THE EFFECTS OF PROLONGED BOUTS OF EXERCISE AND ACUTE ISCHEMIC PRECONDITIONING ON CARDIAC BIOMARKER RELEASE

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

Doctor of Philosophy

August 2017

Abstract

Although habitual exercise can have profound beneficial effects on the cardiovascular system, there is a growing evidence base to suggest that single acute bouts of endurance exercise can lead to the release of cardiac biomarkers of damage and dysfunction. Whether this cardiac biomarker release is indicative of reversible or irreversible cardiomyocyte damage is not clear.

The first study in this thesis employed a new high sensitivity assay to assess cardiac troponin (cTnI) as well as N terminal pro-brain natriuretic peptide (NT-proBNP) before, during and after 2 hr of treadmill exercise in young, healthy but non-athletic participants. No cTnI samples were elevated above the detection limit of the assay during exercise and only one participant had a detectable cTnI value post-exercise (22 ng/L). NT-proBNP levels were elevated more consistently during exercise and recovery.

In the second study, the repeatability of cardiac biomarker responses to 2 hr of treadmill exercise was assessed 1 and 12 weeks after an initial trial (Study 1). The same participant had an elevated cTnI value post-exercise in week 0 (22ng/L) and week 1 (30 ng/L). All other participants had no detectable cTn in trials in week 0 and 1. At week 12 one participant (different to the person in week 0 and 1) had elevated cTnI post-exercise (25 and 38 ng/l). NT-proBNP levels rose with exercise in all trials but peaked earlier in trial 1 and demonstrated great individual variability.

Study 3 and 4 investigated the role that ischemia may play in cardiac biomarker release and cardiac functional changes after endurance exercise. Ischemia was studied indirectly by employing remote ischemic preconditioning (RIPC) in a single-blind crossover research design. In study 3 trained cyclists completed 2 one hour time trials in a controlled laboratory environment with trials preceded immediately RIPC or a SHAM protocol. cTnI values were reduced after the RIPC trial compared to the SHAM during recovery from exercise (significant main effect for trial). NT-proBNP and indices of cardiac function were not mediated by RIPC. Using the same RIPC intervention in study 4 we employed a longer exercise task (160 km cycle) in trained cyclists. We confirmed a partial attenuation of cTnI appearance post-exercise following the RIPC intervention compared to the SHAM. In addition NT-proBNP was lower after the RIPC trial but RIPC did not mediate any change in cardiac function post-exercise. These data reflect the first tentative evidence that ischaemia could be implicated in post-exercise cardiac biomarker release.

Although exercise can lead to cardiac biomarker appearance, and ischaemia may be implicated mechanistically, the rapid appearance and removal of these biomarkers as well as the limited impact on cardiac function still supports a reversible insult to the cardiomyocytes.

Acknowledgement

When I started planning for this PhD I contacted an old lecturer of mine (Professor Tom Reilly). He wrote a lovely email on all the different projects that were available and who to contact. It was a long detailed email. When I was an undergraduate student I remember Tom's professionalism, fair play, always willing to help students, never seemed to be too busy. I got to know Professor Reilly on a professional level when he supervised my undergraduate dissertation and on a more personal level when I ran for the university running club and the long trips to Hollymount's international road race. Tom I thank you.

I would like to thank Professor Adrian Lees. Adrian in many ways was very similar to Tom. Very thoughtful, very knowledgeable, professional, always willing to help. He had a deep love of sport & exercise science. Adrian was one of those people that had the ability to give without directly giving. Adrian, like Tom, was softly spoken, had time for everybody, was another true professional, and he did make a difference. I thank Adrian for just being himself. I am lucky to have been taught by him.

Thanking my postgraduate supervisor third is not the traditional way and I hope it does not offend. I would like to thank my supervisor Professor Keith George for his contribution and professionalism, for all the support and encouragement he has gave me during the long months and years I have spent undertaking my PhD. Without his guidance and constant feedback this would have been a very different experience. The

university is very lucky to have staff like Professor Keith George. Thank you for your patience, understanding, guidance and professionalism, I have learned so much.

During my research studies I have been very lucky to have my own daughter on the research team. She was part of the RIPC team. Jemima is just finishing off her A-level exams and has been accepted to read Sport & Exercise Science for the 2017 academic year. A very big thank you to Beth McCarthy, a friend of my daughters, she was also part of the RIPC team. Thank you to Dr. Helen Jones the phlebotomist at LJMU and a big thank you to Dr. Neil Chester for his patience and professional attitude. Nothing was too small or too stupid for Neil. I am lucky to have had the opportunity to work with Neil, Thanks Neil. To the many more people I met at the university I thank you all.

Lastly, I would like to thank my family for all their love and encouragement. To my wife (Anne), I am sorry you had to spend all those lonely hours watching TV by yourself whilst I ran to the study to work on my PhD. At times, you must have thought it was never going to end. Anne thank-you for supporting me throughout this PhD project. It would not have happened without your support. Lastly, I could have spent more time with my other daughter Siobhan, I hope I was not hiding in the study too much. Siobhan has one more year before she starts her university journey reading Psychology.

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Declaration

I hereby declare that this thesis represents my own work which has been completed after registration for the degree of PhD at Liverpool John Moores University, and has not been previously included in a thesis, dissertation submitted to this or other institution for a degree, diploma or other qualification.

Signature:

Date: August 2017

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Chapter 1:

Introduction

1.1. Background Context

Since the 1950s there have been numerous observational and intervention-based scientific studies that have examined the link between physical activity, physical fitness and cardiovascular health. Cardiovascular Disease Statistics (2014) reported that heart and circulatory diseases were responsible for over a quarter of deaths (28%) in the UK in 2012, with one in six male deaths and one in nine female deaths due to coronary heart disease (British Heart Foundation 2017). Importantly, these statistics noted that a total of nearly 74,000 deaths were attributed to a lack of regular physical activity. In addition longitudinal studies, that have followed large sample populations, have shown that there is a protective effect of exercise and/or physical activity for numerous cardiovascular and non-cardiovascular chronic diseases such as diabetes, osteoporosis and many types of cancers (Fletcher et al., 2001). An important additional consequence of this field of research is that groups that change their activity patterns though changes in active occupations or recreational levels have decreased morbidity and mortality rates (Myers et al., 2002). This empirical evidence has led eminent panels such as the American College of Sports Medicine (ACSM) & the American Centres for Disease Control and Prevention (CDC) to provide consensus statements to support the positive link between exercise and positive health status (Pate et al., 1995; Fletcher et al., 2001; Myers et al., 2002). Overall, the current state of knowledge is that the more active, or fit, individuals are the less likely they are to suffer from primary or secondary cardiovascular disease, irrespective of gender, age and/or race/ethnicity (Morris et al., 1953; Paffenbarger et al., 1975; Pate et al., 1995; Myers et al., 2014).

Population-based understanding of the health benefits of exercise may partially explain the global increase in mass participation endurance sporting events (Baggish et al., 2011). Common endurance sporting activities include events such as marathons (42 km), ultra-marathons (161km) sportives (160 km) and multi-sport events such as triathlons (Ironman Triathlon: swim 3.86 km, bike for 180 km and run for 42 km). Event data shows a massive increase in participation in such activities over the last 20-30 years (Hoffman et al., 2011).

It is, however, interesting that exercise prescription for health, often expounded from epidemiological databases and produced by ACSM and CDC (amongst many others), targets exercise prescription at volumes and intensities far below those employed by the amateur or elite athlete training for common endurance and ultra-endurance mass participation events. The consequence of this dichotomy is that some attention is required as to the cardiovascular health benefits when exercise training or physical activity levels go way beyond those supported at a population level that induce positive cardiovascular outcomes. The question that may be posed is whether the relationship between "exercise dose" and cardiovascular health benefit is linear, curvilinear or of some other nature (Whyte et al., 2008). The full nature of the relationship between exercise and health status is still to be determined, with the intensity, duration and volume of physical activity required for optimal cardiovascular health is still to be clarified.

The physical activity guidelines for 19 to 64 years olds to improve cardiovascular health is to either complete 150 minutes of moderate intensity exercise for 150 minutes or 75 minutes of vigorous intensity exercise, or alternatively combination of moderate and vigorous intensity exercise over a week. Interestingly, Blair et al., (1989) concluded that

the positive cardiovascular health effects of exercise may not be extended above an intensity of 10 metabolic equivalents (METS) or 35 ml/kg/min. This exercise intensity may be below the level of which most amateur endurance athletes will need to train to improve performance. If no cardiovascular health benefits are manifested at these exercise doses then a subsequent question is whether very high exercise loads have any negative cardiovascular health consequences?

Contrary to the well-known health benefits of exercise training, there is a growing body of work that suggests exercise can contribute to an acute depression in cardiac function (Stenburg & Saltin, 1964; La Gerche et al., 2012; Sharma et al., 2011; Lord et al., 2015) as well as an acute release of cardiac biomarkers of damage and dysfunction (Shave et al., 2007; Middleton et al., 2007). There is also some tentative evidence that lifelong endurance exercise training can result in chronic changes such as myocardial fibrosis (Wilson et al., 2011; Eijsvogels et al., 2017) and an elevated risk of ECG abnormalities including atrial fibrillation (Karjalainen et al., 1999; Baldesberger et al., 2008; Anderson et al., 2013). Detailed analysis of individual studies provides some development of the issues concerning higher levels of performance and volumes of training. For example, Anderson et al., (2013) noted that in Nordic skiers completing specific events; 1) the higher the exercise intensity the higher the risk of arrhythmia, 2) athletes that completed the event five or more times showed a 29 percentage increase in prevalence of arrhythmia and 3) athlete that had a higher finishing position had a higher risk of arrhythmia. A combination of increasing participation in (ultra)endurance events and the awareness, in both scientific and lay environments, of potential cardiovascular consequences of such high levels of physical activity has resulted in a “living lab” approach to this topic. In essence, many mass-participation (ultra) endurance events have allowed research to take

place alongside participation. This provides unique insights into a small but growing part of the sport/exercise population. Whilst cross-sectional in nature, such as the Anderson et al., (2013) study, unique data are being generated that are prompting on-going research and generating new hypotheses to test.

One topic area that has utilised this "living laboratory" approach is the phenomenon that acute bouts of exercise can lead to either a decrease in cardiac function (normally assessed via echocardiography) or an elevation in blood levels of cardiac biomarkers indicative of cardiac myocyte damage or cardiac dysfunction (Shave et al., 2010). On the basis that cardiac biomarkers have an important role to play in the diagnosis of coronary syndromes, as well as being linked to disease severity, it is somewhat counterintuitive and surprising that there have been numerous reports in the medical and scientific literature of elevated cardiac biomarkers after acute bouts of exercise (Shave et al., 2010). The increase in cardiac troponins (cTnT, cTnI) and/or brain natriuretic peptides (NT-proBNP) with acute exercise has fuelled scientific and media speculation that there may be negative health effects of (ultra) endurance training and competition, although no causal data supports this supposition at this point. To date information available in this sphere is both complex and contradictory with research presenting between 0% (Cummins et al., 1987) and 100% (Middleton et al., 2008) prevalence rates for the elevation of cTnT and cTnI above detection levels of the assay during or after prolonged exercise. This wildly variable and contradictory extent database is difficult to interpret and requires some critical evaluation and further research including a move from "living lab"/field-based approach to biomarker and cardiac function research into a more controlled laboratory environment (Shave et al., 2012).

We are not sure of the mechanism(s) for, or the long-term clinical consequence of exercise-induced cTn or NT-proBNP release. Whether this represents reversible damage or dysfunction to the heart or some minor form of irreversible insult is important to determine. On-going research is needed as to why, when and how cardiac biomarkers become elevated during and after acute exercise bouts. Indeed, without better understanding of this phenomenon there will be continued confusion and concern when advising athletes engaging in different exercise or training strategies. In beginning to understand the reversible/irreversible nature of these insults "clinically-relevant" processes such as ischaemia has been proposed as a potential mechanism in past cardiac biomarker studies but too date there is no available empirical evidence to implicate ischaemia.

Consequently, the primary focus of this thesis is to employ the high sensitivity (hs) cTnI assay, which has the ability to identify lower levels of cTn compared to standard assays, to determine the presence of elevated cardiac biomarkers during and after moderate to high intensity endurance exercise in healthy individuals in a controlled laboratory setting. A secondary focus it to begin to explore a potential mechanistic role for ischaemia in the appearance of cardiac biomarkers during and after exercise in human competing high intensity and high volume exercise.

1.2. Thesis Overview

Initially, this thesis will provide a review of relevant past literature in relation to cardiac biomarker and functional responses to acute endurance exercise. Four empirical studies

are then described. In study one we describe the response of cTnI, using the new hs-cTnI, assay, as well as NT-proBNP to 2 hour of treadmill running exercise in healthy subjects within a laboratory-controlled environment. Study 2 is linked to study 1 in that it specifically addresses the issue of the repeatability of exercise-induced cardiac biomarker release as the same participants completed two further (identical) 2 hr treadmill exercise bouts undertaken 1 and 12 weeks after the first bout (week 0).

Study 3 and 4 both address the potential role of ischaemia in exercise-induced cardiac biomarker release. In an attempt to increase the intensity of exercise, participants in study 3 undertook two 1-hour cycle time trials in a controlled laboratory environment with biomarkers and cardiac function assessed during early recovery. The potential mediating role of ischaemia was addressed by the application of remote ischemic preconditioning (RIPC) before one trial. This was then compared to a SHAM intervention. The final study employed the same research design, outcome measures and RIPC intervention but employed a repeated 160 km cycle ride as the exercise exposure.

This group of studies will provide new insight into exercise-induced cardiac biomarker release, its repeatability, its association with ischaemia and its link to cardiac function. We will add new knowledge to the debate about whether exercise-induced cardiac biomarker release reflects reversible or irreversible damage/decline to cardiomyocytes.

Chapter 2:

Review of the literature

2.1. Definition and History of Cardiac Biomarkers

Historically biomarkers are normally easy-to-access (often blood borne) markers that attempt to reflect, as accurately as possible, patho-physiological processes in the body. Cardiac biomarkers "represent" events in the heart and central cardiovascular system and are normally parameters or indices that are easier to measure or access than some complex cellular or sub-cellular process of importance. Cardiac biomarkers tend to reflect processes such as damage, dysfunction and/or elevated risk of a pathological event. Biomarkers as measures of cardiovascular health status or future risk are, therefore, highly popular and prevalent in medical science.

One of the earliest cardiac biomarkers assumed to reflect the cardiomyocyte damage associated with pathological processes, such as heart attacks, was creatine kinase (CK-MB; Apple et al., 1994). Creatine kinase (CK) is an enzyme involved in the synthesis of ATP from phosphocreatine, anaerobically, when energy is required rapidly in large quantities such as during very short bursts of high intensity exercise. CK is a dimeric molecule composed of M and/or B subunits. These two proteins can form into 3 different isozymes: CK-MM, CK-MB, and CK-BB. CK-BB and CK-MM are located in brain and skeletal muscle, respectively. The detection of cardiac damage and dysfunction was focussed on CK-MB and early exercise studies employed this biomarker. Significant elevations in CK-MB, above reference levels and into the range suggestive of myocardial damage, were observed after marathon running (Ohman et al., 1982; Cummins et al., 1987). An important requirement of any cardiac biomarker is a high specificity and

concentration within cardiomyocytes (Kemp et al., 2004). Disappointingly, there has been evidence to show that CK-MB is also present within skeletal muscle and it is possible the exercise-related skeletal muscle damage can elevate CK-MB, which would reflect a "false-positive" for cardiac damage (Schneider et al., 1995; Collinson et al., 2001). As a consequence, other cardiac biomarkers have been developed over the last 30 years.

Cardiac troponins are part of the thin filaments within the contractile protein of the cardiomyocyte that have defined roles in the calcium-mediated regulation of muscle contractions. Two of the three cTn complex (cTnI and cTnC) are only found within the myocardium (there are small amounts of cTnT in other tissues) and thus cross-reactivity with skeletal muscle is less of a concern. Despite this the first generation cTnT assay used a bovine antibody and there was some evidence of cross-reactivity with skeletal myocytes, making it unclear if the origin of cTnT was from skeletal muscle, cardiac muscle or both (Collinson et al., 2003). The 2nd and 3rd generation cTnT assays used recombinant human antibodies in the assay and this significantly improved cardiac specificity (Collinson et al., 2003). Originally, cTnT was preferred over cTnI as there was, commercially, only one well-defined cTnT assay (Roche) but there were multiple cTnI platforms and assays making vendor-based differences an issue for data interpretation.

Because of the cardiac-specificity of more recent cTn assays any elevation was thought to reflect cardiomyocyte damage and thus became important in clinical decision making (Ford et al., 1999). In a clinical setting, it is speculated that the early release of cTn is

from the cytosolic pool followed by a persistent release from the structural bound protein pool of the cardiomyocyte as the tissue breakdowns during apoptosis/necrosis. Release of cTn from the structural pool is synonymous with irreversible damage whereas release form the cytosolic pool could reflect reversible cardiomyocyte insults (Jeffe & Wu, 2011).

The latest development in cTn assays has been the "high sensitivity" assays for both cTnT and cTnI (hs-cTn - Abbott Diagnostics, Chicago, IL, USA; Elecys, Roche Diagnostics GmbH, Mannheim Germany). The rationale for the development of high sensitivity assays was that the detection limit and the 99th percentile of normal population data with the original assays were largely the same. This meant any detectable cTn value was likely clinically significant and prognostic. Indeed the magnitude of cTn release was significantly correlated with tissue damage, scar size and prognosis (Collinson et al., 2006). With a relatively high detection threshold there was some concern that the "old " assays were under-reporting low levels of cTn release and those low levels of cardiomyocyte damage may be of some clinical relevance. The hs-assays also has the potential to uncover "resting" levels of circulating cTn which was not observed with older assays and whose clinical value is unknown. The new hs-cTn assay can differentiate cTn levels approximately 100 fold lower than the standard assays. (Westwood et al., 2015)William et al (2018) stated that even minor hs-cTn changes can have significant clinical impact at a patient level and, therefore, the ability to access lower levels of hs-cTn is likely to augment the frequency of positive or meaningful results. The challenge is to distinguish false reassurance or alarm of myocardial damage. Frankenstain et al (2011) monitored reference values for cTn in 20 healthy participants at 1 ,2 ,3, and 4 h and then 1, 2 ,3 , and 4 weeks after initial assessment. They concluded that in order to interpret hs-

cTn data over serial measurements the data from this study related to biological variation is highly important. Biol0gical variation was, however, quite low over this timeframe. It would seem pertinent that this assay is adopted in exercise settings where the quantification of baseline data and low magnitude cTn responses to exercise are common. Consequently, in the empirical data chapters in this thesis we employ hs-cTn assays.

B-type natriuretic peptide (BNP) is a 32 amino acid polypeptide that is secreted with N-terminal pro-brain natriuretic peptide (NT-proBNP) by the left and right ventricular cardiomyocytes in response to volume or pressure overload when the "wall", or more specifically the cardiomyocytes, are stretched (Weber et al., 2005). Thus increased levels of BNP (or NT-proBNP) reflects an increase in myocardial wall stress and has been widely used as a biomarker to monitor cardiac disease progression, risk or status in cases where overload of the heart is likely to occur; such as in congestive heart failure (Foote et al., 2004; Scharhag et al., 2008). NT-proBNP has emerged as an excellent diagnostic biomarker in many heart diseases but is most commonly used in heart failure as a routine part of diagnosis, risk assessment and treatment (Gustafsson et al., 2005). A systematic review of clinical studies by Hill (2014) determined the diagnostic and prognostic performance of NT-proBNP (76 articles) reflected excellent sensitivity in the diagnosis of heart failure. The clinical cut-off point for NT-proBNP was set at 0.45 ng/mL for patients <50 year olds and 0.90 ng/mL for 50-75 years old. Most studies related to prolonged exercise linked to elevated NT-proBNP have completed a single measurement at baseline and this does not explore the natural or biological variation that exists with baseline concentrations of NT-proBNP (Zile et al 2016). It has been suggested that repeated biomarker measurements may be required to reflect more accurately the dynamic and progressive nature of the underlying pathophysiological processes, such as mechanical

overload, cardiac fibrosis, and inflammation, and therefore may be more suitable for additional prognostic information (Meijers et al., (2017). Despite this, the increases in NT-proBNP with ultra-endurance exercise are generally much greater than biological variation in resting values.

As well as having a clear clinical role in cardiovascular disease detection and prediction, it has become apparent that the imposition of exercise has been show to result in a transient elevation of both cTn and NT-proBNP in healthy athletes (Scharhag et al., 2005; Scharhag et al., 2008; Nie et al., 2010; Shave et al., 2010). Many issues related to this phenomenon remain unclear. The presence of biomarker release with different populations, undertaking different exercise tasks is described poorly. Likewise the consistency of the exercise response of both biomarkers, the clinical significance (if any) of these elevations and the processes or mechanism(s) that underpin the changes in these biomarkers with exercise is not known. These issues drive the focus and general rationale of this thesis.

2.2. Cardiac Troponin (cTn) and Exercise

Descriptive evidence of exercise induced cTn biomarker elevation has been presented in many empirical studies in the last 30 years including work from our own research group and/or collaborators (Shave el al., 2002; George et al., 2005; Whyte et al., 2005; Middleton et al., 2006; Middleton et al., 2008; Legaz-Arrese et al., 2015). Despite widespread descriptive evidence of cTn presence in the circulation after acute exercise bouts there is evidence of a significant level of between-subject and between-study variability in biomarker response. For example, Mair et al., (1992), who used 2nd

generation cTnT assay after a 230 km cycle ride, noted an elevated value in only 1 out of 28 participants. Bonetti et al., (1996), who employed a 2nd generation cTnT assay after a stage of the Tour d'Italia, reported elevated biomarker levels in 5 out of 28 cyclists. Mair et al., (1997), again using 2nd generation assays, reported no elevation in cTnT or cTnI after a 67 km Alpine marathon using 2nd generation assays. Alternatively, Scharhag et al., (2005), who examined cTnT and cTnI in 105 endurance athletes after a marathon (n=46), 100 km ultra-marathon (n=14) or a long distance mountain bike ride (n=45), noted that cTnI was elevated in 78% of athletes whereas cTnT exceeded its URL in 47% of athletes. Finally, Fortescue et al., (2007), in the largest single study to date, observed a cTn elevation post-marathon running in 328 out of 482 runners. Clearly, there are a set of research design, personal, exercise or environmental circumstances that contribute to such individual heterogeneity in biomarker response to specific exercise stimuli.

Narrative reviews have suggested that the variability in the cTn response to exercise likely reflects inconsistency within the research designs and participants employed in multiple small studies (Shave et al., 2010). Specifically, variation in subject age, sex, fitness, exercise modality, intensity, duration, number of blood draws and type of cTn assay have all lead to diverse data and some confusion (Shave et al., 2010). Most of the original investigations have used different assay types (1st, 2nd, 3rd and 4th generation or hs-cTn assay) and employed small sample sizes in a vast array of settings (laboratory, field, competitive, non-competitive, different environmental stresses), participants (males, females, young, old, athletes, and charity runners) and exercise intensities (walking, jogging, race pace and sprinting). What is of interest is that few of these factors have been independently studied by controlled, manipulated designs to address the importance of individual factors or the synergy between issues. Consequently drawing this evidence

together has been problematic. Despite this, both narrative and structured reviews have attempted to produce an overview of cTn release associated with exercise (Scharhag et al., 2005; Shave et al., 2007).

As a way to summarise the variety of disparate data from smaller individual studies, Shave et al., (2007) completed a meta-analysis that assessed 1120 participants from 26 relevant acute-exercise studies. Table 2.1 documents details from the individual studies and heterogeneity in outcome is noticeable. The key finding from the meta-analysis was that the "event rate", the likelihood of cTnT being detectable (above URL of 0.01µg/1), after acute exercise, was 47%. Running events resulted in a slightly higher event rate of 52% of cTnT compared to cycling of only 27% (Table 2.1).

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Table 2.1. Participants with significant cTn elevation post exercise (adapted from Shave, 2007)

STUDY NAME	EXERCISE MODE	GENDER (% MALES)	MEAN AGE (YEARS)	MEAN BODY MASS (KG)	MEAN DURATION (MIN)	EVENT RATE	LOWER LIMIT	UPPER LIMIT	DATA
Shave et al., (2004)	cycle	100	28	74.9	254	**0.06**	0.00	0.50	1/9
Neumayr et al., (2002)	cycle	100	37	72.5	1342	**0.06**	0.01	0.34	1/16
Shave et al., (2004)	cycle	100	34	77.7	126	**0.13**	0.02	0.54	1/8
Dawson et al., (2005)	cycle	100	31	78.2	240	**0.13**	0.03	0.39	2/16
Neumayr et al., (2005)	cycle	100	34	70.4	550	**0.45**	0.28	0.63	13/29
Scharhag et al., (2005)	cycle	93	36	74.0	360	**0.51**	0.37	0.65	23/45
George et al., (2005)	run	100	21	75.9	85	**0.03**	0.00	0.30	1/20
Shave et al., (2002)	run	100	29	77.0	30	**0.06**	0.00	0.50	1/9
Scharhag et al., (2005)	run	71	44	69.0	607	**0.21**	0.07	0.49	3/14
Shave et al., (2005)	run	86	44	71.5	613	**0.35**	0.22	0.50	15/43
Middleton et al., (2005)	run	100	33	70.4	139	**0.36**	0.14	0.66	4/11
Shave et al., (2003)	run	0	44	57.3	374	**0.43**	0.14	0.77	3/7
Shave et al., (2002)	run	100	33	77.7	301	**0.44**	0.18	0.75	4/9
Scharhag et al., (2005)	run	87	40	73.0	238	**0.52**	0.38	0.66	24/46
Apple et al., (2002)	run	58	38	*	289	**0.53**	0.31	0.73	10/19
Whyte et al., (2005)	run	17	35	75.9	245	**0.62**	0.48	0.74	32/52
Neilan et al., (2006)	run	68	41	71.7	285	**0.63**	0.51	0.74	38/60
Middleton et al., (2006)	run	93	29	75.9	212	**0.64**	0.38	0.84	9/14
Fortescu et al., (2007)	run	67	39	*	*	**0.68**	0.64	0.72	328/482
George et al., (2005)	run	79	33	76.0	256	**0.72**	0.54	0.86	21/29
George et al., (2004)	run	89	36	81.2	256	**0.74**	0.58	0.86	26/35
Rifai et al., (1999)	triathlon	0	43	56.0	715	**0.17**	0.04	0.48	2/12
Shave et al., (2004)	triathlon	100	41	74.0	506	**0.50**	0.32	0.68	13/26
Cleave et al., (2001)	Triathlon	*	*	*	*	**0.56**	0.43	0.67	35/63
Tulloh et al., (2006)	triathlon	95	38	*	686	**0.84**	0.69	0.93	32/38

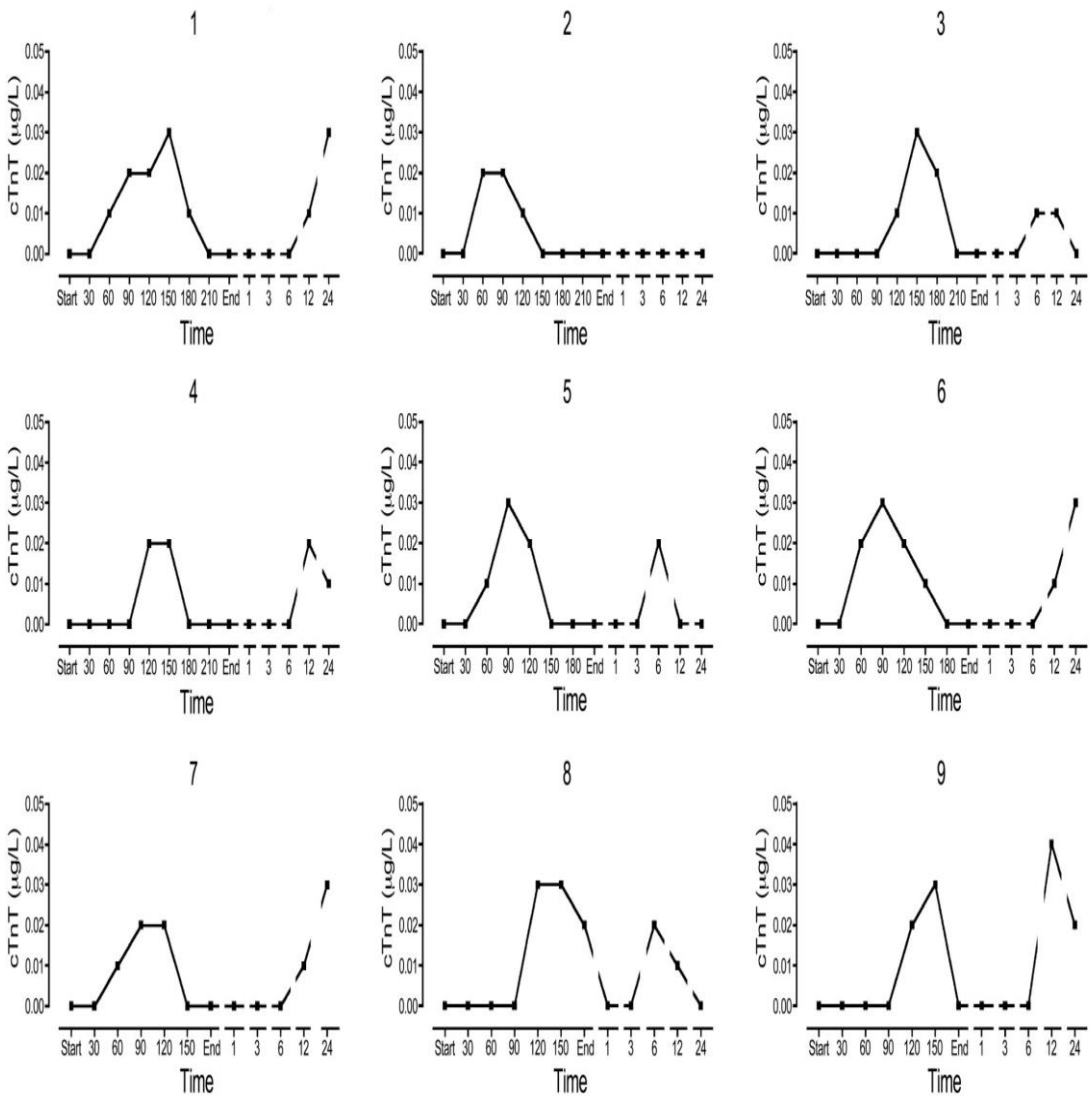
***Data that were unavailable, gender** according to the percentage of men in the study sample, **sample size (total)**

The explanation for running having a higher event rate was not clear although post-hoc analysis also suggested that the longer the event the slightly lower the event rate which likely relates to exercise intensity (specifically higher event rates were noted for marathons compared to longer running events). Whilst informative this meta-analysis did not quantify the precise characteristics of exercise that promote cTn release and/or contribute to the understanding of the significance (clinical) or mechanisms. This work has acted as a general stimulus for on-going study and analysis. Specifically, this analysis and other commentary's raised a couple of key issues that limited data interpretation; (1) single post exercise blood samples (normally immediately after exercise) and (2) variable assay use with limited data using new high sensitivity techniques.

In addressing point (1) Middleton et al., (2007) studied 8 runners who completed a marathon distance run (self-selected pace) on a treadmill. This laboratory-controlled approach allowed blood draws every 30 min (during exercise) and at multiple time points in recovery, (up to 24 hr post run completion). Employing a 3rd generation assay for cTnT, uniquely this biomarker was elevated in all athletes during exercise and in all but one participant at some (but varied) point during recovery (Figure 2.1). There was a moderate quantitative release of cTnT (range 0.02 to 0.04 µg/L) during the first 60 minutes of treadmill running at marathon pace. The data suggested a biphasic response, which may be interpreted as similar to a clinical release, although the absolute levels and rapid turnover would suggest a non-clinical response. This study provided stark evidence of the limitation of many field-based studies that only used pre and a single post-exercise biomarker assessment point. A clear consequence of this study for the work contained in this thesis is that multiple blood draws should be

incorporated into any study design, controlled-laboratory or field-based, to get more accurate insights into cardiac biomarker turnover during and after strenuous exercise.

Figure 2.1. Individual cTnT Release During and After Completion of a Lab-based Marathon (Middleton et al., 2007).



In respect to point (2) Vilela et al., (2014) recently reviewed the available evidence of hs-cTn release in sport and exercise settings. As a newer biomarker the evidence-base is much smaller, but this review and meta-analysis was still timely for on-going work (Table 2.2). Vilela et al., (2014) reported data for a total of 479 participants performing strenuous exercise in studies between 2009 and 2013. The main outcome was a positive response rate for cTn appearance in two-thirds (69.8%) of the participants in the review, irrespective of the exercise mode, intensity and volume undertaken. Further Vilela et al., (2014) noted that (a) baseline values of hs-cTnI were relatively low, (b) hs-cTnI increased during exercise to early recovery, (c) peaked at 3 – 4 hours post exercise, and (d) recovered considerably by 24 hr post-exercise. Evidence of the combined use of the new hs-cTn assay with multiple blood samples is to be commended. Vilela et al., (2014) concluded that it is likely that cTn release with exercise reflected a reversible insult to cardiomyocytes but could not provide insight into mechanism(s) of release. Further, most work to date reported by Vilela et al., (2014) consisted of studies of athletes performing very high exercise volumes and thus placing considerable hemodynamic demands upon the heart. A consequence of this is that there is a clear rationale to assess hs-cTn turnover during and post exercise in less well-trained individuals completing less strenuous exercise, whilst employing multiple blood draws. This is the focus of the first study in this thesis.

Table 2.2. Studies employing hs-cTn assays to assess the cardiac biomarker response to strenuous exercise (adapted from Vilela et al., 2014)

STUDY NAME	MODE	DESIGN	SAMPLE	DATA
Mingels et al., (2009	Running (marathon)	Assessed by highly sensitive cardiac troponin T and commercial cardiac troponin T and I assays pre and post exercise	85	The runners had cTnT concentrations greater than the 86% 99th percentile with the hs-cTnT assay compared to 45% using the Roche 4th generation cTnT assay. Indicating detection depends highly on an assay's limit of detection.
Mingels et al., (2010)	5, 15, 21 and 42 km	) using hs-cTnT assay pre and post exercise.	297	The distance covered was related to the elevation of cTnT post exercise. 86%, median 0.030 µg/L The use of hs-cTnT is a predictor of cTnT post exercise when using hs-cTnT assay after the marathon.
Saravia et al., (2010)	Running (marathon)	Comparison between hs-cTnT and 4th generation cTnT assay during Berlin marathon 2006 pre and post exercise.	78	Post-race fourth generation was significant in 43%, hs-cTnT was positive in all runners.
Scherr et al., (2011)	Running (marathon)	0, 24 and 72 h post marathon	102	hs-cTnT peaked immediately post exercise and showed the rapid removal often seen during exercise. (values returned to normal levels within 72 h).
Lippi et al., (2012)	Running (60 km ultra-marathon	Before and immediately after race	15	Post-race assay was above URL in 12 participants (80%; $P < 0.001$) using both the hs-cTnI.
Tian et al., (2012)	Running (90 min treadmill run, 95% ventilatory threshold)	0, and at 1, 2, 3, 4, 5, 6, and 24 h post-exercise	13	Peak hs-cTnT occurred at 3–4 h post exercise in all runners, but was substantially higher ($P < 0.05$) in adolescents [median (range): 211.0 (11.2–794.5) ng/l] compared with adults [median (range): 19.1 (9.7–305.6) ng/l]. Peak hs-cTnT was followed by a rapid decrease in both groups, although adolescent data had not returned to baseline at 24 h

2.3. NT-proBNP and Exercise

The significance of elevated NT-proBNP during exercise is poorly understood, despite the fact there is a growing database detailing the NT-proBNP response to different exercise bouts (Shave et al., 2007; Table 2.3). For example, Vidotto et al., (2005) assessed NT-proBNP levels before and after a half marathon with little evidence that running a half-marathon altered this cardiac biomarker. Conversely, Scharhag et al.,

(2008) assessed NT-proBNP responses in a large cohort (n=105) healthy athletes with samples taken before, 15 minutes, and 3 hours after a marathon (n = 46), a 100-km run (n = 14), and a mountain bike marathon (n = 45). NT-proBNP increased in all 3 events (P < .001) with the highest increase in the 100-km runners (median increase 200 ng/L; 25th/75th percentile 115/770 ng/L), which differed from the increase in the marathon (97 ng/L; 36/254 ng/L) or the mountain bike marathon (78 ng/L; 37/196 ng/L) (P < .01). Further, 81 of 105 athletes exceeded the URL of NT-proBNP (males/females 88:153 ng/L) after exercise. This suggests a role for duration over exercise intensity (and thus accumulated hemodynamic stress) in the exercise-related rise in NT-proBNP.

Recent studies have attempted to interrogate issues that may mediate NT-proBNP responses to exercise. Legaz-Arrese et al., (2015) assessed the NT-proBNP response to high intensity rowing. In 18 elite and 14 amateur rowers undertaking a 30 min maximal rowing test NT-proBNP rose in all subjects and recovered to baseline by 24 hr post-exercise. There were no significant differences in baseline and peak post-exercise NT-proBNP between groups. This suggests that athlete status does not mediate NT-proBNP responses to exercise. A study by Sahlen et al., (2008) demonstrated a correlation between high baseline levels of NT-proBNP and the amount released during prolong exercise. The participants were 43 senior endurance runners, > 55 years (mean age 61 + 4), who completed a 30 km cross-country race. Whether these results are a consequence of the use of "mature" athletes has yet to be determined. Fu et al., (2010) studied adolescent runners (mean age 16.5 + 1.6 years, N=17), before, immediately after and 4 hours after endurance exercise. 29% of runners had baseline NT-proBNP above URL and these runners had greater absolute increase in NT-proBNP post exercise. Fu et al., (2010) proposed that the higher levels of NT-proBNP may be

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an important part of the myocardium's transition from childhood to maturity. Again more work is needed to test this specific point, possibly with a long-term research design as athlete's age and mature.

A range of studies has demonstrated, like cTn, inconsistencies in the NT-proBNP response to exercise. Again, similar limitations must be noted including many studies adopting an uncontrolled "field" exercise exposure and limited recovery blood samples. Reduced numbers of post-exercise blood samples promotes the possibility of grossly underestimating any peak exercise response (Scott et al., 2009). Whilst narrative reviews have attempted to inform and produce an overview of NT-proBNP responses to prolonged exercise (Shave et al., 2005; Scharhag et al., 2008) there is still a requirement for descriptive research in controlled exercise settings and in different participant groups, whilst employing multiple blood draws. Consequently, in this thesis we will assess NT-proBNP alongside hs-cTn in a range of exercise settings.

Table 2.3. Studies employing NT-proBNP assays to assess the cardiac biomarker response to endurance exercise (adapted from Vilela et al., 2014)

STUDY NAME	EXERCISE MODE	DESIGN	SAMPLE	DATA
Legaz-Arrese et al., (2015)	High intensity rowing (30 min)	Blood draws pre-test and 5 min, 1,3,6,12 and 24 h	Elite (n = 18) Novice (n = 14)	Biomarker rose in all subjects and recovered to baseline by 24 hr post-exercise. Athletic status did not mediate NT-proBNP responses to exercise
Salvagno et al., (2015)	Running, ultramarathon (60 km)	Pre and immediately post-run samples	n = 18	Significant increase after the run for NT-proBNP, 5/18 (28%) values > 125 ng/L.
Fu et al., (2010)	strenuous running for 21 km	Blood draws pre, 0 and 4h post run	n =17 (adolescents)	NT-proBNP increased by 50% post exercise (123 + 51 vs. 193 + ng/L. High levels may indicate myocardium growth. transition
Sahlén et al., (2008)	Running, 30 km cross country race	Pre and immediately post exercise blood draws	n = 43> 55 yr old	A correlation between high baseline levels of NT-proBNP and the amount released during prolong exercise. This age group may have an adverse effect to exercise.
Scharhag et al., (2008)	Mountain bike, 100 km run and marathon	Blood samples 15 min and 3 hours post exercise	bike (n = 45) run (n = 14)	The 100km run had the highest increase of NT-proBNP above URL, 200ng/L. 81 of 105 athletes exceeded the URL
Lippi et al., (2008)	Running half marathon (21 km)	Blood samples 0, 3, 6 and 24 hr post-exercise	n = 17	No participant exceeded URL. Cut off used to define URL 194 ng/L. 0/17
Vidotto et al., (2005)	Runners, half marathon (21 km)	Blood samples were taken pre and 20 min, 2 hr post-exercise	Male (n = 12) female (n = 13)	Running 21 km altered cardiac biomarker elevation.

2.4. Repeatability of Cardiac Biomarker Response to Exercise

There is substantive evidence to show that single bouts of exercise can stimulate the appearance of cardiac biomarkers of damage and dysfunction in the blood stream (Shave et al., 2007; Shave et al., 2010). In considering the clinical implications and importance of this biomarker phenomenon there has been some interest in determining

if this is a consistent or repeatable phenomenon. The basic standpoint is that if this biomarker response is repeatable, predictable and therefore by association an obligatory response to exercise stress, this likely means that it is a physiological phenomenon.

To date only a limited number of studies have identified if the exercise-related appearance of evidence of cTn and/or NT-proBNP is a highly repeatable phenomenon (Middleton et al., 2007; Sahlen et al., 2008;Tian et al., 2014; Wedin & Henriksson 2015). Sahlen et al., (2008) assessed the repeatability of the hs-cTnT and NT-pro-BNP response in mature athletes pre and immediately after a 30 km cross-country race with 3 years between each race. The correlation coefficients for the biomarkers between the two races were r = 0.82 for NT-proBNP and 0.84 (Spearman's rho, both p <0.001). This suggests some level of consistency of response although the intervening 3 years may have led to substantial changes in individual fitness and health that may challenge the true nature of the repeatability of this phenomenon.

Wedin & Henriksson (2015), expanded the research area by using elite, intermittent high-intensity exercise. Participants (n=16) with a median age of 19 years, completed two matches of Scandinavian floorball with 3 months between the two games. Blood was analysed at pre, immediately after and 2 hours post games. The authors noted at 2 h post-match moderate reproducibility for elevated hs-cTnT (ICC = 0.368).

Middleton et al., (2007) and Tian et al., (2014) employed a much more constrained time period for their repeated exercise bouts which likely exerted more control over the

test design and data collection. Middleton et al., (2007) assessed three repeated 15-mile hill runs over three consecutive days with blood draws taken pre, 0, 1, 20 h after each bout of exercise. cTnT was elevated above detection levels in 4 runners 1 h after the first bout of hill running (0.013 – 0.125µg.L^{-1}) but no detectable elevations were observed after the other 2 bouts of running in the same participants. This does not support the concept of a repeatable phenomenon over a short time frame. Tian et al., (2014) employed a controlled laboratory based setting for their study, with blood draws taken at 0 and 3 h post-exercise, with identical 90 min treadmill runs separated by 3 weeks. A significant elevation in hs-cTnT was noted after both trials but a clear "blunting" in the magnitude of response was observed after the second bout of exercise. The authors suggested some sort of "learning effect" could have caused the lower hs-cTnT response in Trial 2. Given the timeframes involved and the reduced cTn response in second exercise bout, this data could reflect a "repeat-bout" effect. This draws parallels with work associated with skeletal muscle fatigue and damage and the release of the CK-MM biomarker after repeat bouts of exercise (normally eccentric) where skeletal muscle damage is assumed. Although descriptive in nature, some researchers have speculated that what is happening to the cardiac muscle is akin to the adaptive response to delayed onset muscle soreness (DOMS) after damaging exercise in skeletal muscle. The "repeat bout" effect is where a single eccentric exercise bout that results in skeletal muscle damage then imparts a "refractory period" and reduced damage occurs in subsequent and similar exercise challenges in the same people and same muscle groups (Cheung et al., 2003). This requires further substantiation with cardiac biomarkers. The general lack of consistency in the data assessing the repeatability of the cardiac biomarker response to exercise drives the rationale for the second study in this thesis using active participants in a laboratory

setting with more experimental control whilst assessing both hs-cTn and NT-proBNP. The potential for a repeat bout effect when exercise is repeated in a short time frame also drives the rationale to have three identical exercise tests with the first two bouts separated by a week and the final bout 12 weeks later that allows repeatability and the repeat bout effect to be assessed.

2.5. Clinical Consequences and Potential Mechanisms of Cardiac Biomarker Release

Despite continuing debate in relation to the exact prevalence of "positive" cTn and NT-proBNP responses during, and after prolonged exercise, the simple presence of this response has raised important concerns for athletes, coaches, physicians and researchers alike (George et al., 2012). Clinically, significant elevations in cTn's and NT-proBNP could be indicative of the pathological processes associated with disease or injury of the myocardium. Exemplars of athletes suffering an acute cardiac arrest during the high stress environment of sports performance are, thankfully, rare. Despite this when such events happen it is very shocking and often generates significant media attention. For the clinician attending a sport event, or even an Accident and Emergency Department receiving athletes post-event, they must by confident in their differential diagnosis. If the presence of cardiac biomarkers after exercise reflects a pathological process this could have negative cardiovascular health consequences, which must be assessed quickly, and accurately (Whyte, 2007).

The appearance of cardiac biomarkers in cases of acute cardiac syndromes and chronic heart failure are well documented. Acute coronary syndrome generally have a biphasic

cTn release, with an initial cTn appearance approximately 2 h after the event followed by extended release that can last for many days (Collinson et al., 2003). Despite this we know biomarker release can occur outside of "frank coronary disease". There are ample case studies of cTn elevation in (for example) tsako-tsubosyndrome (also known as stress cardiomyopathy) or ventricular tachycardia (high heart rate) where there is no documented coronary artery disease and the long term cardiomyocyte damage is likely negligible. Consequently, the exercise response may be clinically irrelevant and reversible and simply reflective of a heightened cardiac workload.

Despite the standpoint noted above there is still very little data (especially in humans) as to what potential mechanisms are at play in this phenomenon. A number of scientists have speculated that exercise-related cardiac biomarker release reflects passive diffusion cross the cell membrane due to an increase in permeability, similar to that seen with the adaptation process in skeletal muscle hypertrophy (Neymayr et al., 2004). This might suggest a reversible mechanism that is assisting in cardiac remodelling (McNeil et al., 1992; Scharhag et al., 2006; Shave, 2010). Although the mechanical process of heart remodelling remains unclear, Reactive oxygen species (ROS) have been linked to both cardiac damage and cardiac remodelling (Dennis et al., 2014). Reactive oxygen species are a group of highly reactive molecules, which have the potential to modulate several biological processes as well as cause tissue damage and dysfunction. Their effects can be beneficial or deleterious, depending on the concentrations produced and the site of production. For example, Schiattarella (2017) indicated that there is a positive correlation between specific markers of oxidative stress and cardiac remodelling in patients with coronary disease.

Another speculative idea for the cause of cTn elevation with exercise is the release of integrins caused by the increased myocardial stretch during prolonged exercise (Koller, 2009). Integrins are transmembrane receptors that assist the passage of cTn molecules out of the cardiomyocytes (Hessel et al., 2008). Hessel et al., (2008) hypothesised that the stretch-related process stimulates the release of cTnT & cTnI with the help of integrins. Despite this speculation there is no empirical data assessing the role of any potential mechanisms in cardiac biomarker release with exercise in humans (Dawson et al., 2003; Viela et al., 2014).

Another potential mechanism for cTn or NT-proBNP release with exercise is excessive right ventricular overload. Evolving research has expanded our understanding of how prolonged exercise, such as marathon running and ultra-endurance events, places a disproportionate afterload on the RV (Shave et al., 2008). Evidence indicates a moderate correlation between a reduction in RV ejection fraction and increase in cTn and NT-proBNP after exercise, but with little or no relation to changes in LV function (La Gerche et al., (2014). Research data suggests that a greater relative increase in pulmonary afterload with exercise places the RV under a disproportionate increase in stress during prolonged exercise leading to potential overload and stress/injury in the RV

2.5.1. Ischaemia and Cardiac Biomarker Appearance

In a clinical setting one of the mechanisms that underpins cTn elevation is cardiomyocyte ischaemia that leads to a cascade of events resulting in cell death and biomarker release (Shave et al., 2010). It has been speculated that "sub-clinical" ischaemia may contribute to exercise-related biomarker release (Dawson et al., 2003) although this has not been directly tested as a hypothesis in a human-exercise setting.

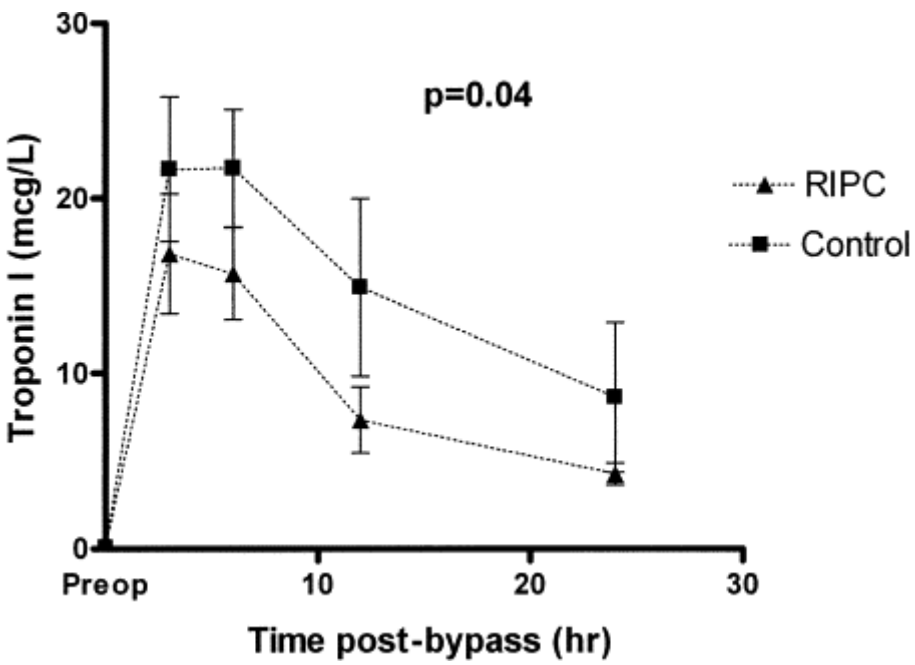
Recently the development of ischemic preconditioning has presented an opportunity to indirectly evaluate the role of ischaemia in exercise-related cardiac biomarker appearance. Ischemic preconditioning (IPC) developed from the pioneering work by Murry et al., (1986) when dogs were exposed to 4 x 5 minute of coronary artery occlusions, too brief in themselves to cause myocyte death, interspersed with five-minutes of reperfusion. This was compared to a control period with no meaningful IPC. An ischemic event akin to a cardiac arrest was then induced in all animals. The results of the study demonstrated that; (1) dogs that received the IPC had a 75% reduction in ischaemia in the secondary ischemic insult, and (2) the dogs that received IPC had a much smaller infarct size.

Since the early 1990s there have been a range of clinical and physiological studies assessing the value and utility of IPC. A key development from the perspective of clinical application has been the use of remote ischemic preconditioning (RIPC) where the ischemic stimulus is presented in a different artery (e.g. a limb) to that where the assessment of effect of the ischemic preconditioning is still measured (i.e. the heart). Przyklenk et al., (1994) pioneered the development of RIPC by showing that brief occlusion of the circumflex artery rendered the myocardium served by this artery as resistant to further incidents of ischaemia. More importantly, canines underwent 4 x 5 circumflex artery occlusion with 5 minutes of reperfusion, then 1 hour of sustained left anterior descending coronary artery occlusion and 4.5 hours of reperfusion. Their study showed that the IPC to the circumflex artery provided some protection against myocardial damage after left anterior descending artery occlusion. This implied that the protection from ischaemia/reperfusion can occur at a point distant to the site of

IPC. Indeed, further studies in animals showed that even when IPC was applied to a major arteries in distant organs (e.g. hind limb, lower leg, kidney) the infarct size in the heart was still reduced and thus protection against subsequent myocardial injury was evident (Aon et al., 2010). The exact mechanism(s) of ischemic preconditioning remains obscure.

As a consequence of these developments noted above Cheung et al., (2003) employed RIPC in a study in children undergoing repair to congenital heart defects. Four 5-minute cycles of RIPC were applied to produce lower limb ischaemia-reperfusion in 17 children. Lower cTnI release was then observed compared to the control ($p=0.04$) during surgery. The authors suggested the reduction in cTnI with surgery after RIPC could indicate a possible protective effect of RIPC on subsequent myocardial injury (see Figure 2.2).

Figure 2.2. Pre and post-operative levels of troponin I after the application of remote ischemic preconditioning (RIPC) or a control (from Cheung et al., 2006).



Systematic reviews and meta-analyses have confirmed that RIPC can reduce the release of cTn in response to an "event". Takagi et al., (2008) performed the first meta-analysis on RIPC in cardiovascular surgery and included data on 184 patients. They demonstrated a statistically significant reduction in cTn after RIPC relative to a control group/procedure. Alreja and Bugano (2012) pooled 17 clinical trials in a systematic review and meta-analysis to evaluate the effect of RIPC on myocardial and renal injury. Patients had lower levels of cTnT or cTnI after RIPC compared to a control group/trial. Pilcher et al., (2012) produced a systematic review and meta-analysis of the cardioprotective effects of RIPC in open cardiac surgery in 10 studies with a total of 693 subjects. RIPC reduced cTn levels 12 hours after surgery compared with the control group. The fixed and random effects differences were 0.35 (95% CI 0.19 to

0.51) and 0.53 (95% CI 0.18-0.88) which indicates changes that are reflect meaningful cardioprotection.

Translation from clinical studies to humans performing exercise has only been attempted once to date (Messaoudi et al., 2013) but forms a potentially interesting and simple way to indirectly assess the role (if any) of ischaemia in the post-exercise elevation in cardiac biomarkers. The study by Messaoudi et al., (2013) measured hs-cTnI at multiple time points (0, 1, 2 ,3 ,4 and 8 h) post 70 min of cycling at 80% of maximum heart rate followed by exercise at 95% of maximum heart rate until exhaustion. NT-proBNP was measured 1 and 3 h post exercise. The bout of cycling was preceded by RIPC or a control intervention. The key outcomes were that hs–cTnI significantly increased, reaching a peak after 4 h post exercise but that RIPC did not mediate a significant change in hs-cTnI levels after exercise. This led the authors to suggest that ischaemia was unlikely to play a major role in the aetiology of cTn appearance after exercise of this limited magnitude. There are a number of methodological issues in RIPC studies generally, and the Messaoudi et al., (2013) study specifically, that require consideration and standardisation in future RIPC studies. For example, the time, location and repetition of procedure for pressure cuff placement on limbs is highly variable. Salvador et al., (2016) completed a systematic review and meta-analysis on RIPC and exercise performance in 19 studies. Many of the 19 studies adopted the RIPC protocol used by de Groot et al., (2010) where a pressure cuff was placed on both thighs and inflated for 5 minutes followed by 5-minute reperfusion period. The ipsilateral limb was then subjected to the same protocol. This process was repeated 4 times for a total RIPC exposure of 40 minutes. Salvador et al., (2010) proposed at least 3 cycles of hypoxic-ischemic cycles were

necessary to achieve a protective response although empirical support for this is still required. There is a need for the correct cuff sizes to be used to allow for complete arterial blood flow blockage and there is also some variation in the pressure level employed to prevent blood flow (Dempsey et al., 1999). The location of the pressure cuff has been varied in a range of studies. Some studies have used one cuff on one limb, sometimes the thigh (83%), sometimes the forearm (17%). Loukogergakis et al., (2007) noted a greater effect when ischemic reperfusion occurred on the thighs with blood flow disruption applied alternatively to both limbs. Salvador et al., (2010) speculated that the more muscle exposed to ischaemia the greater the effect and consequently proposed that pressure cuffs should be used bilateral (left forearm and right thigh, then right forearm and right thigh) rather than unilateral. Finally, most single blind cross-over studies have used a SHAM control to help understand the treatment effect. In RIPC studies to date it has been common to simply use a very low blood pressure, such as 20 mmHg, well below diastolic pressure and thus not disruptive of blood inflow or outflow to the limb. Whether this is an effective SHAM requires evidence-based data in all studies as participants may notice the difference in cuff pressures applied. Despite these limitations with the use of this protocol as a "placebo", no specific alternatives have been proposed or tested.

These studies and technical considerations provided the primary focus, or rationale, for studies 3 and 4 in this thesis, applying RIPC (or SHAM) prior to repeated bouts of cycling in the lab (3) or the field (4). This will provide insight into the role of ischaemia in the cardiac biomarker release with exercise that is currently lacking or controversial.

2.6. The Link between Cardiac Biomarker Appearance and Cardiac Functional Changes Post-exercise.

Saltin & Stenburg (1964) were the first to describe a potential decline in intrinsic left ventricular (LV) contractile function as a consequence of prolonged exercise in the presence of unaltered haemodynamic loading. This study prompted ongoing investigation into the immediate or short term effects of prolonged exercise upon cardiac function that continue to this day (e.g. Lord et al., 2016; Eijsvogels et al., 2017; Oxborough et al., 2018; Lord et al., 2018); . Most studies have been supportive of these initial findings if the exercise exposure is significantly large and strenuous (see reviews; Shave et al., 2008; Oxborough et al., 2010). Douglas et al., (1987) termed this phenomenon "cardiac fatigue" and this has been broadly adopted within recent literature (e.g. George et al., 2011; 2012).

In their meta-analysis Middleton et al., (2006) revealed an overall post-exercise reduction in left ventricular ejection fraction (EF), end-systolic pressure-volume ratio (SBP/ESV) ratio, and peak early to atrial diastolic filling velocity ratio (E/A). This suggests a transient decrease in both LV systolic function and diastolic filling as a consequence of undertaking acute bouts of prolonged exercise (greater than 2 hr duration). Further work has also reported similar changes in the right ventricle (RV) after prolonged exercise (Oxborough et al., 2011; La Gerche et al., 2012; La Gerche et al., 2014)).

A significant amount of research work in relation to "cardiac fatigue" has been descriptive with the aim of understanding what exercise (duration, mode etc.) and

participant (age, sex, fitness etc.) factors may augment the appearance of changes in cardiac function. This clearly parallels work with acute exercise and the cardiac biomarker response.

Again, as with cardiac biomarkers, an important consideration of this apparent decrease in LV and RV function after prolonged exercise is related to putative mechanism(s) (Dawson et al., 2003). Mechanistic work is often limited in human studies, especially those based in the field where whole organ assessment of cardiac function via non-invasive imaging has been the primary outcome variable. On the basis of an early case study (Rowe 1992) and case-series data (Siegel et al., 1984) some have postulated that post-exercise changes in LV function were a consequence of cardiomyocyte damage, somewhat akin to how cardiac function declines consequent to the ischaemia and cellular damage that occurs with myocardial infarction (Collinson et al., 2003). The work by Rowe (1992) who reported on Sy Mah's autopsy (an athlete who set a world record of 524 marathons but died of non-cardiac cause) and identified fibrosis of the papillary muscle with the absence of atherosclerosis. Rowe speculated the fibrosis could have been caused by exercise-inducted vasospasm caused by localised ischaemia.

Consequently, the concept of exercise-induced cardiac damage (EICD) was conceived ***alongside*** exercise induced cardiac fatigue (EICF) and the assessment and association of both phenomena have been descriptively studied in parallel in recent years. Whether one (EICD) leads directly to the other (EICF) remains a controversial issue. In human work we are limited to assessing the correlation between evidence of EICF and EICD

and in the vast majority of cases there has been no clear link or association. Despite this, Neilan et al., (2006) reported a significant correlation between cTn elevation and reduced RV function. Rifai et al., (1999) using participants from the Hawaii Ironman triathlon associated elevated cTn with qualitatively poor echo scores. Ohers have not identified a direct relationship between elevated cTn and permanent cardiac function (George et al., 2009; Siegel et al., 1988). This area requires further examination due to differential findings in past work.

The studies proposed in this thesis related to the impact of RIPC on cardiac biomarker appearance provide an ideal scenario for assessing the relationship between EICF and EICD and whether both phenomenon have a relationship to ischaemia. Our understanding is still at its development phase in part due to the fact that it is challenging to study the structure of the human heart. Non-invasive imaging advances such as novel echocardiographic techniques have helped to open up new research directions to help to improve our understanding of what happens to the myocardium during and after prolonged exercise. This provides the specific rationale for the inclusion of measures of cardiac function in studies 3 and 4.. Specifically we will look at global measures of LV and right ventricular function as well as regional myocardial function using tissue Doppler assessment (George et al., 2005).

2.7. Summary

The clinical literature clearly supports the notion that injury to the myocardium (or cardiac dysfunction) is associated with the release of significant levels of specific biomarkers such as cTnI/cTnT. Further chronic heart failure has been strongly

associated with high levels of circulating NT-proBNP. In both clinical scenarios cardiac function is also depressed. The fact that acute bouts of prolonged strenuous exercise may produce similar changes in cardiac biomarkers and function is, superficially, counter-intuitive but supported by a substantial empirical database. Understanding why these phenomenon happen is important in our appreciation and debate of the positive and negative effects of exercise on the myocardium. Contradictory outcomes in some studies to date also requires on-going enquiry. Further, there is a need for clear policies from the extensive research in this area to help inform sports medical staff when working in this field with athletes who present with elevated cardiac biomarkers or mildly depressed cardiac function after an exercise bout.

With the development of hs-cTn assays as well as developing novel measures of cardiac function it is still imperative to define what happens in different bouts of exercise, in different populations. This current thesis will seek to assess the nature of hs-cTnT and NT-proBNP responses to acute exercise and the repeatability, or potential repeat bout effect, associated with this phenomenon. Further, we wish to begin to explore potential mechanistic issues behind these phenomena. Whilst, ischaemia has long been "touted" as a key mechanism empirical data does not yet exist in humans to link these issues.

The proposed studies will help to inform the continuing question as to whether "elevation of cardiac biomarkers or a depression in cardiac function after exercise" represents a reversible or irreversible insult to the heart.

2.8. Study Hypotheses

STUDY1: Hypothesis 1: Running for 2 hours on a motorised treadmill at moderate intensity will reveal a rapid turnover of cTnI, using the new high sensitivity assay, and NT-proBNP in moderately active adults**.**

STUDY 2: Hypothesis 2a: Cardiac biomarker responses to the same exercise bout employed in STUDY 1, are highly repeatable after a period of 7 days between exercise tests in the same participants.

STUDY 2: Hypothesis 2b: Cardiac biomarker responses to the same exercise bout employed in STUDY 1, and Hypothesis 2a, will be highly repeatable after a period of 12 weeks between the first and last exercise test in the same participants.

STUDY 3: Hypothesis 3: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 1-h strenuous cycle time trial exercise is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.

STUDY 4: Hypothesis 4: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 160 km cycle trial is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.

Chapter 3:

Study 1: Is there a rapid turnover of hs-cTnI and NT-proBNP during and after 2 h of prolonged moderate intensity exercise in healthy active individuals?

3.1. Introduction

Most of the existing literature related to the release of cardiac biomarkers (cTnI/T and/or NT-proBNP) with acute bouts of prolonged exercise have employed limited pre-post designs attached to field based competitions with older assays in elite athletes (Shave et al., 2010). Though valued, much of the inconsistent data derived from these studies may be attributed to variations in the design employed, the participants studied and/or the assays used (Shave et al., 2010). It has been postulated that the competitive nature of these original research studies made it difficult to control many confounding variables such as the exercise dose and environmental stress (Apple et al., 2002; Laslett et al., 1996; Siegel et al., 2001; Lippi et al., 2008).

In an important step forward, Middleton et al., (2008) performed a controlled laboratory-based study in 9 athletes who completed a self-paced treadmill marathon. In tandem with this design, cTnT was analysed from blood draws at 30 min intervals during exercise, as well as immediately and at 1, 3, 6, 12 and 24 h after exercise. All of the athletes had a detectable rise in cTnT during exercise and 8/9 runners had detectable cTnT during recovery. This data would suggest all athletes performing substantial bouts of prolonged exercise might release cTn into their circulation and thus it could be considered as a normal/physiological or even obligatory release. To date this design and experiment has not been replicated with newer assays (e.g. hs-TnI)

and in different participant groups and this provides part of the rationale for the initial study.

The potential insight generated by a combination of a controlled exercise environment (exposure), the ability to assess lower levels of cTn using the new hs-cTnI assay and the recruitment of healthy but untrained participants is unique. Consequently, the aim of the first study in this thesis is to document the kinetics of hs-cTnI and NT-proBNP in response to 2 hr of endurance exercise in a controlled laboratory setting in young, untrained participants. Blood samples will be collected during exercise and into early recovery. If hs-cTnI and NT-proBNP levels appear rapidly during prolonged exercise (i.e. within 2 hours) as well as drop below baseline within 3 hours post exercise, this could indicate a more rapid release and turnover than that which occurs with AMI. This would support reversible cardiomyocyte damage and/or sub-clinical haemodynamic stress as the overarching mechanism underpinning biomarker release with exercise.

STUDY1: Hypothesis 1: Running for 2 hours on a motorised treadmill at moderate intensity will reveal a rapid turnover of cTnI, using the new high sensitivity assay, and NT-proBNP in moderately active adults**.**

3.2. Methods

Participants

In line with Middleton et al., (2008) study, nine healthy and active male participants (mean + SD; age 21 + 2 yr; stature 1.80 + 0.04 m; body mass 77.2 + 15 kg) volunteered to take part in the study. Ethical approval was obtained from Liverpool John Moores University Ethics Committee prior to data collection. Participants were recruited from the Cheshire Regiment's 1st team football squad (see Table 3.1). All participants provided written informed consent. The participants received a participant information sheet and were verbally informed of the procedures involved in the testing protocol, with any associated risks and their rights to withdraw from the study at any point clearly outlined. All participants completed a health related questionnaire prior to undertaking any test (Appendix 1) to screen for medical conditions and/or a family history of cardiovascular disease. We specifically recruited participants with moderate levels of fitness who could complete the tasks required but who were not currently, or in the last 3 years, training for endurance competition

Table 3.1. Participants' characteristics

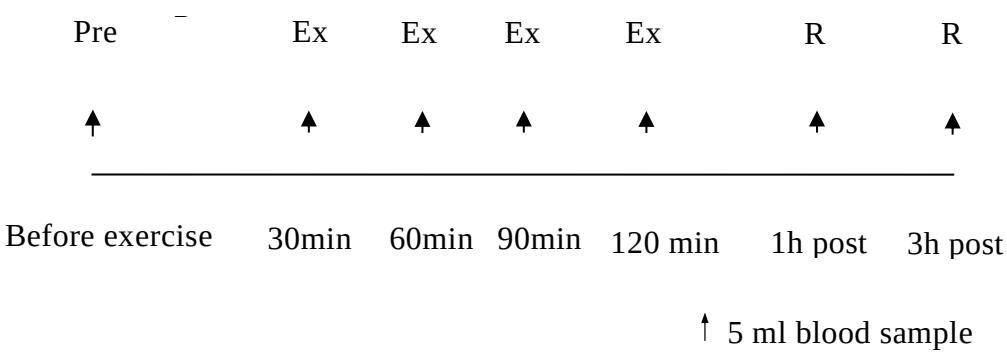
Variables	Subjects' characteristics
Men	9
Physical activity (h/wk)	15 + 0.4
Running activity (km/week)	4 + 4
Competitive running experience (km/week)	0
Marathon or ultra-distance experience	0
Treadmill running experience (km/week)	2 + 3

Data are presented as mean + SD

Protocol

To improve familiarisation participants were asked to complete 2 visits to the laboratory at the Tom Reilly Building Liverpool John Moores University and be ready to start for 10am. At visit 1 participants initially had their height and body mass measured and then performed a graded treadmill running test to volitional exhaustion. This test determined treadmill speed and target heart rate for the prolonged exercise trial in visit 2. At visit 2, which was a minimum of 7 days after visit 1, the endurance trial was performed. This lasted 120 min at 70 to 80% of peak heart rate that was determined from the initial maximal treadmill test. Blood samples for the determination of hs-cTnT and NT-proBNP were performed at baseline (rest), at 30, 60, 90 and 120 min of exercise and then 1 and 3 hours post-exercise (Figure 3.1). All participant were directed not to exercise intensely in the 48 hours before attending the laboratory. Participants were asked to eat breakfast before attending the study and to avoid caffeine and alcohol for 24 hr before the assessments. Participants were allowed to drink water before and during exercise *ad libitum*, to maintain hydration. The test occurred at least 3 hours after the participant's last meal.

Figure 3.1. Schematic of the blood collection protocol.



The height (m) and body mass (kg) of each participant was measured using a standard stadiometer (Seca, Germany) and electronic scales (Seca, Germany), respectively. At the first visit after a general warm up of walking on the treadmill and stretching, the treadmill speed was set at an initial speed of 6 $km.h^{-1}$ and the gradient set at 1%. During the test the running speed was increased in increments of 1 $km.h^{-1}$ every minute until 13 $km.h^{-1}$ was reached. The treadmill speed was then kept constant and the gradient was increased 1% every minute until the test was voluntary terminated. Exercise HR was measured with an HR monitor (Polar S810i; Kempele, Finland) at 5 s intervals and maximum HR was recorded as the highest 30 s average value.

In the prolonged treadmill test a general warm up of walking on the treadmill and stretching was performed before the treadmill speed was gradually increased to the target speed. Timing of the 2 hr trial and HR were recorded from this point. During the run the subject was aware of the time, distance and speed. The prolonged exercise trial was completed at 70 to 80% of maximum heart rate determined from the initial maximal treadmill test. Heart rate data was continuously recorded throughout the exercise trial and the participants were allowed to make small changes in treadmill speed for their own comfort at any stage.

Blood Sampling and Analysis

Blood samples were collected via an in-dwelling cannula. A small butterfly needle was inserted in to a vein with a cannula in the arm and left there for the duration of the exercise so that a total of 7 x 5 ml blood samples were collected using serum gel vacutainer (Becton Dickinson UK Ltd, Oxford UK). After the sample had been

collected and removed the cannula was flushed with 10mL of 0.9% NaCl. The insertion and maintenance of the venous cannula was performed by qualified staff. Patency of the cannula was checked regularly and saline flushes used if necessary. Collected whole blood samples was left to clot for approximately 60 min and then centrifuged at 1000 g at 18^{o}C for 10 min. The serum was then drawn off with a disposable pipette (Effendorf, Hamburg, Germany), samples were divided into 3 aliquot parts, and stored at -80^{o}C for later analysis. Participants were encouraged to drink during exercise *ad libitum* to maintain euhydration.

Cardiac troponin I was analysed using the hs-cTnI ADVIA Centaur CP TnI Ultra assay. The assay is a three-site sandwich immunoassay using direct chemilominometric technology. A "Binary Lite" reagent was used to reduce non-specific binding. The antibodies in the ancillary reagent bind to troponin I in the sample based on guidelines from the Clinical & Laboratory Standards Institute. The market versions of the ADVIA Centaur assay have a lower detection limit that equates to the 99th percentile of a healthy population (20 ng/L). The assay for our laboratory has an established diagnostic cut-off of 30 ng/L and values above this are considered to represent myocardial damage, with the current accepted cut-off for diagnosis of acute myocardial infarction (AMI) at 50 ng/L.

The IMMULITE 2500 analyser was used for the quantitative measurement of the NT-proBNP assay with an incubation of 10 minutes. It is a two site chemiluminescent immunometric assay. Based on guidelines (Siemens 2007) the IMMULITE 2500 analyser employs a lower detection limit that equates to the 95th percentile of a healthy

population (50 ng/ml with a linear calibration range of 35,000 ng/ml). Values above 125 ng/mL in patients younger than 75 years old reflects a clinically relevant outcome.

Statistical Analyses

Power analyses are problematic when some biomarkers are undetectable at rest or post-exercise. Consequently, we adopted an approach to recruit a motivated sample whose size was similar to previous studies. The final sample consisted of 9 subjects, comparable to Middleton et al., (2008: n = 9) and Mousavi et al., (2009: n =8). All cohort data are reported as mean + SD unless otherwise stated. hs-cTnI data were analysed individually and descriptively due to the likelihood of undetectable values at rest. All NT-proBNP data (pre, during and post-exercise) were analysed using repeated measures one-way ANOVA and post-hoc pair-wise comparisons as appropriate. All analyses were performed using Statistic Package for Social Science for windows version 17.0 (SPSS v17 for Windows, SPSS Inc IL, USA).

3.3. Results

All participants completed the 2 h motorised treadmill test at a mean speed of 8.0 + 0.8 km/h and mean heart rate of 142 ±17 beats.min^{-1} (71 + 8% of measured maximum heart rate).

Table 3.2 details individual hs-cTnI values at each blood draw for all participants. All cTnI values were below the assay detection limit of 20 ng/L at baseline. During exercise no cTnI was detected in any sample. Post-exercise cTnI remained undetectable

in 7 of the 8 participants. The single participant with detectable hs-cTnI post exercise had a minor increase at post 60 min (22 ng/L) with a maximum value at 180 min post-exercise (24 ng/L). No value exceeded the clinical cut-off of 50 ng/L at any sample point.

Table 3.2. Individual data for hs-cTnI (ng/L)

SUBJECTS	PRE	EX 30	EX 60	EX 90	EX 120	R 60	R 180
1	<20	<20	<20	<20	<20	<20	<20
2	<20	<20	<20	<20	<20	<20	<20
3	<20	<20	<20	<20	<20	<20	<20
4	<20	<20	<20	<20	<20	**22**	**24**
5	<20	<20	<20	<20	<20	<20	<20
6	<20	<20	<20	<20	<20	<20	<20
7	<20	<20	<20	<20	<20	<20	<20
8	<20	<20	<20	<20	<20	<20	<20
9	<20	<20	<20	<20	<20	<20	<20

Pre=pre exercise sample, Ex=exercise samples, R=recovery samples, numbers are minutes through each component of the protocol

There was a significant increase in NT-proBNP compared to baseline levels at all sample points ($P<0.001$).Values at EX120, R60 and R120 were higher than EX30 with heterogeneity of response noted as mean values increased ($P > 0.05$). Mean and SD for NT-proBNP peaked at R60 post-exercise. Mean NT-proBNP values at 180 min post-exercise was still close to the 125 ng/l clinical cut-off and raised above baseline levels (Table 3.3). The subject (4) with detectable cTn post-exercise had an exaggerated NT-proBNP response with a peak (365 ng/L) 60 min post-exercise although there was then a rapid drop at 180 min post-exercise (65 ng/L). The participants who were presented with cTn levels below URL had a highly variable NT-proBNP response, with no clear pattern of high or low responders.

Table 3.3. Individual and mean cohort data for NT-proBNP (ng/L)

SUBJECTS	PRE	EX 30	EX 60	EX 90	EX 120	R 60	R 180
1	80	90	120	138	194	210	170
2	64	46	43	58	89	120	158
3	40	75	90	120	147	107	87
4	36	50	88	121	223	**365**	**65**
5	38	50	75	83	102	127	161
6	43	55	76	104	137	155	210
7	50	63	90	62	55	132	86
8	41	59	73	86	58	96	77
9	52	76	83	89	120	203	66
MEAN	**49**	**63**	**82**	**96**	**125**	**168**	**120**
SD	**14**	**15**	**20**	**27**	**57**	**84**	**54**

Pre=pre exercise sample, Ex=exercise samples, R=recovery samples, numbers are minutes through each component of the protocol
*Significant increase in NT-proBNP compared to baseline levels at all sample points (P<0.001).

3.4. Discussion

To the authors knowledge this is the first study to utilise the new hs-cTnI assay as well as the NT-proBNP assay in a controlled laboratory trial with healthy recreationally active participants, to determine the cardiac biomarker response to 2 h of prolonged running on a motorised treadmill. The key findings were that; 1) detectable cTnI was observed in only 1/9 participants in the early recovery period, and 2) a progressive rise in NT-proBNP across exercise and early recovery was noted in all subjects, with partial recovery 3 hr post-exercise.

The current study's observation of only sporadic (1/9) and very low (sub-clinical cut-off of 30 ng/l) values for hs-cTnI in response to 2 h of treadmill running are somewhat at odds with the prevailing literature (Middleton et al., 2008). Despite this, similar small percentages of "cTnI-responders" have been reported in field-based studies of

competitive endurance running (Abasher et al., 2010) and study-to-study variability is well noted in narrative reviews (Shave et al., 2010) and meta-analyses (Shave et al., 2007). In adopting a research design with multiple blood draws to more fully describe the cardiac biomarker kinetics during and post-exercise we were surprised that we did not see similar cTn data to that published by Middleton et al., (2008). Middleton et al., (2008) described a cTnT increase in all of the athletes completing a treadmill-based marathon in athletes. The consistency of response, subject-to-subject was notable leading the authors to suggest that cTnT release with prolonged exercise may be an obligatory and physiological response. We can only speculate as to the discrepancy between the current study and Middleton's data but some details are worthy of note. Middleton et al., (2008) studied highly trained athletes completing a treadmill-marathon as quickly as possible (these participants would have been more highly trained and exercising at a higher absolute and relative intensity for c. 3.5 hrs). These differences (training status and exercise exposure) may provoke different cTn responses to prolonged exercise (Shave et al., 2010) but have rarely been systematically studied in controlled environment. There is some suggestion that higher exercise intensities and trained participants are more likely to demonstrate a cTn response to prolonged exercise but we note that Eijsvogels et al., (2010) reported positive cTn responses in low fit participants undertaking prolonged walking-exercise, albeit using an older assay. Notably Middleton et al., (2008) also used a 3rd generation cTnT assay, as opposed to hs-cTnI, and different detection limits may be relevant (Eijsvogels et al., 2010).

There is also some in-consistency when reporting cTnI versus cTnT in response to exercise with the two molecules having different molecular weights possibly impacting

their “exercise-response” (Klinkenberg et al., 2016). Further cTnT was at a maximum 2 h post-exercise vs. 5 h post-exercise for cTnI.

The implications that we draw is that exercise-induced cTnI release is likely dependent on a range of potentially interacting factors (assay, fitness status, exercise volume) and as such is extremely difficult to predict. The sporadic release and the sub-clinical threshold response when cTnI was detectable do not appear to support irreversible cell death as the cause of any change in cTnI in this specific sample undertaking the specific exercise stimulus.

The appearance and rise of NT-proBNP in response to the current exercise exposure was variable. NT-proBNP data rose in all participants during exercise with some evidence of recovery early post-exercise in most subjects. Evidence from other researchers suggest that NT-proBNP values for healthy untrained and athletes are not elevated under resting conditions but can be substantially increased after prolonged endurance events (Scott et al., 2009). High intensity exercise, in healthy individuals, over shorter events, such as 30 to 60 min, has also resulted in an increase in NT-proBNP (Vidotte et al., 2005; Lippi et al., 2008). Consequently, the rise in NT-proBNP is the current study supports previous research.

To date, most of the available literature related to NT-proBNP release with exercise comes from pre - and post-endurance event data in well-trained athletes (e.g. Scott et al., 2009). To the best of our knowledge this study represents one of the first attempts

to detail NT-proBNP kinetics in response to controlled but prolonged exercise exposure in healthy and recreationally active young participants. A progressive rise with exercise duration points to the importance of this facet of exercise and supports some previous data (Nie et al., 2011; Carranza et al., 2011) in different participant groups (children) and various sports. The fact that most data were elevated above clinical cut-off limits is consistent with previous data (Scott et al., 2009) but must be interpreted, clinically, with some caution. Whilst mean cohort data peaked at 60 min post exercise (169 ng/L) this over simplifies a heterogeneous individual response and clearly by 180 min post exercise the NT-proBNP mean data was dropping back towards baseline.

This suggests two things; 1) individual variability is likely a complex and composite response to multiple between subject differences (age, fitness, health) that have not been adequately studied previously, and 2) rapid recovery of NT-proBNP from an exercise stress suggests a transitory and likely physiological response likely mediated by the increased cardiac stretch that occurs with exercise exposure as a means to increase preload and thus stroke volume during endurance exercise.

If NT-proBNP release is obligatory with endurance exercise exposure one might hypothesise that repetitive bouts of exercise should provoke the same NT-proBNP response. This has not been studied to date and will be the focus of the next Chapter. Likewise, the response of cTn to the same bout of exercise, in the same subjects (repeatability) has rarely been studied before in a controlled environment. This would likely inform the debate about the clinical importance of cTn changes with exercise and

whether it reflects reversible or irreversible cell death and provides a secondary rationale for the next study.

Limitations

The limited fitness of the participants and lack of familiarisation employed in the current study led to lower exercise intensities and volumes being completed in the 2 hr trial. This may have led to reduced cTnT data. The phlebotomist had difficulties taking blood from some participants and it may be a good idea to reiterate the importance to the participants that they come to the laboratory euhydrated. The use of multiple blood draws and 2 hours on the treadmill will be continued in the next Chapter.

3.5. Conclusion

In conclusion, moderate intensity prolonged running in healthy males resulted in a limited release of cTn (as detected by a new hs assay) as well as a significant, and exercise-duration dependent elevation in NT-proBNP. The rapid elevation and removal during early recovery suggest these changes have limited clinical relevance and possibly reflect normal physiological processes.

Study 2 will develop this work by employing and repeated, identical exercise bouts in the same participants 1 and 12 weeks after this first exercise exposure. This will provide an attempt to address the issue of repeatability of exercise induced cardiac biomarker elevation.

Chapter 4:

Study 2: The short and long-term repeatability of the hs-cTnI and NT-proBNP response to a 2 h bout of prolonged moderate intensity running in healthy active individuals.

4.1. Introduction

There is ample evidence that single bouts of exercise (across the intensity and duration domains, in a range of participant groups and in different settings) will lead to the appearance of biomarkers of cardiac insult/damage and stress (Shave et al., 2007; 2010). We have added to this database in the previous chapter, utilising a new hs-cTnI assay as well as providing greater insight into the exercise and recovery kinetics of NT-proBNP. Further we utilised multiple blood sample times to document the kinetics of the biomarker responses in healthy non-athletic adults that has rarely been described previously (Eijsvogels et al., 2010). We described limited hs-cTnI release and a more consistent increase and recovery in NT-proBNP. Given the nature of the kinetic data we proposed that these biomarker changes represented low or negligible levels of clinical risk and probably reflected normal physiological adaptation to endurance exercise.

We, and others, have conceived the idea that if these responses were highly repeatable this would provide further evidence of the obligatory or physiological nature of these biomarker responses. Somewhat surprisingly, the repeatability of cardiac biomarker responses to repeated exercise bouts, in a controlled environment is scarce (Nie et al., 2011). Nie et al., (2011) looked at the effect of repeat endurance runs (same speed and HR) on the same day, approximately 4 hours apart. This does not reflect the ideal

design to assess repeatability due to the limited recovery period between exercise bouts and the potential for a time of day effect (Chan-Dewar et al., 2013). Despite this, the data from Nie et al., (2011) indicated that the cTnT response to the second bout of exercise was qualitatively similar yet quantitatively "blunted". Whilst speculative, it may suggest that reversible cardiomyocyte damage from bout one is attenuated in a subsequent bout. Whether this is the case with exercise performed 1 and 12 weeks after an initial exercise trial is not known. The plan to utilize 2 repeat bouts at different times allows us to explore the potential for a "repeat-bout" effect when performing the same exercise stimulus.

Repeatability of cardiac biomarker responses to exercise has received very little attention (Tian et al., 2012). If the exposure was repeatable this study may help with our understanding of the impact or mechanisms involved in cTn elevation during exercise. Consequently, the specific aims of this second study were; (1) to analyse the cardiac biomarker response to two identical bouts of exercise performed exactly 7 days apart in a controlled environment (termed Week 0 and Week 1)**,** and (2) compare the cardiac biomarker response to a further exercise bout 12 weeks after the initial exercise exposure.

STUDY 2: Hypothesis 2a: Cardiac biomarker responses to the same exercise bout employed in STUDY 1, are highly repeatable after a period of 7 days between exercise tests in the same participants.

STUDY 2: Hypothesis 2b: Cardiac biomarker responses to the same exercise bout

employed in STUDY 1, and Hypothesis 2a, will be highly repeatable after a period of 12 weeks between the first and last exercise test in the same participants.

4.2. Methods

Participants

Eight healthy and active male participants (mean + SD; age 22 + 2 years old; stature 1.80 + 0.04 m; body mass 82.6 + 11.3 kg) volunteered to take part in the study. All participants were asked to complete three similar (speed, HR) prolonged runs on a motorised treadmill under the same conditions in week 0, seven days later in week 1 and then after a further 12 weeks. These participants are the same as those reported in Chapter 3 and were asked to maintain a consistent approach to diet and physical activity over the duration of the study. Ethics approval was granted by Liverpool John Moores Ethics Committee and all participants provided written informed consent before participation.

Eight participants exercised at a constant (over the 3 bouts) relative intensity for 120 min on a motorised treadmill that equated to 70 to 80% of peak heart rate, which was determined from the initial maximal treadmill test from Study 1. Specific controls were placed upon the repeated runs including the same; time of day, diet and hydration, prior exercise and environmental conditions. Venous blood was drawn, to determine hs-cTnI and NT-proBNP, at baseline (rest), at 30 min, 60 min, 90 min and 120 min of exercise and then 1 hour and 3 hours post-exercise for both trials. Heart rate data was recorded continuously throughout all treadmill runs.

Protocol

All assessment and data analysis protocols were consistent between Week 0, Week 1 and Week 12 and thus are reported in the previous Chapter.

Statistical Analyses

All data are reported as mean ± SD unless otherwise stated. hs-cTnI was analysed individually and descriptively due to the likelihood of undetectable values at baseline. Repeatability was assessed qualitatively by assessing those participants who had detectable values in all three trials.

All NT-proBNP data (pre, during and post-exercise) were analysed using repeated measures (for time and trial) two-way ANOVA and post-hoc pair-wise comparisons as appropriate. This approach determined the systematic changes in NT-proBNP kinetics. For the purposes of assessment of random variance in this biomarker, peak NT-proBNP values for all subjects in each of the trials were compared using Pearson product-moment correlation coefficients. All analyses were performed using Statistic Package for Social Science for windows version 17.0 (SPSS v17 for Windows, SPSS Inc IL, USA).

4.3. Results

The 8 participants completed exercise tests in week 0 and week 1. Two participants did not complete the 12 week study due to sports related injury. The 8 participants who completed the Week 0 treadmill run did so at a mean speed of 7.6 + 0.7 km/h and heart rate of 148 + 17 beats.min^{-1} (72 + 9% of measured maximum heart rate). The same participants completed the treadmill run in Week 1 at a mean speed of 7.4 + 0.5 km/h and a mean heart rate of 137 + 20 beats.min^{-1} (64 + 8% of actual heart rate maximum). The 6 participants who completed the run in Week 12 had a mean speed of 7.5 + 0.4 km/h and a mean heart rate of 141 + 3.2 beats.min^{-1} (65 + 8% of maximum heart rate). For the 6 runners who completed all tests there was no significant difference in speed and HR across the 0 and 12 week trials (> 0.05).

Table 4.1 demonstrates the cTnI responses to three treadmill exercise bouts for the participants who completed the tests. In Week 0, Week 1 and Week 12 cTnI values were below the clinical assay detection limit of 50 ng/L, in all participants, at baseline and during exercise.

Table: 4.1. Individual data for hs-cTnI in Week 0, Week 1 and week 12 (ng/L)

SUBJECTS	WEEK	PRE	EX 30	EX 60	EX 90	EX120	R 60	R 180
1	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**-**	**<20**	**<20**	**<20**	**<20**
	12	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
2	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
	12	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
3	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
	12*	**-**	**-**	**-**	**-**	**-**	**-**	**-**
4	0	<20	<20	<20	<20	<20	**22**	**24**
	1	**<20**	**<20**	**<20**	**<20**	**<20**	**30**	**33**
	12	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
5	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**-**	**<20**	**<20**	**<20**	**<20**
	12	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
6	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
	12	**<20**	**<20**	**<20**	**<20**	**<20**	**25**	**38**
7	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
	12*	**-**	**-**	**-**	**-**	**-**	**-**	**-**
8	0	<20	<20	<20	<20	<20	<20	<20
	1	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**
	12	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**	**<20**

Pre=pre exercise sample, Ex=exercise samples, R=recovery samples, numbers are minutes through each component of the protocol
* subjects that did not complete the 12 week run

There was only one participant (4) with detectable cTnI in both the week 0 and week 1 trials and the variability in actual concentrations was very small (during week 0 at 180 min there was a value of 24 ng/l and at week 1 there were detectable cTnI at 60 and 180 min of recovery, 30 and 33 ng/l). All other participants had non-detectable values in both weeks before, during and after exercise, reflecting consistent outcomes at a

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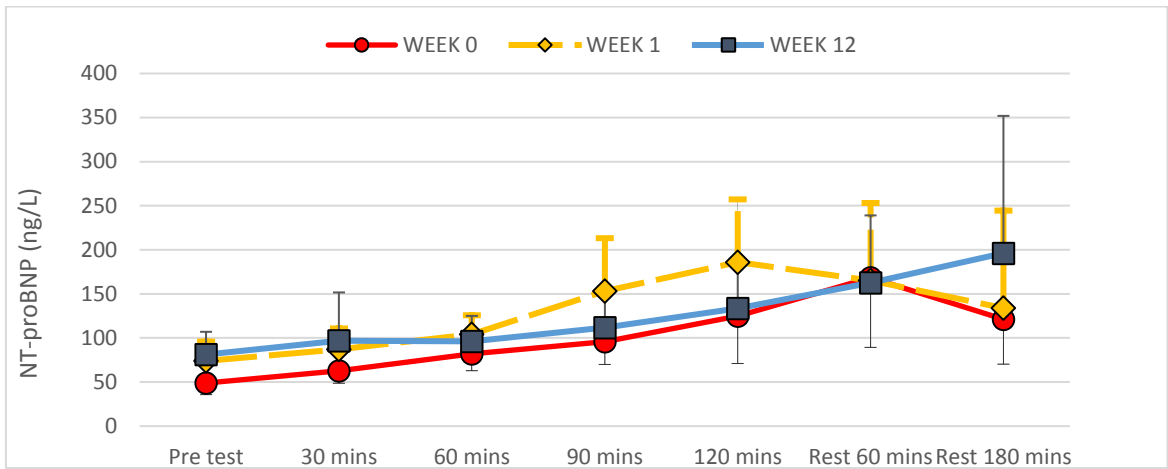
global level (responder vs. non-responder). In week 12 the "responder" in week 0 and 1 (4) had no detectable cTnI throughout the trial. One different participant (6) had detectable hs-cTnI of 25 and 38 ng/l (the highest value reported in the study) at 60 and 180 min of recovery in Week 12 but had not had any detectable values in week 0 and 1.

Figure 4.1 and Table 4.2 demonstrate the cohort and individual NT-proBNP response to the three identical exercise bouts. There was a main effect of exercise duration ($P<0.001$) as NT-proBNP rose with exercise duration in all weeks. Slightly higher values at rest were noted during week 12 but there was no main effect of week ($P=0.429$). Despite this there was a significant interaction effect ($P<0.004$) with data in Week 1, generally higher and peaking earlier (Ex 120) compared to a later peak for both Week 0 (R 60) and Week 12 (R180). The maximum concentration of NT-proBNP was 388 ng/L with 21 values out of 64 that exceed the clinical cut off value (125 ng/l). This was quite marked within and between individuals in the NT-proBNP response to the 3 exercise trials. Peak NT-proBNP levels were compared within individuals across trials. Correlation coefficients between trials 0 and 1, 0 and 12 and 1 and 12 were small to moderate in nature ($r = 0.036$, $r = 0.23$ and $r = 0.39$ respectively; all $P>0.05$).

Figure 4.1. Mean cohort data for NT-proBNP responses in Week 0, Week 1 and Week 12



Pre=pre exercise sample, Ex=exercise samples, R=recovery samples, numbers are minutes through each component of the protocol

Table 4.2. Individual NT-proBNP data in Week 0, Week 1 and Week 12 (ng/L)

Subjects	Week	Pre	Ex 30	Ex 60	Ex 90	Ex 120	R 60	R 180
	0	80	90	120	138	194	210	170
1	**1**	**75**	**88**	**94**	**100**	**150**	**175**	**183**
	12	**124**	**203**	**107**	**88**	**90**	**56**	**70**
	0	64	46	43	58	89	120	158
2	**1**	**90**	**122**	**136**	**185**	**210**	**147**	**66**
	12	**83**	**55**	**90**	**110**	**134**	**151**	**88**
	0	40	75	90	120	147	107	87
3*	**1**	**115**	**129**	**81**	**107**	**87**	**120**	**80**
	12	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	0	36	50	88	121	223	365	65
4	**1**	**83**	**69**	**115**	**249**	**330**	**388**	**406**
	12	**105**	**122**	**135**	**183**	**226**	**283**	**306**
	0	43	55	76	104	137	155	210
5	**1**	**56**	**67**	**83**	**106**	**141**	**93**	**107**
	12	**31**	**44**	**48**	**60**	**86**	**96**	**108**
	0	50	63	90	62	55	132	86
6	**1**	**40**	**72**	**133**	**245**	**194**	**163**	**34**
	12	**62**	**40**	**95**	**116**	**137**	**231**	**494**
	0	41	59	73	86	58	96	77
7*	**1**	**56**	**63**	**87**	**105**	**131**	**108**	**93**
	12	**-**	**-**	**-**	**-**	**-**	**-**	**-**
	0	52	76	83	89	120	203	66
8	**1**	**78**	**84**	**96**	**124**	**241**	**127**	**99**
	12	**58**	**98**	**124**	**106**	**131**	**158**	**142**

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Pre=pre exercise sample, Ex=exercise samples, R=recovery samples, numbers are minutes through each component of the protocol.

4.4. Discussion

The aim of this study was to analyse the repeatability of hs-cTnI and NT-proBNP responses to three similar prolonged runs on a motorised treadmill, with 1 and 12 weeks between bouts. The key finding were: 1) detectable hs-cTnI was identified in the same single runner in Week 0 and Week 1 trials and for the remaining 7 participants no detectable hs-cTnI was observed in either runs – suggestive of gross levels of repeatability [responders vs. non-responders], 2) at week 12 the responder in Week 0 and Week 1 did not demonstrate an elevated cTn although one different participant did who had not had a detectable level in week 0 and 1 providing limited support for a repeat bout effect, and 3) individual NT-proBNP responses were quite variable although an exercise duration related elevation in NT-proBNP was noted in all weeks although the magnitude of the response was somewhat augmented after the Week 1 run and the recovery kinetics were delayed in Week 12. Individual variation in peak NT-proBNP data suggest this phenomenon is not highly repeatable.

The current study's observation of low number of detectable cTnI values in response to 2 h of repeat prolonged runs (1/8) for Week 0; Week 1 (1/8) and Week 12 (1/7) is somewhat in line with the prevailing literature where moderate intensities and prolonged exercise is accomplished (Abasher et al., 2010; Shave et al., 2010). In one of the few repeated exercise trials, Nie et al., (2011) reported similar low levels of cTnT after treadmill running in trained adolescents. There is, however, as noted in the previous chapter some disparity between the small number of detectable cTnI samples in comparison to a previous controlled laboratory study (Middleton et al., 2008) with

multiple assay points during and post-exercise. Middleton et al., (2008) demonstrated an elevation in cTnT in all subjects at some point during exercise or recovery. The lower response rate of only 1 participant per trial in the current study likely reflects a combination of assay type, exercise intensity and duration as well as training status of the participants.

The current data reflects a largely repeatable phenomenon in a gross (responder vs. non-responder) sense. The subject that had levels above clinical cut off during Week 0 also had elevated levels during Week 1 and participants that did not respond to elevated cTn during Week 0 did not during Week 1. The participant that responded during Week 0 and Week 1 but not Week 12 may indicate that there was a repeat bout effect after 7 days between the Week 0 and Week 1 but not after 12 weeks. Alternatively, the lack of response in week 12 may reflect a change in training status. It was difficult to manage what the participants did during the 12 weeks between trails. The subject that had higher readings during Week 0 and Week 1 may have changed the intensity and duration of training during the 12 weeks. This lack of evidence of a "repeat bout" effect during 60 min of treadmill running indicates that cardiac tissue responses to exercise do not match those of damaging skeletal muscle responses. This may be attributable to the fact that damaging exercise for skeletal muscle often contains a high eccentric component which is hard, if not impossible, to replicate in cardiac tissue.

There was some evidence of cohort consistency with the analyses of NT-proBNP as all participants had elevated NT-proBNP during the three trials. There were, however,

quite marked week-to-week differences in data in the same individual. Week 1 did show a slightly augmented profile and then reduces to similar concentrations achieved during Week 0 which may indicate evidence of a repeat bout effect after an identical exercise bout 7 days before and 12 weeks to reduce the repeat bout effect. The altered R 180 in week 12 may be as a result of a reduced sample size affecting statistical evidence. Correlation coefficients for peak NT-proBNP data were low to moderate, likely indicative of substantial variability in the individual response to repeated bouts of exercise.

To the best of our knowledge this is the first study to look at the repeatability of cardiac biomarker response to prolonged exercise (with repeated blood draws) with 1 and 12 weeks separating repeated bouts in a controlled environment. Data for the hs-cTnI assay provide some support for a cardiac biomarker release being a repeatable phenomenon. It would be somewhat presumptuous to say that the replication of one positive hs-cTnI assay definitively supports this as a repeatable phenomenon at an individual level, but this does reflect the balance of responders and non-responders. The lack of individual repeatability in NT-proBNP data means that one-off tests in participants must be treated with some caution as exercise may provoke quite profound effects.

There were a number of NT-proBNP values above the clinical cut-off (125 ng/l). Clearly in the absence of other clinical indicators (including a general lack of hs-cTnI) and rapid removal in many cases during recovery this again likely represents a physiological process. That said, in situation of clinical presentation after exercise

(maybe in a race medical tent) presentation with a high NT-proBNP (if assayed) should be interpreted in the full knowledge of likely recent exercise exposure.

Limitations

The use of eight participants for week 0 and 1 and 6 participants for week 12 may have restricted statistical significance. The detection limit for the hs-cTn assay should be lowered in further work in an attempt to uncover resting cTn levels and lower levels of exercise response.

4.5. Conclusion

In conclusion, three similar bouts of prolonged treadmill running in healthy untrained males in weeks 0, 1 and 12 resulted in a limited (gross level) repeatable cTnI response, with no real evidence of a repeat bout effect. NT-proBNP rose with exercise and into recovery in all weeks but peak values were slightly higher in Week 1 and 12 with a delayed recovery in Week 12. Interestingly individual NT-proBNP could vary quite markedly in specific individuals and correlational analysis suggests that this is quite a variable phenomenon.

In study 3 the potential mediating role of ischaemia will be evaluated by the application of remote ischemic preconditioning (RIPC) before one trial compared to a SHAM intervention. Participants will undertake two 1 hour cycle time trials in a controlled laboratory environment with biomarkers and cardiac function assessed during early recovery. Of note the detection level for cTn will be lowered.

Chapter 5:

Study 3: The impact of remote ischemic preconditioning on cardiac biomarkers and functional response to endurance exercise.

This chapter has already been published and copyright assigned

Cocking, S., Landman, T., Benson, M., Lord, R., Jones, H., Gaze, D., Thijssen, D.H.J. and George, K (2017). The Impact of Remote Ischaemic Preconditioning on Cardiac Biomarker and Functional Response to Endurance Exercise, *Scandinavian Journal of Sports Medicine*, 10, 1061-1069.

doi: 10.1111/sms.12724.

Chapter 6:

Study 4: The impact of remote ischemic preconditioning (RIPC) on the cardiac biomarker and cardiac functional response to a 160 km cycle ride.

6.1. Introduction

In the previous chapter, we employed a repeated measures research design to assess the impact of RIPC (compared to a SHAM protocol) on the cardiac biomarker and functional responses during the early recovery period after a 60 min cycle time-trial. The key outcome was a significant main effect of trial on cTnT values. cTnT values were lower in the RIPC trial than the SHAM trial across the recovery period although there was no significant time-by-trial interaction. Due to the lower levels of cTnT during recovery in the RIPC trial it was concluded that this provided the first, albeit limited, support for a role of ischaemia in the appearance of cTnT post-exercise. Clearly further exploratory work was required.

Initially, it would be sensible, in any follow-up study, to address the potential limitation(s) of the previous Chapter, specifically related to the limited volume of the exercise challenge. Whilst there is evidence that shorter bouts of exercise may result in cTnT appearance during recovery (Shave et al., 2010) there is a much larger evidence-base with the potential for a more marked individual and cohort cTnT response with a greater exercise challenge (Douglas et al., 1987; Scott et al., 2009; George et al., 2010; Shave et al., 2010). In constraining the exercise in the previous chapter to a laboratory-base, for valid reasons of control of the environmental challenge (for example), the exercise task was limited to what could be comfortably completed and acceptable to the participants. The adoption of a 60 min TT as the exercise exposure is

at the lower end of exercise duration and volumes employed in previous exercise-related biomarker studies (Dawson et al., 2003; Scharhag et al., 2007; Shave et al., 2010). The use of the hs-cTnI assay in the current study, rather than cTnT used in the last study, allows for more direct comparisons with Chapters 3 and 4 and the continued use of a lower detection limit (now available in the hs-cTnI assay) will help in our understanding of cTn elevation at the lower level of detection. By adopting a field-based exercise protocol in the current chapter the exposure to exercise is closer to replicating descriptive studies of cTn and NT-proBNP appearance in prolonged endurance-based athletes (Neilan et al., 2006; Scott et al., 2009). A greater exercise exposure (duration and volume) places the heart under much greater and sustained hemodynamic and metabolic load and potentially provides an ideal research design to further evaluate the impact of RIPC, and by association the role of ischaemia, on cTn appearance with exercise.

We have retained the repeated measures research design with a SHAM comparison to maintain internal validity whilst the change in exercise exposure and setting increases external (ecological) validity. We also retained the technical approach to RIPC that was pioneered in the seminal study of Murry et al., (1986) and has been developed by recent research in this field (Whittaker & Przyklenk et al., 1994; Whittaker & Przyklenk, 2011). Specifically the application of a RIPC on both legs and both arms in a repetitive fashion over 40 min, prior to the exercise exposure, is targeted at maximising the RIPC stimulus. Consequently, this chapter will monitor the release of cardiac biomarkers of damage (hs-cTnT) and dysfunction (NT-proBNP) as well as non-invasive measures of cardiac function after repetitive 160 km endurance cycling bouts, for regionally competitive club cyclists, in a real-world environment. If the elevation of

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biomarkers and the reduction in cardiac function are (partially) related to ischaemia we would hypothesise a higher and more divergent response in hs-cTnI, NT-proBNP and indices of cardiac function after a 160 km cycle ride (c. 6 hr) preceded by SHAM as opposed to RIPC.

STUDY 4: Hypothesis 4: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 160 km cycle trial is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.

6.2. Methods

Participants

Nine male competitive cyclists (Mean + SD; Age 40.2 + 6.2 yr; Body mass 76.0 + 9.5 kg; Height 175.8 + 6.9 cm; BMI 22.0 + 1.4) were recruited via personal contact with a local cycling club. Recruitment was on the basis that all participants could complete a 160 km cycle trial at an average pace of 32 to 33 km/h, in order to facilitate a team cycling exposure that would reflect a club "race-day", as well as being available and able to complete two rides over an identical course 7 days apart. All participants were screened using a medical health questionnaire to determine the presence of personal or an early family history of cardiovascular disease. All participants were free from current illness or injury and self-reported no significant interruptions or alterations in training within the last 4 weeks. The study received Liverpool John Moores Ethics Committee approval and all subjects provided written informed consent after due consideration of the participant information sheet.

Table 6.1. Study 3 participants' characteristics (n = 9)

Variables	Subjects' characteristics
Men	9
Physical activity (h/wk)	15 + 1
Cycling activity (km/week)	120 + 4
Competitive cycling experience (yrs)	8 + 3
Participants with ultra-distance experience (cycling > 6hrs)	9

Data are mean + SD

Protocol

In a randomised, controlled single-blind crossover design participants completed two field based 160 km cycle ride. Participants received either a RIPC or SHAM protocol immediately before completing each cycle ride. Both cycle rides started at 9:30 am to reduce the effects of circadian variation (Atkinson and Reilly, 1996). The two trials were separated by a minimum of 7 days recovery. Although we could not control environmental exposure for both rides the cyclists were asked to ride at the same intensity irrespective of ambient conditions. All participants were able to self-monitor heart rate and performance data (speed, distance etc.) in a continuous manner during both rides. For both rides the participants were asked to refrain from exercise as well as caffeine and alcohol intake for the 24 hr prior to each trial. Participants were asked to arrive at the laboratory on both test days at 7 am fully hydrated and having consumed their personal standardised breakfast that was repeated for both trials. To help with familiarity and practicality the participants contributed to the design of the 160 km route. Immediately after RIPC or SHAM riders began the 160 km cycle rides. The riders rode together to achieve the highest average pace for each trial whilst being encouraged to make small adjustments to their route to attempt to match similar exercise exposure (duration and intensity) on both rides. The average elevation gain for

the two rides was planned at 735 metres. Heart rate, speed, calories and elevation were continually assessed using their own individual bike tools (Polar, T31; Garmin 500 or 550 models) and uploaded to an online programme for further analysis (www.Strava.com). Participants were instructed to take in fluids and food ad-libitum.

Before (PRE), immediately (POST-0H) and 1 hr into recovery (POST-1H) after both cycle trials venous blood samples (to determine cardiac biomarker levels) and an ultrasound echocardiogram (to assess cardiac function) were completed.

Remote ischemic preconditioning (RIPC) and SHAM procedure

Remote ischemic preconditioning was performed in the supine position using the bilateral occlusion technique to achieve acute limb ischaemia in line with the methods employed in the last chapter and during a running exercise study by Bailey at al., (2012). In the present study participants were randomised into either RIPC or SHAM groups for the first cycle trial and then they had the reverse procedure in the second trial. Automated pressure cuffs (Hokanson, Washington, USA) and manual pressure cuffs (Accuson, UK) and was consistent with the approach adopted in the previous chapter. Two blood pressure cuffs were rapidly inflated to 220 mmHg on the ipsilateral upper right bicep and lower left thigh to block arterial inflow for 5 minutes and then rapidly deflated. Cuffs were then changed to the opposite limbs (left bicep and lower right thigh) before inflating to 220 mmHg again. This protocol was repeated 4 times (total time of ischaemia reperfusion was 40 minutes). For the SHAM protocol the participants followed the same protocol as above but cuffs were only inflated to 20 mmHg which did not disrupt arterial blood flow.

Blood Sampling Procedure

A trained phlebotomist withdrew 5 ml blood samples using repetitive venepuncture to assess cTnI and NT-proBNP. At each blood draw 5 mL was taken from the antecubital vein with the subject in the sitting position, samples were collected using serum gel vacutainer (Becton Dickinson UK Ltd, Oxford UK). After the whole blood had been collected it was left to clot for approximately 60 min and centrifuged at 1000 g at 18^{o}C for 10 min. The serum was then drawn off with a disposable pipette (Effendorf, Hamburg, Germany), samples were divided into 3 aliquot parts, and stored at -80^{o}C for later analysis.

Cardiac Troponin

In the previous Chapter a hs-cTnT assay was employed whereas in this study we used a hs-cTnI assay. The use of this assay allowed the lower end to be "opened up" with the view of identifying lower levels of cTnI. Whilst many studies have employed assessments of both cTnI and cTnT there have been small differences in data from the assays (Rifai et al., 1999; Urhausen et al., 2004). Most of these differences should be negated by a hs-assay. Given the absolute cardiac specificity of cTnI and the use of the hs-cTnI assay in Chapters 3 and 4 we felt it made sense to move to this assay when the new lower detection limit became available. Serum samples were analysed in a single session for the presence of cTnI using the hs - TnI-Ultra assay for the ADVIA Centaur (Siemens Healthcare Diagnostics, Frimley, UK). The detection limit of the instrument was 0.006 µg/L, upper limit 50 µg/L. The manufacturers claim was 10% CV at 0.03

μg/L with a 99th centile of 0.04 μg/L. The current accepted cut-off for diagnosis of AMI is 50 ng/L.

N-terminal pro-B-type natriuretic peptide (NT proBNP): NT proBNP levels was assessed with the chemiluminescent sandwich immunoassay using the Immulite 2500 (Siemens Healthcare Diagnostic Frimley, UK). The minimum detection limited for this assay is 20 ng/L with a maximum of 35000 ng/L. The inter-assay % coefficient of variation was 5.0 to 4.0% with a range of 40.9 to 32096 ng/L.

Cardiac Function

A trained cardiac sonographer followed the guidelines set by American Society of Echocardiography (Lang et al., 2015). A 2 dimensional, transthoracic echocardiogram was performed with the participant resting in the left lateral decubitus position. Acquisition and analysis were performed using VividQ and EchoPac (GE Medical, Norway). All images were optimised with gain, compression, and dynamic range to enhance myocardial definition. Given time constraints during the trial we focussed on fewer images and outcome variables compared to Chapter 5. Apical 2- and 4-chamber views allowed the examination of LV end-diastolic (LVEDV) using the Simpson Biplane method. This facilitated the estimation of LV ejection fraction (EF). Stroke volume (SV) was determined by subtracting LV end-systolic volume from LVEDV.

LV diastolic filling was assessed using pulsed-wave Doppler echocardiographic recordings from the apical 4-chamber view. Specifically, spectral Doppler envelopes

characterized from a 4 mm sample volume allowed measurement of peak flow velocity of the early diastolic rapid filling E wave and peak flow velocity of the late diastolic filling wave due to atrial contraction (A). The ratio of E wave to A wave (E/A) was calculated. The same apical view was used for tissue Doppler assessment of peak myocardial tissue velocities in the basal septum and basal LV lateral wall at the level of the mitral annulus as well as the basal RV lateral wall at the level of the tricuspid annulus. Peak systolic (S'), peak early diastolic (E') and peak late atrial diastolic (A') myocardial velocities were recorded.

Before exercise and at all measurement points post-exercise, arterial blood pressure was recorded using an automated sphygmomanometer (Dinamap, GE Medical, Wisconsin, USA). Body mass was recorded using an electronic weighing scale (SECA, Germany).

Statistical Analyses

Statistical analysis was completed using IB SPSS 23.0 (SPSS, Chicargo, Illinois) software. All data are reported as mean ± SD and statistical significance is set at $P < 0.05$. Two-way repeated measures ANOVA (time [3 levels] - PRE vs. 0 vs 1 h post-exercise, intervention [two levels - RIPC vs. SHAM) was used to test the changes during the 160 km continues bike ride for the primary and secondary outcome parameters and whether these changes occur when exercise preceded by RIPC or SHAM. When there was a significant main or interaction effect post-hoc analysis was used, using the Bonferroni test, to correct for multiple comparisons. Repeated

measures t-test was used to analyse environmental and performance data associated with trial 1 vs. trail 2 as well as RIPC vs. SHAM trials.

6.3. Results

160 km Cycle Rides

Eight participants completed both trials with a single participant unable to take part in the group cycle for trial 2. Seven cyclists completed both trials together with the final participant cycling with the group during trial one and then completing the second trial alone. In this case, the exercise time and average heart rate was held constant between trial 1 and 2 but this resulted in a decrease in distance completed in the second trial due to lack of pacing support in a group setting. Performance data and ambient conditions for the two trials are contained in Table 6.2 as well as a comparison between RIPC and SHAM trials. Whilst there are some small performance differences between trial 1 and 2 it should be noted that average and maximal HR were similar between the two trials and there was little performance difference between RIPC and SHAM trials.

Table 6.2. Performance data and ambient conditions between trial 1 and 2 as well as RIPC and SHAM trial for the 160 km cycle rides

	TRIAL 1	TRIAL 2	P-VALUE	RIPC	SHAM	P-VALUE
TIME (HR:MIN)	04:47 + 0:5	05:14 + 0:19	< 0.05	05:04 + 0:17	04:59 + 0:18	NS
DISTANCE (KM)	154	171	< 0.05	164 + 14	161 + 9	NS
AVERAGE SPEED ($KM.H^{-1}$)	32 + 1	32 + 1	NS	32 + 1	32 + 1	NS
MAXIMUM SPEED ($KM.H^{-1}$)	71 + 2	62 + 1	< 0.05	66 + 4	68 + 6	NS
AVERAGE HR ($BT.MIN^{-1}$)	140 + 13	139 + 12	NS	139 + 11	140 + 13	NS
MAXIMUM HR ($BT.MIN^{-1}$)	169 + 13	169 + 19	NS	169 + 17	169 + 14	NS
ENERGY EXPENDED (CAL)	3696 + 322	3844 + 709	NS	3827 + 160	3624 + 653	NS
MIN TEMPERATURE (O^C)	16	16	NS	16	17	NS
MAX TEMPERATURE (O^C)	17	18	NS	18 + 1	18 + 1	NS
MEAN WIND SPEED (MPH)	7	12	-	10 + 3	8 + 2	NS
WIND DIRECTION	W	WNW	-	-	-	-
RELATIVE HUMIDITY (%)	64	64	-	64	64	NS
BAROMETRIC PRESSURE (HPA)	1011	1024	NS	1019 + 7	1016 + 7	NS

Pre=pre exercise sample, Ex=exercise samples, R=recovery samples, numbers are ng/L through each component of the protocol

Cardiac Biomarkers

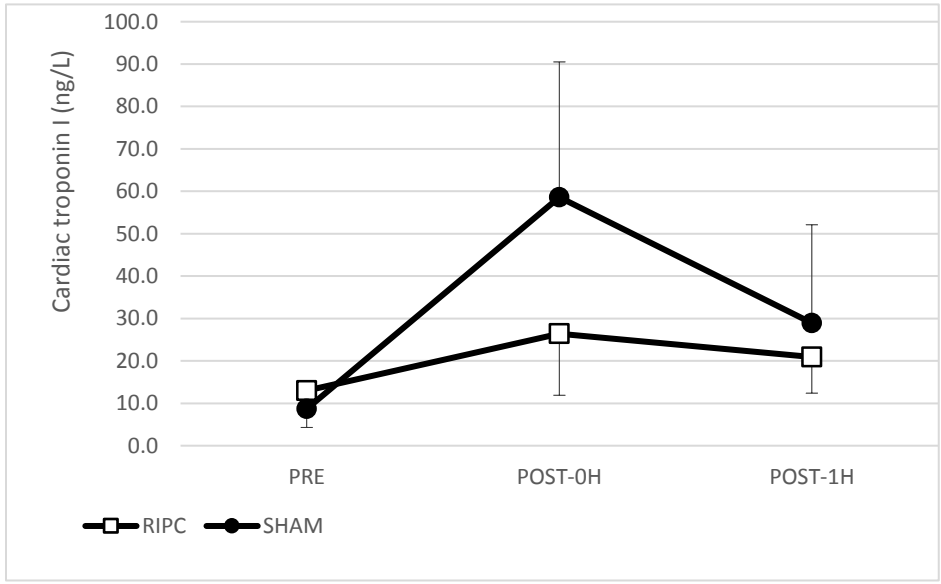
There was a significant main effect of trial (P = 0.001) with data for hs-cTnI lower in the RIPC trial compared to the SHAM trial (independent of time). There was a significant main effect of time (P = 0.043) with hs-cTnI values initially rising during early recovery (Figure 6.1.). There was a significant interaction (trial * time) effect (P

= 0.018) that facilitated pairwise comparison at POST-0H and POS-1H where hs-cTnI values were lower after the RIPC trial at both time points compared to SHAM (P < 0.05, Fig 6.1a). The maximum concentrations of hs-cTnI occurred POST-0H (RIPC 26 + 15 ng/L vs. SHAM 59 + 32 ng/L; see Table 6.2). Evidence of individual heterogeneity is worthy of note. In all PRE samples only one cyclist had an hs-cTnI value above the clinical cut-off for myocardial damage (RIPC trial) but his values reduced across the rest of the trial. In post-exercise samples individual variability was again noted with a maximal value of 100 ng/L (SHAM trial). Four (RIPC trial) and six (SHAM trial) cyclists presented hs-cTnI values above the cut-off for myocardial damage at POST-0H but in all but one case a reduction was noted at the subsequent POST-1H sample point. In the other participant there was an increase in hs-cTnI at POST-1H (POST-0H 35 ng/L vs. POST-1H 82 ng/L).

There was a significant main effect of intervention for NT-proBNP data with values in RIPC trial lower than in the SHAM trial (P = 0.001; Fig 6.1b); There was a significant main effect of time (P = 0.001) with values rising from PRE to post-exercise. The interaction (trial * time) was also significant (P = 0.007) with pairwise post-hoc comparisons revealing lower NT-proBNP data at both POST-0H and POST-1H in the RIPC trial. Maximal values for NT-proBNP occurred POST–0H for SHAM (RIPC 100 + 47 ng/L vs. SHAM 123 + 52 ng/L; Figure 6.1b: Table 6.2). Although individual heterogeneity in NT-proBNP data was observed all participants values were below the clinical cut-off value with the highest (194 ng/L) observed at POST-1H in the SHAM trial. This subject completed this trial alone and was the only participant to see his NT-proBNP value rise from POST-0H to POST-1H.

Figure 6.1. hs-cTnI (a) and NT-proBNP (b) values at PRE, POST-0H and POST-1H exercise time points during both RIPC (open squares) and SHAM (solid circles) trials.

(a)



(b)

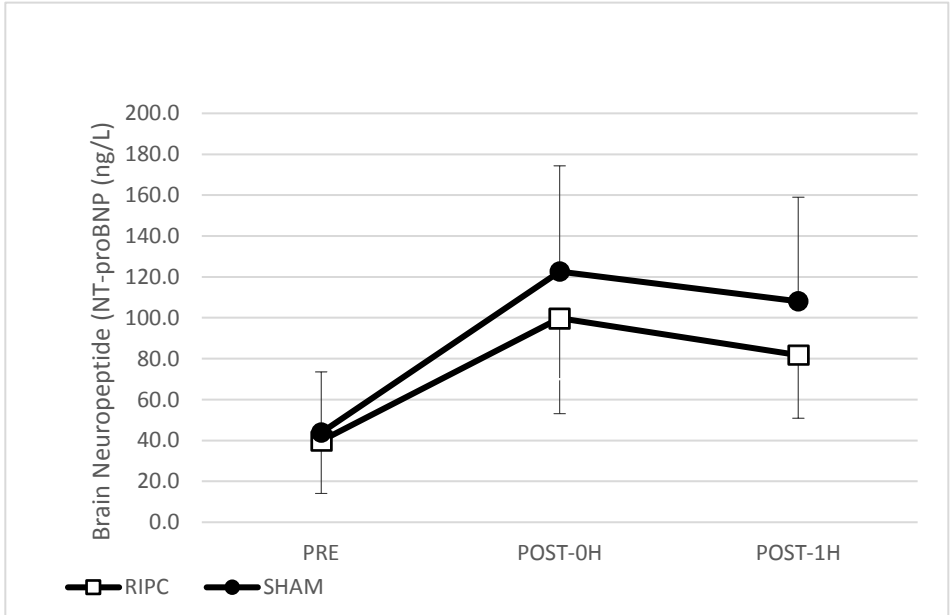


Table 6.3. Cardiac loading and rate changes at PRE, POST-0H & POST-1H time points during both RIPC and SHAM conditions in competitive cyclists in both trials (n=8, mean ± SD).

	INTERVENTION			P VALUES	
	PRE	**POST-0H**	**POST-1H**		
HR (BEATS/MIN)					
RIPC	63 ± 4	69 ± 7	64 ± 3	Trial	0.864
SHAM	64 ± 6	70 ± 4	62 ± 4	Time	0.200
				Trail x time	0.113
SBP (MMHG)					
RIPC	124 ± 14	125 ± 10	119 ± 8	Trial	0.553
SHAM	134 ± 9	125 ± 8	120 ± 7	Time	0.236
				Trail x time	0.291
DBP (MMHG)					
RIPC	71 ± 6	74 ± 10	71 ± 4	Trial	0.190
SHAM	85 ± 8	72 ± 6	67 ± 9	Time	0.213
				Trial x time	0.230
LVEDV (ML)					
RIPC	146 ± 14	132 ± 18	130 ± 32	Trial	0.124
SHAM	146 ± 16	136 ± 12	128 + 24	Time	0.164
				Trial x time	0.871

Abbreviations: HR, Heart rate; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; LVEDV, left ventricular end-diastolic wave

There was no significant main effect (trial or time; both P>0.05) and no significant interaction effect for LVEDV (a surrogate of preload), HR, and blood pressure (P>0.05; Table 6.3).

There were no significant main effects or interaction term for any index of LV systolic function (EF, SV and S' [all wall segments]; P>005; see Table 6.4).

Table 6.4. Measure of left ventricular systolic function at PRE, POST-0H and POST-1H exercise time points during both RIPC and SHAM conditions in regionally competitive cyclists in both trials (n=8, mean + SD).

	INTERVENTION			P VALUES	
	PRE	**POST-0H**	**POST-1H**		
LV EF (%)					
RIPC	51 ± 15	58 ± 14	54 ± 16	Trial	0.289
SHAM	52 ± 11	58 ± 12	58 ± 19	Time	0.122
				Trial x time	0.452
SV (ML)					
RIPC	72 + 22.7	79 + 16.1	79 + 21.1	Trial	0.927
SHAM	76 + 16.7	75 + 13.8	74 + 27.2	Time	0.663
				Time x time	0.359
LV SEPTAL S' ($M.S^{-1}$)					
RIPC	9 + 1	9 + 2	9 + 1	Trial	0.119
SHAM	8 + 1	9 + 2	9 + 2	Time	0.911
				Trial x time	0.709
LV LATERAL WALL S' ($M.S^{-1}$)					
RIPC	11 + 3.6	12 + 3.0	12 + 3.5	Trial	0.160
SHAM	13 + 2.8	13 + 2.8	13 + 2.8	Time	0.149
				Trial x time	0.454

Abbreviations: LV EF, left ventricle ejection fraction; SV, stoke volume; S, septal; LS, lateral septal.

A main effect of time was evident for E ($P<0.05$), with peak inflow velocity dropping immediately post-exercise in both trials (Table 6.5). There was no main effect of trial and no significant interaction term for E. Data for peak A flow velocity were not mediated by time, trial or interaction. There was a substantial drop in E/A ratio immediately post-exercise, which contributed to a main effect of time as well as a significant interaction effect. Pairwise comparison of E/A data suggested a lower value at Post-0H in the RIPC trial compared to the SHAM trial with little difference at Post-1H. There was no main effect of trial, time or interaction for LV septal or lateral wall E' ($P>0.05$, Table4). Data for peak LV septal or lateral wall A' flow velocity were not mediated by time, trial or interaction.

Table 6.5. Measure of right ventricular systolic and diastolic data at PRE, POST-0H and POST-1H exercise time points during both RIPC and SHAM conditions in regionally competitive cyclists in both trials (n=8, mean + SD)

	INTERVENTION			P VALUES	
	PRE	**POST-0H**	**POST-1H**		
LV E ($m.s^{-1}$)					
RIPC	83 ± 8	69 ± 11	69 ± 13	Trial	0.165
SHAM	82 ± 15	68 ± 16	71 ± 13	Time	0.035*
				Trial x time	0.073
LV A ($m.s^{-1}$)					
RIPC	55 ± 10	60 ± 12	54 + 12	Trial	0.291
SHAM	54 ± 8	66 ± 7	61 + 8	Time	0.115
				Trial x time	0.0.72
LV E/A					
RIPC	1.53 ± 0.26	1.34 ± 0.51	1.16 ± 0.30	Trial	0.058
SHAM	1.57 ± 0.52	1.04 ± 0.24	1.23 ± 0.36	Time	0.047*
				Trial x time	0.022*
LV SEPTAL E' ($m.s^{-1}$)					
RIPC	12 ± 2	10 ± 4	10 ± 3	Trial	0.162
SHAM	10 ± 3	10 ± 4	9 ± 3	Time	0.128
				Trial x time	0.787
LV SEPTAL A' ($m.s^{-1}$)					
RIPC	9 ± 2	8 ± 2	10 ± 1	Trial	0.175
SHAM	9 ± 1	10 ± 3	9 ± 2	Time	0.239
				Trial x time	0.951
LV LATERAL Wall E' ($m.s^{-1}$)					
RIPC	14 ± 4	13 ± 4	13 ± 4	Trial	0.824
SHAM	13 ± 3	13 ± 3	13 ± 4	Time	0.981
				Trial x time	0.975
LV LATERAL Wall A' ($m.s^{-1}$)					
RIPC	9 ± 3	10 ± 2	9 ± 3	Trial	0.593
SHAM	9 ± 3	9 ± 2	9 ± 2	Time	0.958
				Trial x time	0.363

Abbreviations: LV E and LV A wave, Left ventricle, early (E) to late (A) wave; late diastolic filling; LV E/A, is the ratio of the early (E) to late (A) ventricular filling waves. Septal E' and Septal A'; Septal early (E') to late (A'); LV' lateral E' and A', early (E') and late (A') lateral wall.

Table 6.6. Measure of right ventricular systolic and diastolic data at PRE, POST-0H and POST-1H exercise time points during both RIPC and SHAM conditions in regionally competitive cyclists in both trials (n=8, mean + SD).

	INTERVENTION			P VALUES	
	PRE	**POST-0H**	**POST-1H**		
RV FREE WALL S' ($m.s^{-1}$)					
RIPC	11 ± 1	13 ± 3	13 ± 3	Trial	0.109
SHAM	12 ± 2	13 ± 4	14 ± 4	Time	0.499
				Trial x time	0.324
RV FREE WALL E' ($m.s^{-1}$)					
RIPC	13 + 2	9 + 3	11 + 5	Trial	0.852
SHAM	11 + 3	12 + 3	10 + 2	Time	0.050
				Trial x time	0.467
RV FREE WALL A' ($m.s^{-1}$)					
RIPC	11 + 3	14 + 4	15 + 3	Trial	0.203
SHAM	13 + 3	15 + 4	15 + 3	Time	0.157
				Trial x time	0.056

Abbreviations: RV S', right ventricle septal; RV E' and RV A' waves, right ventricle early (E); to late (A)

There was no significant main effects of time or trial and no significance interaction effect for RV free wall S', E' and A' (P>0.05, Table 6.6).

6.4. Discussion

In the previous chapter we concluded that exposure to RIPC (versus SHAM) before exercise may reduce the hs-cTnI response to a subsequent laboratory-based, controlled 60 min cycling exposure. This study extended the previous chapter by employing an exercise exposure of significantly greater volume that was more representative of a field-based cycling event. The principal findings from the current study are: a) hs-cTnI was reduced after 160 km of cycling that followed exposure to RIPC compared to a

SHAM trial; (b) NT-proBNP was also lower following 160 km of cycling after RIPC compared to SHAM; and (c) cardiac function was relatively well preserved after a 160 km cycle ride and post-exercise changes were not mediated by RIPC or SHAM.

Cardiac Biomarkers

Pre exercise levels of cTnI were similar in both trials and were at the same quantitative level as seen in previous chapters in that all but one subjects had hs-cTnI levels below clinical cut-off for myocardial infarction for week 2 (31 ng/L). This suggests that the other participants presented for testing in an appropriate stage of recovery from previous prolonged exercise training bouts. The one subject that was above URL may not have been in a complete state of rest/recovery before the trial (may not have rested as requested between studies). In both trials hs-cTnI rose post-exercise with a drop-off toward baseline in early recovery (POST-1H). This kinetic of response was consistent between individuals and similar in both RIPC and SHAM trials. This is a very similar finding to a range of laboratory and field-based studies including those using cycling as an exercise stimulus (Scharhag et al., 2005; Middleton et al., 2008; Skadberg et al., 2017). The post-exercise levels of hs-cTnI were greater at POST-0H than in previous studies within this thesis, which may be as a result of between study differences in exercise volume as well as the fitness status of the participants (Shave et al., 2008). Specifically, the use of competitive cyclists and an exercise exposure that lasted for 5 hours at optimal "race" pace helped to produce higher levels of hs-cTnI after both SHAM and RIPC trials compared to data observed in the previous chapter. This would support the concept that exercise duration or volume is more important in relation to exercise-associated elevations in cTn that exercise intensity (Dawson et al., 2003).

The most striking finding in relation to the hs-cTnI data was the significant attenuation of values observed after the RIPC trial compared to the SHAM trial. We noted a significant main effect of trial as well as a significant time by trial interaction. This supports and extends the findings in the previous chapter and suggests that ischaemia (the effects of which are potentially offset via prior RIPC) may play some role in the hs-cTnI response to prolonged exercise. It should be noted that like the previous chapter the current data does not support the findings of Massaoudi et al., (2013) who did not observe a mediating effect of RIPC on cTnI (or NT-proBNP). The contradictory nature of the between-study outcomes could be due to a number of factors; exercise mode, intensity, duration and volume; participant's fitness status; RIPC exposure; or a combination of factors.

The explanation for the lower hs-cTnI after exercise in the RIPC trial implicates ischaemia to some extent but the fact that hs-cTnI was still elevated over baseline in the RIPC suggest other mechanisms may be at play in both trials. Whilst our current research study cannot directly implicate potential ischemic pathways that are altered by RIPC we make the following limited speculation. Ischaemia has a number of direct and indirect effects on the cardiomyocyte that include a reduction in intracellular ATP an increase in protons and an upregulation in glycolysis (Piper et al., 2004) that together can negatively affect the Na/ Ca^{2+} pump which results in an increase in Ca2+ (Gateau-Roesch & Argaud, 2006). Alongside a rise in reactive oxygen species (ROS) the increase in intracellular Ca^{2+} can lead to an opening of mitochondrial membrane pores therefore increasing membrane permeability called mitochondrial permeability

transition pores (mPTP). If this happens in RIPC where ischaemia is followed by reperfusion and re-oxygenation, some level of protection against the development of mPTP is assumed. Cardiomyocyte protection occurs in the first few minutes of oxygenated reflow and it has been suggested that a range of pathways and mechanisms contribute to the protection of cellular integrity. Possible pathways include the upregulation of the signalling protein kinase C (PKC). When an inhibitor to PKC-ε is employed, cardiomyocyte protection is reduced (Murphy & Steebergen, 2008). Jin et al., (2004) suggested that sphingosine 1 phosphate (S1K) may also have a protective role as an inhibition of S1K reduced the cardioprotective effects of RIPC in mice. We can speculate that the increase in hs-cTnI observed in the SHAM trial was due to the lack of RIPC and the upregulation of signalling pathways that prevent or reduce ischemic damage and processes such as mPTP. Critically, the different mechanisms and their contributions involved in cardioprotection after RIPC remain unclear and therefore requires verification, likely in animal model studies.

Although still to be confirmed, the rapid elevation and clearance of the cTn that occurs, as well as the quantities measured indicate reversible cell damage as opposed to irreversible cell death. There is a general agreement that the presence of elevated cTn is not an indication of irreversible damage (Shave et al., 2010) but likely reflects cTn release from the unbound "cytosolic" pool rather than contractile apparatus. As well as a role for ischaemia, other mechanisms should be briefly noted as hs-cTnI did rise post-exercise after a SHAM procedure that would seem to rule out ischaemia as a lone mechanism. There are a number of different non-ischemic mechanisms, such as a stunned myocardium, blebs or myocardial swelling buds (Kloner et al., 1974; Sage et al., 1988) and in specific studies environmental stressors such as heat (Whiticar et al.,

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2008) and high altitude influences (Boos 2013; Mellor et al 2013). It is speculated that these potential mechanisms could be related to a rise in reactive oxygen species (ROS) that is known to occur from the metabolic processes of aerobic prolonged exercise (Di Meo and Venditti 2001: Sahlin et al., 2010). It is believed that elevated lipid cells membrane peroxidation may result in myocyte cell membrane damage or dysfunction (Ju 2001).

These potential mechanisms require further study. Both the last chapter and this present study may be the first to indirectly show the role of ischaemia in exercise induced hs-cTnT and hs-cTnI but some caveats are important. Firstly, there was no evidence of ischaemia on post exercise ECG traces (associated with the echocardiogram) and secondly there were no other signs and symptoms of acute coronary syndromes. The potential role of ischaemia still requires further clarification.

The peak post-exercise hs-cTnI occurred at POST-0H but was quite heterogeneous between individuals. This is also similar to past work (Middleton et al., 2007; Regwan et al., 2010; Vilela et al., 2014). Some values were above clinical cut-offs for ACS (Thygesen et al., 2013) but the subject that had a hs-cTnI concentration 5 x the clinical cut-off criteria (100 ng/L at POST-0H) had one of the lowest hs-cTnI levels in the group by POST-1H. In the absence of cardiac dysfunction, ECG changes associated with ACS and, any other signs and symptoms of cardiovascular disease we would suggest that the post-exercise elevation in hs-cTnI is likely of minimal clinical significance.

Unlike the previous chapter, NT-proBNP increased significantly post-exercise in both trials. This corresponds with past endurance-exercise studies (Scharhag et al., 2008; Lippi et al., 2008; Scott et al., 2009; Tian et al., 2014). The rise of the biomarker may be associated with stretch of the atria and the hemodynamic overload associated with exercise. Hamasaki (2016) proposed in their extensive review that NT-proBNP release was linked primarily to exercise duration rather than intensity. Neumayer et al., (2005) believed the elevation of NT-proBNP was a physiological endocrine response to mitigate against myocardial stress. Hamasaki (2016) proposed that NT-proBNP release with endurance exercise may have cardio protective and growth regulatory effects on the myocardium. A novel funding was that the NT-proBNP response to exercise was decreased after RIPC, despite the fact that in event and post event cardiac function data do not suggest different hemodynamic stress (and thus myocardial stretch) between trials. This phenomenon has not been described before and was not observed in the previous chapter. Whether and how RIPC has some protective effect in terms of signalling pathways to NT-proBNP release is difficult to determine in the current research design and requires further research.

Cardiac Function

There were no meaningful post-exercise changes in LV or RV systolic function observed in either trials (RIPC or SHAM). Consequently this data is in agreement with the findings of previous chapter and other empirical work (Goodman et al., 2001; Tian et al., 2014), but contrasts with others who have observed evidence of changes in left ventricular systolic function in a range of exercise settings (Upton, et al., 1994; Shave

et al., 2002; Middleton et al, 2006; Ostariz et al., 2013). This would suggest that cardiac functional integrity is well preserved after 160 km cycling and this possibly reflects the training status and nature of cycling (load supported) when compared to most evidence of cardiac fatigue that comes from ultra-endurance running (George et al., 2009; Scott et al., 2009).

The transient decline in peak E diastolic flow velocity that occurred at POST-0H confirms similar data from the previous study and seems to be a highly consistent finding in both lab and field based work that has assessed diastolic function after endurance exercise (Middleton et al., 2007; Oxborough et al., 2010; Ostariz et al., 2013). Interestingly changes in diastolic flow parameters were not exactly matched by regional assessment of tissue velocities during diastole in either the LV or RV that is somewhat at odds with previous research (Williams et al., 2007). Whilst the qualitative direction of change for E' and A' matched data for flow velocities the magnitude of change and low sample size may have contributed to a lack of statistical significance. Changes in early diastolic filling velocities have been linked to a reduction in preload, alterations in heart rate and/or some intrinsic mechanism related to cardiomyocyte relaxation. It is difficult to provide irrefutable evidence for any of these other than an increase in HR is usually accompanied by an increase in E which is not what has happened here (Giannaki et al., 2008). At POST-0H there was a significant correlation between delta E and delta LVEDV (r = .49) partially suggesting a role for preload in the decline in early filling velocities.

A link between cardiomyocyte damage and changes in LV/RV function, proposed by Dawson et al., (2003), is not supported here as the changes in hs-cTnI that were augmented in the SHAM trial were not matched by bigger changes in E. This increase in the biomarkers and the manipulation by RIPC seen in this present study and the lack of a reduction in LV systolic function may indicate different mechanisms or the activation of these mechanisms in an order that facilitates protection of the myocardium. Other intrinsic mechanisms such as altered intercellular Ca^{2+} handling are impossible to evaluate in the current research setting. These areas require further investigation.

Implications

Based on the transient nature and quantity of the biomarkers observed post-exercise it is unlikely that the elevation of these biomarkers are indicative of long-term irreversible damage or dysfunction in the myocardium (Shave et al., 2010; La Gerche et al., 2012). It is highly likely that post-exercise changes in cardiac biomarkers have no immediate clinical consequence, however, due care and consideration should always be given to an elevated hs-cTnI in any clinical assessment. The evidence that RIPC did have an effect on two prominent cardiac biomarkers, that are well established for the diagnosis of myocardial infarction (hs-cTnI) and heart failure (NT-proBNP), should prompt further investigation employing a range of RIPC protocols, different exercise settings and/or different participant groups.

Limitations

The relatively small sample size (N=8) has an impact on the generalisability of the findings. The blood pressure (20 mmHg) employed in the SHAM trial made it challenging to adequately blind subjects to their treatment, and could influence the performance of participants. The combined use of manual and automatic pressure cuffs for RIPC may have provided some minor alterations in treatment exposure.

6.5. Conclusion

The use of RIPC prior to a 160 km cycle ride significantly reduced the magnitude of both hs-cTnI and NT-proBNP release post-exercise when compared to a SHAM trial. This suggests some putative role for ischaemia during exercise-induced changes in hs-cTnI. How RIPC mediates NT-proBNP data is not clear. There was no evidence of change in systolic cardiac function post-exercise in both trials. The decline, post-exercise, in early diastolic inflow (E) was consistent between trials but linkage between functional depression and biomarker elevation was not apparent.

Chapter 7:
General Discussion

7.1. Overview of Study Aims and Hypotheses

At the beginning of the thesis and then is specific studies we stated the following hypotheses. The outcome to these hypotheses is summarised in Table 7.1. This provides the brief backdrop to some generic issues worthy of discussion at this stage of the thesis

STUDY1: Hypothesis 1: Running for 2 hours on a motorised treadmill at moderate intensity will reveal a rapid turnover of cTnI, using the new high sensitivity assay, and NT-proBNP in moderately active adults.

STUDY 2: Hypothesis 2a: Cardiac biomarker responses to the same exercise bout employed in STUDY 1, are highly repeatable after a period of 7 days between exercise tests in the same participants.

STUDY 2: Hypothesis 2b: Cardiac biomarker responses to the same exercise bout employed in STUDY 1, and Hypothesis 2a, will be highly repeatable after a period of 12 weeks between the first and last exercise test in the same participants.

STUDY 3: Hypothesis 3: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 1-h strenuous cycle time trial exercise is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.

STUDY 4: Hypothesis 4: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 160 km cycle trial is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.

After reflection on the entire thesis, we identified the following overarching issues worthy of some comment within and between studies;

Lab Versus Field Studies

Many of the research studies to date (see meta-analysis by Shave et al., 2007) employed research designs with continuous but variable intensity exercise undertaken during competitive races in the "field". The two primary reasons for these types of study were; (1) easy access to well-motivated groups of compliant athletes, and (2) the ability to assess the impact of extremes of exercise prescription (e.g. Ironman Triathlons, 100 mile races, multi-day events). This approach provided a "rich" set of data and was the foundation of Shave's meta-analysis and further narrative reviews (Oxborough et al., 2010).

Despite positive aspects to this "living-lab" approach, there are limitations. The competitive nature of the events assessed meant that access to participants during the race was challenging and, therefore, blood collections only occurred pre- and post-race. Occasionally some extra blood samples were collected into early recovery (Scott et al., 2009) but this was not the "norm". Based on the data generated from the landmark study of Middleton et al., (2007) there are serious concerns about the limited insight

provided by only two blood draws and biomarker analyses. Whilst informative, and certainly allowing often unremitting volumes of exercise to be studied, the preponderance of field-based studies places a significant challenge to our broader understanding and knowledge of exercise-related biomarker work.

We choose to move our focus for the first three studies of this thesis from the field into controlled laboratory-based research designs to partially address the imbalance in previous research. Laboratory studies allow some element of replication and extension of the landmark laboratory-based treadmill study of Middleton et al., (2007) that pioneered multiple blood draws and was extended by other groups, notably in Spain (Legaz-Aresse et al., 2012) and Macau/China (Nie et al., 2012), to provide a better reflection of biomarker "kinetics". The ability to take blood samples during exercise and into the recovery period was an important element of the laboratory-based studies in this thesis.

Interestingly, despite the adaption of multiple blood draws with and after exercise in our laboratory-based studies the positive biomarker response (specifically cTn) and the magnitude of any individual rise in cTn was very limited. This likely reflects a combination of; (a) a low exercise dose, (b) a less trained group of participants, (c) a high assay detection limit in studies 1 and 2, and (d) all of the previous points acting synergistically. Points (a) and (b) are largely the limitations of laboratory-based studies that must be balances against the limitations of field work already noted.

To confirm the interplay between exercise and RIPC from study 3 we made the decision to move back to the "field" for study 4. This facilitated a much greater exercise dose and this illuminated a clearer role for ischaemia. Much larger cohort and individual cTn responses were observed in both trials of study 4. This highlights the value of exercise volume in cardiac biomarker studies.

In summary, the first three studies attempted to take advantage of the strengths of using a laboratory environment in the belief that it allowed for more precise conclusions about biomarker responses during controlled exercise exposure. Although a valid approach prolonged exercise in the laboratory does bring its own challenges. Feedback from the participants alluded to the difficulty of exercising in an environment when it is not the participant's natural competitive or training environment and thus exercise volume was reduced. The use of a field-based environment in the final study demonstrated a greater biomarker response. These studies do not suggest that laboratory or field based studies have distinctive benefits and limitations and should be combined, as appropriate, in on-going research.

Fitness of the Participants

This issue was raised briefly in the last section but is worth considering separately. Specifically this PhD contained 2 studies with less-well trained participants (Chapter 3 and 4) as well as 2 studies with well-trained cyclists (RIPC work in Chapter 5 and 6). In previous research there has generally been a focus on highly trained endurance athletes (e.g. Scott et al., 2009) although there is ample data from "charity" runners at events like Marathons (Shave et al., 2005; Fortescue et al., 2007). Fortescue et al.,

(2007) did note, in a large and diverse sample completing the Boston Marathon, that inexperience and younger age were the strongest predictors of cTn elevation.

Whilst “fitness” is a difficult construct to measure or report accurately, and different studies have only attempted self-report descriptive indices (Fortescue et al., 2007), it is logical that differences in fitness status should be considered as relevant for biomarker studies. We know that fitness status markedly effects cardiac structure, function and electrical activity so it may be postulated to also influence the ability to withstand cardiomyocyte insult in prolonged exercise exposures (George et al., 2011). Unpicking participant fitness from the exercise dose within most field studies is also extremely difficult. Despite this, and again with knowledge of changes in assay detection limits, we would tentatively support the notion that less well-trained participants, undertaking smaller bouts of exercise, are likely to show smaller changes in cardiac biomarkers post-exercise.

The biggest changes in cTn and NT-proBNP came in study 4 which combined both the high training status of the athletes with the biggest exercise dose in this thesis. The cTn and NT-proBNP data were substantially higher in this study than all the 3 previous datasets. Whether this is exercise volume or fitness status cannot be explicitly unpicked post hoc. It is likely a combination of these factors such that these athletes have the ability to hold an elevated exercise intensity over a prolonged period we believe would contributed to continued hemodynamic stress on the myocardium that resulted in cTn and NT-proBNP elevations post-exercise.

In summary, in our studies we noted that cTn responses were more common in more experienced/trained participants completing more prolonged exercise, compared to less experienced participants doing less work. The issue of interpreting this data is that it is quite difficult to un-pick participant experience from exercise volume (intensity) and also biomarker detection limits employed (which varied between studies). Whilst our work agrees with some past work it disagrees with other studies (e.g. Fortescue et al., 2007; Eijsvogels et al., 2010) and this suggests that further study is required to evaluate these specific issues on biomarker response to exercise.

The use of Different cTn assays and Detection Levels

To lower detection levels we used the new hs-cTnI and hs-cTnT which are both widely used within a clinical setting for the diagnosis of AMI and other diseases related to cardiac muscle injury (Westwood., 2015). There is speculative evidence that a reason for differences in exercise detection response may be as a result of the differences in molecular weights between hs-cTnI (24 kDa) and hs-cTnT (35.4 kDa) (Eijsvogels et al., 2010) as well as differences in assay detection levels (Shave et al., 2010). With hs-assays it may be that inter-biomarker differences may be reduced.

For the first 2 studies in this PhD hs-cTnI was the cardiac biomarker of choice (alongside NT-proBNP). These showed only very modest and infrequent elevations in hs-cTnI above the assay detection limit of 20 ng/L (study 1 POST-1H 22 ng/L increased by POST-3H to 24 ng/L, study 2 POST-1H 25 ng/L increased by POST-3H

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to 38 ng/L). This detection limit is lower than old 1-4th generation assays but still too high for the combination of athlete group and limited exercise exposure.

Study 3 in this PhD employed the hs-cTnT (rather than hs-cTnI used for studies 1 and 2) following the same blood draw analyses times as study 1 and study 2 (immediately post exercise and 1 h and 3h). The use of this assay hs-cTnT assay allowed the lower end to be "opened-up" and thus to report the assay to a much lower detection threshold (3 ng/L). Changes in cTn values (higher in the SHAM trial) included POST-0H 20 ng/L that increased by POST-1H to 22 ng/L and POST-3H to 25 ng/L. These data are still low and similar to the small number of positives in studies 1 and 2 but the overall kinetics were much easier to observe in all participants. Despite this the low magnitude and rapid clearance of cTn values did not indicate irreversible changes to the myocardium as would be observed after an AMI.

Study 4 went back to using hs-cTnI now that we could lower the detection level on this assay in line with study 3. cTn values were higher in this study (maximum concentration occurred POST-0H (RIPC 26 ng/L vs. SHAM 59 ng/L) all but one case showed reduction at the subsequent POST-1H sample point but this likely represented the longer exercise exposure and training status as much as the specific assay. Again the limited magnitude and rapid turnover of cTn does not indicate, match the kinetics observed in AMI.

In summary, the cTn detection threshold used in our studies for cTn varied but were still lower that older assays. That said opening up the assay capabilities provided extra insight in low exercise-dose studies. These assays should be used in exercise-biomarker studies moving forward.

Mechanisms and evidence for (ir)reversible cardiomyocyte damage with exercise.

The last 2 chapters in this PhD employed echocardiography alongside biomarkers in an attempt to link changes in cardiac function to biomarker appearance. Studies 3 and 4 did not show changes in LV systolic function which is in agreement with many other studies that have employed 1-5 hr of endurance exercise exposure (Oxborough et al., 2012). A study that employed cycling for 50 – 80 min to exhaustion at anaerobic threshold showed no change to cardiac function (Palatini et al., 1994). This would indicate that systolic function is preserved during this type of exercise exposure. Changes in diastolic function post-exercise in study 3 and 4 were generally of a small magnitude, recovered quickly and were not mediated by RIPC and SHAM. Thus, these changes had limited clinical significance and were not aligned with any biomarker changes. A post-exercise, transient decline in diastolic function has been postulated to be due to a decrease in preload, ischaemia and damage, and/or other intrinsic processes (Oxborough et al., 2010). This study was not able to provide irrefutable evidence of the role ischaemia as a key precursor for post-exercise changes in diastolic function as the effect was similar in both RIPC and SHAM trials.

Whilst studies 1 and 2 did not point to specific mechanisms underpinning cardiac biomarker release the data, in totality, did not point to irreversible cardiac insult.

Studies 3 and 4 demonstrated a reduction in cTn levels after the RIPC test, compared to the SHAM trial, and this indicates that ischaemia is potentially part of the mechanistic cascade mediating the elevation of cTn during prolonged exercise. The evidence that cTn is also elevated in the SHAM trial indicates that the kinetics of cTn will be complex and will most probably be associated with a number of interrelated mechanisms. The idea of disproportionate functional change to the RV and elevated hs-cTn and NT-proBNP with little or no change to the LV is in line with La Gerche et al., (2014). Although there is disparity with the mechanism of remodelling reactive oxygen species has been linked as a potential precursor (Schiattarella (2017). Future studies will need to evaluate a number of mechanisms related to exercise associated changes in a range of different biomarkers. Whilst it is still open to debate whether the release of cTn following exercise irrefutably reflects reversible or irreversible cardiac damage. The weight of evidence would support reversible and minor (sub-clinical) insults to cardiomyocytes. This is supported by:

1) Small magnitude of release, and
2) Limited time-period for elevation. Clinically associated elevations has shown that cTn levels in the blood have high levels of cTn above URL and can last for 21 days, whilst exercise associated elevations are lower and return below URL within 24 hours.
3) Whilst a link between cardiomyocyte damage and cardiac function change was proposed by Dawson et al., (2003) this was not supported during this thesis.
4) No other clinical signs and symptoms were observed in any trial,

5) Repeatability (whilst not elegantly supported) does suggest a physiological stimulus.

Despite all of these statements there is still a need for due care and attention to any evidence of elevated cTn or NT-proBNP in a post-exercise setting until a range of investigations reduces the risk of a cardiac event.

Table 7.1. Overview of individual study aims

Study title	Chapter	Hypotheses'	Results	Conclusion
Is there a rapid turnover of hs-cTnI and NT-proBNP during and after 2 h of prolonged moderate intensity exercise in healthy active individuals?	Chapter 3	STUDY1: running for 2 hours on a motorised treadmill at moderate intensity will reveal a rapid turnover of cTnI, using the new high sensitivity assay, and NT-proBNP in moderately active adults.	For the first study hs-cTnI was the cardiac biomarker of choice (alongside NT-proBNP). These showed only very modest and infrequent elevations in hs-cTnI above the assay detection limit of 20 ng/L (study	In conclusion, moderate intensity prolonged running in healthy males resulted in a limited release of cTn (as detected by a new hs assay) as well as a significant, and exercise-duration dependent elevation in NT-proBNP. The rapid elevation and removal during early recovery suggest these changes have limited clinical relevance and possibly reflect normal physiological processes.
The repeatability of the hs-cTnI and NT-proBNP response to a 2 hr bout of prolonged moderate intensity running.	Chapter 4	STUDY2a: Cardiac biomarker responses to the same exercise bout employed in STUDY 1, are highly repeatable after a period of 7 days between exercise tests in the same participants.	Detectable hs-cTnI was identified in the same single runner in Week 0 and Week 1 trials and for the remaining 7 participants no detectable hs-cTnI was observed in either runs – suggestive of gross levels of repeatability [responders vs. non-responders],	Three similar bouts of prolonged treadmill running in healthy untrained males in weeks 0, 1 and 12 resulted in a limited (gross level) repeatable cTnI response, with no real evidence of a repeat bout effect.
The impact of remote ischemic preconditioning on cardiac biomarkers and functional response to endurance exercise	Chapter 5	STUDY 3: Hypothesis 3: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 1-h strenuous cycle time trial exercise is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.	Absolute hs-cTnT levels were lower after RIPC than SHAM across all time points post-exercise (main effect for trial: $P < 0.05$), but there was no main effect of time or time-by-trial interaction (both $P > 0.05$). Maximal values for hs-cTnT occurred POST-3H (RIPC: 20 ± 9 ng/L vs. SHAM: 25 ± 8 ng/L, $P = 0.08$). No main effect of time, trial or time-by-trial interaction was observed for NT-proBNP (all $P > 0.05$).	Reduced serum hs-cTnT values were observed after a 60 minute cycling TT that was preceded by RIPC when compared to a SHAM protocol. This provides the first, indirect, evidence that ischaemia is implicated as a mechanism in changes in hs-cTnT post-exercise.
The impact of remote ischemic preconditioning (RIPC) on the cardiac biomarker and cardiac functional response to a 160 km cycle ride.	Chapter 6	STUDY 4: Hypothesis 4: Exercise-related changes in cTn, NT-proBNP and cardiac function will be attenuated when a 160 km cycle trial is preceded by RIPC in comparison to a time trial proceeded by a SHAM treatment.	There was a significant main effect of trial ($P = 0.001$) with data for hs-cTnI lower in the RIPC trial compared to the SHAM trial (independent of time). There was a significant main effect of time ($P = 0.043$) with hs-cTnI values initially rising during early recovery. There was a significant interaction (trial * time) effect ($P = 0.018$) that facilitated pairwise comparison at POST-0H and POS-1H where hs-cTnI values were lower after the RIPC trial at both time points compared to SHAM ($P < 0.05$).	The use of RIPC prior to a 160 km cycle ride significantly reduced the magnitude of both hs-cTnI and NT-proBNP release post-exercise when compared to a SHAM trial. This suggests some putative role for ischaemia during exercise-induced changes in hs-cTnI

7.3. Future Research Direction

Continued work is required to further our understanding of the mechanisms involved in cTn release during exercise. The role of the myocyte plasma membrane could be a plausible place to begin to look at this in animal models.

There is still a need to improve our understanding of exercise modality, participant's fitness and environmental conditions. Without better management of these variables it will be a challenge to further develop our understanding of responders and non-responders (those that release cTn and those that do not). This present study and many other research groups are showing that we have responders and non-responders during prolonged exercise. There is a need to identify if non-responders do become responders after long-term training. This could be achieved with the aid of a longitudinal investigation(s) on the impact of repeat bouts of prolonged exercise working with large sample size of high performing young and veteran endurance athletes to develop our understanding of responders and non-responders.

7.4. Conclusion

For this dissertation, I tested 6 hypotheses: 1) The running for 2 hours on a motorised treadmill at moderate intensity will reveal a rapid turnover of cTnI, using the new high sensitivity assay, for cTnI and NT-proBNP, **REJECTED**; 2) Cardiac biomarker responses are highly repeatable after 7 days, **ACCEPTED**; 3) Cardiac biomarker responses will be highly repeatable after 12 weeks, **REJECTED**; 4) Exercise-related

changes in cTn, NT-proBNP and cardiac function can be attenuated when 1-h strenuous cycle exercise is preceded by RIPC, **ACCEPTED**; 5) Higher and more divergent response in hs-cTnI, NT-proBNP after a 160 km cycle ride (c. 6 hr) preceded by SHAM as opposed to RIPC, **ACCEPTED**; 6) Indices of cardiac function after a 160 km cycle ride (c. 6 hr) preceded by SHAM as opposed to RIPC, – **REJECTED**.

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Appendix 1.

Consent, Health Questionnaire, Participant Information and Risk Assessment forms

CONSENT / ASSENT FORMS

The examples of the consent / assent forms given below are a minimum requirement which are suitable for most studies but may need alterations to be commensurate with individual study requirements. The participant is consenting to everything described in the text of the participant information sheet.

For some studies a fuller itemised consent form may be needed to cover other important issues especially where additional elements are optional for the participant for example:

- Additional invasive tests
- Additional questionnaires, interviews or focus groups with sub-samples of the study population
- Consent to the use of audio/video-taping
- Consent to the use of verbatim quotes or photographs
- Transfer of data to countries outside of the EEA
- Consent to the removal and storage of human tissue samples for future, undefined research

Applicants should not that where a study involves the participation of children under the age of 16, parental consent should always be sought and, in the case of medical research or other invasive studies, parental

consent should be sought from young people up to the age of 18. For social science based research young people aged 16 years and older are generally capable of giving their own consent; however as a research you will need to assess the individual's capacity to consent, depending on their maturity and understanding. Where researchers are recruiting young people aged 16 – 18 years old without parental consent they should, where appropriate, encourage the young person to inform their parent or guardian of their participation.

Where the proposed study involves the removal, storage or use of human tissue samples relevant information should be included in the participant information sheet and explicit consent for the removal, storage and / or use of the tissue samples sought in the consent form. Where applicable the explicit consent for the following should also be sought:

- Genetic testing
- Storage for future research (generic consent)
- Use for commercial research

Where a study involves the administration of a questionnaire or survey a signed record of consent is not required for completion of the questionnaire/survey as long as it is made clear in the information sheet that completion of the questionnaire/survey is voluntary. Under these circumstances return of the completed questionnaire is taken as implied consent.

In such cases the REC would expect a statement to be included at the start of the questionnaire/survey where the respondent confirms that they have read the participant information sheet and are happy to complete the questionnaire.

Participation in any other interventions within the same study eg interviews, focus groups must be supported by obtaining appropriate written consent.

Signatories to the consent should be those who are involved in the consent process eg the participant (or guardian/carer), the researcher or his/her representative delegated to take consent.

Where a consent form is to be signed by a participant at home and returned by mail to the researcher 2 copies should be provided both of which are returned and countersigned by the researcher and one copy posted back to the participant.

All consent forms must include the logo of the lead and any other participating institutions. All consent forms should be version dated in the header/footer to ensure that the most recent approved version is used.

LIVERPOOL JOHN MOORES UNIVERSITY

EXERCISE-INDUCED CARDIAC BIOMARKER RELEASE: REVERSIBLE OR IRREVERSIBLE CARDIAC DAMAGE?

Mark Benson – Research Institute of Sport & Exercise Sciences

1. I confirm that I have read and understand the information provided for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason and that this will not affect my legal rights. ☐

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

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

Name of Participant Date Signature

Name of Researcher Date Signature

Name of Person taking consent Date Signature

(if different from researcher)

Note: When completed 1 copy for participant and 1 copy for researcher

Researchers details

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HEALTH and TRAINING QUESTIONNAIRE

The impact of ischemic preconditioning (IPC) on the cardiac biomarker response to endurance exercise

The initial part of the questionnaire is to record any current or recent (last 2 weeks) self-report indications of ill health. This question (only) will be repeated before all endurance exercise bouts to check you have not been ill during the last 2 weeks as this could affect the results of the study.

Section one: Health Screen **(circle answer)**

1. Have you felt generally unwell within the last 2 weeks? Yes No

If yes, please specify _____

2. Have you taken any prescription or non-prescription medication because of signs and symptoms of ill-health within the last 2 weeks Yes No

If yes, please state what medication you have taken _____

3. Have you taken any days off work due to ill-health in the last 2 weeks Yes No

If yes, please specify _____

4. Have you missed any training due to ill-health in the last 2 weeks Yes No

If yes, please specify _____

Section two: Personal Information

5. Name:

6. DOB: Age:

7. Height: Weight:

8. Address _____

_____

_____

9. Telephone number:

Home:

Mobile:

10. Email:

Section three: Medical History (adapted from PARQ) **(circle answer)**

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

If yes, please give details, including dates _____

_____

_____

12. Have you ever experienced pains in your chest? Yes No

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

_____

_____

13. Do you ever faint or suffer from severe dizziness? Yes No

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

_____

_____

14. Has a doctor ever said your blood pressure was too high? Yes No

If so, please give any details you can _____

_____

15. Has a doctor ever told you that you had a joint or bone problem that might be exaggerated or aggravated by exercise? Yes No

If so, please give details,

_____

_____

16. Is there any good physical reason, not mentioned here why you should not take part in this physical activity challenge? Yes No

If so, please give details,

_____

_____

17. Do you suffer from any medical condition that hasn't been mentioned in the previous questions? Yes No

If so, please give details,

_____

_____

18. Has any member of you close family (siblings, parents, grandparents) suffered from early onset cardiovascular disease (before 45 years of age in men and 55 in women)? Yes No

If so, please give details,

_____

_____

Section Four: Training, Competition and Lifestyle

19. What is your highest level of competitive performance/achievement?

Please provide a brief description?

_____

_____

20. How long (years) have you been competing at this level?

_____

_____

21. Do you smoke? Yes No

What do you smoke (please circle) cigarettes cigars pipe

If yes,

How long have you smoked for? _____

How many per day? _____

Have you ever smoked? Yes No If yes,

When did you smoke? _____

How many per day? _____

22. Do you currently follow any specific diet restrictions (e.g. gluten free, vegetarian)? Yes No

If yes please provide details

_____

23. Do you take any dietary supplements? Yes No

If yes, please specify what you take and the frequency _____

_____

24. What is your current training load (can you provide details of number of sessions, type of sessions, duration and intensity of sessions)?

Monday _____

_____

Tuesday _____

_____

Wednesday_____

_____

Thursday_____

_____

Friday_____

_____

Saturday_____

_____

Sunday_____

_____

25. How long (months, years) have you been training at this level?

_____

_____

26. Please detail any further information you would like to tell us _____

_____

_____

_____

_____

Participant signature: _____

Thank you for completing this questionnaire

LIVERPOOL JOHN MOORES UNIVERSITY

Title of Project: EXERCISE-INDUCED CARDIAC BIOMARKER RELEASE: REVERSIBLE OR IRREVERSIBLE CARDIAC DAMAGE?

Mark Benson – Research Institute of Sport & Exercise Sciences

Introduction

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

1. What is the purpose of the study?

The purpose of the following studies is to contribute new data to the on-going debate about the clinical relevance of exercise-induced release of cardiac biomarkers into the blood and whether this reflects reversible or irreversible damage to the heart of humans during and after prolonged exercise. These issues require further exploration to inform athletes, coaches, scientists and clinicians about the cardiovascular risks and benefits of engaging in prolonged and strenuous exercise.

2. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do you will be given this information sheet and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw will not affect your rights/any future treatment/service you receive."

3. What happens if I take part in test 1 but become ill before one of the others tests?

All participants will be screened (self-report signs and symptoms) for illness prior to all exercise bouts. If you are feeling unwell or have very recently (within last 2 weeks) been ill the test(s) will not commence on that day. To assess current or recent ill health we will ask you if you have had to have time off work/training, you recently took or are currently taking medication and you have a general feeling(s) of ill-health.

4. Is there any exclusion criteria?

Yes, one or more from the list below:

a. *Early (close male relatives under 45 years and close female relatives under 55 years of age) family history of cardiovascular disease*
b. *Current musculoskeletal injury*
c. *Younger than 18 or older than 45 years*
d. *Not able to complete 2 hour running at 70 to 80% max running speed*
e. *Self report current or recent (last 2 weeks) ill health*
    i. *general feeling of ill health*
    ii. *use of medication*
    iii. *having to take days off work or training*
f. *currently training for a marathon*

g.

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

The study will involve visiting the Sports Science laboratories at LJMU on a number of occasions after you have read this form, asked any questions and provided a written informed consent form. The testing sessions will involve:

- *Initially we will measure your cardiorespiratory fitness and anaerobic threshold on a treadmill ($V\dot{}\dot{}O_{2max}$ tests). This is a graded intensity test. During these tests you will be required to exercise to exhaustion whilst wearing a mouthpiece that will monitor how much oxygen you use during exercise. You will also be asked to wear a heart rate monitor. This trial should last about 20 minutes.*
- *After this initial trial you will be asked to complete 3 endurance trials. This will mean a total of four visits to the laboratories. In each of the endurance trials you will exercise for 120 min at 70 to 80% of peak running speed that will be determined from the initial maximal treadmill test.*
- *For all tests, you will arrive at the lab in the morning (9 -10 am) after a light breakfast.*
- *Before exercising you will undergo a cardiac scan, have your urine osmolality assessed, be weighed and have your blood pressure taken. You will have your skin temperature recorded, your core temperature assessed, using an aural probe that is placed just inside the outer ear, and a heart rate monitor fitted. A small needle will be put in a vein in your arm and left there for the duration of the exercise so that a total of 7 blood samples of 5 ml can be collected (total blood withdrawn is 35 ml). This approach called "cannulation" means we don't have to keep putting needles in to your arm every time we need to take a blood sample.* Thirty-five ml represents a small amount of blood (equivalent to 1/4 of a small wine glass) and the use of the cannula will reduce the potential for bruising from repeated needle entries. The insertion and maintenance of the venous cannula will be performed by qualified staff.
- *During exercise we will continuously measure heart rate, blood pressure, oxygen uptake, core and skin temperature.*
- *Upon completion of the exercise protocol the same data collection procedures taken at rest will be completed immediately, 1 and 3 hours post-exercise. Please note that on the days you undertake the endurance trials you will be in the laboratories for the morning during which you will complete the endurance trial and the pre, during-exercise, post and 1 hr post blood samples will be taken. You are then free to leave but you will need to return briefly to the laboratories in the afternoon to provide a 3 hr post-exercise blood sample. The researcher looking after you will let you know the exact time you need to return on the day (see timeline below for exercise and blood samples)*

6. Are there any risks / benefits involved?

The subjects will wear a safety harness during the max tests as there may be a risk of falling during the max test and gloves and lab coats will be worn during the blood collection process to reduce the chances of infection.

There is a small risk of infection due to a needle/cannula being used to take blood samples. This may also result in bruising at the site of needle insertion. Running may result in a level of muscle damage/soreness if the athlete is unaccustomed to the intensity and/or duration of the exercise. This can last between 24 and 72 hours.

You will gain valuable information on your current state of fitness, the efficacy of your training regime and useful insights into your responses to prolonged running. Knowledge of your own physiological responses to exercise over longer distance will be useful in your preparation for competition (or training).

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

Your participation and all your data will be treated confidentially.

If you have any questions relating to any of the techniques used during the research study please feel free to contact me to discuss this further. Participation in this research study is voluntary and you are free to withdraw at any time without prior explanation.

Mark Benson MSc

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Generic Risk Assessment
New

Building…… Tom Reilly Building School/Service Department…Sports Science Location…… Human Performance Laboratory

Activity…… Research testing …… Date of Risk Assessment……16/04/2016……

Assessment carried out by…Mark Benson …. Signed……..

Persons consulted during the Risk Assessment Professor Keith George and Andrew Hulton (Technician)

STEP 1 What are the Hazards?	**STEP 2** Who might be harmed and how?	**STEP 3 (a)** What are you already doing?	**STEP 3 (b)** What further action is needed?	**STEP 4** How will you put the assessment into action?
Spot hazards by • *Walking around the workplace* • *Speaking to employees*	*Identify groups of people. Staff and students are obvious, but please remember* • *Some staff/students have particular needs*	*What is already in place to reduce the likelihood of harm, or to make any harm less serious.*	*Compare what you are already doing with good practice. If there is a gap, please list what needs to be done.*	*Please remember to prioritise. Deal with the hazards that are high risk and have serious consequences first.*

• *Checking manufacturers instructions* - Venipuncture injury (including bruising around the sample site) - Infection from blood sample or sampling procedure - Muscular soreness from exercise - Dehydration/heat injury from prolonged exercise	• *People who may not be present all the time* • *Members of the public* • *How your work affects others if you share a workplace* Subjects - All of points raised in STEP 1 Researchers - Infection (see STEP 1)	- All personnel involved with venepuncture and handling blood samples are fully trained. - Subjects will be fully familiarised with exercise. - A cut-off core temperature of 39.5°C will be adhered to and water/fluids will be allowed ad libitum	- No further (new) action is required. - These are points of good practice that will be adopted during the study.	Action by whom …… …… …… …… …… …… …… …… …… …… …… ……	Action by when …… …… …… …… …… …… …… …… …… …… …… ……	Done? …… …… …… …… …… …… …… …… …… …… ……

STEP 5 REVIEW DATE:……• Review the assessment to make sure you are still improving

• If there is a significant change, or there has been an accident, check the Assessment

and where necessary,