, 1992). An additional 5 ml of water per kg BM was ingested during exercise to ensure similar hydration between trials. All trials were conducted at the same time of day in order to avoid the circadian variation in internal body temperature (Reilly and Brooks, 1986). During both trials participants were required to run at 65% of their measured VO_{2PEAK} at a gradient of 0%. To provide an environment replicable to outdoor conditions whereby the movement of air aids convective and evaporative heat loss (Jones and Doust, 1996; Saunders *et al*., 2005), for both trials a fan was placed 2 metres in front of participants, with a wind speed relative to the participants running speed.

On arrival at the laboratory, nude BM was recorded to the nearest 0.1 kg (model 875, Seca, Hamburg, Germany) and a rectal probe was inserted. SBP (a measure of afterload) and DBP was measured after 5 minutes rest in a supine position using a Dinamap automated BP monitor (Critikon Corporation, Tampa, USA). Each participant then underwent a resting echocardiographic examination, as described in Section 3.2.2. A venous cannula was inserted into the brachial vein and 5 ml of saline was flushed through the cannula to keep it patent each time a blood sample was drawn. Each 5 ml blood draw was made into a serum gel tube and the blood was left to clot. After being centrifuged serum was aliquoted and stored (–80°C) for subsequent analysis of cTnI and NT-proBNP. cTnI was measured using an ADVIA Centaur TnI-Ultra assay within the Elecsys 1010

automated batch analyser (Roche Diagnostics, Lewes, Sussex), with a sensitivity range of 0.006-50 µg.L^{-1}. The assay coefficient of variation was 20% (functional sensitivity) at 0.015 µg.L^{-1} and 10% at 0.03 µg.L^{-1}. The upper reference limit (99^{th} percentile) of an apparently healthy population is 0.04 µg.L^{-1} (Uettwiller-Geiger *et al*., 2002). NT-proBNP was determined using an Elecsys NT-proBNP electrochemiluminescent immunoassay (ECLIA) on the Roche Elecsys 1010 (Roche Diagnostics, Lewes, Sussex), with an analytical range of 5 – 35,000 ng.$L^{-1}$and intra-assay and inter-assay imprecision of 0.7-1.6% and 5.3-6.6%, respectively (Vidotto *et al.*, 2005). NT-proBNP levels below the detection limit were recorded at the lower limit of 19 ng.L^{-1}. The upper reference limit of an apparently healthy population is 125 ng.L^{-1} (Silver *et al.*, 2004).

T_c was measured using a rectal probe and data logger (1000 Series Squirrel Data Logger, Grant Instruments, Cambridge, UK). Skin temperature (T_{sk}) was also measured using the data logger and stainless steel mounted skin thermistors (EUS-U-VS5-0, Grant Instruments, UK) placed at four sites (sternum, forearm, thigh and calf) on the right side of the body using a single layer of waterproof tape. A mean weighted T_{sk} was then calculated using the method of Ramanathan (1964; See Equation 5.1). Heat storage was calculated from the equation employed by Havenith *et al.* (1995) whereby values were calculated from changes in T_c (ΔT_c) and T_{sk} (ΔT_{sk}) from resting values at 10, 20, 30, 40, 50 and 60 min of exercise. Where C_b is the specific heat capacity of the body tissue (3.49 $J.g^{-1}.{}^{o}C^{-1}$; See Equation 5.2). A HR monitor (Polar A1, Polar Electro, Finland) was fitted and worn throughout the 60 min trials. RPE (Borg, 1970) was also measured during both trials. Two BLa measurements were drawn from the finger using the Lactate Pro (LT-1710, Arkray Factory Inc, KDK Corp, Japan) and both values averaged. At the 30 min collection point, participants straddled the treadmill for blood samples and BLa measures to be taken. The difference in nude BM between PRE and POST-exercise, corrected for

the volume of fluid ingested and urine output was used to calculate exercise-induced BM loss.

Equation 5.1 Mean weighted T_{sk}

T_{sk} = (0.3 x sternum) + (0.3 x forearm) + (0.2 x thigh) + (0.2 x calf)

Equation 5.2 Heat storage

Heat Storage ($J \cdot g^{-1}$) = ($0.8\Delta T_c + 0.2\Delta T_{sk}$) x C_b

5.2.3 Data Analysis

Descriptive data are presented as mean ± SD and were supported by assessing the mean difference (mean diff) and 95% confidence interval [95% CI]. The Shapiro-Wilk test was applied to all data in order to assess for normal distribution. All PRE and POST-exercise variables, excluding cTnI, were analysed using a Two-way (Condition: HEAT-COOL x Time: PRE, POST, 1 h POST, 2 h POST) repeated-measures ANOVA. Sphericity was analysed by Mauchly's test of sphericity followed by Greenhouse-Geisser or Huynh-Feldt adjustment where appropriate. Where any differences were identified, pairwise post-hoc comparisons with a Bonferroni correction were used in order to show where they lay. The change in PRE to POST BM was analysed using a Student's paired *t*-test. Cardiac troponins were analysed descriptively because of the likelihood of undetectable PRE-exercise values. All statistical analyses were conducted using IBM SPSS Statistics 22 (IBM Corp., Armonk, NY, USA). An alpha level of $p < 0.05$ was considered statistically significant. Furthermore, effect size using Cohen's *d* and partial eta squared (η_p^2) were calculated, as described in Section 4.2.3. Cardiac biomarkers were also compared to the upper reference limits of an apparently healthy population.

5.3 Results

Six male recreational athletes (mean ± SD; age 29 ± 7 years, height 1.84 ± 0.04 m, BM 76.7 ± 6.0 kg and VO_{2PEAK} 59.2 ± 3.5 mL.kg^{-1}.min^{-1}) completed both trials, with two participants being withdrawn due to T_c reaching the safety cut-off criteria. A large difference in BM loss was observed between conditions (HEAT: 1.6 ± 0.3 kg, COOL 0.8 ± 0.2 kg; mean diff = -0.9; $t_{(5)}$ = 5.545, p = 0.003; 95% CI [-1.3, -0.5]; *d* = 2.26), with relative BM loss being significantly greater in the HEAT (2.1 ± 0.5%) compared to the COOL (1.0 ± 0.2%). A large interaction between condition and time for exercise HR was observed ($F_{(2.450,12.252)}$ = 775.723, p = 0.022; η_p^2 = 0.50), whereby HR was significantly higher in the HEAT from 30 min onwards (Figure 5.1a). A large effect was seen across time ($F_{(2.079,10.397)}$ = 128.639, p < 0.001; η_p^2 = 0.96) with 0 min being lower than all exercise time points (p < 0.001). An insignificant large interaction between condition and time for T_c was observed, however, T_c was significantly higher in the COOL at 20 mins only. An insignificant large effect was seen between conditions for T_c, whilst a large significant effect across time was observed ($F_{(1.319,6.596)}$ = 46.761, p < 0.001; η_p^2 = 0.90) with post-hoc analysis revealing a progressive increase in T_c from 0 min onwards (Figure 5.1b). A large interaction between condition and time was observed for T_{sk} ($F_{(2.098,10.488)}$ = 52.427, p < 0.001; η_p^2 = 0.83), whereby T_{sk} was higher in the HEAT from 10 min onwards, with both T_{sk} in both conditions reaching a steady-state level (Figure 5.1c). This was supported with a large effect for condition (mean diff = 6.62; $F_{(1.000,5.000)}$ = 545.350, p < 0.001; 95% CI [5.89, 7.35]; η_p^2 = 0.99), whereby HEAT was higher than COOL (Figure 5.1c), but no effect for time. Heat storage demonstrated no interaction between condition and time, with a large effect for condition (mean diff = 4; $F_{(1.000,5.000)}$ = 109.115, p < 0.001; 95% CI [4, 6]; η_p^2 = 0.96), whereby HEAT was higher than COOL. A large

effect for time was also seen ($F_{(1.000,5.000)}$ = 29.482, p = 0.001; η_p^2 = 0.86) with post-hoc analysis revealing a progressive increase in heat storage from 20 min onwards (Figure 5.1d). BLa demonstrated no significant interaction between condition and time, nor effect of condition or time (Figure 5.1e). A large significant interaction between condition and time was displayed for RPE ($F_{(5,25)}$ = 7.462, p < 0.001; η_p^2 = 0.60), whereby HEAT was higher than COOL from 20 mins onwards (Figure 5.1f). A large effect was observed for condition ($F_{(1.000,5.000)}$ = 9.853, p = 0.026; 95% CI [0.24, 2.38]; η_p^2 = 0.66) with HEAT being higher than COOL (Figure 5.1f). A large effect for time was also demonstrated ($F_{(1.588,7.940)}$ = 13.944, p = 0.003; η_p^2 = 0.74), however, post-hoc analysis revealed no differences within time points.

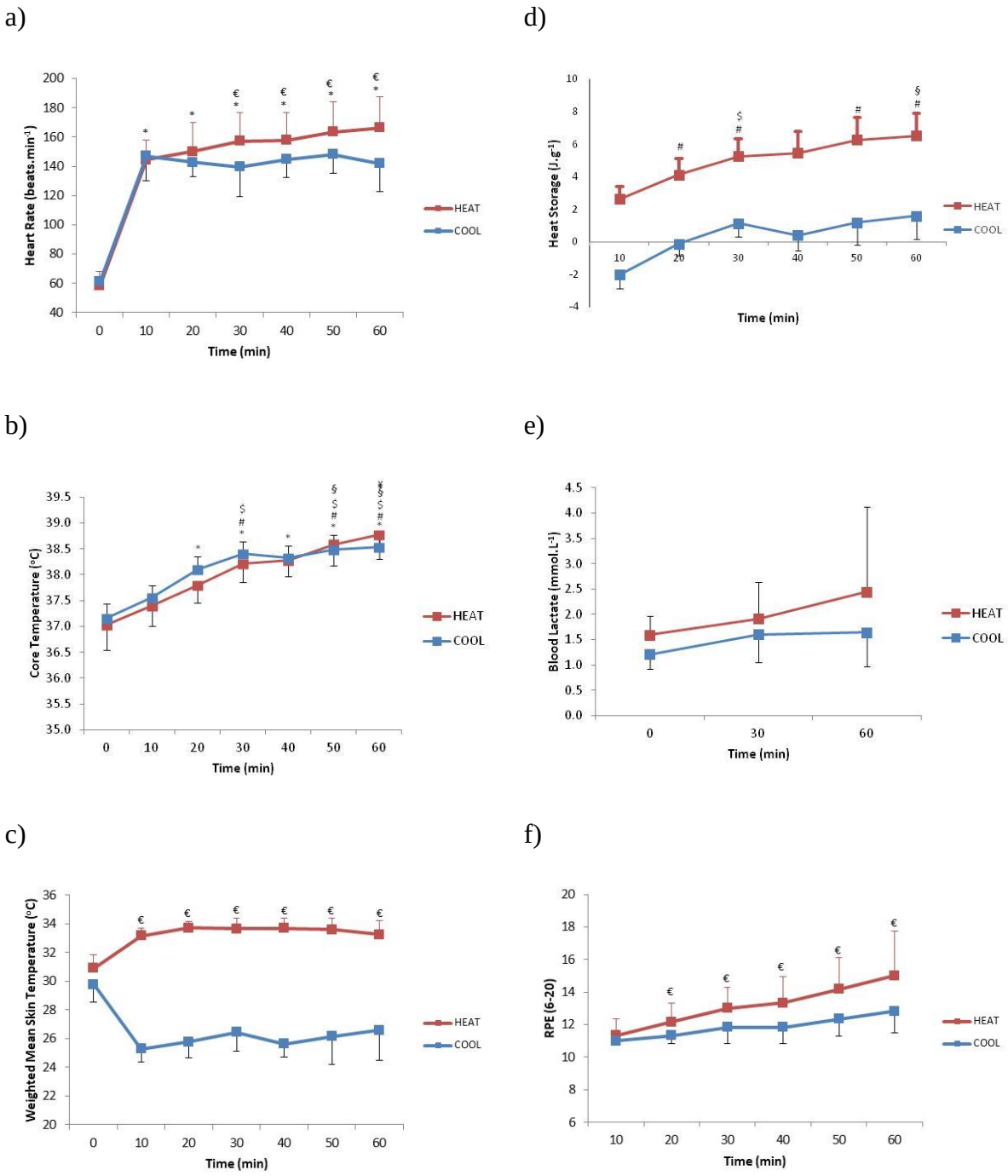


Figure 5.1 The mean ± SD of a) HR, b) T_c, c) T_{sk}, d) Heat Storage, e) BLa and f) RPE across assessment time points in both trials (n = 6). * Significantly different to 0, $p < 0.05$. # Significantly different to 10, $p < 0.05$. $ Significantly different to 20, $p < 0.05$. § Significantly different to 40, $p < 0.05$. ¥ Significantly different to 50, $p < 0.05$.

€ Significantly different between HEAT and COOL.

5.3.1 Echocardiographic Data

5.3.1.1 Loading

LVEDV showed a large insignificant interaction between condition and time, with a small insignificant effect for condition and time. HR showed a large insignificant interaction between condition and time. A large effect between conditions as well as across time ($F_{(3,15)}$ = 13.765, p < 0.001; η_p^2 = 0.73) was observed, with post-hoc analysis revealing POST to be higher than PRE (mean diff = 19; p = 0.004; 95% CI [8, 30]; Table 5.1) and 2 h POST (mean diff = 11; p = 0.043; 95% CI [0, 22]; Table 5.1). An insignificant large interaction between condition and time was observed for SBP. A large insignificant effect for condition but significant for time ($F_{(3,15)}$ = 4.287, p = 0.023; η_p^2 = 0.46; Table 5.1) was displayed for SBP, with post-hoc analysis revealing no differences between time points. Similar data was apparent for DBP, where a moderate insignificant interaction between condition and time was shown, with an insignificant large effect for condition and time (Table 5.1). E:E' showed an insignificant moderate interaction between condition and time, with an insignificant large effect for condition and a moderate effect for time also shown (Table 5.1).

Table 5.1 LV loading across assessment points in both conditions (n = 6, mean ± SD).

	HEAT				COOL			
Variable	PRE	POST	1 h POST	2 h POST	PRE	POST	1 h POST	2 h POST
LVEDV (ml)	181±21	173±26	178±31	168±25	181±21	152±26	183±13	171±15
HR (beats.min^{-1})	53±8	78±9	68±14	63±4	53±8	67±9	59±6	60±10
SBP (mmHg)	122±6	118±6	118±7	120±11	122±6	114±4	114±5	118±5
DBP (mmHg)	67±3	65±5	66±3	67±3	67±3	64±4	63±4	66±1
E:E’	6±2	6±1	6±1	6±2	6±2	6±1	6±1	6±1

LVEDV: left ventricular end diastolic volume, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, E:E’: peak early trans-mitral flow/peak early myocardial tissue velocity. PRE, POST, 1 h POST and 2 h POST: before, immediately after, and one hour and 2 hours after each run, respectively.

5.3.1.2 LV Systolic Function

A large insignificant interaction was observed between condition and time for EF, with a post-hoc power analysis of 0.3. A large insignificant effect for condition was also observed. A significant large effect was displayed for time ($F_{(3,15)}$ = 3.102, p = 0.058; η_p^2 = 0.38) with post-hoc analysis revealing a reduction POST compared to PRE (mean diff = 6; p = 0.027; 95% CI [-11, -1]; Table 5.2). A tendency for POST exercise values to be lower during the HEAT condition was observed (Figure 5.2a). An insignificant moderate interaction between condition and time for SV was apparent as well as a small insignificant effect for condition. A large significant effect for time ($F_{(3,15)}$ = 3.953, p = 0.029; η_p^2 = 0.44) was observed, however, post-hoc analysis revealed no significant comparisons (Table 5.2). Some individual variation was apparent, with some individuals demonstrating elevated levels POST, whilst others showed reduced values (Figure 5.2b). SBP/LVESV displayed a large insignificant interaction between condition and time. Small insignificant effects for condition as well as time (Table 5.2) were observed for SBP/LVESV, with a varied individual response (Figure 5.2c). S' showed an insignificant large interaction between condition and time. A moderate insignificant effect for condition and a large insignificant effect for time was apparent for S' (Table 5.2), with a small variation between participants (Figure 5.2d).

Table 5.2 LV systolic function across assessment points in both conditions (n = 6, mean ± SD).

	HEAT				COOL			
Variable	PRE	POST	1 h POST	2 h POST	PRE	POST	1 h POST	2 h POST
EF (%)	53 ± 3	44 ± 6	53 ± 5	48 ± 6	53 ± 3	50 ± 5	52 ± 4	50 ± 6
SV (ml)	96 ± 14	77 ± 18	95 ± 18	80 ± 7	96 ± 14	77 ± 14	95 ± 6	86 ± 13
SBP/LVESV ($mmHg \cdot ml^{-1}$)	1.46 ± 0.15	1.26 ± 0.19	1.48 ± 0.30	1.43 ± 0.38	1.46 ± 0.15	1.56 ± 0.35	1.31 ± 0.16	1.41 ± 0.23
S’ ($cm.s^{-1}$)	8 ± 1	7 ± 1	8 ± 1	8 ± 1	8 ± 1	8 ± 1	8 ± 0	8 ± 1

EF: ejection fraction, SV: stroke volume, SBP/LVESV: systolic blood pressure/left ventricular end systolic volume, S’: peak systolic myocardial tissue velocity. PRE, POST, 1 h POST and 2 h POST: before, immediately after, and one hour and 2 hours after each run, respectively.

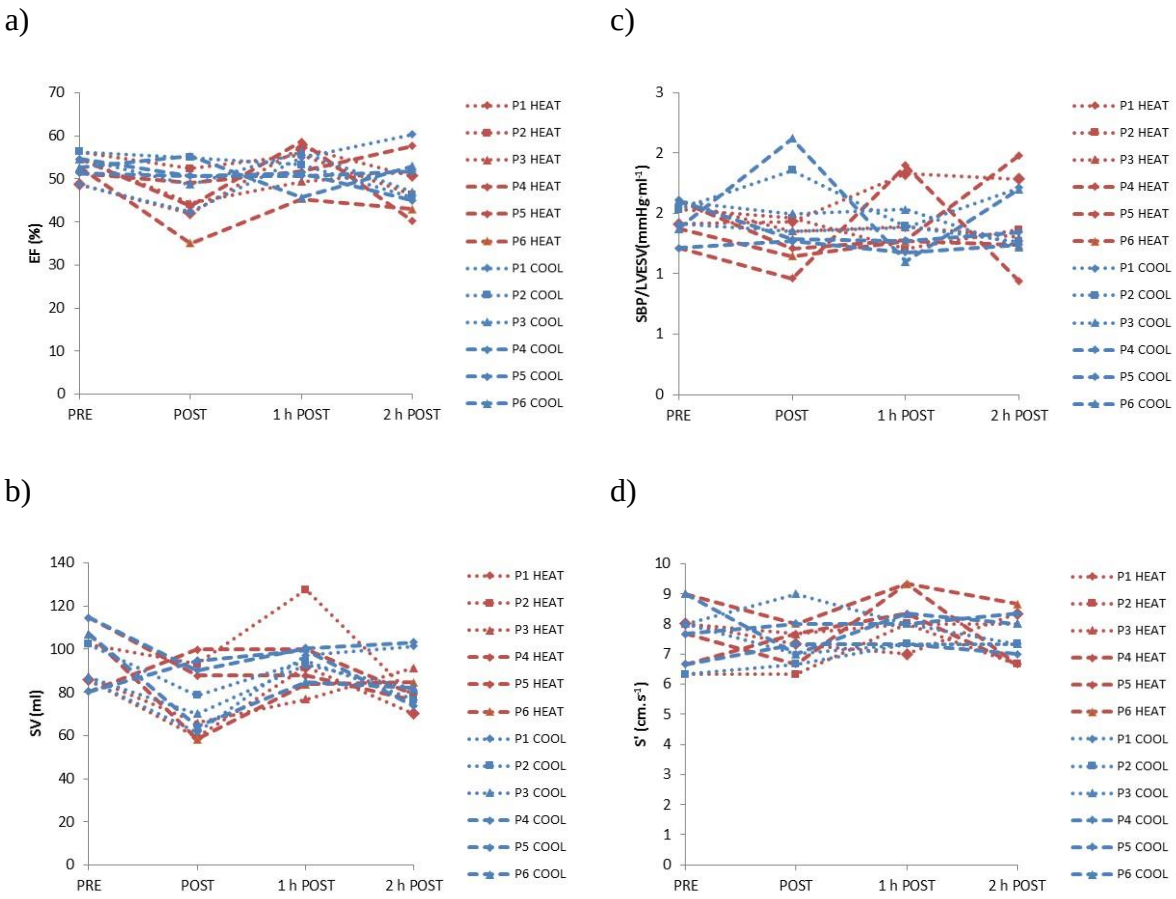


Figure 5.2 Individual heterogeneity for LV systolic function across assessment points in both conditions where a) EF, b) SV, c) SBP/LVESV and d) S' (n = 6). PRE, POST, 1 h POST and 2 h POST: before, immediately after, and one hour and 2 hours after each run, respectively.

5.3.1.3 LV Diastolic Function

E showed a large insignificant interaction between condition and time, with a small insignificant effect between conditions. A large insignificant effect for time was also observed (Table 5.3), with some variation between participants (Figure 5.3a). A moderate insignificant interaction between condition and time was demonstrated for A. A large insignificant effect for condition was seen for A, with a large insignificant effect for time (Table 5.3). Participants showed a much greater variation across time points, with some demonstrating large elevations, whilst others displayed large reductions (Figure 5.3b). As

a result, E:A showed a moderate insignificant interaction between condition and time, with a post-hoc power analysis of 0.1. A large insignificant effect for condition as well as time was observed (Table 5.3), with a varied response between participants was also shown for E:A (Figure 5.3c). E' showed a large insignificant interaction between condition and time. An insignificant large effect for condition as well as a moderate insignificant effect for time was also seen for E' (Table 5.3). Participants with a higher PRE value tended to show a larger reduction in E' POST, whilst those with a lower E' tended to show an increased or maintained E' value POST (Figure 5.3d). A large insignificant interaction between condition and time was apparent for A', with a small insignificant effect for condition. A large insignificant effect for time for A' was also observed , however, post-hoc analysis revealed POST to be significantly lower than 1 h POST (mean diff = 1; p = 0.013; 95% CI [-3, 0]; Table 5.3). A' showed a varied response between the participants across time points (Figure 5.3d). As a result, E':A' showed a large insignificant interaction between condition and time. A large insignificant effect for condition as well as time was also seen for E':A' (Table 5.3), with some participants showing a small variation across time points, whereas others showed a much larger variation (Figure 5.3f).

Table 5.3 LV diastolic function across assessment points in both conditions (n = 6, mean ± SD).

	HEAT				COOL			
Variable	PRE	POST	1 h POST	2 h POST	PRE	POST	1 h POST	2 h POST
E ($cm.s^{-1}$)	76 ± 7	66 ± 9	72 ± 5	72 ± 9	76 ± 7	71 ± 8	70 ± 7	70 ± 8
A ($cm.s^{-1}$)	44 ± 7	45 ± 11	52 ± 5	50 ± 6	44 ± 7	42 ± 9	45 ± 9	45 ± 8
E:A	1.76 ± 0.29	1.56 ±0.43	1.41 ± 0.20	1.47 ± 0.23	1.74 ± 0.27	1.74 ± 0.34	1.65 ± 0.48	1.64 ± 0.48
E' ($cm.s^{-1}$)	13 ± 4	11 ± 2	13 ± 3	12 ± 3	13 ± 4	13 ± 2	13 ± 3	13 ± 2
A' ($cm.s^{-1}$)	7 ± 2	7 ± 1	8 ± 1	7 ±1	7 ± 2	6 ± 1	8 ± 1	7 ± 1
E':A'	1.95 ± 0.64	1.73 ± 0.49	1.69 ± 1.42	1.67 ± 0.50	1.95 ± 0.64	2.26 ± 0.79	1.62 ± 0.45	1.73 ± 0.35

E: peak early trans-mitral flow velocity, A: peak atrial filling velocity, E:A: early to late diastolic filling ratio, E': peak early diastolic myocardial velocity, A': peak late diastolic myocardial velocity, E'/A': peak early to late myocardial tissue velocity ratio. PRE, POST, 1 h POST and 2 h POST: before, immediately after, and one hour and 2 hours after each run, respectively.

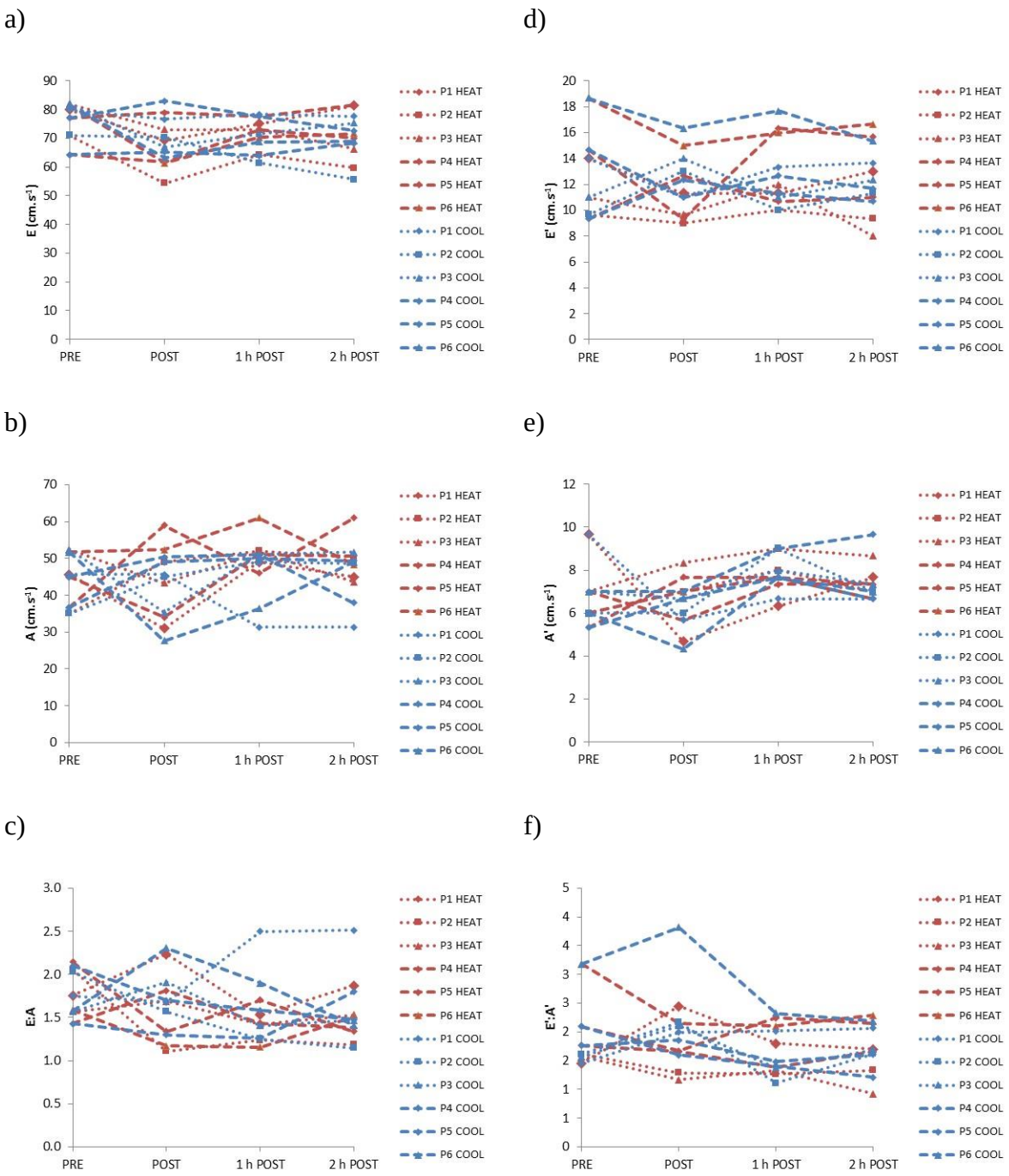


Figure 5.3 Individual heterogeneity for LV diastolic function across assessment points in both conditions where a) E, b) A, c) E:A, d) E', e) A' and f) E':A' (n = 6). PRE, POST, 1 h POST and 2 h POST: before, immediately after, and one hour and 2 hours after each run, respectively.

5.3.2 Cardiac Biomarkers

No detectable cTnI was present in serum samples taken PRE-exercise in both conditions (Figure 5.4). Within the HEAT condition, cTnI was elevated in runner 1 at 1 h POST (0.06 µg.L^{-1}) and 2 h POST (0.11 µg.L^{-1}), exceeding the upper reference limit, and in runner 6 at 2 h POST (0.03 µg.L^{-1}). Participant 1 also displayed an elevation in cTnI within the COOL condition at 2 h POST (0.03 µg.L^{-1}). All other participants had no detectable values across all time points in either condition. Data for NT-proBNP was below the upper reference limit for a healthy population for all runners PRE-exercise (Figure 5.5) and remained below this limit across all time points. A small insignificant interaction between condition and time was observed for NT-proBNP, with a large insignificant effect being observed for time. NT-proBNP showed a large significant effect for condition (mean diff = 6; $F_{(1.000,5.000)}$ = 11.085, p = 0.021; 95% CI [1.37, 10.63]; η_p^2 = 0.69), with higher levels being observed within the HEAT condition at with values ranging from 19.96-109.94 ng.L^{-1} compared to the COOL condition 19.96-106.56 ng.L^{-1}.

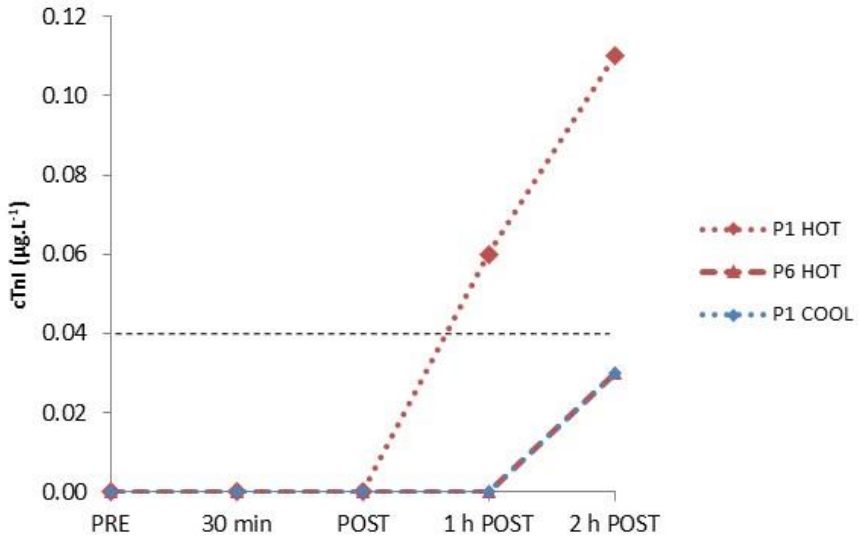


Figure 5.5 Individual data for cTnI across assessment points in both conditions. The dotted line represents the 99th percentile of an apparently healthy population (≤ 0.04 µg.L-1; Uettwiller-Geiger *et al*., 2002; n = 2).

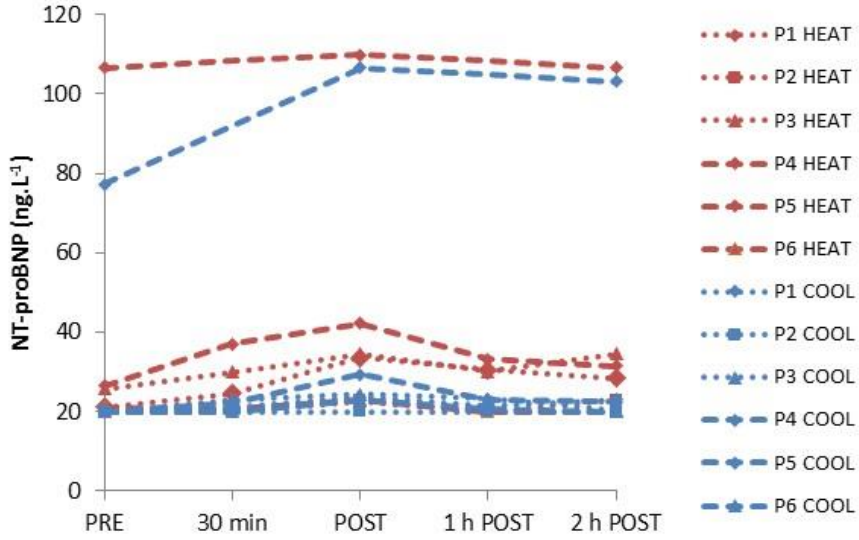


Figure 5.6 Individual data for NT-proBNP across assessment points in both conditions (n = 6).

5.4 Discussion

The present study represents the first to compare exercise in a controlled hyperthermic and normothermic environment upon cardiac function and markers of cardiac damage,

one of the extraneous factors suggested to precipitate changes in ventricular function and biomarker appearance (Chapter 4; Gresslien and Agewall, 2016; Oxborough *et al.*, 2010; Sedaghat-Hamedani *et al.*, 2015). The key findings indicate that some measures of systolic function were reduced POST-exercise compared to PRE, with no consistent evidence of diastolic dysfunction. A tendency for a larger reduction in both systolic and diastolic function within the HEAT condition was apparent, with a marked heterogeneity between participants in response to both conditions and exercise time points. cTnI was elevated above detection levels in two participants POST-exercise within the HEAT and one participant POST-exercise within the COOL, again demonstrating heterogeneity between participants and conditions. There was no significant change in NT-proBNP across time points, however, values were significantly greater during the HEAT condition as opposed to the COOL condition.

RPE was significantly higher within the HEAT trial likely reflecting a greater T_{sk}. The magnitude of cardiovascular drift was significantly greater during the HEAT condition, a common finding observed during exercise in hot environments compared to normothermic conditions (Rowell *et al.*, 1966; Lafrenz *et al.*, 2008). It is likely that a peripheral vasodilation (evidenced by the elevated T_{sk}), stimulated in an effort to dissipate heat within the HEAT condition, may have led to a reduction in central blood volume during exercise (Coyle and Gonzalez-Alonso, 2001). It is also likely that the rate of intravascular fluid loss through sweat production was also a confounding factor to the observed difference in cardiovascular drift as evidenced by the greater loss of BM during the HEAT trial compared to the COOL. A combination of blood volume redistribution and intravascular fluid loss encountered during the HEAT trial may have resulted in a reduction in venous return (Rowell *et al.*, 1966, 1969), therefore, leading to an elevated HR in order to maintain cardiac output in the HEAT. Therefore, it is likely that the total

myocardial work performed during the HEAT trial would have been greater than that performed during the COOL. It cannot be disregarded, however, that the change in BM may also be due to metabolic factors such as substrate loss and muscle breakdown (Maughan *et al.*, 2007). Despite evidence of different cardiovascular stress in the HEAT and COOL trial we should acknowledge that similar BLa concentrations were observed between conditions despite a greater heat gain and BM loss (~2%) within the HEAT, suggestive that any change in blood flow to the working muscle may be minimal. Likewise, T_c was similar between both trials suggesting that the moderate exercise intensity allowed for a maintenance of exercise performance (completion of the exercise at the desired intensity) as well as temperature regulation. This is also supported by the similar rate of increase in heat storage between both conditions.

5.4.1 LV Systolic Function

Systolic function (EF) was significantly reduced POST-exercise compared to PRE in both conditions with a tendency for function to be lower within the HEAT condition. The average 5% reduction in EF POST within the COOL is likely attributed to participant and technical variability, however, the 16% reduction within the HEAT trial is greater than what would be expected from measurement variability. It is possible that the greater BM loss in the HEAT compared to the COOL may partially explain some of the changes in EF. It is also likely that the rate of fluid loss through sweat production, along with peripheral vasodilation within the HEAT may have led to a reduction in venous return and therefore, reduced LV preload. As a consequence, a significantly elevated exercise HR was observed within the HEAT in order to maintain cardiac output, resulting in a greater myocardial work performed within the HEAT through a greater sympathetic activity. It is suggested once a tolerable sympathetic load/threshold has been exceeded for a prolonged period of time, a desensitization or down regulation of cardiac β-

andrenergic receptors (Banks *et al.*, 2010; Oxborough *et al.*, 2010) and autonomic responsiveness (Seiler *et al.*, 2007) may occur. Whilst the duration of the current study may not be prolonged enough to exceed this threshold, it may explain the tendency for cardiac function to be reduced further within the HEAT in this study and in the previous Chapter.

The changes in EF and SV were not mimicked by a significant reduction in S', likely due to the localised nature of S'. Therefore, it is likely that changes in systolic function were a result of a combination of reduced filling time, fluid loss and blood volume distribution rather than a shift in the inotropic state of the myocardium (Shave *et al.*, 2004). This is supported by the insignificant changes in SBP/LVESV (a surrogate of LV contractility) after both trials. The current and previous data (Scharhag *et al.* 2006) suggests that 60 minutes of exercise rarely results in a decrement in systolic function, and whilst a tendency for function to be reduced more in the HEAT exists, the exercise duration is unlikely to unmask any effects of environmental temperature on systolic function. Whilst it is pertinent to assess the impact of environmental temperature in a setting where a greater volume of exercise is performed, this is unlikely to occur in a controlled laboratory setting and thus be problematic to make comparisons in a field based setting.

5.4.2 LV Diastolic Function

Within the present study, small and sporadic changes in indices of diastolic function were observed with no significant impact of 60 minutes of running and no mediating effect of environmental temperature. The lack of significance in the current study may be due to the study being underpowered through a low sample size as a result of the difficulty in recruiting participants for prolonged exercise in a laboratory. A high variance exists for diastolic flow and tissue velocities compared to other functional parameters, thus a larger

sample size is often necessary for an appropriate statistical power. As a marker of left atrial pressure, E:E' was not significantly reduced following prolonged exercise. Myocardial tissue velocities were also not altered by the exercise or related to measures of HR or loading, therefore, suggesting any possible changes in preload and the exercise undertaken did not influence filling patterns.

5.4.3 Cardiac Biomarkers

No detectable cTnI was present in serum samples taken pre-exercise and is likely explained by the training status and control of exercise exposure prior to this laboratory-based study. Detectable cTnI values were observed in two participants after the HEAT trial and one after the COOL trial, with peak values being demonstrated at the 2 h POST assessment point. Whilst cTn has been detected in individuals in as little as 30 minutes exercise (Middleton *et al.,* 2006; Shave *et al.,* 2010), it is suggested an elevation in cTn in response to exercise is evident in all individuals, but may not always be detected due to the time of sampling (Gresslien and Agewall, 2016). Therefore, within the current study, we cannot rule out that detectable values in other participants may have been missed due to blood sampling time. In one participant the peak value was greater than the upper reference limit for a healthy population, however, below the cut-off for AMI, indicative of minimal cardiac damage within the present study. Whilst the exact mechanism for cTn release is unknown (Gresslien and Agewall, 2016; Shave *et al.* 2010), previous studies suggest it is likely to be a physiological response, due to the high percentage of participants with increased cTn post-exercise, low absolute levels and a rapid normalization of values, representing cTn release from the "unbound" cytosolic reservoir (Gresslien and Agewall, 2016; Shave *et al.* 2010). Within the present study, it was not possible to evaluate the normalization of elevated values due to the absence of blood sample collection at either 24 or 48 hours post-exercise.

Data for NT-proBNP were below the upper reference limit for a healthy population for all participants PRE-exercise and remained below this limit across all time points. Within the present study, a greater magnitude of cardiovascular drift was evidenced within the HEAT, signifying a greater total myocardial work performed during the heat trial. Coupled with a greater NT-proBNP concentration within the HEAT trial, a complex interaction between exercise intensity and duration may exist which is not surprising since NT-proBNP is elevated in response to volume overload and myocyte stretch (Shave *et al*., 2007b). This is further supported by the high concentrations of NT-proBNP demonstrated in Chapter 4. In contrast to cTnI, NT-proBNP demonstrates a half-life of around 120 minutes, being eliminated mainly through the kidneys (Hall, 2004), therefore, any peak values were likely to have been detected within the sampling points of the present study.

5.4.4 Limitations

A controlled laboratory-based study design was chosen to allow the appropriate control and comparison of two environmental conditions in a repeated measures design. The exercise duration selected ensured participants completed both trials before an unethical T_c was reached and exercise was terminated. As a consequence, a limitation of the study is the exercise dose performed, which may have limited the expression of cardiac functional change and the appearance of cardiac biomarkers in comparison to other studies. This also reduced the ability to differentiate between the two environmental conditions. Therefore, future studies should consider increasing the volume of the exercise (either intensity or duration) to evaluate whether environmental temperature mediates changes in cardiac function and markers of damage. As previously mentioned, another limitation was the low participant number which reduced the statistical power of the study. The measurement variance in flow and tissue velocities, coupled with

participant heterogeneity may explain the lack of statistical significance within the present study. Data collection points were limited to immediately, 1 and 2 h post exercise. Therefore any transient elevations in cTn which could have been observed with more frequent analysis may have been missed whilst it was also unclear when the elevated cTnI normalised.

5.5 Conclusion

Both LV systolic and diastolic function were not significantly altered following 60 min of running in either a controlled normothermic or hyperthermic environment. The release of cardiac biomarkers was limited, with a tendency for markers to be higher in the hyperthemic condition. The results also presented a high degree of individual variation to the exercise stimulus in both trials of the study, but overall suggests the CV system of the participants coped well with the exercise stress imposed.

Participation in prolonged exercise, such as training for and participating in endurance and ultra-endurance events is becoming increasingly popular, particularly in environmentally stressful conditions. The method of pre-cooling immediately prior to exercise in thermally stressful environments, is popular and has been shown to be an effective means of reducing physiological strain, prolonging performance and improving perceptual tolerance. The impact of pre-cooling and subsequent prolonged exercise in a thermally stressful environment upon cardiovascular function has yet to be examined. Therefore, the next Chapter will investigate the effect of pre-cooling prior to a single bout of steady state exercise within a hyperthermic environment upon cardiac function and markers of cardiac damage.

CHAPTER 6

The effect of whole body pre-cooling upon left ventricular function and cardiac biomarker release after prolonged steady state exercise in laboratory controlled hyperthermic environment.

6.1 Introduction

Despite a large amount of evidence to support LV systolic and/or diastolic dysfunction and an elevation in cardiac biomarkers, contradictory evidence does exist (Chapter 2), likely due to the differences in research design, sample size, data interpretation, participant heterogeneity, intensity and duration of exercise and environmental factors (Chapter 4; Gresslien and Agewall, 2016; Oxborough *et al.*, 2010; Sedaghat-Hamedani *et al.*, 2015). Most of the existing literature also employs a field-based design, whereby the nature of these studies has not allowed for a controlled approach to the determination of the impact of environmental strain on cardiac function. To the best of our knowledge only two lab-based, and thus controlled studies, have investigated the impact of environmental temperature upon LV function and cardiac biomarker release. Shave *et al*. (2004c) suggested that ambient temperatures may play some part in exacerbating the post-exercise response of LV function and cardiac biomarker release, attributed to the greater myocardial work performed in the 19^{o}C trial than the 0^{o}C trial. In Chapter 5 we did not observe any changes in systolic or diastolic function, independent of changes in preload after 60 min of running in ambient temperatures of either 13^{o}C or 30^{o}C. Therefore, suggesting a depression in LV function may have an exercise duration threshold even in times of high thermal stress. A tendency for function to be reduced following the 30^{o}C trial was apparent, with cTnI being detectable in one participant following the 13^{o}C trial and two participants following the 30^{o}C trial, thus warranting further laboratory-based investigation with a greater exercise duration. A limitation of Chapter 5 was the limited post-exercise data collection points which may have missed any transient elevations in cTn which could have been observed with more frequent analysis. Therefore, the present study will employ more frequent assessment points post-exercise, which will be more indicative of the normalisation of any elevated values.

The method of pre-cooling immediately prior to exercising in thermally stressful environments has been shown to be an effective means of reducing physiological strain, prolonging performance and improving perceptual tolerance (Cuddy *et al*., 2014). A maintained SV and attenuated HR during steady state exercise after pre-cooling has been previously observed (Hessemer *et al.*, 1984; Lee and Haymes, 1995; Schmidt and Brück, 1981), suggesting a reduced cardiovascular strain following pre-cooling. Therefore, cold water immersion will be employed as a method of manipulating the cardiovascular response to prolonged exercise by reducing the participants T_c.

At present, no study has specifically examined the manipulation of T_c through pre-cooling, prior to exercise in a hyperthermic environment upon cardiac function and markers of cardiac damage. Therefore, the aim of this study was to examine the effect of pre-cooling, prior to exercise in a hyperthermic environment, upon cardiac function, specifically EF and E:A, and markers of cardiac damage (cTnI and NT-proBNP). It was hypothesised that performing prolonged exercise in a hot environment, preceded by pre-cooling may reduce the myocardial load during exercise, resulting in a maintained LV function and a reduced appearance of cardiac biomarkers in comparison to a control trial.

6.2 Method

6.2.1 Participants and Study Design

Ten male recreational athletes (mean ± SD; age 29 ± 7 years, height 1.76 ± 0.08 m, body mass 72.8 ± 11.6 kg and VO_{2PEAK} 54.1 ± 7.9 mL.kg^{-1}.min^{-1}) were recruited for the study. All participants were healthy and free of known or early family history of cardiovascular disease, injury or illness and were not taking medications. Ethical approval was obtained from Coventry University prior to the start of data collection. Before the study commenced, all participants provided written informed consent.

A preliminary laboratory session was used to determine the VO_{2PEAK} of the athletes and to allow familiarization with the treadmill and all other equipment. In two subsequent laboratory sessions, participants completed identical 90 min runs (at 65% VO_{2PEAK}) in the heat (32.4 ± 0.9ºC and 46.8 ± 6.4% RH) preceded by either a seated control (CON) or a pre-cooling (P-C) condition for 60 minutes in a randomised, counterbalanced crossover design. Trial 1 and 2 were separated by at least one week to avoid heat acclimation (Barnett and Maughan, 1993).

Baseline assessment occurred 1 h prior to each seated condition (PRE), with post-condition assessments being taken immediately following the completion of the condition (0 min). Post-trial assessments were then carried out immediately after run completion (POST) and then 1 (1 h POST), 2 h (2 h POST), 4 h (4 h POST) and 24 h (24 h POST) into recovery. During PRE and post-trial testing measurements included; BM, HR, BP, echocardiographic imaging, venous and fingertip blood sampling. T_c, T_{sk} and HR were monitored throughout the protocol. At 15 min intervals throughout the exercise protocol, participants provided thermal and exertional ratings. Fingertip blood samples for BLa were obtained PRE and at 30 min intervals throughout the exercise protocol.

6.2.2 Protocols

VO_{2PEAK} protocol and measurements:

The VO_{2PEAK} test and both 90 min trials were completed upon a HP-Cosmos treadmill (Nussdorf-Traunstein, Germany), as described in Section 5.2.2.

Experimental Procedures:

Prior to the initial experimental trial, participants arrived at the laboratory at least 3 h postprandial having refrained from alcohol and caffeine for 12 h and strenuous activity

during the previous 24 h. All participants recorded dietary intake for 48 h prior, replicating this diet before the subsequent experimental trial. A volume of 5 ml of water per kg BM (363 ± 63 ml) was consumed 2 h before arriving at the laboratory to achieve euhydration (Montain and Coyle, 1992). An additional 7 ml of water per kg BM (508 ± 89 ml) was ingested during exercise to ensure similar hydration between trials. All trials were performed at the same time of day in order to avoid the circadian variation in internal body temperature (Reilly and Brooks, 1986). During both trials participants were required to run at 65% of their measured VO_{2PEAK} at a gradient of 0%. To provide an environment replicable to outdoor conditions whereby the movement of air aids evaporative and convective heat loss (Jones and Doust, 1996; Saunders *et al*., 2005), for both trials a fan was placed 2 m in front of participants, with a wind speed relative to the participants running speed, measured at 1.3 m above the treadmill belt (Figure 6.1).

Figure 6.1 Experimental setup

On arrival at the laboratory, nude BM was recorded to the nearest 0.1 kg (model 875, Seca, Hamburg, Germany) and a flexible translucent PVC rectal probe was inserted (Grant Instruments, UK). Following 5 min rest in a supine position, SBP (a surrogate of afterload) and DBP was measured using a Dinamap automated BP monitor (Critikon Corporation, Tampa, USA). Each participant then underwent a resting echocardiographic examination, as described in Section 3.2.2.

Whole blood samples were collected by venepuncture without venostasis from an antecubital vein using a 21G butterfly syringe into a serum gel tube and the blood was left to clot. After being centrifuged at 1500 *g* for 10 min, serum was aliquoted and stored (– 80°C) for subsequent analysis of cTnI and NT-proBNP (See Section 3.2.1). PV changes were estimated from changes in haemoglobin (Hb) and haematocrit (Hct; Dill & Costill, 1974; Maughan *et al.*, 2001) from fingertip blood samples. Fingertip samples were collected into heparinized microhematocrit tubes (Hawksley and Sons, Sussex, UK) and microcuvettes (HemoCue AB, Angelholm, Sweden) for the determination of Hct (Hawksley 1560 Microhematocrit reader) and Hb (HemoCue hemoglobin meter; HemoCue Inc, Lake Forest, California, USA), respectively. Results of cTnI and NT-proBNP were corrected for percentage change in PV.

T_c and mean weighted T_{sk} was measured, as described in Section 5.2.2. Heat storage was calculated from the equation employed by Havenith *et al.* (1995) whereby values were calculated from changes in T_c (ΔT_c) and T_{sk} (ΔT_{sk}) from PRE compared to values at 15 min intervals during exercise (See Equation 5.2). A HR monitor (Polar A1, Polar Electro, Finland) was fitted and worn throughout the exercise protocol. Participants also rated their thermal sensation (ASHRAE, 2004), thermal comfort (ASHRAE, 2004) and perceived exertion (RPE; Borg, 1970). A capillary sample was drawn for determination

of BLa concentration (Biosen HbA1c, EKF-diagnostic GmbH, Germany) in order to assess physiological stress, whereby participants straddled the treadmill for BLa measures to be taken. The difference in nude BM between PRE and POST-exercise, corrected for the volume of fluid ingested and urine output was used to calculate exercise-induced BM loss.

Pre-cooling and control seated conditions

During the P-C condition, participants underwent a pre-cooling manoeuvre by means of water immersion for 60 min prior to the exercise trial. Pre-cooling involved each participant being seated and submerged in water at the height of the participant's sternal notch (Figure 6.2). A water temperature of 20.3 ± 0.3^{o}C was used in order to avoid some of the comfort issues associated with cold water immersion (Quod *et al.*, 2006). Every 10 mins, participants were required to make a slight movement of the arms and legs to avoid a 'warm pocket' of water developing close to the skin. Upon leaving the water, participant's towel dried and dressed. The seated control condition (CON) involved the participants remaining seated for 60 min in a laboratory (ambient temperature 20.2 ± 1.7^{o}C and RH 60.2 ± 2.5%). For consistency, the exercise protocol commenced 10 min following the end of both seated conditions.

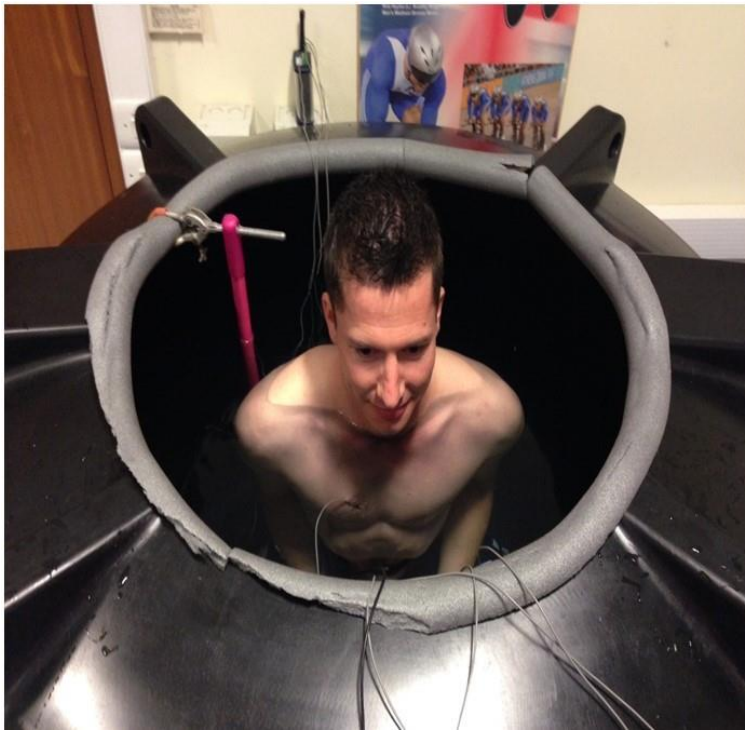


Figure 6.2 Participant undergoing the pre-cooling procedure

6.2.3 Data Analysis

Data are reported as the mean ± SD and were supported by assessing the mean difference (mean diff) and 95% confidence interval [95% CI]. The Shapiro-Wilk test was applied to the data in order to assess for normal distribution. All variables except BM loss, PV loss and cTnI were assessed using a Two-way (Condition x Time) repeated-measures ANOVA. Sphericity was analysed by Mauchly's test of sphericity followed by Greenhouse-Geisser or Huynh-Feldt adjustment where appropriate. Where any differences were identified, pairwise post-hoc comparisons with a Bonferroni correction were used in order to show where they lay. The change in PRE to POST BM and PV was analysed using a Student's paired *t*-test. cTnI were analysed descriptively because of the likelihood of undetectable PRE-exercise values. All statistical analyses were conducted using IBM SPSS Statistics 24 (IBM Corp., Armonk, NY, USA). An alpha level of $p < 0.05$ was considered statistically significant. Furthermore, effect size using Cohen's *d* and

partial eta squared (η_p^2) were calculated, as described in Section 4.2.3. Cardiac biomarkers were also compared to the upper reference limits of an apparently healthy population.

6.3 Results

Eight participants (mean ± SD; age 28 ± 6 years, height 1.76 ± 0.08 m, BM 72.6 ± 12.5 kg and VO_{2PEAK} 53 ± 6 mL.kg^{-1}.min^{-1}) completed both trials, with two participants being withdrawn due to T_c reaching the safety cut-off criteria. BM loss was similar between conditions (CON: 2.2 ± 0.6 kg, P-C: 2.1 ± 0.4 kg), with relative BM loss being similar between CON (3.2 ± 1.1%) compared to P-C (2.9 ± 0.6%). PV loss was also similar between conditions (CON: 8 ± 8, P-C: 6 ± 10). A small insignificant interaction between condition and time for exercise HR was observed. There was an insignificant small effect between conditions, whilst a large significant effect was seen across time ($F_{(1.955,13.688)}$ = 251.210, $p < 0.001$; η_p^2 = 0.97). Post-hoc analysis revealed 0 min to be lower than all exercise time points ($p < 0.001$; Figure 6.3a). A progressive increase in HR was apparent with 15 min being significantly lower than the subsequent time points ($p < 0.01$), with a number of time points being significantly lower than the final 90 min time point (Figure 6.3a). A large significant interaction between condition and time for T_c was observed ($F_{(1.962,13.737)}$ = 11.723, p = 0.001; η_p^2 = 0.63) whereby T_c was significantly higher between 0 and 45 min in the CON condition compared to P-C (Figure 6.3b). T_c was significantly higher following CON compared to P-C to a large extent (mean diff = 0.40; $F_{(1.000,7.000)}$ = 7.375, p = 0.030; 95% CI [0.05, 0.75]; η_p^2 = 0.51). A large significant effect was seen across time for T_c ($F_{(1.348,9.437)}$ = 83.661, $p < 0.001$; η_p^2 = 0.92) with post-hoc analysis showing significant differences between all comparisons apart from PRE to 15 min and 75 min to 90 min (Figure 6.3b). A reduction in T_c following the P-C condition was apparent, with an increase in T_c in both conditions to a similar point at exercise cessation.

A large significant interaction between condition and time was observed for T_{sk} ($F_{(2.461,17.224)} = 5.651$, $p = 0.010$; $\eta_p^2 = 0.45$), whereby T_{sk} was significantly higher at 0 and 15 min following CON compared to P-C (Figure 6.3c). A large significant effect for condition (mean diff = 0.96; $F_{(1.000,7.000)} = 33.887$, $p = 0.001$; 95% CI [0.57, 1.35]; $\eta_p^2 = 0.83$), whereby CON was higher than P-C was seen for T_{sk}, as well as a large significant effect for time ($F_{(7,49)} = 43.509$, $p < 0.001$; $\eta_p^2 = 0.86$; Figure 6.3c). Heat storage demonstrated a large significant interaction between condition and time ($F_{(1.490,10.430)} = 13.196$, $p = 0.002$; $\eta_p^2 = 0.65$), whereby heat storage was significantly different at 0 min (Figure 6.3d). An insignificant moderate effect was seen for condition, however, a large effect for time was observed ($F_{(1.900,13.302)} = 129.432$, $p < 0.001$; $\eta_p^2 = 0.95$) with post-hoc analysis showing significant heat gain between 0 and 60 min, with all possible interactions being below $p = 0.017$, after which there was a plateau in heat gain. A large insignificant interaction between condition and time was apparent for BLa, with a large insignificant effect between conditions. A moderate significant effect was observed for time ($F_{(4,28)} = 12.127$, $p < 0.001$; $\eta_p^2 = 0.63$; Figure 6.3e), with post-hoc analysis showing increases from PRE at 30 min (mean diff = 0.6; $p = 0.032$; 95% CI [0.1, 1.2]) and 60 min (mean diff = 1.2; $p = 0.001$; 95% CI [0.6, 1.8]). BLa at 30 min was also lower than 60 min (mean diff = 0.6; $p = 0.044$; 95% CI [0, 1.1]).

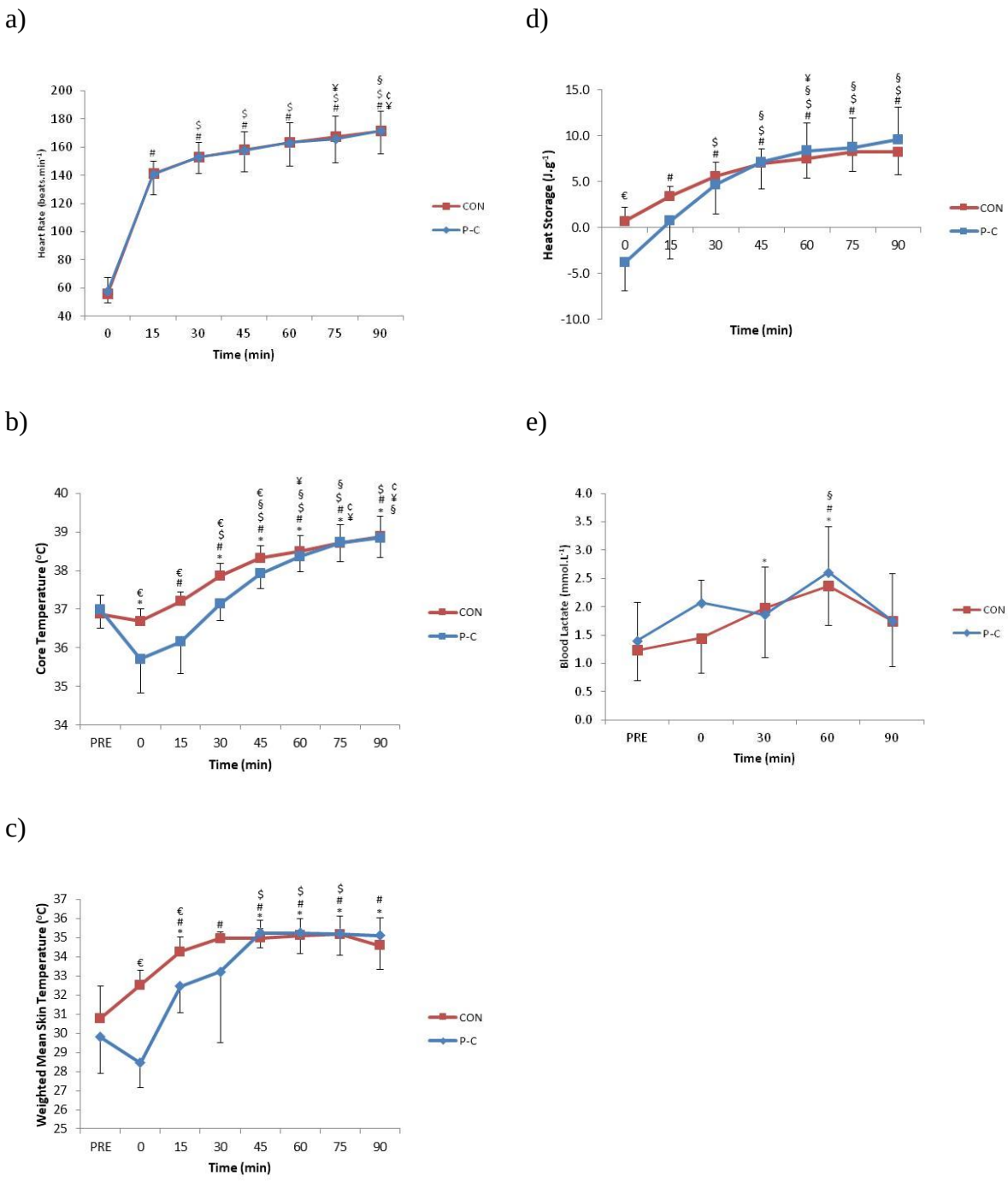
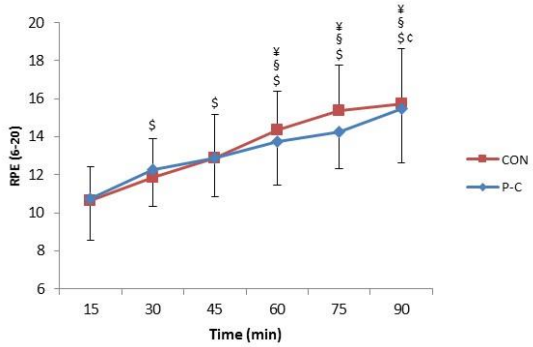


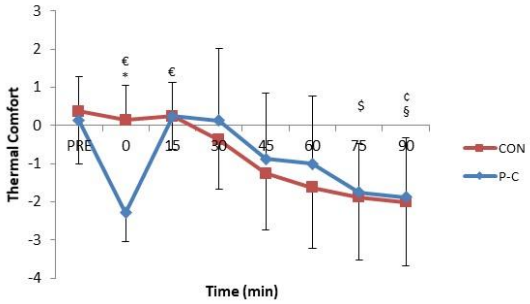
Figure 6.3 The mean ± SD of a) HR, b) T_c, c) T_{sk}, d) Heat Storage and e) BLa across assessment time points in both trials (n = 8). * Significantly different to PRE, $p < 0.05$. # Significantly different to 0, $p < 0.05$. $ Significantly different to 15, $p < 0.05$. § Significantly different to 30, $p < 0.05$. ¥ Significantly different to 45, $p < 0.05$. ¢ Significantly different to 60. € Significantly different between CON and P-C, $p < 0.05$.

RPE demonstrated a large insignificant interaction between condition and time, with a large effect for condition shown. A large significant effect for time was seen for RPE ($F_{(1.662,11.636)}$ = 34.434, p < 0.001; η_p^2 = 0.83), with post-hoc analysis revealing a progressive increase in RPE (Figure 6.4a). A large significant interaction between condition and time was observed for thermal sensation ($F_{(7,49)}$ = 6.407, p < 0.001; η_p^2 = 0.48; Figure 6.4b), with CON being significantly higher than P-C at 0 and 15 min. Thermal sensation was found to be higher within the CON trial compared to the P-C as a large significant effect for condition was found (mean diff = 1; $F_{(1.000,7.000)}$ = 7.936, p = 0.026; 95% CI [0, 1]; η_p^2 = 0.53). A large significant effect for time has also been found ($F_{(2.339,16.373)}$ = 65.259, p < 0.001; η_p^2 = 0.90), with post-hoc analysis revealing a progressive increase in thermal sensation. A large significant interaction was found between condition and time for thermal comfort ($F_{(3.011,21.079)}$ = 3.994, p = 0.021; η_p^2 = 0.36), with a significant difference at 0 and 15 min (Figure 6.4c). An small insignificant effect was seen for condition, whilst a large significant effect for time was found ($F_{(1.810,12.669)}$ = 8.021, p = 0.007; η_p^2 = 0.36), with post-hoc analysis revealing a progressive decline in thermal comfort.

a)



c)



b)

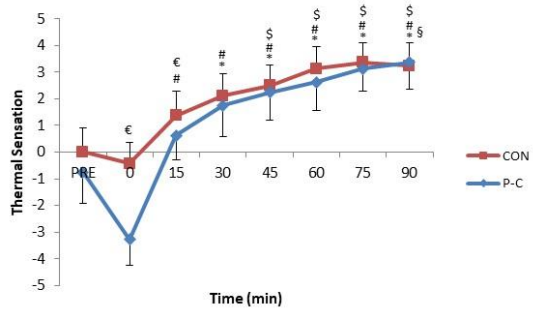


Figure 6.4 The mean ± SD of a) RPE, b) Thermal Sensation and c) Thermal comfort across assessment time points in both trials (n = 8). * Significantly different to PRE, $p < 0.05$. # Significantly different to 0, $p < 0.05$. $ Significantly different to 15, $p < 0.05$. § Significantly different to 30, $p < 0.05$. ¥ Significantly different to 45, $p < 0.05$. ¢ Significantly different to 60, $p < 0.05$.

6.3.1 Echocardiographic Data

6.3.1.1 Loading

LVEDV demonstrated an insignificant moderate interaction between condition and time as well as an insignificant moderate effect for condition. A large significant effect for time was shown ($F_{(5,35)} = 6.018$, $p < 0.001$; $\eta_p^2 = 0.46$), with post-hoc analysis revealing POST to be lower than PRE (mean diff = -15; $p = 0.005$; 95% CI [-25, -5]) and 2 h POST (mean diff = -18; $p = 0.041$; 95% CI [-35, -1]), and 1 h POST lower than 2 h POST (mean

diff = -10; p = 0.043; 95% CI [-20, 0]; Table 6.1). HR showed an insignificant moderate interaction between condition and time, with an insignificant small effect for condition also seen. A large significant effect for time was observed ($F_{(5,35)}$ = 8.610, p < 0.01; η_p^2 = 0.55), with post-hoc analysis revealing POST to be higher than PRE (mean diff = 29; p = 0.015; 95% CI [6, 52]), 2 h POST (mean diff = 22; p = 0.036; 95% CI [1, 42]), 4 h POST (mean diff = 22; p = 0.015; 95% CI [4, 39]) and 24 h POST (mean diff = 25; p = 0.002; 95% CI [10, 40]; Table 6.1). A large insignificant interaction for SBP was observed between condition and time, whilst a moderate effect for condition was seen. A large effect for time was demonstrated ($F_{(5,35)}$ = 6.791, p < 0.001; η_p^2 = 0.49), with post-hoc analysis revealing 1 h POST to be lower than 4 h POST (mean diff = -10; p = 0.004; 95% CI [-17, -4]) and 24 h POST (mean diff = -10; p = 0.048; 95% CI [-19, 0]; Table 6.1). DBP demonstrated a large insignificant interaction between condition and time, with a large insignificant effect for condition as well as time (Table 6.1). A moderate insignificant interaction between condition and time was observed for E:E', with a small insignificant effect for condition and moderate insignificant effect for time (Table 6.1).

Table 6.1 LV loading across assessment points in both conditions (n = 8, mean ± SD).

Variable	CON						P-C					
	PRE	POST	1 h POST	2 h POST	4 h POST	24 h POST	PRE	POST	1 h POST	2 h POST	4 h POST	24 h POST
LVEDV (ml)	155±24	146±35	150±26	164±22	155±28	148±22	157±24	136±18	146±19	154±16	150±21	153±24
HR (beats.min^{-1})	59±15	89±17	83±28	65±14	65±13	66±20	62±15	90±16	71±15	70±9	70±5	63±13
SBP (mmHg)	130±8	124±11	122±11	129±13	132±13	130±9	132±10	125±7	120±12	121±12	132±11	131±13
DBP (mmHg)	70±8	74±15	66±11	72±13	70±11	70±13	71±10	65±11	64±11	65±10	71±11	69±10
E:E’	7±1	7±3	6±1	6±1	6±1	7±1	7±1	6±2	6±2	6±1	7±1	6±1

LVEDV: left ventricular end diastolic volume, HR: heart rate, SBP: systolic blood pressure, DBP: diastolic blood pressure, E:E’: peak early trans-mitral flow/peak early myocardial tissue velocity. PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively.

6.3.1.2 LV Systolic Function

EF demonstrated a large insignificant interaction between condition and time, with a post-hoc power analysis of 0.7. A small insignificant effect for condition and a large significant effect for time was observed ($F_{(2.166,15.161)}$ = 7.413, p = 0.005; η_p^2 = 0.51), with post-hoc analysis revealing PRE to be higher than 2 h POST (mean diff = 5; p = 0.038; 95% CI [0, 9]; Table 6.2). Some individual variation was apparent, with some individuals demonstrating elevated levels POST, whilst others showed reduced values (Figure 6.5a). A large interaction between condition and time for SV was observed ($F_{(5,35)}$ = 2.771, p = 0.033; η_p^2 = 0.28), with 24 h POST being lower in the CON trial compared to P-C (mean diff = -10; p = 0.024; 95% CI [-19, -2]). A small insignificant effect for condition was shown, whilst a large significant effect for time was found ($F_{(5,35)}$ = 9.637, p < 0.001; η_p^2 = 0.58), with post-hoc analysis revealing PRE to be higher than POST (mean diff = 17; p = 0.034; 95% CI [1, 33]), 1 h POST (mean diff = 14; p = 0.031; 95% CI [1, 28]) and 4 h POST (mean diff = 6; p = 0.008; 95% CI [2, 10]; Table 6.2). The majority of participants demonstrated a similar post-exercise response (Figure 6.5b). A moderate insignificant interaction between condition and time, with a small insignificant effect for condition was found for SBP/LVESV. A large significant effect for time was observed ($F_{(5,35)}$ = 6.511, p < 0.001; η_p^2 = 0.48), with post-hoc analysis revealing PRE to be higher than 2 h POST (mean diff = 0.28; p = 0.045; 95% CI [0.01, 0.55]; Table 6.2), with a varied response between participants (Figure 6.5c). S' demonstrated a moderate insignificant interaction between condition and time, whilst a large insignificant effect for condition was seen. A large significant effect for time was found ($F_{(5,35)}$ = 2.615, p = 0.041; η_p^2 = 0.27; Table 6.2), however, post-hoc analysis revealed no significant differences with individual variation apparent in response to exercise and condition (Figure 6.5d).

Table 6.2 LV systolic function across assessment points in both conditions (n = 8, mean ± SD).

Variable	CON						P-C					
	PRE	POST	1 h POST	2 h POST	4 h POST	24 h POST	PRE	POST	1 h POST	2 h POST	4 h POST	24 h POST
EF (%)	55±5	48±7	48±7	52±7	52±4	49±4	55±5	49±6	47±5	49±4	52±5	54±6
SV (ml)	85±19	72±27	74±22	85±22	81±20	73±16	86±16	66±13	69±14	75±9	79±18	83±20
SBP/LVESV	1.89±	1.68±	1.59±	1.67±	1.80±	1.75±	1.89±	1.72±	1.56±	1.55±	1.88±	1.89±
($mmHg \cdot ml^{-1}$)	0.21	0.16	0.21	0.29	0.23	0.18	0.36	0.20	0.14	0.21	0.29	0.31
S' ($cm.s^{-1}$)	8±1	9±1	9±1	9±1	9±1	8±1	8±1	9±2	9±1	10±1	9±1	9±1

EF: ejection fraction, SV: stroke volume, SBP/LVESV: systolic blood pressure/left ventricular end systolic volume, S': peak systolic myocardial tissue velocity. PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively.

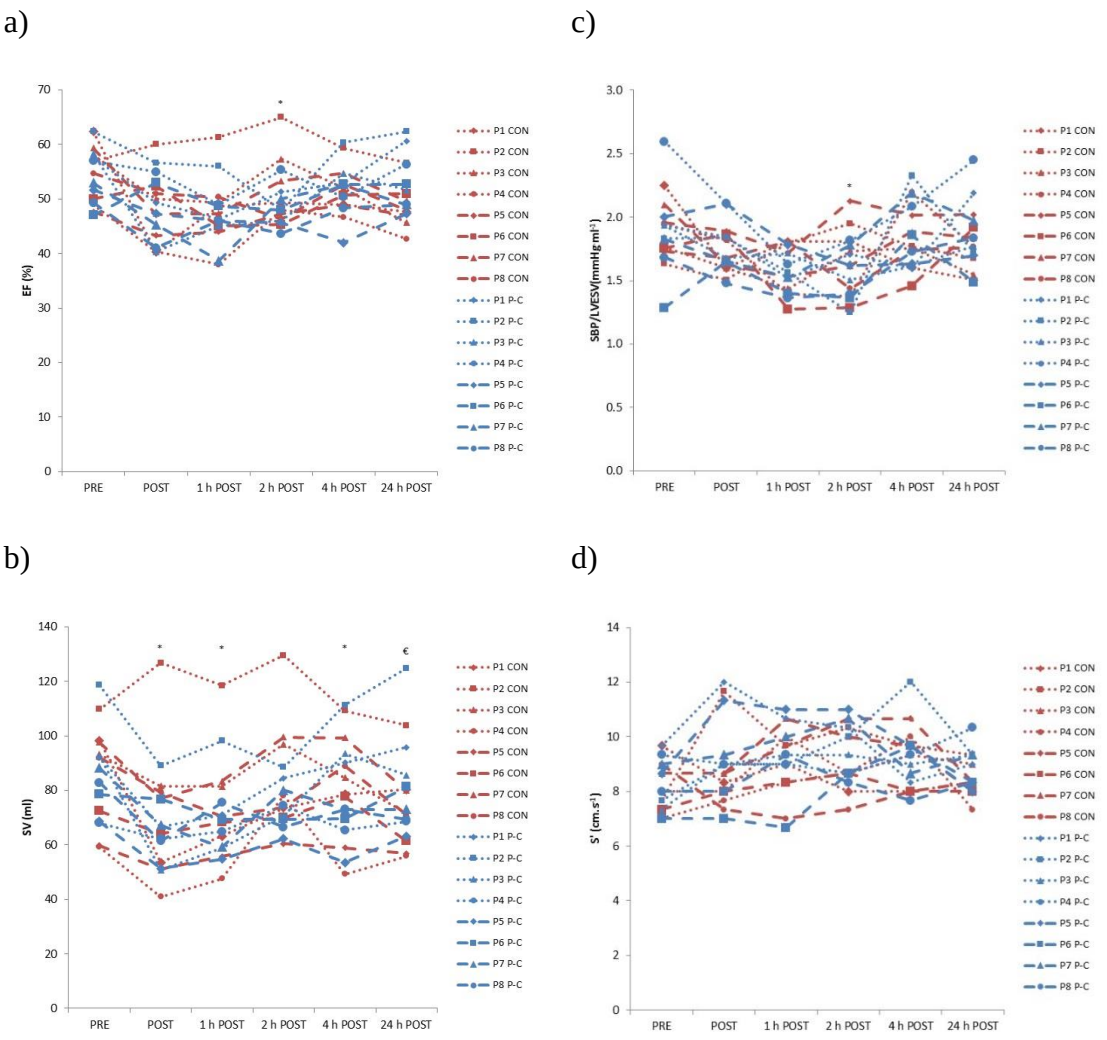


Figure 6.5 Individual heterogeneity for LV systolic function across assessment points in both conditions where a) EF, b) SV, c) SBP/LVESV and d) S' (n = 8). PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively. * Significantly different to PRE. € Significantly different between CON and P-C, $p < 0.05$.

6.3.1.3 LV Diastolic Function

A large insignificant interaction between condition and time for E was observed, with a small insignificant effect for condition. A large significant effect for time was seen ($F_{(5,35)} = 12.145$, $p < 0.001$; $\eta_p^2 = 0.63$; Table 6.3), however, post-hoc analysis revealed no significant differences. The majority of participants demonstrated a decrease in response

to both conditions post-exercise (Figure 6.6a). A large interaction between condition and time was seen for A ($F_{(5,35)}$ = 2.802, p = 0.031; η_p^2 = 0.29), however, post-hoc analysis revealed no differences between conditions at any time points. A large insignificant effect for condition was shown, with a large significant effect for time ($F_{(5,35)}$ = 3.535, p = 0.011; η_p^2 = 0.34; Table 6.3), whilst again post-hoc analysis showed no significant differences. Some individual variation was apparent, with some individuals demonstrating elevated levels POST, whilst others showed reduced values (Figure 6.6b). As a consequence, E:A demonstrated a large insignificant interaction between condition and time, with a post-hoc power analysis of 0.3. A moderate insignificant effect for condition was also shown. A large significant effect for time was found ($F_{(5,35)}$ = 14.638, p < 0.001; η_p^2 = 0.68; Table 6.3), with post-hoc analysis revealing PRE to be higher than POST (mean diff = 0.82; p = 0.047; 95% CI [0.01, 1.62), 1 h POST (mean diff = 0.66; p = 0.008; 95% CI [0.18, 1.14]) and 2 h POST (mean diff = 0.60; p = 0.012; 95% CI [0.13, 1.07]). POST was significantly lower than 24 h POST (mean diff = -0.7; p = 0.017; 95% CI [-1.32, -0.13]), as was 1 h POST (mean diff = -0.57; p = 0.009; 95% CI [-1.00, -0.15]) and 2 h POST (mean diff = -0.51; p = 0.017; 95% CI [-0.93, 0.09]). The majority of participants demonstrated a decrease to both conditions post-exercise (Figure 6.6c). E' demonstrated an insignificant moderate interaction between condition and time and a small effect for condition. A large significant effect for time was observed ($F_{(5,35)}$ = 15.378, p < 0.001; η_p^2 = 0.69; Table 6.3) with post-hoc analysis showing POST was significantly lower than PRE (mean diff = -3; p = 0.012; 95% CI [-5, -1]), 1 h POST (mean diff = -2; p = 0.019; 95% CI [-3, 0]), 2 h POST (mean diff = -2; p = 0.006; 95% CI [-3, -1]), 4 h POST (mean diff = -3; p = 0.001; 95% CI [-4, -1]) and 24 h POST (mean diff = -3; p = 0.003; 95% CI [-5, -1]). 1 h POST was also significantly lower than 24 h POST (mean diff = -1; p = 0.026; 95% CI [-3, 0]). The majority of participants again demonstrated a decrease to both

conditions post-exercise (Figure 6.6d). An insignificant small interaction between condition and time was observed for A'. Large but insignificant effects for both condition and time were also seen (Table 6.3). However, post-hoc analysis showed a significant increase in A' at 1 h POST compared to PRE (mean diff = 2; p = 0.026; 95% CI [0, 3]) and 24 h POST (mean diff = 1; p = 0.047; 95% CI [0, 3]). A' showed an apparent individual variation post-exercise (Figure 6.6e). As a result, E':A' showed an insignificant moderate interaction between condition and time. A moderate insignificant effect for condition was seen, whilst a large significant effect for time was observed ($F_{(5,35)}$ = 13.911, p < 0.001; η_p^2 = 0.67). Post-hoc analysis revealed PRE to be significantly higher than POST (mean diff = 0.69; p = 0.014; 95% CI [0.14, 1.23]), 1 h POST (mean diff = 0.60; p = 0.001; 95% CI [0.30, 0.90]) and 2 h POST (mean diff = 0.49; p = 0.022; 95% CI [0.07, 0.90]). POST was significantly lower than 24 h POST (mean diff = -0.60; p = 0.015; 95% CI [-1.08, -0.12]), whilst 1 h POST was significantly lower than 4 h POST (mean diff = -0.24; p = 0.024; 95% CI [-0.45, -0.03]) and 24 h POST (mean diff = -0.51; p = 0.007; 95% CI [-0.88, -0.15]). Similar to other variables, the majority of participants again demonstrated a decrease in response to both conditions post-exercise (Figure 6.6f).

Table 6.3 LV diastolic function across assessment points in both conditions (n = 8, mean ± SD).

Variable	CON						P-C					
	PRE	POST	1 h POST	2 h POST	4 h POST	24 h POST	PRE	POST	1 h POST	2 h POST	4 h POST	24 h POST
E ($cm.s^{-1}$)	84±11	60±19	67±15	69±15	74±12	77±15	85±12	57±18	63±17	71±14	78±12	82±11
A ($cm.s^{-1}$)	43±11	45±7	48±16	48±12	49±14	38±9	42±7	55±15	47±11	49±11	46±12	45±11
E:A	2.11±0.67	1.42±0.60	1.48±0.47	1.51±0.48	1.65±0.58	2.12±0.67	2.11±0.55	1.16±0.53	1.40±0.48	1.50±0.40	1.81±0.54	1.91±0.52
E' ($cm.s^{-1}$)	12±1	9±2	11±2	11±2	12±2	12±2	13±2	10±1	11±2	11±2	11±2	13±1
A' ($cm.s^{-1}$)	7±1	8±3	8±2	8±2	8±2	7±1	7±1	9±2	9±2	8±2	8±1	7±2
E':A'	1.97±0.46	1.40±0.66	1.42±0.30	1.52±0.55	1.66±0.49	1.83±0.48	1.96±0.55	1.17±0.36	1.32±0.29	1.44±0.33	1.57±0.40	1.94±0.61

E: peak early trans-mitral flow velocity, A: peak atrial filling velocity, E:A: early to late diastolic filling ratio, E': peak early diastolic myocardial velocity, A': peak late diastolic myocardial velocity, E'/A': peak early to late myocardial tissue velocity ratio. PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively.

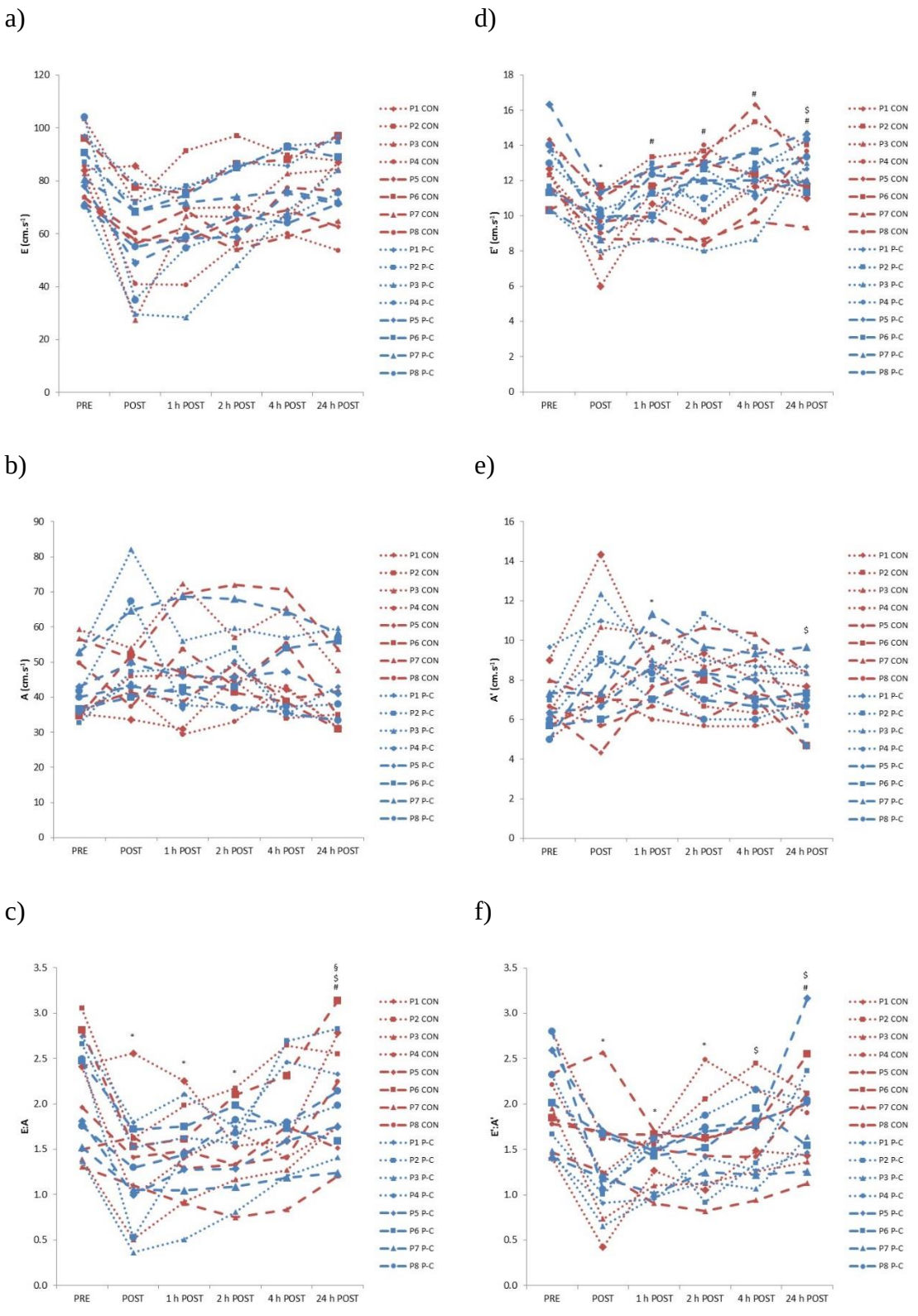


Figure 6.6 Individual heterogeneity for LV diastolic function across assessment points in both conditions where a) E, b) A, c) E:A, d) E', e) A' and f) E':A' (n = 6). PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively. * Significantly different to PRE,

$p < 0.05$. # Significantly different to POST, $p < 0.05$. $ Significantly different to 1 h POST, $p < 0.05$. § Significantly different to 2 hr POST, $p < 0.05$.

6.3.2 Cardiac Biomarkers

cTnI was not detectable in serum samples taken PRE-exercise in both conditions (Figure 6.8). However, by the 2 h POST assessment, all participants demonstrated detectable values, with all but one participant (participant 1 CON) demonstrating values above the upper reference limit by the 4 h POST assessment. Whilst this time point displayed a number of peak values, some participants demonstrated a peak in cTnI at the 24 h POST assessment. Within and between participants, there was no consistency between peak cTnI values and the time points they occurred at. Data for NT-proBNP was below the upper reference limit for a healthy population for all participants PRE-exercise (Figure 6.9) and remained below this limit for 6 out of 8 participants across all time points. Participant 5 demonstrated elevations above the upper reference limit within the P-C condition, whilst participant 6 demonstrated elevations above this limit within both conditions. A small insignificant interaction between condition and time was observed for NT-proBNP. A small insignificant effect for condition as well as an insignificant large effect for time was also found.

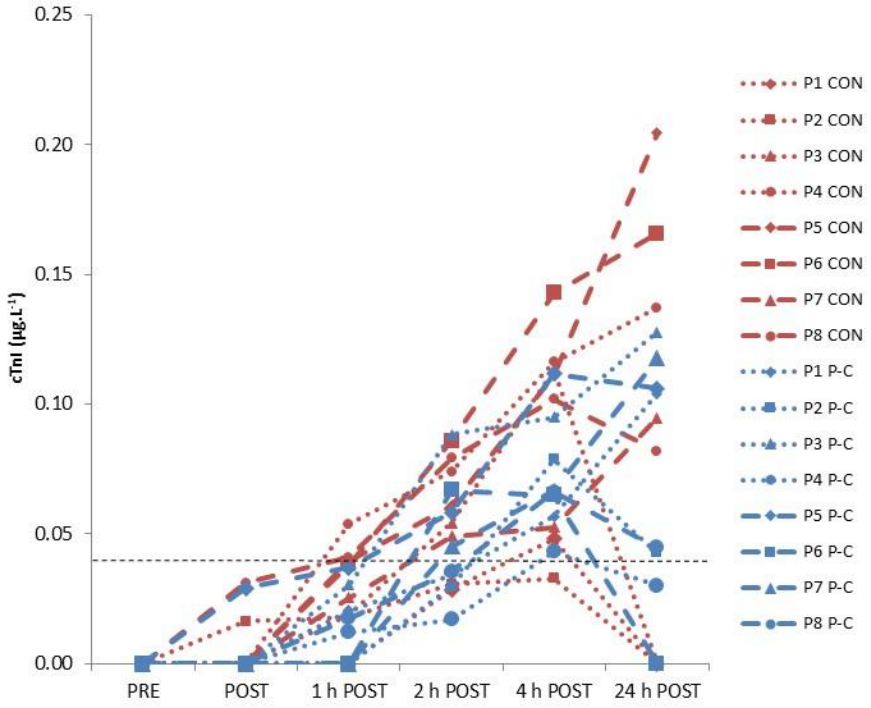


Figure 6.8 Individual data for cTnI across assessment points in both conditions. The dotted line represents the 99th percentile of an apparently healthy population (≤ 0.04 µg.L^{-1}; Uettwiller-Geiger *et al.*, 2002; n = 8). PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively.

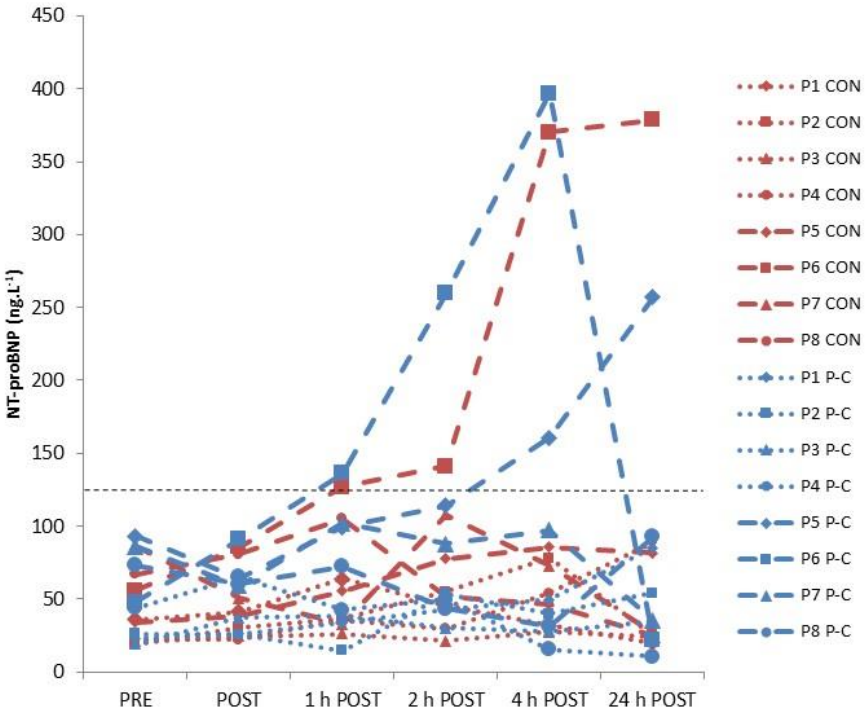


Figure 6.9 Individual data for NT-proBNP across assessment points in both conditions. The dotted line represents the upper reference limit of an apparently healthy population (125 ng.L-1; Silver et al., 2004; n = 8). PRE, POST, 1 h POST, 2 h POST, 4 h POST and 24 h POST: before, immediately after, and 1 hour, 2 hours, 4 hours and 24 hours after each trial, respectively.

6.4 Discussion

The present study represents the first attempt to manipulate T_c through pre-cooling, prior to exercise in a controlled hyperthermic environment, and then assess the impact upon LV cardiac function and markers of cardiac damage. The findings indicate that some measures of systolic function were reduced POST-exercise compared to PRE, whilst consistent evidence of diastolic dysfunction was apparent. Similar changes in cardiac function between conditions were observed, with a marked heterogeneity between participants in response to both conditions and exercise time points. cTnI was elevated above detection levels in all participants in both conditions, demonstrating heterogeneity between participants and conditions. There was no significant change in NT-proBNP

across time points, nor was there a difference between conditions. Therefore, overall there was no effect of pre-cooling upon measures of cardiac function and damage.

The key focus of this study was to compare the thermal and cardiovascular stress of exercise in a hyperthermic environment and any potential effect of pre-cooling on LV cardiac function and biomarker appearance during recovery. Therefore, initial consideration as to whether the two exercise bouts presented differing thermal and cardiovascular stresses must be considered. The pre-cooling intervention significantly reduced T_c and T_{sk} at the start of the P-C trial compared to CON and delayed the rise in T_c and T_{sk} during the first 30 min of exercise, with most differences disappearing before the end of the protocol. The P-C trial also demonstrated a greater heat storage compared to CON trial during first 30 min. It was apparent in the P-C trial that the lowering of T_{sk} prior to exercise had increased the temperature gradient between the skin and the environment in comparison to the CON trial. As the environmental temperature was greater than T_{sk}, it is therefore possible that the use of pre-cooling increased the potential for heat gain through convection, aided by the airflow produced by the fan. Whilst airflow was similar between conditions, it has been shown to aid heat exchange through convection and evaporation (Saunders *et al*., 2005). Therefore, it may well explain the greater increase in T_{sk} and heat gain in the first 15 min of the P-C trial in comparison to the CON. Overall it is likely that whilst there was a significant difference in thermal stress between trials, this was of a limited duration, evidenced by a similar $T_{c\ and}$ T_{sk} at the end of both trials.

BM and PV loss between conditions was similar, however, this can be attributed to metabolic factors such as substrate loss and muscle breakdown (Hoffman *et* al., 2018; Maughan *et al.,* 2007) and may be a result of the amount of heat lost through the

evaporation of sweat. Heat loss through sweat is largely determined by metabolic heat production and environmental heat load (Neilsen, 1996), both of which were the same for both trials within this study.

The present study also found no HR difference between conditions, despite the difference in T_c up to 45 mins, with a similar magnitude of cardiovascular drift as exercise continued. The increase in T_{sk} across both trials demonstrates a decrease in evaporative cooling efficiency and likely an increase in peripheral vasodilation and cutaneous blood volume (González-Alonso *et al*., 1999), resulting in a decrease in central blood volume during exercise (Coyle and Gonzalez-Alonso, 2001). It is also likely that the similar rate of intravascular fluid loss through sweat production was also a confounding factor to the observed similarity in cardiovascular drift between trials. A combination of blood volume redistribution and intravascular fluid loss may have resulted in a reduction in venous return (Rowell *et al.,* 1966, 1969), therefore, leading to an elevated HR in order to maintain cardiac output in both trials. It is likely that the total myocardial work performed was similar between trials. This is also reflected by a progressive increase in RPE as exercise duration increases, with no significant difference in RPE between trials. Thermal comfort and sensation also only differed between trials immediately following the cooling or controlled procedure. Similar BLa concentrations were observed between conditions, suggestive of a maintained muscle blood flow during both trials. The similar BLa, HR and RPE would, therefore, suggest similar physiological stressors between trials and likely explains the lack of difference in LV function and cardiac biomarkers.

6.4.1 LV Systolic Function

Systolic function was significantly reduced at POST-exercise time points compared to PRE in both trials, with no difference in function between conditions apart from a

reduction in SV 24 h POST in the CON trial. It is possible that the reduction in PV seen in both trials may partially explain some of the change in systolic function. The average change in EF was similar between trials (12% reduction in CON, 11% reduction in P-C), likely due to the similarity in cardiovascular load between conditions. The change in EF is within a range expected from participant and technical variability and may partially be due to an increase in peripheral vasodilation and cutaneous blood volume (González-Alonso *et al*., 1999). Therefore, it is probable that the rate of fluid loss through sweat production, along with peripheral vasodilation may have partly led to a reduction in venous return and therefore reduced LV preload. A significant decrease in SBP/LVESV was apparent 2 hr POST exercise. Whilst a small amount of change could be attributed to measurement variability, a change of up to 16% is unlikely due to measurement error alone. Research by Charkoudian *et al*., (2003) has suggested 90 min of cycling can result in an decreased post-exercise SBP through exercise induced dehydration of 1.6% BM compared to a saline infused maintenance of hydration. Therefore, the decrease in SBP/LVESV in the present study may be due to a decreased SBP post-exercise through changes in hydration status or alternately post-exertional hypotension (MacDonald, 2002). This is further supported by an increase in S' suggesting an increased inotrophy, however, S' is localized in nature by only being representative of the septal wall.

6.4.2 LV Diastolic Function

Changes in diastolic filling parameters can be affected by changes in loading and HR (Burns *et al*., 2007; Dawson *et al*., 2003; Oxborough *et al*., 2010) which may have an impact on the current findings due to the speculated changes in blood volume distribution, particularly immediately POST-exercise. Myocardial tissue velocities did not appear to follow the same pattern of change as measures of HR or loading, suggesting the alteration in LV filling pattern may only be partially related to blood pool redistribution induced by

exercise and heat stress, supported by a lack of change in left atrial driving pressure (E:E'). The decrease in E, with little or no compensation in A, reflects an intrinsic alteration in LV pressure decay due to impaired relaxation/myocardial stiffening during diastole, compliance and atrial contractility (Middleton *et al*., 2006a).

There were no differences in diastolic variables between trials, however, given the limited difference in thermal burden and cardiovascular stress as previously discussed (determined *post hoc*), this is to be expected. The insignificant reduction in diastolic function found in Chapter 5 and by Scharhag *et al.* (2006) following 60 minutes exercise, compared to an increased duration and apparent diastolic dysfunction in the present study and that by Tian *et al*. (2014) suggest a minimum duration dependant threshold for diastolic functional changes. The E:A ratio decreases did not exceed what would be expected due to measurement variability but were significantly reduced in some participants below the clinical cut-off for diastolic dysfunction (less than 1.0; Lang *et al.*, 2015), however, changes appeared to be transient in nature as a recovery in diastolic measures before 24 hr POST was observed. No participants reported any clinical signs and symptoms and changes did not appear to impact upon exercise performance, suggestive of limited clinical significance.

6.4.3 Cardiac Biomarkers

cTnI was undetectable in all serum samples taken PRE-exercise and is likely explained by the training status and control of exercise prior to each trial in this laboratory based study. Detectable cTnI values were observed in all participants by the 2 h POST assessment point, with all but one participant (participant 1 CON) demonstrating values above the upper reference limit for a healthy population by the 4 h POST assessment point. It is pertinent to note here that across both trials only 3 participants demonstrated

elevated cTnI levels immediately after exercise cessation, highlighting the importance of repeated measures for cTn post-exercise. The current study also revealed a peak in cTnI in some participants at 24hr POST-exercise, whilst others showed a normalization in cTnI at the same time point. Typically a normalization in cTn is expected after 24 (Middleton *et al.*, 2006b; Shave *et al.*, 2004c) to 48 hours (Melanson *et al*., 2006) post-exercise. Therefore, it may be speculated that a true peak value may have occurred between the 4 h POST and 24 h POST exercise data collection points, however, it was not possible to evaluate the normalization of elevated values in all participants due to the absence of blood sample collection at 48 hours post-exercise. There was no clear evidence of an effect of pre-cooling on cTnI with participant peak values varying across trials. The lack of difference between cardiovascular stress i.e. exercise intensity and thermal stress between trials may explain the current findings. A study by Tian *et al.* (2014) suggested a blunting effect of cTn increase in the second exercise trial compared to the first. This was not apparent in the present study given there was no order effect of the trials on cTnI. The findings from the current and previous studies suggests the likely mechanisms for cTn release to be a physiological response due to the high percentage of participants with increased cTn post-exercise, low absolute levels and a rapid normalization of values, representing cTn release from the "unbound" cytosolic reservoir rather than necrosis (Gresslien and Agewall, 2016; Shave *et al.* 2010). The individual variation demonstrated within the current study, however, warrants further investigation.

Data for NT-proBNP was below the upper reference limit for a healthy population for all participants PRE-exercise and remained below this limit for 6 out of 8 participants across all time points, with no significant increase across time. Any small changes in NT-proBNP likely reflect the limited hemodynamic stress of a 90 min exercise bout at a relatively moderate intensity. Serrano-Ostáriz *et al,* (2011) suggest that once an

exercising HR exceeds 100-120 beats.min^{-1}, exercise duration exerts a bigger influence on NT-proBNP release (Legaz-Arrese *et al.* 2011). This is supported by the higher NT-proBNP concentrations in the present study compared to smaller concentrations following 60 min running in Chapter 5. The findings from Chapter 5, however, suggested a more complex interaction between exercise intensity and duration given a greater NT-proBNP concentration was found within a hyperthermic environment compared to a normothermic environment. As NT-proBNP is elevated in response to volume overload and myocyte stretch (Shave *et al*., 2007b), this finding was attributed to a greater total of myocardial work within the heat. Within the present study there was no difference in NT-proBNP concentration between trials, supporting the earlier statement that there was a limited difference in cardiovascular stress between trials. It is unclear why 2 participants demonstrated elevated concentrations at the 4 hr POST or 24 h POST assessment points. NT-proBNP demonstrates a half-life of around 120 minutes (Hall, 2004), therefore, any peak values were likely to have been detected within the earlier sampling points of the present study.

6.4.4 Limitations

Within the present study, a controlled laboratory-based design was chosen to allow the appropriate control and comparison of a hyperthermic environment with the manipulation of T_c in a repeated measures design. The volume of exercise, whilst controlled for duration and intensity, was greater than that in Chapter 5, however, was lower than the stimulus reported in previous field-based studies which have evidenced cardiac dysfunction and elevated cardiac biomarkers (Chapter 3 and 4; George *et al.* 2005; Whyte *et al*. 2005). The exercise duration was selected to ensure the majority of participants completed both trials before an unethical T_c was reached and exercise was terminated. Whilst this may have limited the exercise dose performed, 2 of the initial 10 participants

were unable to complete the exercise duration due to an elevated T_c, leading to an underpowered EF and E:A. Therefore, whilst the duration of exercise may have limited the expression of any systolic functional changes, it allowed the evaluation between trials of the same running speed and exercise duration. Another consequence of a controlled laboratory-based design is the use of a fixed intensity and duration exercise protocol. Whilst such protocols lack the ecological validity for the application to athletic environments, it has been previously noted that systolic and diastolic functional changes as well as elevations in markers of cardiac damage are influenced by exercise intensity and/or duration (Gresslien and Agewall, 2016; Middleton *et al.*, 2006a; Oxborough *et al*., 2010). The completion of a fixed distance would enable a comparison between the effectiveness of pre-cooling given that the intensity and duration would differ between trials, but also typically pre-cooling allows for participants to exercise at a higher intensity to elicit a similar HR (Ross *et al*, 2013). Within the present study, a fan was used to allow for adequate airflow which aids evaporative and convective heat loss (Jones and Doust, 1996; Saunders *et al*., 2005) to replicate outdoor conditions. It has more recently been suggested that an adequate airflow can provide the same benefits as pre-cooling, with no extra benefit when the strategies are combined, demonstrated by no difference between T_c and T_{sk} after 15 min of fixed intensity cycling (Morrison *et al*., 2014). It may be possible that the use of a fan dampened the thermal and cardiovascular benefits that have been observed in other pre-cooling studies. Whilst cold water immersion is suggested to be the most effective method of individual pre-cooling (Bongers *et* al., 2015; Jones *et al*., 2012) and is a popular strategy employed by some athletes prior to competition (Périard *et al*., 2017), it may not be the most practical method due to issues such as time, cost, access and transportation (Ross *et al*, 2013). The present study also found a limited benefit of pre-cooling, particularly with regards to reducing cardiovascular stress. Future studies may wish to consider other methods of manipulating cardiovascular strain to evaluate the

impact upon cardiovascular function and markers of cardiac damage i.e. per-cooling (during exercise) and post-exercise cooling.

6.5 Conclusion

Diastolic, but not systolic function was significantly reduced following 90 min of running in a hyperthermic environment, with no difference apparent between pre-cooling and control conditions. The release of cTnI was evident in all participants following both trials, with a small release of NT-proBNP, again with a limited effect of pre-cooling. The results also presented a high degree of individual variation between participant's responses to the exercise stimulus in both trials of the study. Pre-cooling appeared to have no beneficial or adverse effect on the cardiovascular parameters measured in this study, therefore pre-cooling may be more useful as a perceived comfort/performance benefit. The findings from the present study extend the limited previous work evaluating the effect of environmental temperature on cardiovascular function and markers of cardiac damage.

CHAPTER 7

General Discussion

7.1 Introduction

Participation in single, or multi-day, prolonged bouts of endurance exercise has proved increasingly popular over recent years (Knoth *et al*., 2012) and provides a substantial cardiovascular challenge. A growing evidence-base suggests that the physiological demands of maintaining a high cardiac workload for a prolonged period may result in a transient impairment in cardiac function, evidenced by a reduction in LV contractile function and/or relaxation during recovery (Douglas *et al.*, 1990b; George *et al.*, 2004, 2005; Middleton *et al.*, 2007a; Shave *et al.*, 2004a, 2004c; Vanoverschelde *et al.*, 1991). An elevation in cardiac biomarkers such as cTn, a biomarker of cardiomyocyte cell insult, immediately following acute bouts of prolonged exercise in apparently healthy individuals has also been widely documented (Shave *et al*., 2007a). There is no consensus regarding the prevalence, mechanisms and clinical management of exercise-induced cTn release (Shave *et al*., 2010). A combination of factors such as exercise duration, intensity and volume, participants training experience, as well as environmental factors have been suggested to precipitate changes in ventricular function and biomarker appearance (Gresslien and Agewall, 2016; Oxborough *et al.*, 2010; Sedaghat-Hamedani *et al.*, 2015).

The main purpose of this thesis was to examine the effect of single and repeat bouts of prolonged exercise and environmental temperature upon LV function and cardiac biomarker release. In all four studies presented in this thesis, healthy individuals were examined over a variety of different exercise challenges. This chapter first summarises the findings of each study, followed by an overarching discussion of developing issues. A consideration on limitations related to the studies and potential future directions are presented, before the significance of the findings and a reflection on potential mechanisms involved.

7.2 Synopsis of Findings

All four studies focused on LV function and cardiac biomarker release in response to prolonged exercise, with the findings summarised in Table 7.1. The first two empirical studies concentrated on the design of repeated exercise bouts, an area that has received little attention in the extant literature but represents greater accumulated cardiovascular work. The findings from these studies suggested that the CV system of amateur cyclists and well trained runners coped well with the accumulated exercise stress imposed by repeated day exercise. Studies two-four focused on the effect of a hyperthemic environment, another novel area of study which may also represent a greater cardiovascular work. Within a field based environment, study two demonstrated clear evidence of cardiac dysfunction and an increase in cardiac biomarkers. We also combined the use of controlled laboratory studies, of which there are few in the literature with an environmental challenge. The participants in study three coped well with the exercise stress, however the exercise dose in the laboratory likely negated any meaningful impact upon cardiac function and biomarker release. The final study was the first to use an intervention within this area of study, however, pre-cooling appeared to have no beneficial or adverse effect on the cardiovascular parameters measured.

Table 7.1 Summary of study findings

	Participants	Intensity of Exercise	Environmental Conditions	LV cardiac function	Cardiac biomarkers	Hypothesis
Study 1: Repetitive road cycling over 10 days (1666 km	Recreationally trained cyclists	Self-paced	8.2–18.8^{o}C 39-82% RH	Systolic and diastolic dysfunction was present and remained 22 h into recovery. Limited support for a cumulative change in both systolic and diastolic function.	Highly individual pattern of cTnI and NT-proBNP appearance	REJECTED
Study 2: 1) Single day 37 km 2) Multi-stage ultramarathon, 250 km over 5 days	Well trained long distance runners	Self-paced	30–40^{o}C 31-40% RH	Decrease in systolic and diastolic function Depression in systolic and diastolic function, with limited support for an accumulative effect.	Elevation in cardiac biomarkers cTnI and NT-proBNP Sporadic changes in cTnI and NT-proBNP, with limited support for an accumulation	1 - ACCEPTED 2 - REJECTED 3 - REJECTED
Study 3: 60 min normothermic trial 60 min hyperthermic trial	Recreationally trained athletes	65% VO_2peak 65% VO_2peak	13^{o}C 30^{o}C	Systolic and diastolic function were not significantly altered in either condition.	Limited release of in cTnI and NT-proBNP, with a tendency for markers to be higher in the hyperthemic condition	REJECTED
Study 4: Control + 90 min running trial Pre-cooling + 90 min running trial	Recreationally trained athletes	65% VO_2peak 65% VO_2peak	32.4 ± 0.9^{o}C, 46.8 ± 6.4% RH	Diastolic, but not systolic function was significantly reduced following 90 minutes of running, with no difference between pre-cooling and control conditions.	cTnI was evident in all participants following both trials, with a limited release of NT-proBNP that was not mediated by pre-cooling. High degree of individual variation between participant's responses to the exercise stimulus in both trials.	REJECTED

7.3 Overarching Themes

Reflection on the thesis as a whole allowed for the development of items of discussion that were beyond the scope of single studies. Consequently we have highlighted four overarching themes to explore.

7.3.1 Accumulation of Changes in Cardiac Function and Markers of Cardiac Damage over Repeated Bouts of Exercise

A major focus of this thesis was to extend our understanding of the impact of exercise on cardiac function and biomarker release from a single exercise exposure model to multi-day exercise model(s). Alterations in cardiac function and biomarker release have been well documented following a single bout of exercise (Appleman *et al.* 2014; Douglas *et al.*, 1990b; Eijsvogels *et al.*, 2014a, 2015; George *et al.*, 2004, 2005; Middleton *et al.*, 2007a; Shave *et al.*, 2004a, 2004c; Vanoverschelde *et al.*, 1991). Many athletes, however, engage in repeated bouts of exercise either during competition, through the repetitive element of training, or a combination of the two. Therefore, the evaluation of single bouts of prolonged exercise provides limited information into the practical understanding of cardiac dysfunction and biomarker release in different exercise settings. Despite the common occurrence of repeated bouts of activity, there are only a small number of studies that have focused upon the cardiovascular consequences of engaging in repeated bouts of endurance exercise (Appleman *et al.*, 2015; Bonetti *et al*., 1996; Corsetti *et al*., 2012; Denissen *et al*., 2012; Middleton *et al*., 2007b; Oosthuyse *et al*., 2012; Shave *et al*., 2002b; Williams *et al*., 2009, 2011). A number of these studies have minimal assessment points during the events (Appleman *et al.*, 2015; Bonetti *et al*., 1996; Corsetti *et al*., 2012; Denissen *et al*., 2012; Shave *et al*., 2002b; Williams *et al*., 2009), restricting the evaluation of an accumulative effect.

The current thesis (Chapters 3 and 4) demonstrated no strong evidence for a cumulative impact of repeated exercise exposures on cardiac function and biomarker release in recreational cyclists in a normothermic environment nor in well-trained runners in a hyperthermic environment. As highlighted previously, Chapter 3 demonstrated the presence of systolic and diastolic dysfunction 22 hours into recovery; similar to findings by Williams *et al.* (2009) who noted evidence of diastolic dysfunction 2 days post exercise. Chapter 4 suggested that whilst parameters of systolic and diastolic function were reduced, they recovered partially, between exercise bouts suggesting limited clinical implication. Previous research into single day exercise has suggested that training status may mediate cardiac functional responses (Middleton *et al.*, 2006a), therefore, it may be speculated that the prolonged dysfunction evidenced in Chapter 3 may well be a related to training status and required further investigation. Low concentrations of cardiac biomarkers in Chapter 3 were likely due to the low exercise intensity (Stewart *et al.*, 2016). Transient elevations in cTnI were evident in Chapter 4 with elevated samples after one stage followed by an undetectable level at the next sample point. Therefore, it is more likely that elevated cTnI levels are representative of a physiological rather than a pathological response (Eijsvogels *et al*., 2011, 2015). The participants in Chapters 3 and 4 appeared to cope well with the volume of exercise required to complete the events and appeared to have sufficient recovery opportunities, thereby minimising any accumulation occurring.

7.3.2 Field Based versus Laboratory Based Studies of Cardiac Function and Cardiac Biomarker Release

Previous research within this area has employed a broad range of research designs, however, these can largely be categorized into either 1) a field-based competitive endurance event (typically a single post-exercise assessment) or 2) a controlled

laboratory-based bout of steady-state endurance exercise. The choice of the research setting is highly dependent upon the research question proposed, and can impact upon the internal and external validity of the experiment. A laboratory setting usually allows for the tighter control of extraneous variables, thus increasing the likelihood that the observed results are due to the experimental manipulation, increasing the internal validity of the study (Thomas *et al.*, 2015). The limitation(s) of a controlled laboratory setting can, however, impact on the external validity (ability to generalize results to other participants, settings, measures etc.) of the results, which a field-based study can offer. Whilst the results of a laboratory-based study can be generalized to a larger population if the sample is representative, this generalization may only apply to the specific laboratory situation and cannot be applied to a real-world setting, reducing the ecological validity and practical significance of the study (Thomas *et al.*, 2015). Within the present thesis, we adopted both laboratory and field based research designs to answer the specific research aims and hypotheses of individual studies.

Studies one and two (Chapters 3 and 4) were field based investigations, designed to develop the theme of repeated exposures to exercise. Study one indicated that LV systolic and diastolic function were depressed in some cyclists, although this was highly variable both between and within cyclists as were elevations in cardiac biomarkers, with a general lack of a cumulative effect. The lack of data collection prior to each cycling day in study one limited data interpretation, which was addressed in the design of study two.

Study two highlighted the need to investigate the effect of environmental temperature (See Section 7.3.3) and thus we also adopted controlled laboratory based studies in studies three and four in the present thesis. Environmental conditions during field-based studies are often not reported or can be highly variable across an event and therefore, do not allow

the effect of environmental temperature to be investigated. The direct comparison in a repeated measures design required a laboratory setting to control the environmental exposure as well as other confounding variables such as exercise intensity. It is likely that the reduced exercise dose in study 3 limited any meaningful impact of cardiac function and biomarker release compared to study two. The extended duration of the exercise dose in study four not only supports the theory that diastolic functional changes precede systolic changes (Middleton *et al*., 2006a), but the apparent diastolic dysfunction also highlighted a minimum duration dependant threshold for diastolic functional changes exists.

Whilst exercise in a laboratory provides a controlled environment, exercise typically occurs in near still wind conditions unless equipment is used to produce an adequate wind speed. The presence of wind speed can influence the capacity of heat loss through both convection and evaporation (Nielsen 1996; Saunders *et al*., 2005). Saunders *et al*., (2005) demonstrated that wind still conditions or very low winds speeds during exercise, can lead to excessive heat storage through a reduced capacity of the environment to absorb the heat produced, demonstrated by higher T_c, T_{sk}, HR and RPE. Within the present thesis, the use of fans to replicate outdoor conditions (Jones and Doust, 1996) was made in a bid to increase the ecological validity of the laboratory studies. The use of a set pace of exercise increases the internal validity of the laboratory studies, however does limit the relevance to competitive situations and the exercise dose.

7.3.3 The Impact of Environmental Temperature

Many athletes engage in bouts of exercise in hot, cold, humid and high altitude environments, however limited information upon the effect of environmental temperature upon changes in LV function and biomarker appearance exists (Bonetti *et al*., 1996;

Dávila-Román *et al.*, 1997; Douglas *et al.*, 1987; Gaudreault *et* al., 2013; Neumayr *et al.*, 2001, 2002; Rifai *et al*., 1999). The limitations of a number of these studies are associated with the field-based nature of the studies, whereby a lack of control exists, particularly with no ability to manipulate environmental conditions during ultra-endurance events making it difficult to draw conclusions. With only one lab-based study focusing on the effect of environmental strain upon cardiac function and damage in a normothermic and hypothermic environment (Shave *et al*., 2004c), the current thesis provides novel insights into the impact of exercise within a hyperthermic environment. Chapter 5 demonstrated only a tendency for greater cardiac functional or biomarker changes in a hyperthermic compared to a normothermic environment, with Chapter 6 showing no differences between conditions when T_c was manipulated through pre-cooling prior to exercise in a hyperthermic environment. A clear finding from both Chapters 5 and 6 was that systolic function was not altered independently of a reduction in preload. It is probable that the rate of fluid loss through sweat production, along with peripheral vasodilation may have led to a reduction in venous return and therefore, reduced LV preload. It is also apparent that the duration of the exercise was too small of a stimulus to induce changes in systolic function. The short duration of the exercise and timing of assessments in Chapter 5 may also confounded data interpretation with regards to diastolic function and cardiac biomarkers as again the exercise stimulus was found to be too short to induce significant changes, making it difficult to evaluate the impact of environmental temperature. Overall the participants in Chapters 5 and 6 appeared to cope well with the volume of exercise and environmental conditions required to complete the exercise trials. Future studies should attempt to utilise greater durations of exercise when evaluating the effect of the environment upon cardiac function and biomarkers.

7.3.4 Individual Variation

Throughout this thesis it is apparent significant intra and inter-individual variation existed in response to the exercise demands placed upon the participants which is concurrent with other previous single (Eijsvogels *et al.*, 2015; Scott *et al*., 2009) and multi-day studies (Williams *et al*., 2011). Whilst some participants appeared to cope well with the exercise demands, others demonstrated a greater level of cardiac dysfunction and/or changes in cardiac biomarkers. A number of personal and exercise related factors have previously been implicated with both phenomena which will be discussed below, however, within the present thesis the importance of these factors is a matter of speculation.

A novel finding from Chapter 4 was a reduction in global systolic function (EF and SV) following exercise of a near marathon distance in a hyperthermic environment. Previous literature typically suggests no changes in systolic function, as measured by EF, observed following marathon distances (George *et al*., 2006; Middleton *et al*., 2006b; Whyte *et al*., 2005), however, the duration of the exercise was much greater in Chapter 4, which has previously been implicated in the aetiology of systolic dysfunction (Middleton *et al*., 2006a; Oxborough *et al*., 2010). A meta-analysis found exercise duration and fitness status had a significant impact upon changes in EF. Untrained individuals completing moderate duration exercise (> 3 hours) and trained individuals completing ultra-long duration exercise (> 10 hours) demonstrated a reduction in EF of 4.5% and 6% respectively (Middleton *et al*., 2006a), whereas more recently Serrano Ostariz *et al*. (2013) found no association between race duration or training status and systolic dysfunction following 206 km cycling. Within the present thesis it was clear that an exercise duration threshold existed for systolic function, although at present it is unclear what this is, given that no changes were observed which were unrelated to loading following short duration exercise (Chapters 5 and 6). Similarly, the insignificant

reduction in diastolic function found in Chapter 5 and by Scharhag *et al.* (2006) following 60 minutes exercise, compared to an increased duration and apparent diastolic dysfunction in Chapter 6 and that by Tian *et al*. (2014) suggest a minimum duration dependant threshold for diastolic functional changes (> 60 min; Middleton *et al.*, 2006a). Furthermore, training status could not explain any variations in post-exercise response (Middleton *et al*., 2006a). Decreases in systolic and diastolic function have also been found to be unrelated to age, weight loss or exercise intensity (Serrano Ostariz *et al*., 2013; Stewart *et al.*, 2016). Future research should consider factors which may explain individual variation in changes in LV function following prolonged exercise.

The high inter-individual variation of cTn release in a field-based environment has been previously reported (Shave *et al*., 2007a), which has been partially attributed to the large variation in exercise parameters such as intensity and duration, given that most competitive environments are based on the completion of set distances. Serrano-Ostáriz *et al*. (2009) reported that cTnI was mediated by exercise duration and intensity with lower concentrations reported following shorter duration or lower intensity exercise. It is pertinent to note that the exercise durations used within the study were particularly short (45 min to 180 min) in comparison to previous research and intensities of 85% and 95% of individual anaerobic threshold may underestimate the typical competitive running speeds. More recently, Eijsvogels *et al.* (2015) noted that a longer exercise duration was significantly related to higher cTnI levels but could only explain a small proportion of the individual variance in post-race levels. Within the present thesis, there was no association between exercise duration or running speed with cTnI release in Chapter 4, similar to findings from Fortescue *et al*. (2007), however, pacing strategies within Chapters 3 and 4 may have impacted upon the present findings. Whilst Chapters 5 and 6 in the present thesis used a controlled exercise intensity and duration, high inter-individual variation

was still apparent, implicating factors other than exercise characteristics. A lower participant age has also been associated with higher post-exercise cTnI concentrations (Eijsvogels *et al.*, 2015; Fortescue *et al*., 2007; Mingles *et al*., 2009), a finding often concomitant with a lower training experience (Fortescue *et al*., 2007; Mingles *et al*., 2009), however, other studies have found no link between age (Mehta *et* al., 2012; Shave *et al*., 2004a) or training experience with cTn release (Scheer *et al*., 2011). Future research should consider possible exercise, personal and environmental factors associated with cTn release.

Previous research has suggested those participants who covered less training distance per week demonstrated a greater elevation in NT-proBNP (Neilan *et al*., 2006a). A large difference in participant weekly training distance in the present thesis was apparent i.e. Chapter 4, and could partially explain the inter-individual variation. It has been previously suggested the increase in NT-proBNP is associated with exercise duration (Serrano-Ostáriz *et al.*, 2011), supported in the present thesis by greater concentrations of NT-proBNP in Chapters 3 and 4 in comparison to the shorter durations of Chapters 5 and 6. The similarity of concentrations of NT-proBNP in Chapters 5 and 6 between participants when exercise duration and intensity were controlled may also provide evidence for the influence of exercise duration on NT-proBNP release. NT-proBNP release was not associated with exercise duration in Chapter 4 phase 1, suggesting other factors are also implicated. A complex interaction of baseline (age, gender, volume overload, HR, blood pressure; Chen *et al.*, 2008), prior exercise (training history; Neilan *et al.*, 2006a) and exercise-related factors (exercise duration; Serrano-Ostáriz *et al.*, 2011) are suggested to explain the variability in levels of biomarkers (Legaz-Arrese *et al.*, 2011).

Training history and fitness has been implicated with both cardiac dysfunction and biomarker elevation, however, it may also interlink with a participants response to exercise in differing environmental temperatures. The rate of body heat storage is dependent upon the rate of metabolic heat production and the rate of heat dissipation (Mora-Rodriguez, 2012). An improvement in VO_2max through training results in an enhanced heat dissipation by lowering the T_c threshold for skin vasodilation and sweating (Nadel *et al*., 1974). When participants are working at the same relative intensity (i.e. 65%VO_2peak), those with a greater experience in endurance training will be working at a higher absolute workload than less trained individuals, thereby generating more metabolic heat. The higher exercise workload will also result in an increased cutaneous blood flow and sweat rate in the trained participants (Nadel *et al*., 1974). Within in a normothermic environment, the higher metabolic heat production is compensated by the higher heat dissipation resulting in heat balance (Mora-Rodriguez, 2012). Within a hyperthermic environment, the metabolic heat production can exceed the capacity of the environment to accept heat, resulting in a greater heat accumulation in well trained participants. Therefore, within the present thesis, training status of the participants in Chapters 4 to 6 may impact upon the CV compromise between maintaining thermal equilibrium and the exercise intensity, which could explain some of the individual variation apparent.

7.4 Limitations and Future Research

7.4.1 Experimental Design

The experimental design of a study is largely dependent upon the aim of the research question in focus. As previously discussed, the setting of the research environment (laboratory versus field based) can also impact upon the study design. In Chapter 3, the reluctance of participants and time constraints did not allow for daily pre-exercise data

collection points, which confounded data interpretation when evaluating if an accumulation of across the cycle challenge was apparent. The research design was improved in Chapter 4 to include pre and post-exercise assessment points each day which provided more support for the pattern of changes in cardiac function and biomarkers. A limitation of both Chapters 3 and 4 was that it was not possible to determine the time point in which LV systolic and diastolic function or cardiac biomarkers peaked or returned to pre stage 1 values. Whilst cTn has been detected in individuals in as little as 30 minutes exercise (Middleton *et al.*, 2006; Shave *et al.,* 2010), it is suggested an elevation in cTn in response to exercise is evident in all individuals, but may not always be detected due to the time of sampling (Gresslien and Agewall, 2016). A peak in cTnI has previously been reported after 3-6 hours (Lippi *et al*., 2012; Scharhag *et al.,* 2006; Shave *et al.*, 2010) with a normalization after 24 (Middleton *et al.*, 2007a; Shave *et al.*, 2004c) to 48 hours (Melanson *et al.*, 2006). Therefore, it may well be that the concentrations of cTn may have been underestimated in Chapters 3 and 4. In Chapters 5 and 6 we included multiple sample points in an attempt to monitor the kinetics of cTnI release. Chapter 6 provided a greater number of post-exercise assessment points compared to the majority of research within this area and this approach should be adopted moving forward to help fully understand biomarker kinetics and the association with other variables (person, exercise and environment).

The volume of exercise was controlled (for duration and intensity) in Chapters 5 and 6 but was lower than the stimulus reported in previous field-based studies, which have provided the more robust and consistent evidence of cardiac dysfunction and elevated cardiac biomarkers (George *et al.* 2005; Whyte *et al*. 2005). The exercise duration in Chapter 5 limited the expression of both systolic and diastolic functional changes. The exercise duration of both Chapters was selected to ensure participants completed both

trials before an unethical T_c was reached and exercise was terminated prematurely, allowing the evaluation between trials of the same running speed and exercise duration. Whilst the duration of the exercise limited the exercise stimulus, it did highlight a minimum duration dependant threshold for diastolic functional changes.

7.4.2 Sample Size

The sample sizes in some Chapters within the present thesis were small, which may have led to a reduced statistical power. The measurement variance in flow and tissue velocities, coupled with participant heterogeneity may explain the lack of statistical significance, particularly within Chapter 5. Increasing the sample size would increase the statistical power. Chapter 3 may also be considered as a series of case studies (n = 3), however the nature of ultraendurance exercise often means recruitment is difficult, particularly when coupled with invasive techniques such as venous blood sampling.

7.4.3 In-event Data

Through the completion of this thesis it is apparent that alterations in cardiac function and elevations in cardiac biomarkers are highly variable: person to person, measurement to measurement and to the posed exercise stimulus itself. In Chapter 4, the reluctance of participants did not allow for a measurement of exercise intensity making it difficult to draw conclusions in relation to the acute cardiovascular stress of the exercise bouts. The varied terrain and different pacing strategies of runners (i.e. walking uphill sections and running downhill) may have placed variable hemodynamic loading and stress on the cardiac muscle at irregular intervals, permitting periods of recovery in some runners (Denissen *et al*., 2012). The recording of exercise intensity may, therefore, provide valuable insight into the variable cardiovascular load involved in field-based events. Whilst Chapter 3, and data from Williams *et al*. (2011), demonstrated a heterogeneous

response between participants, this cannot be solely be attributed to exercise intensity. Data from Chapters 5 and 6 support this notion in that whilst relative exercise intensities were consistent across participants and trials, factors other than total myocardial work and thermal strain are implicated. Further research should focus on elucidating the factors that precipitate changes in ventricular function and biomarker appearance.

In Chapter 5 we collected blood samples during the exercise bouts in an attempt to monitor the kinetics of cTnI and NT-proBNP release. Whilst cTn has been detected in individuals in as little as 30 minutes exercise (Middleton *et al.*, 2006; Shave *et al.*, 2010), it is possible that the pause during the exercise in Chapter 5 in order to obtain a blood sample may have led to a short recovery period from the exercise stimulus.

7.4.4 Future Research

A number of areas for future research have been highlighted within the individual chapters as well as within this general discussion. It is clear from the current thesis that a number of recommendations when designing research studies within this area should be considered i.e. including multiple assessment points post-exercise (section 7.4.1), the use of greater participant numbers (section 7.4.2) and collection of in exercise data (section 7.4.3). As mentioned in section 7.3.3, this thesis provided novel insights into the effect of a hyperthermic environment upon cardiac function and biomarkers, however, due to the limited exercise dose utilised in the present thesis, this remains an area for future research. Future studies should firstly attempt to utilise greater durations of exercise when evaluating the effect of the environment upon cardiac function and biomarkers. Whilst Chapter 6 used the method of pre-cooling to manipulate T_c, the cardiovascular benefits were found to be attenuated after 20-25 mins of exercise (Bolster *et al*., 1999), highlighted by similar exercise HR, T_c and T_{sk}. Future studies may wish to consider other methods of

manipulating cardiovascular strain to evaluate the impact upon cardiovascular function and markers of cardiac damage i.e. per-cooling (during exercise) and post-exercise cooling. Per-cooling methods such as cold water/slurry ingestion, water spray cooling or cooling packs (Bongers *et al.*, 2017) may have larger potential benefit on thermoregulation, particularly during exercise in a hyperthermic environment compared to pre-cooling methods. An attenuation of T_c through per-cooling would allow serve to maintain central blood volume, limiting the magnitude of cardiovascular drift and therefore reducing the cardiovascular strain of the exercise bout. The method of post-cooling allows the reduction of T_c, T_{sk} and/or muscle temperature directly post-exercise (Bongers *et al.*, 2017), which would also directly impact upon the cardiovascular load post-exercise. Whole body cryotherapy has been shown to have no negative impact upon cardiac function after a weekly course of daily cryotherapy sessions (Banfi *et al*., 2009), however its effect upon cardiac function immediately post-exercise has yet to be evaluated. Whilst a number of methods of pre-cooling and post-cooling are available, future research should also take into account methods which are practical for use in a field-based competitive setting.

7.5 Clinical Implications

Part of the rationale for research within this area is based upon the concern that prolonged exercise may have a deleterious effect upon the health of the heart. The findings of this thesis contribute towards highlighting possible clinical implications of the exercise demands but also provoke some discussion of the possible mechanisms behind changes in cardiac function and elevations in markers of damage.

7.5.1 Cardiac Function

Within the present thesis, changes in systolic function were apparent following multi-day exercise (Chapters 3 and 4) and longer (Chapter 4) but not shorter duration single-day exercise (Chapters 5 and 6). A number of participants in Chapters 3 to 6 demonstrated post-exercise EF below the lower limit for normal LV systolic function (52%; Lang *et al.,* 2015). Within Chapter 3, similar exercise HR's across each day, suggest alterations in EF from the previous day did not seem to impact upon exercise performance. Chapter 4 demonstrated the change in EF appeared to be transient as EF typically recovered partially, if not fully by the PRE-exercise measurement point each day suggesting a limited impact in the short term, however the long term effects of prolonged exercise deserves future research.

Similarly, changes in diastolic function were also apparent following multi-day exercise (Chapters 3 and 4) and longer (Chapters 4 and 6) but not shorter duration single-day exercise (Chapter 5). The E:A ratio was significantly reduced in some participants below the clinical cut-off for diastolic dysfunction (less than 1.0; Lang *et al.,* 2015) in Chapters 3 to 6. This appeared to be transient in nature as a recovery in diastolic measures before 24-hr POST was observed in Chapter 6 and is consistent with other studies (Shave *et al*., 2004a) alongside a recovery between stages in Chapter 4. Overall, no participants reported any clinical signs and symptoms and changes did not appear to impact upon exercise performance either during or on subsequent exercise days, suggestive of limited clinical significance.

The mechanisms responsible for exercise induced changes in cardiac function are not fully understood. Whilst echocardiography is a useful tool in identifying changes in cardiac function, it provides a limited insight in identifying any mechanisms involved.

The use of statistical analysis can identify some of the relationships between changes in loading which may account for some of the alterations in functional parameters (i.e. Chapters 5 and 6). Two potential mechanisms have been proposed as intrinsic, non-load related mechanisms behind changes in cardiac function: 1) Myocyte damage (Neilan *et al.,* 2006b) and 2) β-andrenergic receptor downregulation (Hart *et al.,* 2006). Previous single day studies have suggested an association between cTn release and cardiac function (Neilan *et al.,* 2006b; Rifai *et al.,* 1999) but this cannot be confirmed within the present thesis as the relatively consistent changes in systolic and diastolic function occurred alongside marked individual variability in cTnI release. This would support two separate phenomena between changes in cardiac function and elevations in cardiac biomarkers with potential different mechanisms. During exercise, an increase in sympathetic activity via the neurotransmitters adrenaline and noradrenaline stimulate the β-andrenergic receptors in the myocardium, resulting in an increased chronotropic, inotropic and lusitropic effect on the LV (Opie, 2004). A tolerable sympathetic load/threshold has been suggested (Stewart *et al.,* 2016), which if exceeded for prolonged periods of time could induce a desensitization or down regulation of cardiac β-andrenergic receptors (Banks *et al.,* 2010; Oxborough *et al.,* 2010) and autonomic responsiveness (Seiler *et al.,* 2007) leading to a reduced force of contraction and impaired rate of LV relaxation. This is thought to be a result of the sustained exposure/accumulation of circulating catecholamines during prolonged exercise (Seals *et al.,* 1988) and may, therefore, explain the minimum duration dependant thresholds for both systolic and diastolic dysfunction observed in the current thesis.

7.5.2 Cardiac Biomarkers

As previously discussed a highly individual variation in cTnI release was apparent in the present thesis linked to a number of personal and exercise related factors. It has been

proposed there are 6 potentially interlinked mechanisms to explain elevated cTn levels: 1) an increased membrane permeability allowing unbound cTn in the cytosolic pool to enter the circulation; 2) cellular release of proteolytic troponin degradation products whilst membrane integrity remains; 3) formation and release of membranous blebs during temporary ischaemia of cardiac cells; 4) stimulation of myocyte turnover; 5) myocyte necrosis; and 6) increased rate of apoptosis (Eijsvogels *et al*., 2015; Gresslien and Agewall, 2016). The combination of results within the present thesis highlight a number of findings: transient elevations in cTnI evident in phase 2 of Chapter 4 with elevated concentrations after one stage followed by a rapid normalization of undetectable concentrations at the next sample point, a high percentage of participants with increased cTn post-exercise (Chapter 6) and low absolute levels of cTn (Chapter 3 and 4). With the absence of any clinical signs and symptoms of an adverse cardiovascular event, the elevations in cTnI are more likely to represent an acute physiologic perturbation, most likely representing cTn release from the cytosolic reservoir (Gresslien and Agewall, 2016; Shave *et al.* 2010) as opposed to underlying cardiac damage or pathology (Eijsvogels *et al*., 2011, 2015; Shave *et al.*, 2010). The present thesis along with a number of other studies did not report an association between cTnI and NT-proBNP values (Herrmann *et al*., 2003; Scheer *et al*., 2011), suggesting the release of these biomarkers represents different phenomena.

It is suggested an increased NT-proBNP during and after exercise is related to a growth-regulating property of BNP, leading to myocardial adaptation in healthy athletes (Scharhag *et al*., 2006), rather than a sign of heart damage. Concentrations of NT-proBNP were increased above clinical cut-off values in Chapter 3 and phases 1 and 2 of Chapter 4, which would be suggestive of LV dysfunction in a clinical setting (Shave *et al*., 2007). Given that NT-proBNP concentrations were below the clinical cut-off value following 22

hours of recovery in Chapter 3, the changes in NT-proBNP are more likely to represent the elevated cardiovascular work within both Chapters 3 and 4 as NT-proBNP is elevated in response to volume overload and myocyte stretch (Shave *et al*., 2007b). This is further supported by the fact NT-proBNP concentrations were near to or below the upper reference limit of a healthy population following the shorter exercise durations of Chapters 5 and 6.

7.6 Conclusions

The present thesis contributes to the existing knowledge related to LV cardiac function and markers of cardiac damage following prolonged exercise. The first aim of this thesis was to identify whether repeated day exercise would mediate a cumulative decrease in LV function as well as a cumulative increase in cardiac biomarker concentrations. The findings from Chapters 3 and 4 indicate that whilst evidence of cardiac dysfunction and an elevation in cardiac biomarkers exists, there was limited support for a cumulative change in both systolic and diastolic function and markers of damage. The second aim of this thesis was to examine the impact of prolonged exercise in a hyperthermic environment upon cardiac function and levels of cardiac biomarkers. Whilst Chapter 4 provided the additional stress of a hyperthemic environment, the field-based setting did not allow for the control of other exercise-related parameters. Chapters 5 and 6 showed a limited effect of environmental temperature or manipulating T_c and T_{sk} (via pre-cooling) prior to exercise in a hyperthermic environment, however, further research with exercise of a longer duration in this area is warranted. Overall, the impact of acute endurance exercise upon cardiac function and biomarker appearance is transient in nature and more likely represent an acute physiologic perturbation as opposed to underlying cardiac damage or pathology. Throughout this thesis it is apparent inter-individual variation exists in response to the exercise demands placed upon the participants, however further

investigation into the related factors are required. Furthermore, no participants reported any clinical signs and symptoms and the observed changes did not appear to impact upon exercise performance either during or on subsequent exercise days, suggesting a limited clinical significance. As the number of individuals participating in endurance exercise continues to increase, medical personnel should be aware of the exercise factors which may lead to alterations in cardiac function and elevations in cardiac biomarkers following prolonged exercise and should check for other symptoms to avoid misdiagnosis of myocardial injury.

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