The Effect of Heat Stress, Dehydration and Exercise on Global Left Ventricular Function and Mechanics in Healthy Humans

A thesis submitted for the degree of Doctor of Philosophy

by

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Abstract

This thesis examined the effect of heat stress, dehydration and exercise on global left ventricular (LV) function and LV twist, untwisting and strain (LV mechanics) in healthy individuals. The primary aim was to identify whether the different haemodynamics induced by heat stress, dehydration and exercise would be associated with alterations in systolic and diastolic LV mechanics as assessed by two-dimensional speckle tracking echocardiography.

Study one showed that enhanced systolic and diastolic LV mechanics during progressively increasing heat stress at rest likely compensate in part for a lower venous return, resulting in a maintained stroke volume (SV). In contrast, heat stress during knee-extensor exercise did not significantly increase LV twist, suggesting that exercise attenuates the increase in LV mechanics seen during passive heat stress. Study two revealed that dehydration enhances systolic LV mechanics whilst diastolic mechanics remain unaltered at rest, despite pronounced reductions in preload. The maintenance of systolic and diastolic LV mechanics with dehydration during knee-extensor exercise further suggests that the large decline in SV with dehydration and hyperthermia is caused by peripheral cardiovascular factors and not impaired LV mechanics. During both, heat stress and dehydration, enhanced systolic mechanics were achieved solely by increases in basal rotation. In contrast, the third study demonstrated that when individuals are normothermic and euhydrated, systolic and diastolic basal and apical mechanics increase significantly during incremental exercise to approximately 50% peak power. The subsequent plateau suggests that LV mechanics reach their peak at sub-maximal exercise intensities. Together, the present findings emphasise the importance of acute adjustments in both, basal and apical LV mechanics, during periods of increased cardiovascular demand.

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Definition of Terms

Anisotropy: The property of being directionally dependent. In this thesis, the term refers to the non-uniform arrangement of left ventricular myofibres that determine myocardial deformation.

Apex: Left ventricular myocardial mass located distal of the papillary muscles.

Afterload: The load that the left ventricular muscle fibres work against during the contraction phase. In this thesis, mean arterial pressure is used as a surrogate of afterload.

Base: Left ventricular myocardial mass located between the mitral annulus and the papillary muscles.

Cardiac output ($L \cdot min^{-1}$): The product of heart rate and stroke volume, forming the total blood volume ejected by the left ventricle per minute.

Catecholamines: Sympathomimetic hormones that stimulate adrenergic receptors. In the context of this thesis, the term refers to adrenaline, noradrenaline and dobutamine, the latter which is the synthetic form of dopamine.

Chronotropy: The rate of left ventricular contraction.

Dehydration: The process of body water loss through sweating. The outcome is typically termed hypohydration. To avoid confusion, for the purpose of this thesis only the term dehydration is used as participants were continuously becoming more and more dehydrated, albeit at a slow rate.

Diastole: Relaxation phase within the cardiac cycle from aortic valve closure to mitral valve closure.

Echocardiography: Ultrasound procedure to assess cardiac structure and function.

Ejection fraction (EF, %): Volume of blood ejected with each ventricular contraction expressed as a percentage of the end-diastolic volume.

End-diastolic volume (EDV, ml): The largest volume of the left ventricle at the end of the filling phase.

End-systolic volume (ESV, ml): The smallest volume of the left ventricle at the end of the ejection phase.

Endocardium: Inner layer of the left ventricular myocardium.

Epicardium: Outer layer of the left ventricular myocardium.

Euhydration: Normal hydration status.

Finometer: A device for continuous, non-invasive assessment of beat-by-beat mean arterial blood pressure.

Haematocrit (%): Volume of red blood cells expressed as a percentage of total blood volume.

Haemoglobin: Iron-containing oxygen-transport protein in red blood cells.

Heat stress: The exposure to high external temperatures that exceed the capacity of the thermoregulatory system to maintain normal body temperatures and result in an increase in core temperature.

Hyperthermia: An elevation in core body temperature, typically of at least 1°C.

Inotropy: Referring to the intrinsic contractile property of cardiac myofibres.

Iso-volumic contraction time (IVC, ms): Phase within the cardiac cycle between mitral valve closure and aortic valve opening. Within IVC, endocardial myofibres contract without concomitant changes in left ventricular volume.

Iso-volumic relaxation time (IVRT, ms): Phase within the cardiac cycle between aortic valve closure and mitral valve opening. Typically, within IVRT myofibres start to relax without concomitant changes in left ventricular volume.

Left ventricular mechanics: In this thesis, 'left ventricular (LV) mechanics' is used as an umbrella term for LV twist, untwisting velocity and strain.

Lusitropy: The rate of myocardial relaxation.

Mean arterial pressure (MAP, mmHg): The average blood pressure exerted against the arterial walls over the course of one cardiac cycle.

Normothermia: Referring to normal ambient temperatures of 20 - 25°C; also used to signify a normal core body temperature of approximately 37°C.

Pericardium: Double-walled sac that surrounds the entire heart. The space between the epicardium and pericardium is filled with lubricated fluid.

Preload: The load that is imposed on the left ventricle at the end of the relaxation phase. The term is used to reflect changes in the amount of venous blood returning to the left ventricle as indicated by end-diastolic volume.

Rotation, basal / apical (Rot., degrees): Index of left ventricular short-axis displacement around the waist of the left ventricle. As viewed from the apex, basal rotation occurs clockwise and is expressed in positive values whereas apical rotation occurs counter-clockwise and is represented by positive values.

Rotation velocity (degrees·sec^{-1}): Peak systolic or diastolic time derivative of rotation.

Strain (%): Index of myocardial deformation, either shortening or lengthening. In this thesis, strain is always expressed as Lagrangian strain indicating that the value is expressed as percentage change from the initial end-diastolic length.

Strain rate (·sec^{-1}): Time derivative of strain, representing the velocity of myofibre shortening or lengthening.

Stroke volume (SV, ml): The volume of blood ejected by the left or right ventricle with each contraction. Unless otherwise stated, in this thesis SV refers to the left ventricle.

Systole: Contraction phase within the cardiac cycle from mitral valve closure to aortic valve closure.

Tau (τ): Time constant of relaxation, representing the time it takes for an index of interest to attain approximately 63.2% of its final value.

Twist (degrees): Index of myocardial deformation caused by the counter-directional rotation of the left ventricular base and apex.

Twist velocity (degrees·sec^{-1}): Peak systolic time derivative of left ventricular twist.

Untwisting velocity (degrees·sec^{-1}): Peak diastolic time derivative of left ventricular twist defined as the largest negative deflection following peak systolic twist velocity.

$\dot{V}O_2$max: Maximal oxygen consumption, typically achieved during whole-body exercise at maximal intensity.

CHAPTER 1

General introduction

1.1 Background

It is well-known that an acute bout of exercise requires comprehensive adjustments in all human body systems including the cardiovascular system (de Marées, 2003). As part of the exercise-induced alteration in cardiovascular function cardiac output increases up to fivefold during maximal effort to deliver the required blood flow to the working musculature, the skin and other metabolically active organs (Levine, 2008, Rowell, 1993). Whilst augmented cardiac output at the onset of exercise is typically accomplished by elevations in both heart rate and stroke volume (SV), studies have demonstrated that the SV response during exercise differs depending on the environmental conditions, hydration status and exercise intensity (Adolph, 1947, Astrand et al., 1964, González-Alonso, 2007, González-Alonso et al., 2008a, Higginbotham et al., 1986).

Previous studies have shown that when exercise is performed in the heat, SV is significantly lower compared with SV during exercise in temperate environments, suggesting altered LV function when the need for thermoregulation is elevated (González-Alonso, 2007, González-Alonso et al., 2008a, Rowell et al., 1966, Lafrenz et al., 2008). When the fluid lost during exercise in the heat is not replaced and individuals become dehydrated, the reduction in SV is further exacerbated. In these conditions, the decline in SV is too large to be compensated for by enhanced heart rate, potentially resulting in a reduced cardiac output (González-Alonso et al., 1995, González-Alonso et al., 1997, Sawka et al., 1979, Hamilton et al., 1991, Montain and Coyle, 1992). Conversely, during incremental exercise in healthy individuals SV increases initially from rest. However, SV then reaches a plateau at approximately 40–50% of the maximal individual oxygen consumption ($\dot{V}O_2max$) (Poliner et

al., 1980, Higginbotham et al., 1986, González-Alonso et al., 2008b, Astrand et al., 1964, Mortensen et al., 2005), the mechanisms responsible for this plateau are presently not known. Whilst the existing data clearly indicate that LV systolic and/or diastolic function is altered when exercise is (i) performed in the heat, (ii) whilst dehydrated or (iii) with increasing exercise intensity, the role of the underpinning LV mechanics has yet to be assessed.

Indices of 'global' LV function such as filling pressures, filling velocities, ejection fraction and systolic and diastolic tissue velocities have provided valuable evidence for altered LV function during exercise with and without heat stress and dehydration. The interpretation of LV function from these parameters is, however, limited because they are more reflective of LV haemodynamics and blood flow than of the underpinning myocardial mechanics (Fonseca et al., 2003). LV twist, untwisting and strain ('LV mechanics') are novel indicators of LV function that provide a mechanistic link between LV systolic ejection and diastolic filling and have been shown to play an important role in the regulation of normal LV function (Sengupta et al., 2006b, Sengupta et al., 2008a, Russel et al., 2009, Shaw et al., 2008, Notomi et al., 2006, Burns et al., 2009b). Systolic LV twist facilitates ejection whilst diastolic untwisting is considered an active process that contributes to an efficient LV filling by creating suction, at rest and during periods of enhanced cardiovascular demand (Notomi et al., 2006, Nelson et al., 2010a, Vendelin et al., 2002, Burns et al., 2009b). Additionally, systolic and diastolic myocardial deformation ("strain") across different LV planes provides further insight into the LV mechanics that underpin LV filling and ejection. Consequently, the changes in SV previously observed during exercise with and without heat stress and dehydration may be associated with alterations in LV twist, untwisting and strain.

To further the current understanding of the differential SV response during heat stress, dehydration and exercise, the aim of this thesis was to examine LV twist, untwisting and strain in healthy individuals during (1) heat stress at rest and during small muscle mass exercise, (2) during the combined challenge of dehydration and elevated body temperatures (hyperthermia) at rest and during small muscle mass exercise and (3) during incremental cycling exercise in normothermic and euhydrated conditions. Accordingly, three experimental studies were completed at Brunel University West London between March 2008 and April 2010.

The following chapter of this thesis provides an overview of the existing literature pertaining to; normal LV function at rest, the role of LV mechanics underpinning normal LV function and the cardiovascular adjustments related to the differential SV response during heat stress, dehydration and exercise. In the third chapter the general methodology employed in all three empirical studies is outlined. The specific study designs and results from each study are presented in chapters four, five and six, respectively. Finally, the overall findings are discussed in chapter seven and the conclusions from the three studies are presented.

CHAPTER 2

Review of literature

2.1 Introduction

Normal LV function is characterised by the interaction between filling and emptying, which ultimately results in SV. Whilst it has been shown that in healthy individuals the SV response to exercise is different depending on environmental temperatures, hydration status and exercise intensity little is known about the role of LV mechanics in these conditions. In addition to indicators of global LV function, LV mechanics have emerged as powerful indices of LV systolic and diastolic function in healthy individuals and in cardiovascular disease (Esch and Warburton, 2009, Sengupta et al., 2008a, Sengupta et al., 2007, Poliner et al., 1980, González-Alonso et al., 2008a).

The following literature review initially describes the normal LV contraction and relaxation sequence of a typical cardiac cycle at rest followed by an outline of the LV myofibre architecture relevant to LV twist, untwisting and strain and a review of the existing findings on LV mechanics in healthy individuals. Finally, the literature pertinent to LV function during heat stress, dehydration and incremental exercise is evaluated and the aims and hypotheses of this thesis are presented.

2.2 Normal left ventricular function

The normal cardiac cycle is made up of a phase of myocardial contraction (systole) and a period of relaxation (diastole), resulting in ejection of blood and filling of the LV, respectively. In order to maintain the continuous alteration between filling and ejection from the same chamber a well-coordinated sequence of pressure and volume shifts must occur.

Figure 2-1 depicts the typical interaction between changes in pressures and volumes within the LV and the aorta over one cardiac cycle (Levick, 2003). The systolic phase is initiated by a brief (~50ms) period of iso-volumic contraction during which shortening of endocardial myofibres and stretch of the epicardial fibres result in a fast increase in intra-ventricular pressure without a change in LV volume (Sengupta et al., 2005, Levick, 2003). Once the pressure in the LV exceeds the pressure in the aorta, the aortic valve opens and blood is ejected into the circulation resulting in a reduction in LV volume. Approximately half way through the systolic period, whilst blood is still being ejected, intra-ventricular pressure starts to decline until it is below that in the aorta and the aortic valve closes. By the end of the ejection phase LV volume has reduced from its end-diastolic volume (EDV) by approximately 68% in the healthy resting human, the remaining ~32% representing the end-systolic volume (ESV) (Levick, 2003). Following the end of systole a rapid fall in intra-ventricular pressure from ~80 mmHg to 0–5 mmHg ensues. This rapid drop in pressure promotes efficient filling and occurs during the iso-volumic relaxation time (IVRT, ~80ms) during which myofibres start to relax prior to any change in LV volume (Sengupta et al., 2005, Levick, 2003). This reduction in intra-ventricular pressure establishes the necessary pressure gradient between the left atrium and the LV to open the mitral valve and initiate early LV filling. Contraction of the left atrium at the end of the diastolic period adds approximately 25% of the total end-diastolic blood volume in the healthy resting human (Levick, 2003). Although the order of events throughout the cardiac cycle of a healthy heart does not change dramatically during enhanced cardiovascular demand, the magnitude of EDV, ESV and SV as well as the duration of events may all be influenced by three factors: preload, afterload and neural activity (Patterson et al., 1914, Sonnenblick, 1962, Bers, 2002).

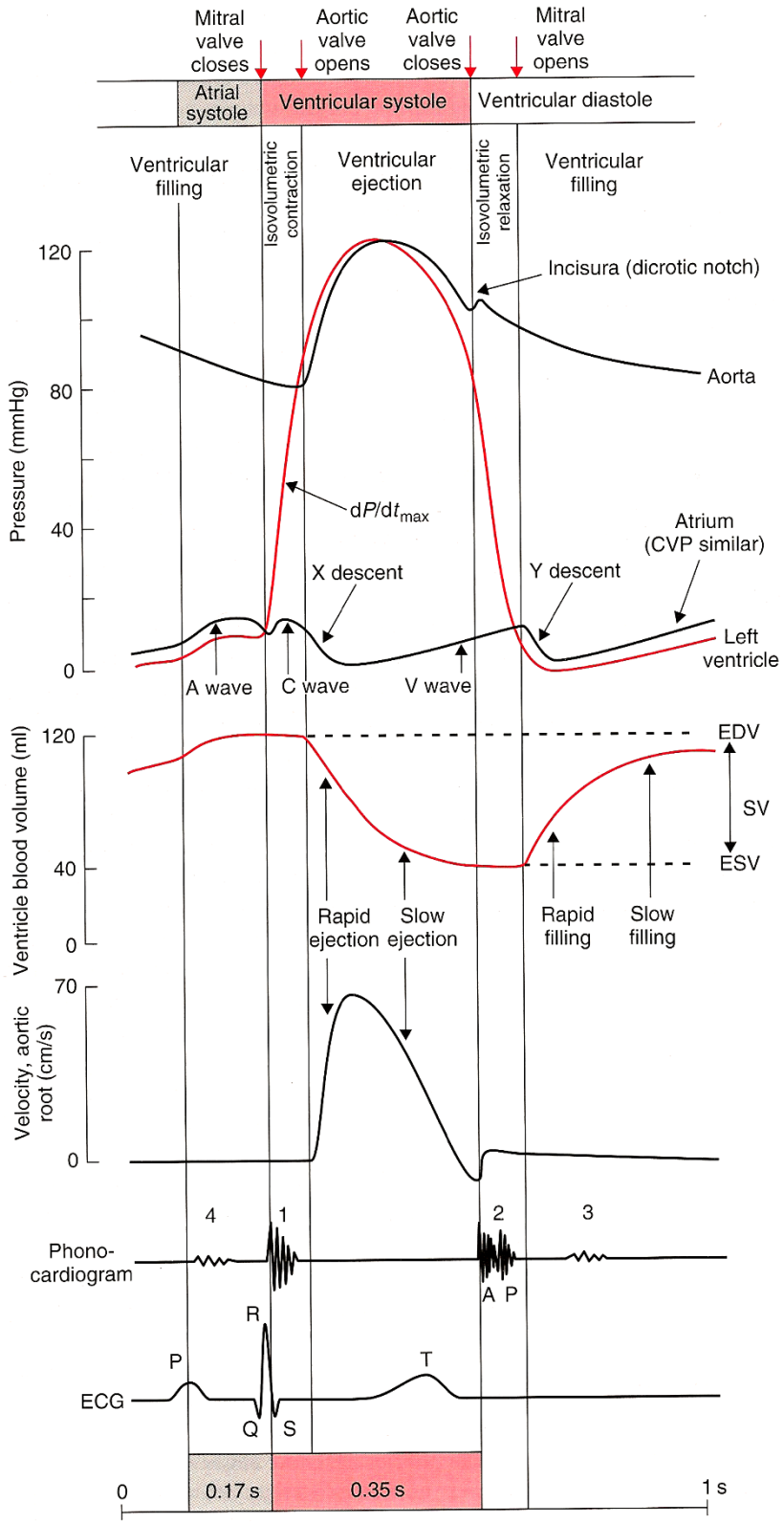

Figure 2-1. The normal left ventricular (LV) cardiac cycle. Interaction between changes in internal and external LV pressures and systolic and diastolic LV volumes are shown. EDV: end-diastolic volume; ESV: end-systolic volume; SV: stroke volume (from Levick, 2003).

2.2.1 Influence of altered preload and afterload on normal left ventricular function

At rest in the standing or upright seated position, the human LV fills with a central venous pressure of approximately 5 mmHg (Levick, 2003, Higginbotham et al., 1986). When an enhanced blood flow is promoted back to the heart, for example in the supine position or during exercise, central venous pressure increases (Higginbotham et al., 1986). An increase in central venous pressure up to 10–12 mmHg is paralleled by a proportional increase in SV. Higher filling pressures, however, do not result in a further increase in SV (Parker and Case, 1979). The augmented SV subsequent to an enhanced preload has been shown to be related to an increase in the tension of cardiac myofibres consequent to their greater stretch at the end of diastole; a phenomenon known as the Frank-Starling mechanism (Frank, 1895, see figure 2-2, Patterson et al., 1914). In addition to the increased SV subsequent to higher filling pressures it has also been shown that a reduction in filling pressure below the normal resting level rapidly reduces SV (Parker and Case, 1979), further demonstrating that an increase and a decrease in preload from normal levels impacts on SV.

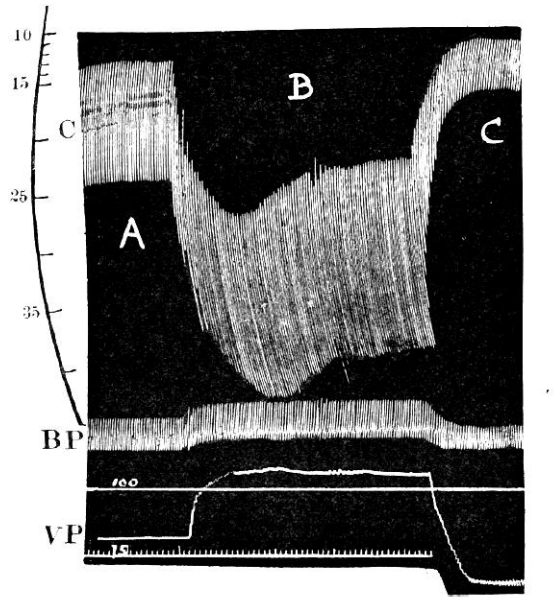

Figure 2-2. Frank-Starling mechanism in a dog heart-lung preparation. The graph illustrates that with increased end-diastolic pressure (VP) left ventricular stroke volume (B) also increases (Patterson et al., 1914).

Similar to the effect of altered preload on LV function a change in LV afterload, which is the resistance that the LV has to work against to eject blood, also impacts on SV (Sonnenblick, 1962, see figure 2-3). A higher afterload reduces the force production of LV myofibres as more of the energy developed during iso-volumic contraction is used to overcome the heightened arterial pressure before actual ejection of blood is possible (Levick, 2003). Thus, progressive increases in afterload inhibit myofibre shortening during the ejection phase, thereby attenuating the reduction in end-systolic volume and resulting in a reduced SV.

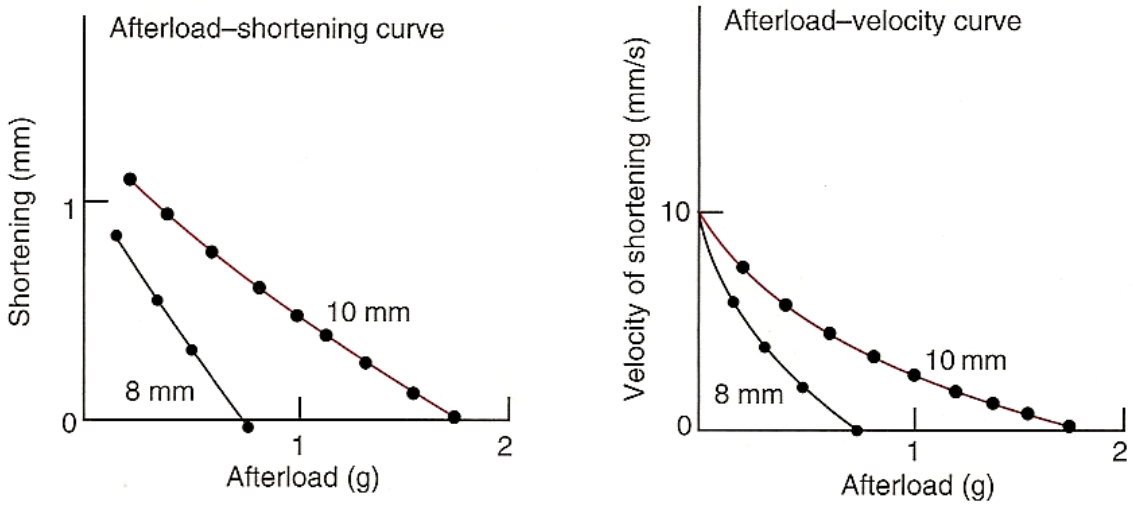

Figure 2-3. Afterload-shortening relationship. Increased afterload during left ventricular contraction results in a reduction in myofibre shortening and the velocity of shortening. When preload is concomitantly enhanced as indicated by increased sarcomere length from 8mm to 10mm, the same afterload results in a greater shortening and velocity of shortening (graph from Levick, 2003 after, Sonnenblick, 1962).

2.2.2 Neural control of normal left ventricular function

The neural input from the sympathetic and parasympathetic branches of the autonomic nervous system presents another factor that influences the rate and force of LV contraction

and relaxation in addition to the previously discussed changes in preload and afterload. In the normal resting state, an electrical wave of excitation from the sinoatrial node triggers an inward current of Ca^{2+} (I_{Ca}) through the sarcolemma into the myocyte which releases further Ca^{2+} from the store in the sarcoplasmic reticulum, a process that has been termed calcium-induced calcium release (Bers, 2002). The free Ca^{2+} within the sarcoplasm then binds to troponin C of the troponin-tropomyosin complex (Levick, 2003) ultimately resulting in crossbridge formation and myocyte contraction. Relaxation occurs when Ca^{2+} is removed either actively by the sarcoplasmic reticulum ATPase pump (~70%) or passively by the Na^{+}/Ca^{2+} exchanger (~28%) (Bers, 2002). At rest, in the healthy human this cycle occurs on average at a rate of approximately 60 times a minute. The resultant heart rate (~60 bpm) reflects the predominant activity of the parasympathetic nervous system and its messenger acetylcholine, which slows the intrinsic pacemaker activity of approximately 100 beats per minute (Jose, 1966). However, when sympathetic activity increases and β_1-adrenergic receptors in the myocardium are stimulated via the neurotransmitters adrenaline and noradrenaline, the rate and force of contraction (positive chronotropic and inotropic effect, respectively) and relaxation (positive lusitropic effect) increase (Opie, 2004). Sympathetic stimulation of β_1-adrenergic receptors triggers the production of cyclic adenosine 3',5'-monophosphate (cAMP) which in turn activates protein kinase A (PKA). Activation of PKA increases the I_{Ca} resulting in a greater Ca^{2+} release from the sarcoplasmic reticulum and, subsequently, enhanced rate and force of contraction (Opie, 2004, Bers, 2002). Increased chronotropy and inotropy are also coupled with a faster re-uptake of Ca^{2+} into the sarcoplasmic reticulum thereby improving myocardial relaxation. This enhanced lusitropic activity is largely achieved by increased phosphorylation of phospholamban, which in the resting state inhibits the sarcoplasmic reticulum Ca^{2+}-ATPase (Bers, 2002, Li et al., 2000).

Importantly, the increase in inotropic and lusitropic state is directly related to the magnitude of sympathetic stimulation (Levick, 2003). However, it must be noted that isolated β_1-adrenergic stimulation such as during administration of an inotropic agent will only result in a small increase in SV, as EDV is concomitantly reduced (Linden, 1968, Levick, 2003). When EDV is maintained at baseline levels, the full extent of inotropic stimulation becomes apparent as SV increases noticeably (Linden, 1968). These findings demonstrate that, similar to augmented preload discussed previously, enhanced myofibre contractility via sympathetic stimulation increases cardiac output by enhancing the force of contraction as well as the rate of contraction and relaxation.

2.2.3 Summary

Stroke volume at rest is the result of the well-coordinated diastolic filling and systolic emptying of the LV, which is achieved by changes in LV pressures and volumes. A transient change in filling pressure or volume (preload) or an altered resistance from the arterial system (afterload) bring about changes in SV mediated by the Frank-Starling mechanism and the afterload-shortening relationship, respectively. Similarly, alterations in neural activity modify the contractility and the rate of myocardial contraction also resulting in a different SV compared with the normal resting state. Whilst the independent effects of changes in preload, afterload and inotropic state on LV function are well-known they do not change in isolation during altered cardiovascular demand such as that seen when body temperatures and hydration status are changed or during exercise. Furthermore, there is increasing evidence that normal diastolic and systolic LV function is underpinned by specific LV mechanics as determined by twist, untwisting and strain.

2.3 Left ventricular mechanics

As outlined in the previous section, LV volumes provide information on the filling and emptying characteristics of the LV. However, LV volumes are merely outcome measures that are not only determined by changes in preload, afterload and contractility but also by the underpinning LV mechanics associated with contraction and relaxation namely; LV twist, untwisting and strain (Fonseca et al., 2003). LV twist, untwisting and strain are measures of the mechanical deformation of the LV as determined by the specific myofibre arrangement across the LV. Assessing LV twist, untwisting and strain permits the quantification of regional and overall LV deformation and, thus, systolic and diastolic mechanical function. Anatomical studies have provided insight into the complexity of the LV architecture that forms the basis for twist, untwisting and strain during different phases of the normal cardiac cycle. This section first outlines the anatomical origin for LV twist, untwisting and strain followed by a detailed definition of the terminology associated with these indices and a review of the current literature examining LV mechanics during altered cardiovascular haemodynamics in healthy individuals.

2.3.1 Left ventricular anatomy and electrical sequence underpinning twist

The healthy human LV has the shape of a prolate or truncated ellipsoid (Adhyapak and Parachuri, 2009, Ashikaga et al., 2004b). This shape is predominantly made up of muscle fibres which are composed of cardiac myocytes (Spotnitz, 2000). Since each cardiac myocyte is only able to actively contract along its long-axis (Spotnitz, 2000), the direction of myocardial movement during systole and diastole is determined by the specific myofibre orientation that forms the LV wall. Previous studies have shown that the fibre orientation

within the LV wall changes continuously from a right-handed helix in the inner wall (subendocardium) to a left-handed helix in the outer muscle tissue (subepicardium) (Schmid et al., 1997, Sengupta et al., 2008a, Sengupta et al., 2007, Greenbaum et al., 1981, Streeter et al., 1969, Spotnitz, 2000, Chen et al., 2005, Takayama et al., 2002). In humans, the fibre angles within these helices range from +60° in the subendocardium to approximately -60° in the subepicardium, with the mid-ventricular wall displaying circumferential fibre alignment (Greenbaum et al., 1981, Streeter et al., 1969, Ingels et al., 1989, see figure 2-4).

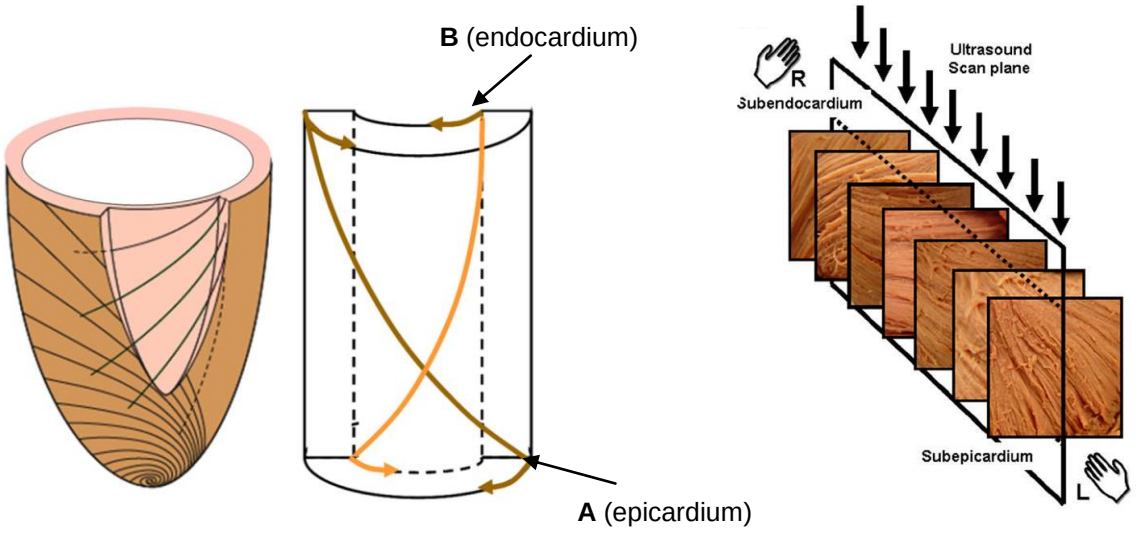

Figure 2-4. Schematic representation of the helical fibre orientation in the human left ventricle. (A) Left-handed helix in the subepicardium and (B) right-handed helix in the subendocardium (modified from Sengupta et al., 2008a, Sengupta et al., 2007).

Consequent to the helical fibre arrangement, contraction of the right-handed endocardial fibres results in counter-clockwise rotation of the LV base and clockwise rotation of the LV apex. In contrast, contraction of the subepicardial fibres results in opposite rotational movements of the base and apex, respectively. Due to the longer lever of subepicardial fibres

overall rotation at the base is clockwise and rotation at the apex is counter-clockwise during systole (Taber et al., 1996).

Preceding the described basal and apical rotation is a heterogeneous electrical activation sequence. Depolarisation of the myocardium begins at the subendocardial apex and moves to the endocardial base followed by activation of the apical epicardium and finally the epicardial base (Sengupta et al., 2006a). Accordingly, during iso-volumic contraction subendocardial fibres shorten first accompanied by concomitant stretching of subepicardial fibres (Ashikaga et al., 2009). In accordance with the Frank-Starling mechanism, this stretching of subepicardial myofibres is thought to increase the subsequent force of myocardial contraction in the epicardial helix (Campbell and Chandra, 2006). However, simultaneous shortening of subendocardial fibres also indicates that the Frank-Starling mechanism presented in section 2.2.1 may be altered when the interaction between subendocardial and subepicardial fibres is modified. As the LV base and apex rotate in opposite directions during systole, contraction is characterised by a twisting motion of the LV around its long-axis. The heterogeneous structure as well as a non-uniform electrical activation sequence means that there is permanent shear strain between subendocardial and subepicardial myofibres (Thompson et al., 2010). Twist and shear strain have been shown to distribute myocardial fibre stress evenly across the LV wall and also improve the efficiency of systolic ejection as indicated by higher ejection fractions (Vendelin et al., 2002).

In diastole, the electrical repolarisation sequence is reversed and mechanical relaxation of subepicardial fibres occurs before lengthening of subendocardial fibres (Sengupta et al., 2006a, Hasegawa et al., 2009). Relaxation results in rapid untwisting or recoil of which approximately 40% occurs during iso-volumic relaxation time (IVRT) (Dong et al., 2001, Notomi et al., 2008). LV untwisting can, thus, be considered an active process important for the generation of ventricular suction beneficial for early LV filling (Rademakers et al., 1992, Notomi et al., 2008). Studies in animals have shown that approximately 90% of LV untwisting precedes mitral valve opening which in turn occurs prior to early LV filling (Notomi et al., 2008) and that LV untwisting correlates with the time constant of relaxation (tau τ) (Dong et al., 2001), further underlining the importance of LV untwisting for normal LV filling. These findings have since been confirmed in humans (Burns et al., 2009b). Although the presented studies demonstrate that LV twist, untwisting and strain are dynamic components of normal LV function, the impact of altered haemodynamics caused by physiological conditions such as heat stress, dehydration and exercise on LV mechanics is poorly understood.

2.3.2 Definition of left ventricular twist and strain indices

As outlined in 2.3.1, the LV architecture predisposes the whole LV to contract with a twisting motion and to relax subsequently by untwisting. Furthermore, LV contraction and relaxation can be quantified by assessing strain (D'Hooge et al., 2000). In relation to these principles of myocardial deformation several twist and strain indices can be measured, however, the terminology relating to the different functional parameters has often been used interchangeably creating confusion in the literature. In order to establish a consistent

terminology within this thesis, all relevant indices are defined as follows and will be used accordingly throughout this document. Abridged definitions of these terms can also be found in the definition of terms section at the beginning of this thesis.

Rotation and rotational velocity

LV rotation refers to the independent rotation at the basal and apical short-axis level, respectively, and is defined as "the angle between radial lines connecting the centre of mass ... to a specific point in the myocardial wall at end-diastole and at any other time during diastole" (Sengupta et al., 2008b). Rotation is expressed in degrees with systolic basal rotation represented by negative values and systolic apical rotation by positive values, respectively (Notomi et al., 2005a). Basal rotation velocity and apical rotation velocity are the time derivatives of their respective rotation, expressed in degrees/sec (Notomi et al., 2005a). In diastole, the reversal of rotation velocity at the LV base and apex is denoted by positive values for basal rotation velocity and negative values for apical rotation velocity.

Twist, torsion and twist velocity

LV twist during systole is the result of the simultaneous clockwise rotation of the LV base and the counter-clockwise rotation of the LV apex. Thus, LV twist is calculated by subtracting the negative basal rotation data from the positive apical rotation data, resulting in positive peak twist (Notomi et al., 2005a). Some authors have suggested normalising twist to the size of the LV chamber in order to account for the greater radius in larger hearts, referring to this parameter as "LV torsion" (Russel et al., 2009). Whilst it is known that a larger LV

has a greater absolute twist, normalising twist to LV length also has the disadvantage of masking the physiological influence of acute changes in LV volumes. In this thesis, the data represent LV twist and not torsion and are expressed in degrees. Similar to rotation velocities, LV systolic twist velocity is the time derivative of the LV twist response and is also expressed in degrees/s (Notomi et al., 2005a).

Untwisting velocity and untwisting rate

The diastolic component of the twist velocity curve is defined as LV untwisting velocity. Peak LV untwisting velocity in this thesis is defined according to Perry et al. (2008) as the "first negative deflection following aortic valve closure". Some studies have referred to peak LV untwisting velocity as "LV untwisting rate" (Wang et al., 2007b). However, other authors have used the term untwisting rate to describe the average untwisting velocity between the peak of LV twist (degrees) and the end of the iso-volumic relaxation time (Takeuchi et al., 2007, Dalen et al., 2010). As both peak LV untwisting velocity and LV untwisting rate provide valuable information regarding LV function both parameters will be reported in this thesis. A graphical representation of basal rotation, apical rotation, twist and their respective velocity traces over the course of one cardiac cycle is shown in figure 2-5.

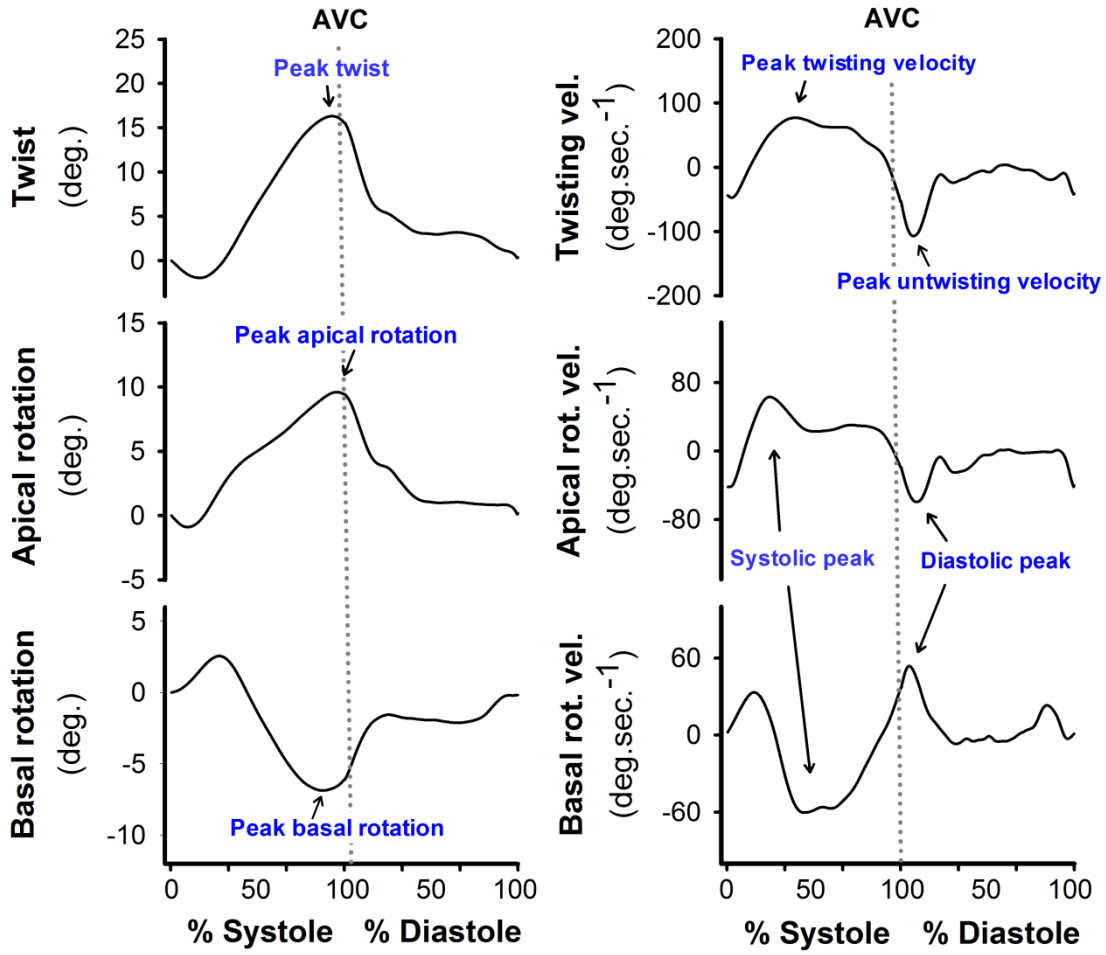

Figure 2-5. Peak left ventricular (LV) twist indices over the course of an entire cardiac cycle. LV basal rotation, apical rotation and twist occur at approximately 90% of the systolic period (left panel) whereas systolic and diastolic rotational velocities occur early in systole and diastole, respectively (right panel). AVC: aortic valve closure; Deg.: degrees; Rot.: rotation.

Strain

In addition to LV twist and untwisting, LV strain and strain rate are indicators of LV myocardial deformation. Although the main focus of this thesis is to examine the role of LV twist mechanics during exercise with and without heat stress and dehydration, myocardial strain and strain rate provide additional valuable information for the interpretation and complete understanding of LV function. LV strain is a measure of myocardial tissue

shortening or lengthening which can either be expressed as natural strain or Lagrangian strain. Natural strain reflects instantaneous deformation with constantly changing reference values during the contraction or relaxation process (D'Hooge et al., 2000). Lagrangian strain is expressed as a percentage of end-diastolic length and subsequent measuring points refer to this initial value (D'Hooge et al., 2000). In this thesis, all strain values are expressed as Lagrangian strain and strain rate represents the velocity of shortening or lengthening (D'Hooge et al., 2000, Teske et al., 2007). Whilst tissue Doppler imaging limits the measurement of strain to the longitudinal planes due to the angle dependence of the Doppler shift, speckle tracking ultrasound enables the assessment of strain and strain rate in the longitudinal, radial and circumferential planes. Longitudinal strain is assessed along the long-axis of the LV, from base to apex. Radial strain represents displacement perpendicular to the longitudinal plane and measures myocardial expansion during systole and thinning during diastole. Circumferential strain occurs perpendicularly to longitudinal and radial strain "around the waist of the ventricle" (Spotnitz, 2000) and indicates the magnitude of short-axis myofibre shortening and lengthening (figure 2-6).

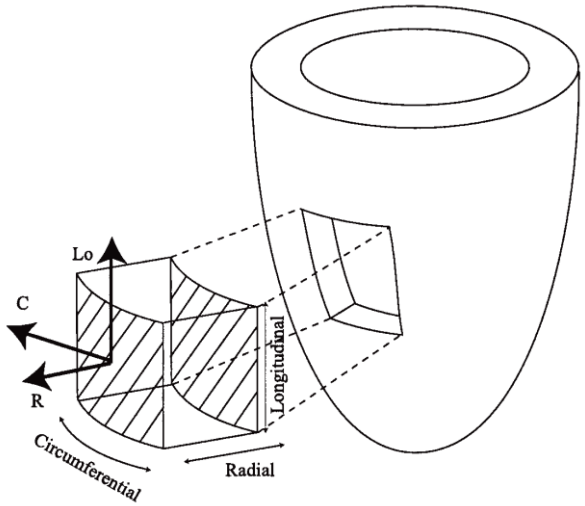

Figure 2-6. Left ventricular (LV) strain indices. LV myofibre strain can be quantified as shortening or lengthening in three different dimensions: the circumferential, longitudinal and radial planes. C: Circumferential; R: Radial; Lo: Longitudinal (from D'Hooge et al., 2000).

Left ventricular (LV) mechanics and twist mechanics

The term LV mechanics has been employed synonymously for different combinations of twist and strain indices. In this thesis, *LV mechanics* comprises the systolic and diastolic components of all LV twist and strain indices. The term *LV twist mechanics*, however, only refers to systolic twist and diastolic untwisting.

2.3.3 Effect of altered preload and afterload on left ventricular twist and strain

Similar to the effects of altered preload and afterload upon EDV, ESV and SV, studies have assessed the impact of changes in cardiac preload and afterload on LV mechanics. Gibbons-Kroeker *et al*. (1995) examined the effect of altered load on LV apical rotation in anaesthetised dogs. Vena caval occlusion reduced preload and afterload and resulted in an increase in apical rotation, whereas both enhanced preload via saline infusion and a higher afterload induced through aortic occlusion reduced apical rotation. The authors concluded that "LV twist at ED [end-diastole] and ES [end-systole] is primarily a function of volume; this relation appears to be unaltered by heart rate, afterload, and contractility" (Gibbons Kroeker et al., 1995). In humans, the combination of lowered preload and afterload via administration of glyceryl trinitrate also results in enhanced LV twist (Burns et al., 2010). Whilst Dong et al. (1999) confirmed the findings by Gibbons Kroeker et al. that a higher afterload decreases LV twist, the studies differ in their findings on the effect of a change in preload upon LV twist. One probable explanation for these conflicting results is that in the studies by Gibbons-Kroeker et al. and Burns et al. the reduction in preload resulted in a concomitant decline in afterload. In contrast, the experimental set-up employed by Dong *et al*. (1999) allowed for the independent manipulation of either preload or afterload. Thus, it

can be assumed that increases in preload improve peak LV twist whereas reductions in preload results in lower systolic twist (Dong et al., 1999).

The effect of reduced preload on systolic twist and diastolic untwisting velocity was further examined in humans by Esch et al. (2010). They showed that lower body negative pressure significantly increases LV untwisting velocity in healthy untrained individuals whilst peak systolic twist did not change. These results indicate an important compensatory function that may attenuate the reduced filling and maintain systolic function despite decreased venous return. Together, the existing studies show that LV twist and untwisting are sensitive to changes in preload and afterload. Since physiological alterations in haemodynamics caused for example by heat stress, dehydration and exercise alter preload and afterload concomitantly, it is difficult to predict LV twist and untwisting in these conditions.

Similar to the influence of altered preload and afterload on LV twist and untwisting, LV strain and strain rate are also preload and afterload dependent. It has been shown that a higher preload via increased blood volume enhances longitudinal strain and strain rate and a rise in afterload reduces these indices (Rosner et al., 2009). Furthermore, as a consequence of the combined effect of a reduction in end-diastolic pressure and end-systolic wall stress in humans, circumferential strain and circumferential and longitudinal strain rate have been shown to increase significantly without affecting longitudinal strain (Burns et al., 2009a). An actual reduction in longitudinal strain was shown following haemodialysis, which reduced

EDV but did not affect ESV (Choi et al., 2008). Thus, similar to the effect of altered loading status on LV twist and untwisting, strain and strain rate are also sensitive to changes in haemodynamics. As outlined previously in section 2.2 normal LV function is not only affected by changes in LV loading conditions but also by alterations in contractility. Accordingly, studies have further assessed the impact of enhanced inotropic state upon LV twist, untwisting and strain.

2.3.3 Effect of altered inotropy on left ventricular twist and strain

In single cardiac myofibres, enhanced sympathetic stimulation results in increased contractility as described in section 2.2.2 (Sarnoff, 1955). Consequently, studies have tried to determine the effect of inotropic stimulation on LV twist and strain. Hansen et al. (1988) showed that a mild increase in inotropic state in cardiac transplant recipients resulted in an enhanced LV twist in the anteroapical and inferoapical segments but not in other LV regions. Studies examining the effects of inotropic stimulation on LV twist in healthy individuals have demonstrated a concomitant increase in peak LV basal and apical rotation and, thus, LV twist (Helle-Valle et al., 2005, Opdahl et al., 2008, Dong et al., 1999, Rademakers et al., 1992). Furthermore, a higher contractile state also increases LV twisting and untwisting velocities, the latter of which has been shown to rise exponentially when ESV is reduced (Rademakers et al., 1992, Wang et al., 2007b). In accordance with this, β_1-receptor blockade with esmolol reduces untwisting velocity, further suggesting a direct influence of inotropic (and chronotropic) state upon LV twist mechanics.

In addition to the effect of sympathetic state upon overall LV twist mechanics, Akagawa et al. (2007) have reported transmural differences following dobutamine administration. They

showed that enhanced inotropic state resulted in a greater endocardial twist compared with epicardial twist. Together with the findings by Hansen *et al*. (1988), these data indicate that LV segments and helices may respond non-uniformly to *β*-adrenergic stimulation. Although there is the possibility that the regional differences described by Hansen *et al*. (1988) and Akagawa *et al*. (2007) may have been influenced by the populations studied and the method of selecting the endocardial and epicardial region, the data fit other reports of a heterogeneous response between the endo- and epicardium at the LV base and apex, respectively (Sengupta et al., 2006a, Hasegawa et al., 2009, Ashikaga et al., 2009). Moreover, the concept of a heterogeneous effect of inotropic stimulation across the LV is supported by the structural heterogeneity (Greenbaum et al., 1981, Akagawa et al., 2007) and a varying *β*-receptor density across the LV (Kawano et al., 2003, Lyon et al., 2008).

Further to the positive effect of enhanced contractility on LV twist indices, studies have shown that systolic circumferential, longitudinal and radial strain and strain rate are also augmented with increased inotropic stimulation (Weidemann et al., 2002, Greenberg et al., 2002, Yue et al., 2009, Paraskevaidis et al., 2008). These findings are not surprising since strain is a measure of fibre shortening/expansion and strain rate is the velocity of this shortening/expansion. Increased contractility, therefore, can be expected to result in enhanced strain and strain rate. Accordingly, some studies have shown that systolic strain rate correlates with LV elastance, an invasive index of intrinsic LV myofibre contractility (Greenberg et al., 2002) whereas diastolic strain rate during IVRT is related to tau (Wang et al., 2007a). As a result of the strong relationship with LV elastance, some authors have concluded that strain rate is a non-invasive measure of intrinsic contractility (Greenberg et

al., 2002, Teske et al., 2007). However, this interpretation may only be true for conditions of maintained load since the magnitude and velocity of LV fibre shortening is not only dependent on intrinsic contractile state but also on the Frank-Starling mechanism as discussed previously (Nesbitt et al., 2009, Rosner et al., 2009). Thus, strain rate can more likely be considered an indicator of overall myocardial contractile state caused by the combined effect of intrinsic contractility and the prevailing haemodynamic load. Consequently, strain and strain rate would provide valuable insight into myocardial deformation during commonly experienced periods of enhanced cardiovascular demand.

2.3.4 Summary

Previous studies have demonstrated that the unique LV geometry facilitates LV twist and that this LV twist is an important determinant of systolic and diastolic function. Further, LV strain and strain rate provide information on myocardial deformation in three different dimensions across the LV. Both, twist and strain have been shown to be sensitive to acute alterations in preload, afterload and inotropic state. Since physiological conditions of altered cardiovascular demand often affect preload, afterload and inotropic state concomitantly, LV mechanics are likely to respond in a condition specific manner. Consequently, assessing LV mechanics may further the current understanding of the SV response during periods of altered cardiovascular demand in healthy individuals.

2.4 Cardiovascular adjustments to heat stress

It is essential for humans to maintain the normal core body temperature (~37 °C) as changes as little as 3–3.5°C can result in injury or even death (Crandall and González-Alonso, 2010, Lim et al., 2008). To achieve such homeostasis the cardiovascular system must respond rapidly to changes in internal and external temperatures by increasing or decreasing blood flow to the skin. Increased skin blood flow enhances convective heat dissipation via sweating whilst a reduction in skin perfusion prevents heat loss (Rowell, 1974). Consequently, during heat stress cardiac output increases to account for the higher skin perfusion (Rowell et al., 1969a). Exercise in the heat presents a further challenge to the cardiovascular system as the increased need for heat dissipation competes with the enhanced demand for oxygen at the level of the working musculature, the heart and the brain (González-Alonso, 2007, González-Alonso et al., 2008a). However, studies have shown that despite the additional need for an elevated skin blood flow cardiac output is the same or even lower during exercise in the heat compared with exercise in normothermic environments (Lafrenz et al., 2008, Rowell et al., 1966, González-Alonso and Calbet, 2003). The existing data consistently show that the lack of an enhanced cardiac output during exercise in the heat is caused solely by a lower SV compared with normothermic conditions. Furthermore, systemic cardiovascular function has been studied extensively and several peripheral adaptations including redistribution of blood and reduction in venous return have been suggested to impact on the SV response during heat stress (González-Alonso et al., 2000, Crandall et al., 2008). The role of LV function and its contribution to altered SV, however, remain incompletely understood.

Although heat stress at rest is less commonly experienced than the combined cardiovascular challenge of exercise in the heat, studies examining the effects of passive heat stress have provided valuable insight into the cardiovascular adaptations required for thermoregulation. Thus, the following section summarises previous findings from studies examining altered systemic haemodynamics and LV function with heat stress at rest followed by an evaluation of the existing evidence of an altered LV function during exercise in the heat.

2.4.1 Haemodynamics and left ventricular function with heat stress at rest

In addition to an enhanced cardiac output with heat stress at rest, the increase in blood flow to the skin required for heat dissipation is further aided by the redistribution of blood away from non-active areas. Studies have shown that a rise in body temperature results in vasoconstriction of the splanchnic region (Escourrou et al., 1982, Rowell et al., 1970, Rowell et al., 1968, Rowell et al., 1971, Rowell, 1974, Crandall et al., 2008), reducing splanchnic blood flow by approximately 30–40 % (Rowell et al., 1970, Rowell et al., 1971). Together with the increase in cardiac output this redistribution of blood prevents the decline in arterial blood pressure that would otherwise be caused by the increase in skin vasodilation as determined by vascular conductance (Wilson et al., 2002).

Several studies have demonstrated that central blood volume, central venous pressure and LV filling pressures are reduced during heat stress at rest, indicating a lower venous return

compared with control conditions (Wilson et al., 2009, Wilson et al., 2007, Rowell et al., 1969a, Crandall et al., 2008, see figure 2-7).

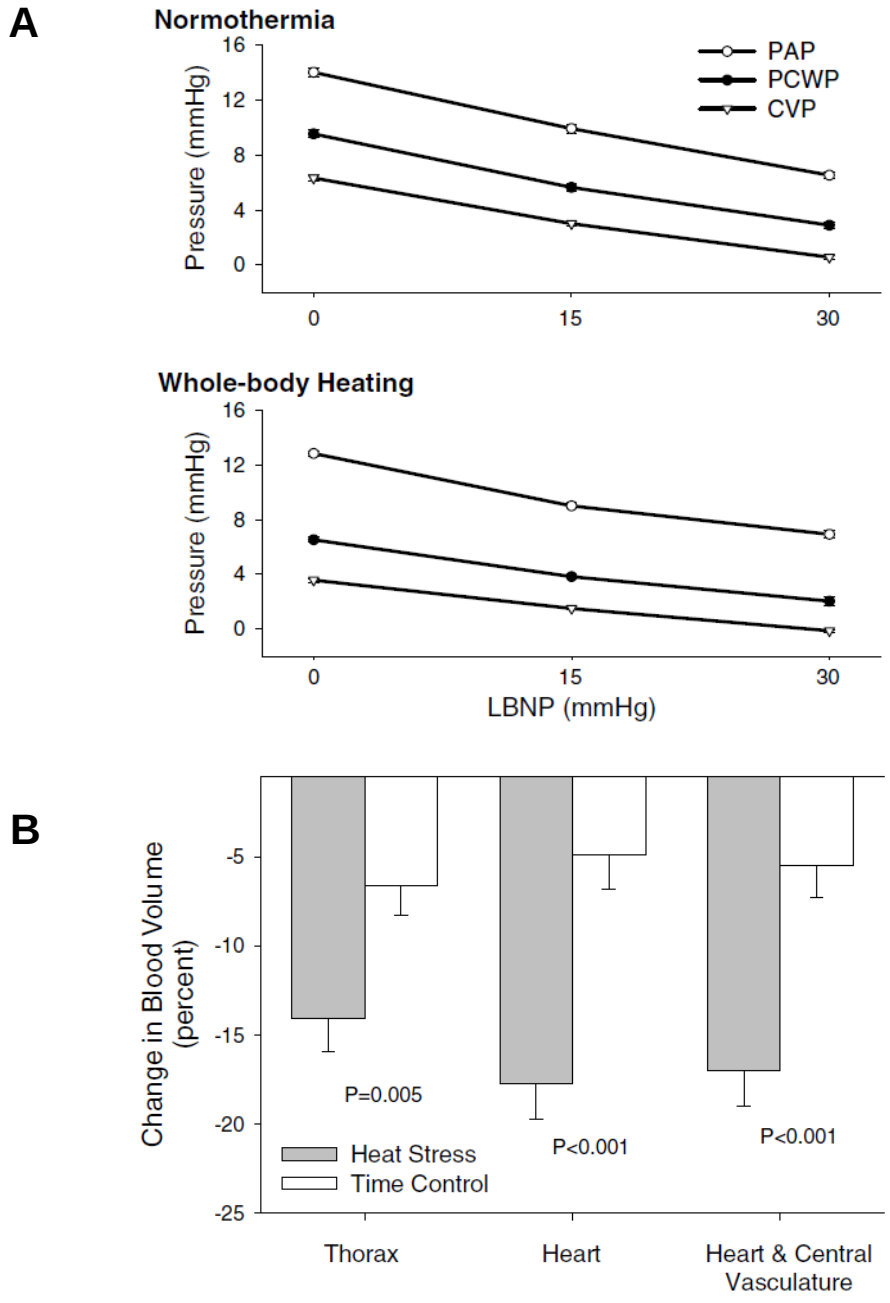

Figure 2-7. Effect of whole-body heat stress on cardiac blood volume. (A) Heat stress reduces central venous pressure (CVP) (modified from Wilson et al., 2007). (B) Heat stress reduces cardiac blood volume (Crandall et al., 2008). LBNP: Lower body negative pressure; PAP: Pulmonary artery pressure; PCWP: Pulmonary capillary wedge pressure.

Whilst the mechanisms for this reduced venous return are still not fully understood, the results indicate that the maintenance of SV during passive heat stress is indicative of an enhanced LV function. In the face of a reduced venous return, maintained SV must be facilitated by enhanced LV systolic/and or diastolic function. In addition to this indirect evidence of an improved LV function, a few studies have reported direct measures of LV function during passive heat stress. Crandall *et al*. (2008) used gamma camera imaging to determine blood volume distribution during heat stress at rest. They showed that EDV is maintained with passive heat stress despite a significant reduction in central blood volume (figure 2-8). Furthermore, the study revealed that ESV decreases significantly with increased body temperatures, resulting in an increased ejection fraction. Although the authors acknowledge that ejection fraction is an imperfect index of LV performance, the data suggest a concomitantly improved LV diastolic and systolic function, which had remained speculative until then. The reduction in ESV is also indicative of an enhanced contractility with heat stress, which has been suggested to be responsible for the maintained SV (Rowell, 1990). However, whilst maintained EDV in the face of a reduced central blood volume is indicative of enhanced LV diastolic function, it does not provide direct evidence for an actual improvement in diastolic function.

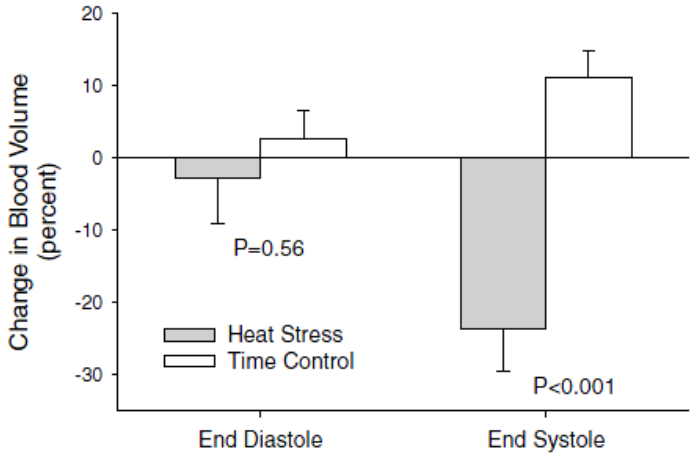

Figure 2-8. End-diastolic volume (EDV) and end-systolic volume (ESV) during passive heat stress. EDV is maintained with passive heat stress whilst ESV is significantly reduced (Crandall et al., 2008).

To further examine the cause of a maintained SV during passive heat stress, Brothers *et al*. (2009) measured LV systolic and diastolic function using echocardiography. They demonstrated that despite a reduction in venous return, early LV diastolic filling velocity and early diastolic myocardial tissue velocity were unchanged with heat stress at rest. The study also showed that LV systolic and late diastolic function was enhanced as indicated by improved systolic tissue velocities and late transmitral inflow velocities, respectively. The authors concluded that the maintenance of SV during heat stress was caused by increased LV systolic and "atrial systolic function" (Brothers et al., 2009). However, maintained early LV filling velocity in the face of a reduced LV filling pressure means that early LV diastolic function must also be enhanced. The mechanisms underpinning enhanced early diastolic function during heat stress at rest were explored by Nelson *et al*. (2010a) who demonstrated that early LV function is indeed enhanced during passive heat stress as reflected by an increase in LV untwisting velocity, irrespective of training status (Nelson et al., 2010b). These data are the first to provide direct evidence of an enhanced LV diastolic function with passive heat stress and underline the importance of assessing LV twist mechanics to detect changes in LV function. Considering that heat stress has been suggested to represent a "hyperadrenergic state" (Rowell, 1990) and that LV twist mechanics have been shown to increase consequent to adrenergic stimulation (Helle-Valle et al., 2005, Opdahl et al., 2008), it is likely that the increase in LV twist mechanics with heat stress at rest is largely mediated by enhanced inotropic state. However, whilst previous studies have provided insight into LV function and LV mechanics with heat stress at rest, the simple pre to post study designs does not reflect the progressive nature of increases in body temperatures experienced by humans in physiologic settings. It remains unknown whether the previously observed improvement in systolic and diastolic LV mechanics is related to the magnitude of heat stress at rest.

Furthermore, LV mechanics have not been explored during the more commonly experienced cardiovascular challenge of exercise in the heat.

2.4.2 Haemodynamics during exercise in the heat

In contrast to the relatively consistent findings from studies examining the cardiovascular response during heat stress at rest, studies assessing the haemodynamic response to exercise in the heat have produced conflicting results. Compared with exercise in normothermic conditions, cardiac output has been shown to be the same (Lafrenz et al., 2008), higher (Rowell et al., 1969b) or lower (Rowell et al., 1966, González-Alonso and Calbet, 2003) during exercise in the heat. Whilst these differences between studies have likely been influenced by different exercise intensities, durations and protocols, all the studies have consistently reported that HR is higher and SV is significantly lower during exercise in the heat. Thus, unlike the evidence that the maintenance of SV during heat stress at rest indicates improved LV function, the lower SV during exercise in the heat compared with exercise in normothermic conditions suggests that LV function may be reduced in these conditions (González-Alonso, 2007, González-Alonso et al., 2008a, Rowell et al., 1966, Lafrenz et al., 2008). With heat stress at rest it was shown that MAP is maintained and, therefore, is unlikely to influence the SV response during a passive thermal challenge. During exercise in the heat, studies have shown that MAP is either maintained or decreased (Lafrenz et al., 2008, Rowell et al., 1969b), suggesting that the lower SV is not caused by an enhanced ventricular afterload.

Similar to heat stress at rest, exercise in the heat results in a reduction in central blood volume compared with exercise in temperate environments (Rowell et al., 1966, Rowell et al., 1969b). Although no measures of LV filling pressures have been reported during exercise in the heat, it can be assumed that venous return is also reduced in comparison with exercise in normothermic environments. It remains unclear, however, why SV is maintained with heat stress at rest but not during exercise in the heat. One possible explanation could be a further reduction in venous return caused by the combined demand for blood flow to the skin and the active skeletal muscles. It has also been suggested that the increased heart rate and consequently reduced filling time may contribute to the reduction in SV in these conditions (Fritzsche et al., 1999). In addition, the changes in haemodynamics and inotropic state during exercise and heat stress likely impact directly on systolic and diastolic LV function, yet at present no data on LV function are available. A reduction in systolic and/or diastolic LV mechanics may explain the lower SV during exercise and heat stress.

2.4.3 Summary

Previous studies have addressed the long-standing question of why SV is maintained during heat stress at rest by showing that LV twist and untwisting are enhanced. It remains unknown, however, whether the improvement in LV twist and untwisting is directly related to the magnitude of heat stress. Furthermore, at present no study has examined LV function during exercise in the heat when SV is significantly lower compared with exercise in normothermic environments. The current data suggest that an impaired systolic and/or diastolic LV function may contribute to the reduction in SV during exercise in the heat. Considering the recently demonstrated importance of improved LV twist and untwisting

during heat stress at rest, impaired LV twist mechanics during heat stress and exercise could potentially explain the reduction in SV previously observed.

2.5 Cardiovascular responses during exercise and dehydration

The above discussed reduction in SV during passive heat stress and the combination of exercise and heat stress is further exaggerated with the additional influence of dehydration (González-Alonso et al., 2008a). During competitive and recreational exercise the limited availability of fluids in hot climatic conditions can result in a severe loss of body fluids, as indicated by reductions in body mass, blood volume or an increase in serum osmolality (Sawka and Noakes, 2007, Nottin et al., 2009, Shave et al., 2009, George et al., 2005, Kozlowski and Saltin, 1964). Previous studies have provided compelling evidence that dehydration during exercise in the heat results in significant perturbations in cardiovascular function in healthy humans compared with euhydrated normothermic and euhydrated heat stressed individuals (for detailed reviews see González-Alonso et al., 2008a, Crandall and González-Alonso, 2010, González-Alonso, 2007). The overall reduction in cardiovascular function with dehydration during exercise in the heat is characterised by an increase in body temperatures and systemic and vascular resistance as well as reductions in muscle blood flow, cardiac output and MAP (González-Alonso, 2007, see figure 2-9). One major factor that contributes to this overall reduction in cardiovascular function is the extensive decline in SV. It has been well-documented that when prolonged exercise in the heat is performed without fluid replacement, SV is reduced significantly more than when the same exercise is performed in the euhydrated state (González-Alonso et al., 1997, Hamilton et al., 1991, Montain and Coyle, 1992, González-Alonso et al., 1995). Despite several studies examining

the potential mechanisms behind this large decline in SV the cause for this phenomenon is still not clear.

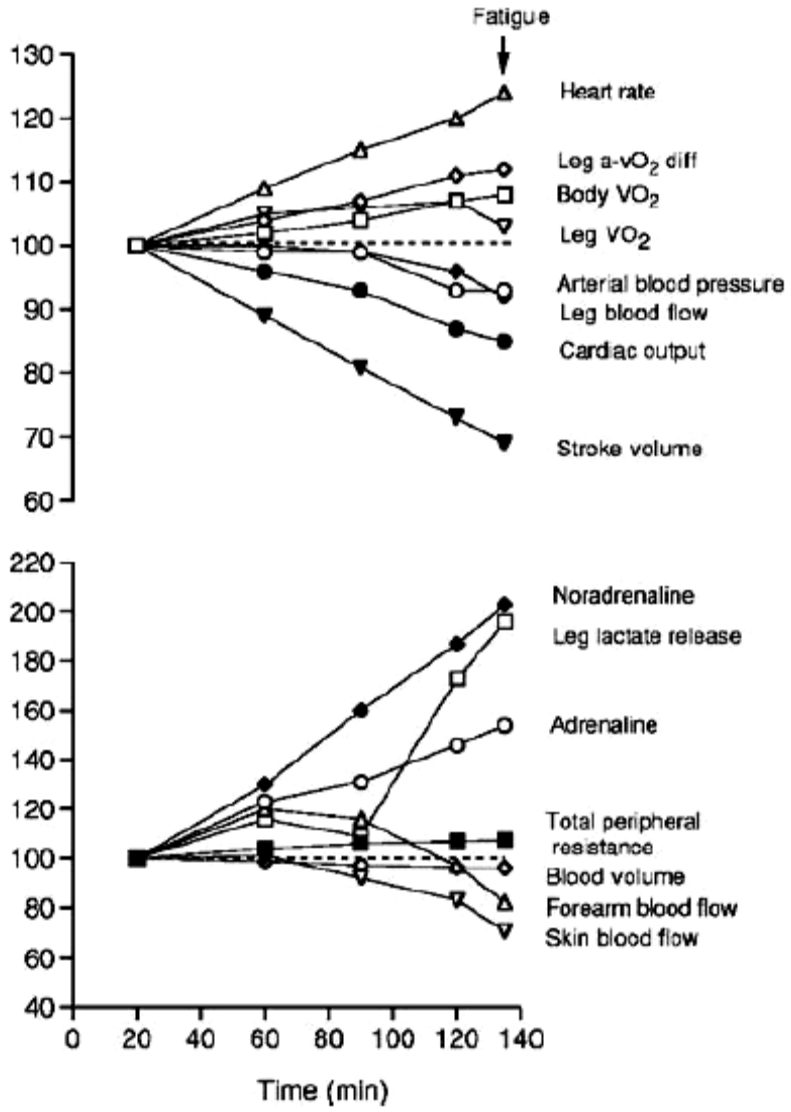

Figure 2-9. Cardiovascular response to exercise in the heat with dehydration. Dehydration causes a significant reduction in cardiovascular function that includes an extensive reduction in stroke volume (top panel bottom line). Data are presented as % change from 20-minute value (from González-Alonso et al., 2008a). a-vO_2 diff: arterio-venous oxygen difference; VO_2: volume of oxygen consumed.

2.5.1 Factors influencing stroke volume during the combined challenge of dehydration and hyperthermia during exercise

In contrast to the large number of studies available on the cardiovascular effects of heat stress at rest, data on the effects of dehydration upon cardiovascular function at rest are scarce. One study has shown that, similar to passive heat stress, dehydration at rest reduces central venous pressure (Kirsch et al., 1986, see figure 2-10). Equally, dehydration during exercise is associated with a reduction in blood volume (Saltin, 1964). Consequently, it is possible that the large reduction in SV with dehydration during exercise in the heat may be related to a reduced venous return. To test this hypothesis, Montain and Coyle (1992) examined the SV response during exercise in the heat when (i) no fluid was replaced, (ii) 80% of sweat loss was replaced by oral ingestion of a carbohydrate-electrolyte solution and (iii) blood volume was restored by venous infusion of a plasma volume expander resulting in a blood volume similar to that when fluid was replaced orally. Despite blood volume expansion the study revealed that SV was only half restored during exercise in the heat with dehydration, indicating that reduced blood volume only accounts for approximately 50% of the reduction in SV. Similarly, increasing central blood volume with supine exercise attenuates the drop in SV and the increase in heart rate despite dehydration and hyperthermia but does not fully offset the decline in SV (González-Alonso et al., 1999a). Further studies have shown that isolating heat stress and dehydration during exercise both result in an ~8% reduction in SV and a maintained cardiac output whereas the combination of heat stress and dehydration during exercise reduces SV and cardiac output by ~20% and 13%, respectively (González-Alonso et al., 1997).

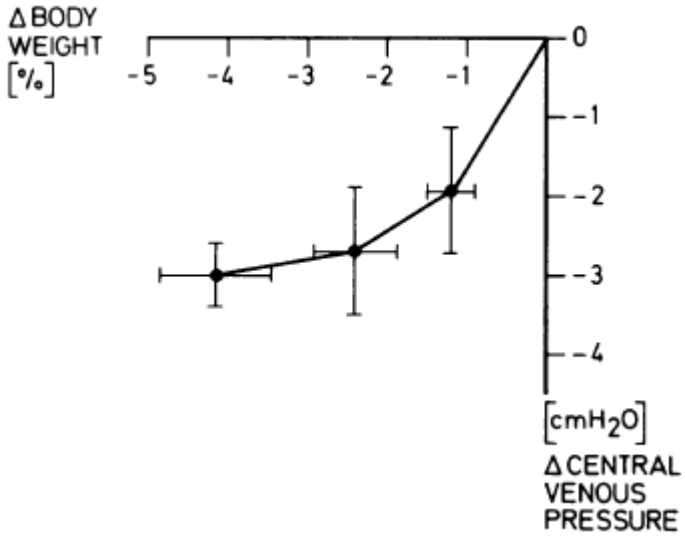

Figure 2-10. Effect of dehydration on central venous pressure at rest. Exercise induced dehydration as reflected by the decline in body mass reduces central venous pressure in resting humans (Kirsch et al., 1986).

The large decline in SV with dehydration during exercise in the heat is also not explained by an enhanced skin blood flow as demonstrated by the reduced SV during exercise in the cold when skin perfusion is minimal (González-Alonso et al., 2000). Rather, it has been suggested that the combined effect of increased heart rate and lowered blood volume may be related to the lower SV caused by dehydration during exercise (González-Alonso et al., 1995, González-Alonso et al., 2000). Whilst these data are in accordance with the previous findings of a higher SV when the rise in heart rate is prevented by β_1–receptor blockade (Fritzsche et al., 1999, Trinity et al., 2010), it remains unknown whether LV diastolic and/or systolic function are actually altered during the combined challenge of exercise, heat stress and dehydration. One study has shown that following prolonged endurance exercise which resulted in ~4.5% dehydration, LV twist, untwisting and strain were significantly reduced

below pre exercise baseline levels (Nottin et al., 2009). Although the authors did not primarily attribute the reduction in LV function to dehydration it is likely that the severe reduction in hydration following exercise has at least in part contributed to the significant decline in LV function. Accordingly, studying the isolated effects of dehydration at rest would further the current understanding of the contribution of hydration status upon reduced LV function during exercise, heat stress and dehydration.

The findings from studies examining LV volumes and the underpinning LV twist mechanics during isolated heat stress at rest clearly demonstrate that maintained SV is facilitated by enhanced systolic and diastolic LV mechanics that compensate for the reduction in venous return (Brothers et al., 2009, Nelson et al., 2010a, Nelson et al., 2010b). The higher heart rate and the lower MAP during dehydration are indicative of an enhanced sympathetic state and reduced afterload, respectively. Thus, enhanced myocardial contractility together with the reduction in afterload should at least in part compensate for the reduced Frank-Starling mechanism caused by decreased venous return. Since SV is, however, extensively reduced, the combination of elevated body temperatures and dehydration may result in an overall impairment of LV mechanics. Similar to the response following prolonged exercise with ensuing dehydration (Nottin et al., 2009), LV mechanics may even be reduced and not be able to compensate for the reduction in preload as seen with heat stress at rest.

2.5.3 Summary

During the combined challenge of exercise, heat stress and dehydration SV is significantly lower than when exercise is performed in the heat with maintained normal hydration. Despite some insight from previous research, the cause for this large decline in SV is still not clear as reductions in blood volume, the redistribution of blood to the skin and reduced cardiac filling times do not fully explain the observed phenomenon. Studies have further shown that systolic and diastolic mechanics are significantly reduced following prolonged exercise that was accompanied by a pronounced dehydration. Thus, the combination of acute dehydration and elevated body temperatures (hyperthermia) during exercise may result in a reduction in LV twist and untwisting. The response of LV mechanics to dehydration and increased body temperatures at rest and in combination with exercise still needs to be studied.

2.6 Left ventricular function during acute dynamic exercise

The review of literature pertaining to heat stress and dehydration has suggested that altered LV mechanics may be involved in the differential SV response previously observed. Little is, however, known about the impact of exercise *per se*. During dynamic exercise the cardiovascular system must meet the increased demand for oxygen in the working musculature. Enhanced oxygen consumption ($\dot{V}O_2$) during exercise is achieved by an improved O_2-extraction from the relatively constant concentration of oxyhaemoglobin in the arterial blood and an increased blood flow to the active muscles (Astrand et al., 1964, Rowell, 1993). The higher blood flow is the result of redistribution of blood by vasoconstriction of vessels in non-active areas, arterial vasodilation in the active musculature and an increase in cardiac output (Rowell, 1993). The increase in cardiac output during the transition from rest

to exercise is achieved by an increase in both heart rate and SV. The rise in heart rate is initiated by a feed forward mechanism termed central command and a feedback mechanism from the mechanoreceptors (Rowell, 1993). Central command increases heart rate within a few beats following the onset of exercise by withdrawal of the parasympathetic vagal inhibition of the pacemaker (Jose, 1966, Nobrega et al., 1995, Lassen et al., 1989). The mechanoreceptors within the active musculature further contribute to the initial increase in heart rate by inhibition of the cardiac vagal tone (Coote and Bothams, 2001). Withdrawal of the parasympathetic influence is thought to occur up to heart rates of approximately 100beats/min (Rowell, 1993). Thereafter, sympathetic stimulation increases via spillage of noradrenaline from vasomotor junction gaps and, to a smaller extent, adrenaline from the adrenal medulla. Adrenaline and noradrenaline bind to the β_1-receptors within the myocardium thereby causing an increase in the rate and force of cardiac contraction and relaxation as described in section 2.2.2.

During incremental exercise, heart rate and catecholamines increase linearly up to the point of volitional fatigue (Galbo et al., 1975, Astrand et al., 1964). The SV response on the other hand is less clear. Some authors have shown that SV first plateaus at approximately 40–50% $\dot{V}O_2$max and remains constant at this level before it actually declines just prior to fatigue, while others believe that SV increases continuously like heart rate, not showing any plateau prior to exhaustion (González-Alonso, 2008, Warburton and Gledhill, 2008).

2.6.1 Stroke volume response during incremental exercise

The initial increase in SV during exercise is mediated by an improved systolic and diastolic function as indicated by an enhanced venous return and a greater myocardial contractility, respectively. LV filling pressures and diastolic LV distension increase at the onset of exercise causing stretch-activation of myofibres according to the Frank-Starling mechanism and subsequently an increase in SV (Poliner et al., 1980, Higginbotham et al., 1986). The magnitude of increase in EDV is dependent on the position and intensity that exercise is performed in. In the supine position at rest SV is higher than in the upright position because of a reduced gravitational influence limiting blood flow back to the right atrium. At peak exercise, however, SV is very similar in the supine and upright position (Poliner et al., 1980, Loeppky et al., 1981, see figure 2-11).

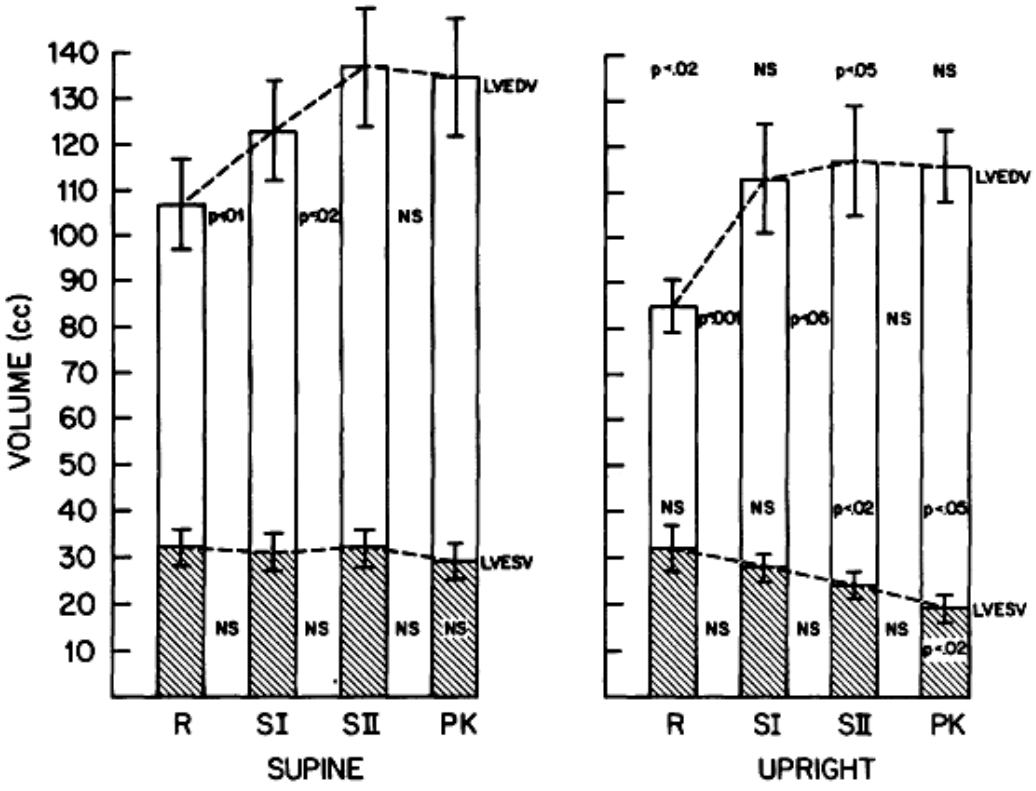

Figure 2-11. Left ventricular volumes at rest (R), during two stages of sub–maximal (SI and SII) and peak exercise (PK). Stroke volume (white bars) increases initially but then plateaus. LVEDV: left ventricular end-diastolic volume; LVESV: left ventricular end-systolic volume (Poliner et al., 1980).

Traditionally, the large increase in venous return during upright exercise is thought to be caused at least in part by the cyclic contraction of the skeletal musculature, thereby promoting emptying of the capacitance vessels and an enhanced blood flow back to the right atrium (Stewart et al., 2004). This long-standing concept of the "skeletal muscle pump" has recently been challenged. González-Alonso *et al*. (2008b) showed that local passive vasodilation in the leg induced by arterial ATP infusion increases cardiac output to a similar extent than that seen during one-legged knee-extensor exercise. Although the study indicates that mechanisms other than the skeletal muscle pump may be able to increase SV during exercise, further evidence is required to determine the exact contribution of the muscle pump to venous return during exercise (Casey and Hart, 2008). Irrespective of the mechanical or biochemical mechanisms of enhanced venous return during exercise, the current data suggest that LV filling pressures increase to the point of volitional fatigue (Higginbotham et al., 1986, González-Alonso et al., 2008b, Mortensen et al., 2005). In addition to this enhanced preload during exercise, improved systolic function also contributes to the initial increase in SV. Stimulation of β-adrenergic receptors results in increased myocardial contractility which in turn enhances the force of contraction and improves LV emptying (Linden, 1968). The same mechanism also improves relaxation and, therefore, LV filling mediated by a more rapid Ca^{2+} re-uptake from the sarcoplasmic reticulum (Opie, 2004).

The presented mechanisms of an enhanced SV with exercise are true for the transition from rest to low intensity exercise. However, during incremental exercise SV typically only increases up to ~40–50% of maximal exercise capacity and then remains at this level until ~90% $\dot{V}O_2$max (Poliner et al., 1980, Higginbotham et al., 1986, González-Alonso et al.,

2008b, Astrand et al., 1964). Thus, the continuously rising cardiac output during incremental exercise above ~50% $\dot{V}O_2$max is solely achieved by a further rise in heart rate.

Although early plateau in SV during incremental exercise and an actual decline in SV prior to fatigue has been contested by authors showing that SV increases continuously up to $\dot{V}O_2$max (Gledhill et al., 1994), the findings from these studies have likely been influenced by the methodology employed, in particular the exercise protocol chosen (Rowland, 2009b, González-Alonso, 2008). The majority of studies agree with the concept of a plateau in SV and studies that have assessed LV volumes during incremental exercise have provided support for this hypothesis. For example, it has been shown that EDV increases initially and then also attains a ceiling, suggesting that preload does not increase further at exercise intensities above 50% $\dot{V}O_2$max (Poliner et al., 1980). The importance of venous return for the SV response during exercise was further demonstrated by the absence of an increase in SV as a result of leg occlusion (Nobrega et al., 1995), supporting the concept of a skeletal muscle pump discussed previously. Accordingly, it is surprising that EDV plateaus during incremental exercise considering that the force of muscle contraction increases progressively up to the point of fatigue (Malek et al., 2009). As a consequence venous return via the muscle pump should increase, even if the relative contribution is smaller than previously estimated (González-Alonso et al., 2008b, Casey and Hart, 2008). Furthermore, the continuously increasing central venous pressure previously shown in healthy individuals does not suggest a reduction in preload, yet EDV does not increase further above exercise intensities higher than ~40–50% $\dot{V}O_2$max (Higginbotham et al., 1986, Mortensen et al., 2005). Thus, at present it appears that the plateau in EDV, and thus SV, may be caused by a reduction in diastolic LV

function. Considering the previously discussed influence of LV untwisting on diastolic filling, it is possible that the plateau in EDV and SV during incremental exercise may be related to an inability of the LV to further increase suction.

The response of systolic LV function as reflected by ESV during incremental exercise is not as clear as that of EDV. Some studies have reported no change in ESV with progressively increasing exercise intensities (Jensen-Urstad et al., 1998, Warburton et al., 2002) whilst others have documented a decrease (Poliner et al., 1980, Doucende et al., 2010). These conflicting findings do not appear to be caused by differences in posture; however, the exact cause for the difference in results is not known. Moreover, it is not possible to determine whether in those studies where ESV decreased, it reached a plateau at sub-maximal exercise intensities. Thus, at present the contribution of changes in ESV to the plateau in SV is not clear. The continuous rise in afterload as indicated by mean arterial pressure may limit the reduction in ESV at higher exercise intensities (Poliner et al., 1980, Higginbotham et al., 1986, Nobrega et al., 1995, Mortensen et al., 2005). If this is indeed the case then this effect should be reflected in an attenuated LV twist as outlined in section 2.3.3.

LV contractility appears to increase progressively during incremental exercise up to the point of fatigue as indicated by enhanced heart rate and circulating catecholamines (Galbo et al., 1975). Thus, a change in myofibre contractility is unlikely the cause for the early SV plateau during incremental exercise. In contrast, reduced LV filling time, consequent to high heart rates, may contribute to the plateau in SV. Indeed, some studies have shown that a

reduction in heart rate via blockade of the β_1-receptors increases SV during exercise (Fritzsche et al., 1999, Trinity et al., 2010). The influence of reduced filling time alone, however, cannot fully explain the early plateau in SV since filling time reduces progressively up to the point of fatigue whereas SV is typically maintained between 50–90% $\dot{V}O_2$max (Higginbotham et al., 1986, Mortensen et al., 2005). Therefore, it is possible that other factors such as a change in LV mechanics may underpin the early plateau in SV during incremental exercise.

2.6.2 Summary

With the onset of exercise, heart rate and SV are increased in healthy individuals. Whilst heart rate continuous to rise up to $\dot{V}O_2$max, SV plateaus at approximately 40–50% maximal exercise capacity. The existing findings suggest that reduced diastolic function may be related to the plateau in SV during exercise intensities exceeding 50% $\dot{V}O_2$max. At present, no study has examined the underpinning response of LV mechanics during moderate to higher exercise intensities. Knowledge of the systolic and diastolic LV mechanics during incremental exercise would greatly improve the existing understanding of cardiac performance during exercise.

2.7 Overall summary

Normal LV function plays a key role in increasing cardiac output during periods of enhanced cardiovascular demand. Recent work has shown the importance of LV twist, untwisting and strain in facilitating filling and ejection during normal LV function at rest and

during altered cardiovascular demand. However, whether changes in LV twist, untwisting and strain are responsible for the differential SV response previously observed during exercise with and without heat stress and dehydration is not known. It is possible that alterations in SV during (i) exercise and heat stress, (ii) exercise with heat stress and dehydration and (iii) incremental exercise may be underpinned by changes in LV twist, untwisting and strain.

2.8 Thesis aims and hypotheses

In view of the presented literature, the overall aim of this thesis was to examine LV twist, untwisting and strain during heat stress, dehydration and incremental exercise in healthy individuals. Three empirical studies were completed based on the following aims and hypotheses.

Study 1

Study aim(s): (1) To examine whether the increase in LV mechanics during heat stress at rest is related to a progressive rise in body temperatures and (2) whether LV mechanics are further altered during the combination of exercise and heat stress.

Research hypothesis 1: LV mechanics will increase progressively with passive heat stress at rest.

Research hypothesis 2: Heat stress during exercise will significantly increase LV mechanics compared with normothermic exercise.

Study 2

Study aim(s): (1) To explore if dehydration at rest causes a reduction in LV volumes and LV mechanics and (2) whether the decline in SV during the combined challenge of dehydration and hyperthermia during exercise is underpinned by a reduction in LV twist, untwisting and strain.

Research hypothesis 1: The combination of dehydration and hyperthermia will significantly reduce LV mechanics at rest.

Research hypothesis 2: Dehydration and hyperthermia during exercise will significantly reduce LV mechanics compared with exercise in a euhydrated and normothermic state.

Study 3

Study aim: To determine whether the previously observed plateau in SV at approximately 40–50% $\dot{V}O_2$max during incremental exercise is underpinned by a concomitant plateau in LV mechanics.

Research hypothesis: During incremental exercise, LV mechanics will be closely related to stroke volume.

CHAPTER 3

General methods

3.1 Introduction

In this chapter, the general methods of data collection and analysis employed in all three experimental studies included in this thesis will be described. Within the general methods the pre-test procedures will be outlined first followed by the test-procedures and statistical analyses. The study design and methods specific to each study will be presented in the respective chapters.

3.2 Pre-test procedures

3.2.1 Ethical approval

Prior to the start of data collection for each study, ethical approval was obtained from the Brunel University School of Sport and Education Ethics Committee or, if required, from the Brunel University Ethics Committee (Appendix I). The procedures employed in this thesis conformed to the code of ethics of the World Medical Association (Declaration of Helsinki).

3.2.2 Participant enrolment

All of the participants volunteered to take part in the studies. Participants were provided with information sheets detailing the exact habituation and experimental procedures. In addition, the procedures were explained verbally and each volunteer was encouraged to ask questions regarding the experiments. Once volunteers had expressed their interest in participating they underwent echocardiographic assessment to examine the quality of images that could be obtained. If images were of a satisfactory quality, that is the entire LV endocardial and epicardial border was clearly visible throughout the entire cardiac cycle,

participants were enrolled in the study. Prior to the start of each experiment participants were asked to fill out a health questionnaire (Appendix II); anyone with a history of cardiovascular disease was excluded from the study. As requested by the Brunel University Ethics Committee a pre-participation 12-lead electrocardiogram (ECG) and manual assessment of resting blood pressure were obtained from all participants of study two (n=8). Based on the ECG and blood pressure results participation was approved by a qualified physician from Ealing Hospital NHS Trust, Southall, UK. All participants (n=27) provided written and verbal consent before the experiment (Appendix IV).

3.2.3 Anthropometry

Participants' free standing stature was determined using a stadiometer and recorded to the nearest 0.1 cm. Body mass was assessed using calibrated electronic scales (SECA model 78, Germany) and recorded to the nearest 0.1 kg, with the participants only wearing their underwear.

3.3 Test procedures

In all three experimental studies of this thesis healthy individuals underwent a series of progressively increasing haemodynamic challenges. Echocardiography was used to assess LV systolic and diastolic function at rest and during exercise with and without heat stress and dehydration.

3.3.1 Echocardiography

Because of its easy and safe application as well as its relatively low cost echocardiography is the most widely used tool to assess cardiac function in the clinical setting and in research (McGowan and Cleland, 2003, Lang et al., 2006). In this thesis, echocardiography was employed to determine changes in LV volumes, timings of cardiac events, twist and strain. The paragraph below briefly introduces some of the physical principles of echocardiography followed by a description of the specific methodology related to image acquisition and image analysis performed to determine LV functional parameters in all three experimental studies.

Principles of echocardiography

The mechanical vibration of objects creates waves that, when travelling through a medium, become audible as sound (Feigenbaum et al., 2005). Ultrasound follows the same principles as audible sound but it occurs at much higher wave frequencies that exceed 20kHz and is, therefore, inaudible to the human ear (Feigenbaum et al., 2005). In some animals such as bats and dolphins the natural use of ultrasound allows for the location of objects without optical guidance (Szabo, 2004). Similarly, echocardiography uses ultrasound to determine the structure and function of the heart. The possibility of creating artificial ultrasound waves for imaging purposes dates back to the discovery of piezoelectricity by the Curie brothers, who demonstrated that piezoelectric crystals vibrate in response to an electrical stimulus, thus creating ultrasound waves (Mould, 2007, Szabo, 2004). When these ultrasound waves are emitted by a transducer and propagate through bodily tissues, the particles in the tissues oscillate in parallel to the line of propagation, creating longitudinal waves (Feigenbaum et al., 2005). During the "listening time" of the transducer returning ultrasound waves from the

tissues interact with the piezoelectric crystals in the transducer and produce an electric signal that is converted into a digital grey-scale image usually displayed in a 90-degree sector (Feigenbaum et al., 2005).

The purpose of cardiac imaging is to obtain both good spatial and temporal resolution. The resolution of the ultrasound image that is created is the result of interaction between the transmitted waves and the tissue properties. Increasing the frequency of the emitted ultrasound waves enhances spatial resolution thus enabling the assessment of smaller structures. However, these higher sound frequencies are more easily attenuated, limiting the depth of tissue that can be accurately represented (Szabo, 2004). Similarly, adjusting the width of the scan sector and the imaging depth will affect the number of frames that can be produced per time. High frame rates are desirable in particular if the aim of the exam is to quantify movement of the valves or tissue (Feigenbaum et al., 2005, Helle-Valle et al., 2005). Consequently, image acquisition must be carried out by adjusting the emitted frequency, sector width, imaging depth and frame rates so that an optimal balance between spatial and temporal resolution is achieved.

Generic procedures for image acquisition

In this thesis, echocardiography was used to assess systolic and diastolic LV function during three experimental studies examining LV function during heat stress, dehydration with hyperthermia and during incremental exercise. Echocardiographic image acquisition and analysis were performed by a single sonographer according to current guidelines for the

assessment of global LV function (Lang et al., 2006). All images were recorded on a commercially available ultrasound machine using an M4S 2–5 MHz probe with the frequency set at 1.7 MHz on transmit and 3.6 MHz on receive (Vivid 7, GE Medical, Horton, Norway). Five consecutive cardiac cycles were saved at end-expiration to minimise lateral displacement of myocardial tissue and to ensure that lung tissue would not obliterate the acoustic window. Off-line analysis of LV function was performed using manufacturer specific software (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0) and all data were averaged over 2-3 consecutive cardiac cycles. Heart rate (HR) was recorded with each image via 3-lead electrocardiogram (ECG) inherent to the ultrasound machine. Two-dimensional echocardiographic images were acquired for the calculation of LV systolic and diastolic dimensions, volumes and ejection fraction. Tissue Doppler images were obtained to determine iso-volumic relaxation time (IVRT). LV systolic and diastolic twist and strain indices were assessed using 2-D speckle tracking echocardiography.

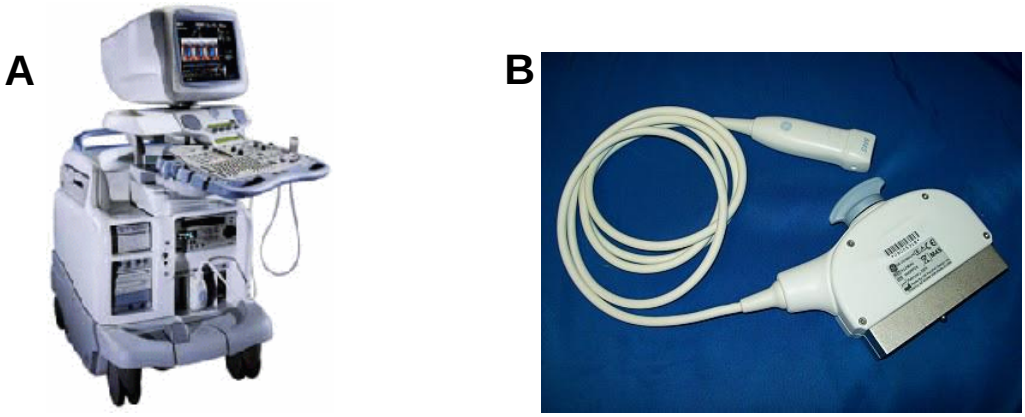

Figure 3-1. Ultrasound system and probe. Images show (A) Vivid 7 ultrasound and (B) 2-D phased-array M4S transducer (both GE Medical, Horton, Norway) used in all three experimental studies.

Left ventricular volumes and iso-volumic relaxation time ("Global LV function")

LV volumes were calculated from one dimensional motion-mode images guided by 2-D parasternal long-axis views. Two-dimensional images were recorded ensuring that both septum and posterior wall were as perpendicular to the ultrasound beam as possible and that the mitral valve was in the centre of the image. From this view, M-mode images were obtained by guiding a single ultrasound beam through the centre of the *chordae tendinae* with the direction of the beam adjusted perpendicularly to the septum and posterior LV wall (Feigenbaum et al., 2005). Internal LV dimensions were measured between the endocardial border of the septum and the endocardial border of the posterior wall in systole and diastole, respectively (figure 3-2). Manufacturer specific software (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0) then calculated end-diastolic volumes (EDV), end-systolic volumes (ESV), stroke volume (SV) and ejection fraction (EF) according to the method by Teichholz et al. (1976). Although the American Society of Echocardiography recommends the Simpson's biplane method as the preferable method to assess LV volumes (Schiller, 1991), the Teichholz method has been validated previously and its known limitations appear to be of significance only in asymmetrically contracting ventricles (Kronik et al., 1979). In this thesis, using the Simpson's biplane method was not feasible due to the limited amount of time available to record echocardiographic images at steady state conditions. Since this method requires acquisition of two separate echocardiographic images, using the Simpson's method prolongs data collection and, therefore, increases the chance of a change in the participants' physiological state from the beginning to the end of the examination. With this in mind and the fact that only healthy individuals with symmetrically contracting left ventricles were studied, the Teichholz method was chosen to determine LV volumes in this thesis.

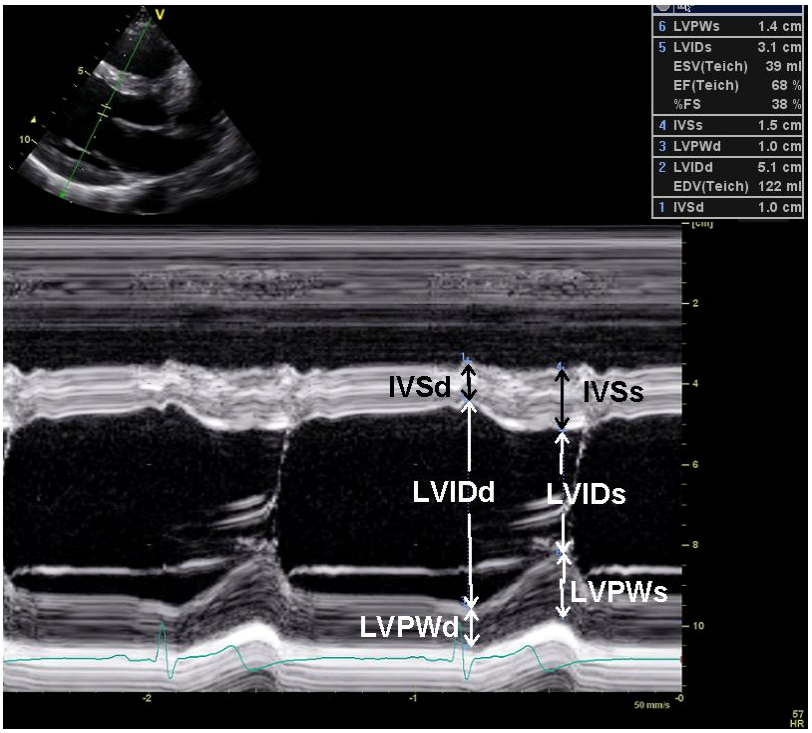

Figure 3-2. Example of an M-mode image and derived measures at rest. LV wall and cavity are displayed along one single scan line (green line) from a parasternal long-axis view. Measurements of inter-ventricular septum (IVS), LV internal diameter (LVID) and LV posterior wall (LVPW) in diastole (d) and systole (s), respectively, are displayed in the upper right panel. From the measured dimensions the software calculated end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF) and fractional shortening (FS).

In addition to the measurement of LV dimensions and volumes for global LV function, cardiac timings were also assessed. Each cardiac cycle can be classified into four time periods; iso-volumic contraction time, systolic ejection time, iso-volumic relaxation time (IVRT) and diastolic filling time. In the three experimental studies for this thesis IVRT was determined from an apical four-chamber view using pulsed wave Doppler imaging of the septal mitral annulus (figure 3-3). Doppler imaging is based on the principle that if the source of sound, in this case myocardial tissue, moves towards the transducer the frequency of sound increases whereas the opposite occurs when the source of sound is moving away from the

transducer (Feigenbaum et al., 2005). Thus, the known myocardial movement during contraction (towards transducer) and relaxation (away from transducer) enables calculation of the velocity of myocardial movement. Apical four-chamber views were recorded with the inter-ventricular and inter-atrial portion of the septum as vertical as possible to ensure alignment with the ultrasound beam. The width of the sector scan was then reduced to only include septal tissue and a pulsed wave Doppler sample volume was placed in the mitral annulus as previously described (Alam et al., 1999). The minimum frame rate was 200 frames per second and care was taken to maintain frame rates within participants and between conditions. From the one-dimensional display of the pulsed Doppler signal peak systolic tissue velocity (S'), early diastolic (E') and late diastolic (A') tissue velocities were identified. IVRT was measured as the time period between the moment when the declining S' initially crossed the baseline until the onset of deceleration of the E' wave (figure 3-3).

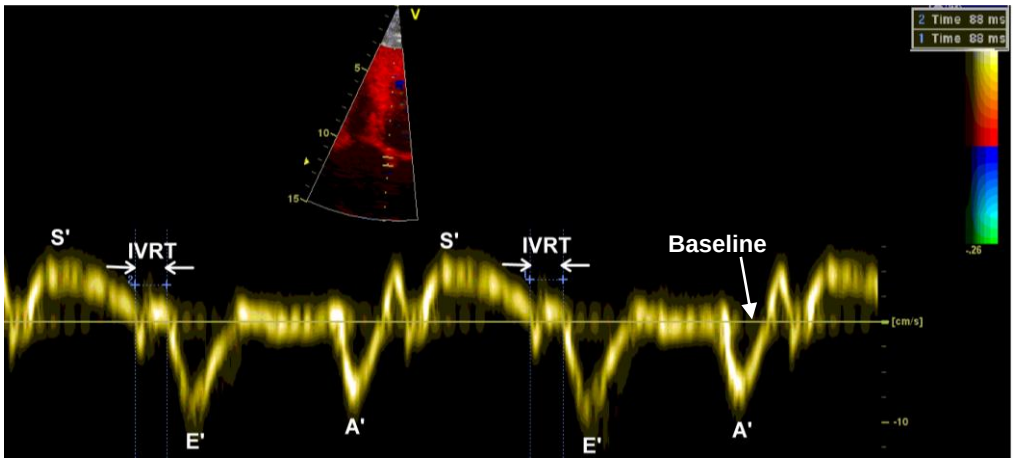

Figure 3-3. Example of the measurement of iso-volumic relaxation time (IVRT). IVRT is measured as the time interval between the end of the contraction phase represented above the baseline and the onset of early tissue relaxation (E'). S': Peak systolic tissue velocity; A': Late diastolic tissue velocity.

Speckle tracking derived left ventricular twist and strain

As outlined in the previous chapter, net LV twist is defined as the difference in counter-directional rotation between the LV base and apex whereas LV strain represents myocardial wall thickening and thinning (Helle-Valle et al., 2005, Notomi et al., 2005a, D'Hooge et al., 2000). Thus, LV twist can be calculated with two-dimensional echocardiography by measuring the short-axis rotation at the LV base and apex separately and subtracting the two from each other (Helle-Valle et al., 2005, Notomi et al., 2005a). LV radial and circumferential strain can also be determined from the same short-axis images as LV rotation and longitudinal strain is assessed from an apical four chamber view (D'Hooge et al., 2000). Several imaging modalities have been shown to reliably measure LV twist and strain including MRI and tissue Doppler imaging (Notomi et al., 2005b, Teske et al., 2007). In addition to these techniques 2-D speckle tracking ultrasound has emerged as a promising tool to quantify LV twist mechanics and strain due to its relatively high frame rates and angle independence. The technical principles of speckle tracking ultrasound are based on the existing properties that make up the normal 2-D grey scale image. Within the human body different tissues have different acoustic impedance thereby affecting the velocity and direction of the ultrasound beam that is passing through the respective tissue. When the ultrasound beam arrives at the junction of two different tissues, some of the ultrasound energy is reflected, some is refracted and a portion continues in a straight line (Feigenbaum et al., 2005). In contrast to targets that are large in relation to the transmitted ultrasound wavelength resulting in specular reflection, small targets produce scattered echoes (fig. 3-4), which provide the ‘texture’ of the 2-D ultrasound images. The term “speckle” is used to describe the unique composition of a large number of such small reflectors within one pixel of the grey-scale ultrasound image (Feigenbaum et al., 2005).

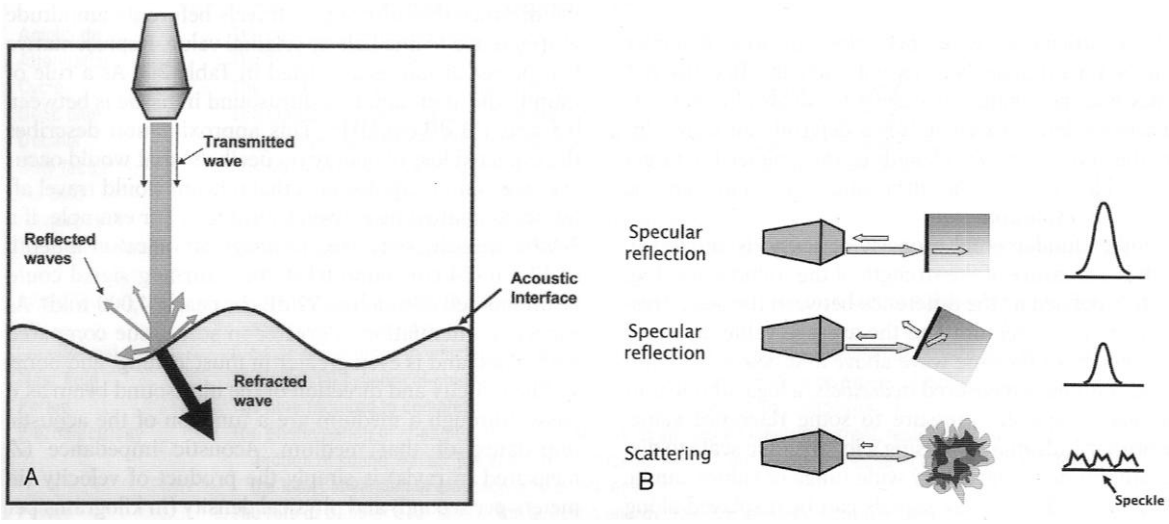

Figure 3-4. Generation of "speckles" within 2-D ultrasound images. When an ultrasound beam propagates onto a small and uneven target the reflected wave produces "speckles" (Feigenbaum et al., 2005).

Specialised software is able to recognise the speckles inherent to every 2-D ultrasound image and track their movement over the course of a cardiac cycle by block-matching and autocorrelation algorithms (Leitman et al., 2004, Wagner et al., 1983). As a result, tracking of the displacement of speckles and their position in relation to other speckles within a region of interest allows for the non-invasive assessment of myocardial rotation, rotation velocity, strain and its time derivative strain rate (Notomi et al., 2005a, Helle-Valle et al., 2005, Teske et al., 2007). Compared with tissue Doppler derived strain this method has the advantage that it is angle independent and that it is not affected by tethering of neighbouring tissues (Edvardsen et al., 2002, Sivesgaard et al., 2009, Ng et al., 2008). Thus, speckle tracking ultrasound enables the assessment of short-axis rotation over the entire myocardium and strain can be determined not only along the longitudinal axis of the LV but also in the radial and circumferential planes (D'Hooge et al., 2000).

Currently, the 2-D speckle tracking software requires that myocardial images are recorded with a frame rate of 40–90 frames per second (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0). Since higher frame rates are beneficial for temporal resolution and essential for accurate tracking of speckles (Helle-Valle et al., 2005), images for analysis of 2-D speckle tracking rotation and strain indices in this thesis were acquired at 80 – 100 frames per second. Further standardisation of image acquisition was assured by keeping frame rates and the imaging depth as constant as possible within individuals and within conditions (Sivesgaard et al., 2009). 2-D parasternal short-axis views for the assessment of basal rotation and strain were recorded at the level of the mitral valve ensuring that there was a clear gap between the posterior mitral leaflet and the inferior segment of the myocardium throughout the entire cardiac cycle. This is important to avoid any overlap of myocardial tissue with valve tissue as this may lead to the software tracking valve tissue instead of the targeted myocardium. With regard to the assessment of apical rotation it has been shown that apical rotation increases the more caudal the 2-D ultrasound image is acquired (van Dalen et al., 2008), thus increasing net LV twist. Accordingly, to calculate the "true" net LV twist from base to apex the apical image must be obtained as close to the apex as possible. This was achieved by adjusting the position of the ultrasound probe 1–2 inter-costal spaces more caudal than the LV basal short-axis view until the image was circular and the LV cavity was displayed just "proximal to the level with end-systolic LV luminal obliteration" (van Dalen et al., 2008). From the stored basal and apical short-axis images LV rotation and strain data were obtained off-line with commercially available software (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0). Within the software application, the operator manually traced the LV endocardial border over the entire myocardium. A region of interest was then

created by the software and its width was adjusted by the user so that the entire LV myocardium was included without exceeding the epicardial border (figure 3-5).

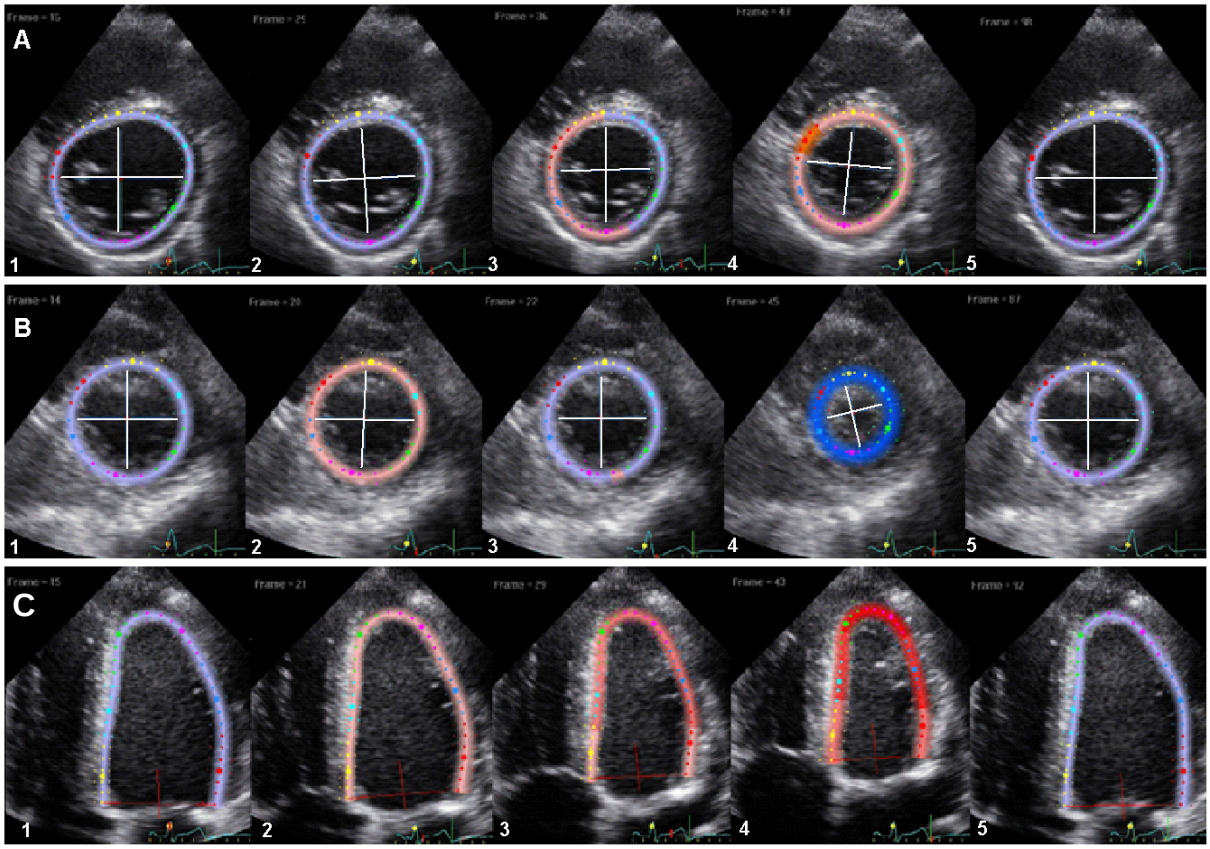

Figure 3-5. Example of two-dimensional left ventricular speckle tracking analysis. A coloured region of interest is superimposed on grey-scale video loops of LV parasternal short-axis images **(A)** at the basal level and **(B)** at the apical level. Examples show still images at (1): end-diastole, (2): iso-volumic contraction, (3): mid-systole, (4): end-systole and (5): end-diastole. Blue region of interest indicates counter-clockwise rotation, red colours indicate clockwise rotation. White crosses demonstrate magnitude of rotation. Note the brief counter-directional rotation during iso-volumic contraction. The net difference in maximal counter-directional rotation of the LV base and apex at the end of systole (4) results in peak LV twist. **(C)** A region of interest is superimposed on the LV myocardium obtained from an apical four-chamber view to assess longitudinal strain. Blue colours indicate relaxed state at the end of diastole (1 and 5). Red colours represent increasing strain over the LV myocardium.

Once the region of interest was set the software divided this area into six equidistant segments according to the guidelines of the American Society of Echocardiography (Lang et

al., 2005, see fig 3-6). The software provided a tracking score of 1–3 for each of the segments with a score of 3 indicating that the software was unable to reliably track the speckles within a segment and a score of 1 indicating that the tracking was successful. Tracking quality was then further checked by visual inspection by the sonographer and, if necessary, corrected for by re-adjusting the region of interest. If myocardial tracking was still not satisfactory in one or more of the six segments, the respective segments were excluded from the final data analysis. In no case were more than three segments excluded from any one image.

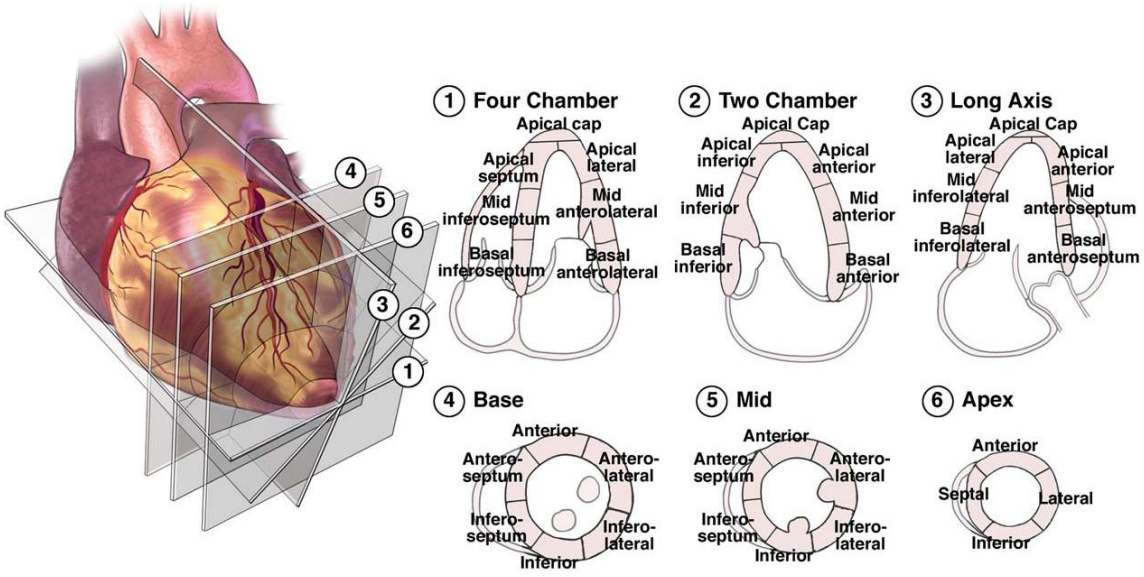

Figure 3-6. Segmentation of the left ventricle. The graphic shows the 17 segment model according to the guidelines of the American Society of Echocardiography (Lang et al., 2005).

Following approval of the tracking by the operator, the software calculated the frame-by-frame rotation and strain results and raw data were exported to a spreadsheet to calculate the mean strain and rotation across all approved segments (Excel, Microsoft Corporation, Seattle, Washington). To account for inter- and intra-individual differences in HR, data were then

normalised to the percentage of systolic and diastolic duration, respectively, also enabling graphical representation of group average data (Burns et al., 2009b). Some investigators have normalised both systolic and diastolic events to the percentage of systolic duration (Nottin et al., 2009, Nottin et al., 2008, Takeuchi and Lang, 2008). This process is probably accurate at rest or when heart rates are only marginally increased. However, at higher heart rates when both systolic and diastolic duration shorten to a different extent it appears appropriate to favour the method suggested by Burns *et al*. (2008b). Normalisation was performed using cubic spline interpolation (GraphPad Prism 5.00 for Windows, San Diego, California, USA). Raw systolic and diastolic frame-by-frame data were interpolated to 300 data points, respectively, resulting in a total number of 600 data points per cardiac cycle. The end of systole was defined as aortic valve closure (AVC), which was automatically determined by the analysis software based on the onset of the QRS complex in the ECG (EchoPAC, GE Medical, Horton, Norway, Version 7.0.0). Changes in the occurrence of AVC were confirmed by tissue Doppler assessment of the mitral annular velocity as described in the previous section.

In order to obtain frame-by-frame twist and twisting velocity values at all systolic and diastolic data points, basal rotation data were subtracted from apical rotation data (Notomi et al., 2005a). Peak untwisting velocity was defined as the first negative deflection following peak LV twisting velocity (Perry et al., 2008). Untwisting rate was defined as the mean untwisting velocity from peak twist to the end of IVRT and calculated as: [peak twist *(deg.)* – twist *(deg.) at* $IVRT_{end}$ *(ms)*] / time from peak twist to $IVRT_{end}$ *(ms)* (van Dalen et al., 2009). From the 600 data points graphical representation of the group average responses in LV twist

and strain indices were created and peak systolic and diastolic values were extracted for statistical analysis (figure 2-5). Time to peak for diastolic twist indices was determined using the frame by frame data obtained from the speckle tracking analysis and expressed as absolute time in milliseconds (ms).

Reliability of echocardiographic measurements

Despite its clear benefits and advantages over other imaging modalities echocardiography has been suggested to be more susceptible to measurement error than alternative imaging tools (McGowan and Cleland, 2003). The main source for measurement error is due to poor image quality caused by inter-individual differences in anatomy and, to a greater extent, sonographer skill (Posma et al., 1996). Thus, much of the measurement error can be reduced by appropriate training of the sonographer and a standardised approach in image acquisition and analysis (Oxborough, 2008). The sonographer for this thesis was trained in image acquisition and image analysis by a qualified cardiac sonographer and followed a systematic procedure according to the current guidelines for echocardiographic image acquisition and analysis (Lang et al., 2006, Oxborough, 2008). To further optimise the quality of images obtained during the three experimental studies, as part of the recruitment process participants were selected following a brief echocardiographic examination. If image quality was considered inferior to the standard required for speckle tracking analysis (i.e. not a clear definition of myocardial borders throughout the cardiac cycle) participants were not enrolled in the studies. To determine the measurement variability of the sonographer for this thesis, within-participant within-day reliability was assessed in nine healthy males. Individuals rested for five minutes in the left lateral decubitus position before the first set of

echocardiographic images was obtained. All images were acquired at end-expiration. Following the complete acquisition of the first set of data (trial 1) and a five minute break, the procedure was repeated (trial 2). Total image acquisition lasted approximately 20 minutes. Data were analysed off-line for LV volumes, IVRT, LV twist and strain indices. Due to the large amount of data generated by speckle tracking analysis (up to 24 different rotation and strain parameters per individual per condition) the results of the reliability study are presented selectively only for the main LV twist and strain parameters relevant to this thesis.

The group mean data for selected 2-D twist and strain results over one entire cardiac cycle are shown in figure 3-7. Although this representation does not allow for the assessment of individual variability in the measurement, the graphs clearly show that the group mean response of nine individuals over the course of an entire cardiac cycle between trial 1 and 2 was almost identical. In fact, for most of the parameters shown, the relevant peaks and the timing of events were the same between the trials, with exception of peak basal rotation which was very mildly reduced during the second trial.

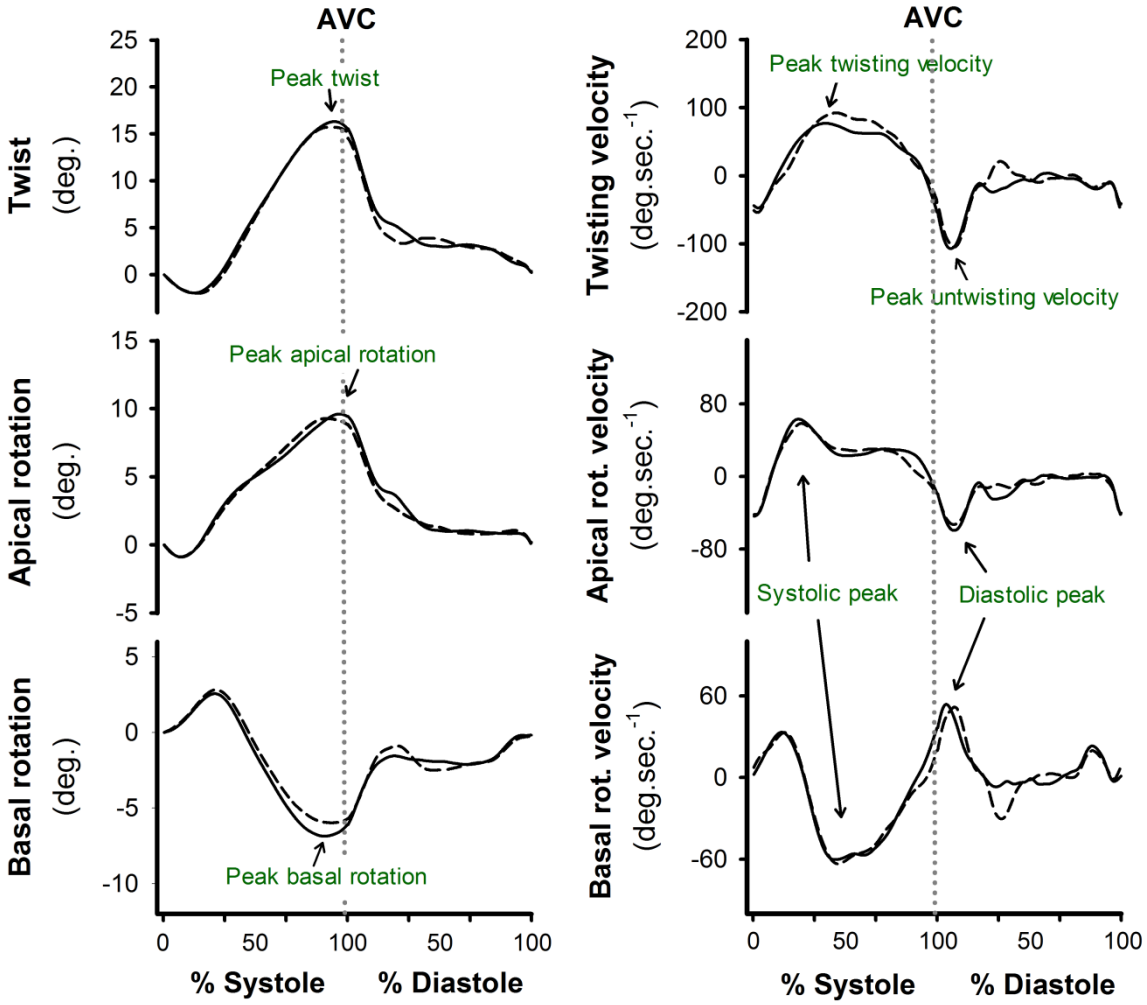

Figure 3-7. Mean group responses for left ventricular (LV) twist indices over the course of a whole cardiac cycle, assessed in two within-day trials (n=9). AVC: aortic valve closure; deg: degrees; rot: rotational; Continuous lines represent trial 1 and dashed lines represent trial 2.

In addition to the representation of group mean data coefficient of variation was calculated to determine the actual reliability of the measurements (Hopkins, 2000). The coefficient of variation for the sonographer of this thesis for LV volumes, IVRT and twist and strain indices is summarised in table 3-1. The results revealed that the variability in assessing LV volumes and IVRT for this sonographer ranged from ~3–13%. These data are in accordance with previously reported intra-observer reliability for ESV and EDV of 3–9% (George et al., 2004,

Otterstad et al., 1997). Moreover, considering the absolute values for each LV index and their respective coefficients of variation, the required absolute change to detect meaningful differences is small, indicating that any change greater than ~5ml or ~6ms can be detected for LV volumes and IVRT, respectively.

Table 3-1. Coefficient of variation (CV) for echocardiographic variables.

LV index	Mean of trial 1 & 2	SD of trial 1 & 2	CV	Absolute change required
End-diastolic volume (ml)	133	±13	3.1%	±4
End-systolic volume (ml)	39	±7	12.6%	±5
Stroke volume (ml)	94	±11	4.4%	±4
Iso-volumic relaxation time (ms)	70	±13	7.9%	±6
Basal rotation (deg.)	-6.9	±2.0	8.6%	±0.6
Apical rotation (deg.)	9.7	±3.8	13.3%	±1.3
Twist (deg.)	16.3	±4.0	13.9%	±2.3
Twist velocity ($deg \cdot sec^{-1}$)	95	±24	25.4%	±24
Untwisting velocity ($deg \cdot sec^{-1}$)	-123	±35	20.1%	±25
Longitudinal strain (%)	-21	±2	5.7%	±1
Basal radial strain (%)	55	±19	25.2%	±14
Apical radial strain (%)	28	±14	36.5%	±10
Basal Circumferential strain (%)	-18	±4	18.7%	±4
Apical Circumferential strain (%)	-24	±5	8.3%	±2

Mean and SD represent group averages and standard deviations from the two within-day trials, respectively (see text for detail). Coefficient of variation (CV) represents variability of measurements between the two trials. Based on the mean and the CV, the respective absolute change that is required to detect meaningful differences between assessments is also shown.

With regard to the measurement variability of LV twist indices, coefficients of variation for LV twist have been reported to range from 8 – 20% (Notomi et al., 2005a, Burns et al., 2010). The present coefficient of variation of ~14% is, therefore, considered acceptable.

Similarly, the coefficient of variation for LV twisting and untwisting velocities of 20–25% was comparable to that previously determined (Burns et al., 2010), meaning that a change in LV twisting and untwisting velocity of approximately 25deg·sec^{-1} or more is required to be considered meaningful. The higher coefficient of variation for LV twisting and untwisting velocity compared with LV twist is a little surprising as all the parameters are derived from the same images and are obtained following an identical procedure by the operator. It is possible that the slightly larger coefficient of variation for twist and untwisting velocities may be related to the algorithm inherent to the processing software as shown previously (Gustafsson et al., 2009). Overall, however, the present data show a good reliability of LV twist indices.

The coefficient of variation for the same sonographer for selected LV strain indices ranged from 6–37%. Previous investigators have reported coefficients of variation for radial and circumferential strain of 5–18% (Oxborough et al., 2009, Cho et al., 2006). In comparison with these previous reliability reports, the variability for some of the present strain indices appears high. However, the absolute change required to see meaningful differences in LV strain was acceptable ranging from 1–14%.

3.3.2 Arterial blood pressure

Mean arterial blood pressure (MAP) pressure was assessed invasively in six participants of studies one and two, respectively. Arterial blood pressure from the remaining participants was obtained non-invasively (study1: n=4; study2: n=2; study3: n=9). Invasive measures of

MAP were obtained from arterial catheterisation. First, a local anaesthetic (Lidocaine Hydrochlorine 2%, Hameln Pharmaceuticals, Gloucester, UK) was administered subcutaneously in the radial aspect of the right wrist. Then, with the aid of a needle and guide wire an arterial line (Leader Cath, Vygon, Ecouen, France) of 1.1 mm inner diameter was advanced ~5 cm into the right radial artery. Following removal of the guide wire the protruding end of the catheter was sutured to the skin and further secured with an adhesive plaster. For the measurement of MAP the catheter was connected to a pressure transducer (Pressure Monitoring Kit, Baxter) that served as the reference for atmospheric pressure and was zeroed 5 cm below the sternal angle. The line was regularly flushed with saline to prevent coagulation. Beat-by-beat MAP was recorded continuously (PowerLab, ADInstruments, Chalgrove, UK) and data were stored on a personal computer for off-line analysis.

Non-invasive blood pressure was measured either by automated sphygmomanometry (study 1) or continuously (study 2) using a beat-by-beat arterial blood pressure monitoring system (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands). For continuous assessment a finger cuff was placed around the middle phalanx of the middle finger of the right hand. In order to estimate aortic pressure similar to that assessed by the invasive method, MAP was corrected for the difference in atmospheric pressure between the location of the finger and the aorta. The beat-by-beat arterial pressure waveform was recorded continuously (PowerLab, ADInstruments, Chalgrove, UK) and data were stored on a personal computer for off-line analysis.

Analysis of both, invasively and non-invasively obtained blood pressure data was performed using specialised data analysis software (Chart Version 5.5.6, ADInstruments, Chalgrove, UK). Data were synchronised with echocardiographic image acquisition. All data points recorded during echocardiographic image acquisition (~10 minutes for studies one and two, respectively, and 2 minutes for study three) were selected and the software calculated the average MAP for the given time period based on the pressure waveform.

3.4 Statistical analysis

Statistical analyses were performed using commercially available software (STATISTICA, StatSoft, Inc., 2002, Version 6). For the detection of differences, alpha was set *a priori* to 0.05. Effects of heat stress, dehydration, rehydration or metabolic demand were assessed using repeated measures analysis of variance (ANOVA). Post hoc comparisons of group differences were performed using paired samples t-tests with Bonferroni correction applied for multiple comparisons. Relationships of mean group responses for each condition within each study were determined using Pearson's product moment correlation. All data in tables and text represent mean ± standard deviation.

CHAPTER 4

Study 1

Effect of progressive heat stress on global left ventricular function and mechanics at rest and during small muscle mass exercise

4.1 Introduction

In heat stressed humans, reductions in central venous pressure (Rowell, 1974, Wilson et al., 2007), central blood volume (Crandall et al., 2008) and right ventricular volume (Nelson et al., 2010a) indicate a lowered venous return to the heart. However, this reduction in venous return does not appear to compromise stroke volume (SV) as SV is largely maintained (Rowell et al., 1969a, Crandall et al., 2008). Accordingly, during passive heat stress systolic and/or diastolic left ventricular (LV) function must be enhanced to compensate for the reduction in venous return. Indeed, systolic and late diastolic tissue Doppler and transmitral inflow velocities have been shown to be increased during passive heat stress (Brothers et al., 2009). Recently Nelson et al. (2010a) further showed that early diastolic function is also enhanced as reflected by an increase in peak LV untwisting velocity. As discussed in detail in chapter 2 of this thesis, LV twist is the result of counter-directional rotation of the LV base and LV apex during ventricular contraction and has been shown to facilitate improved LV filling and ejection during exercise (Notomi et al., 2006, Doucende et al., 2010, Burns et al., 2008a). With the onset of myocardial relaxation twist is reversed resulting in LV untwisting or recoil (Notomi et al., 2006, Burns et al., 2010) and the velocity of LV untwisting contributes to the generation of intra-ventricular "suction" required for ventricular filling (Notomi et al., 2008, Dong et al., 2001). Furthermore, increased LV twist and untwisting during passive heat stress likely improves LV ejection and filling and, thus, contributes to the maintenance of stroke volume previously described (Nelson et al., 2010a). Although these findings highlight the importance of assessing LV twist and untwisting during passive heat stress, previous studies have only examined one level of hyperthermia. Elevations in body temperature are, however, progressive in nature and enhanced core temperatures are preceded by increases in skin temperatures. Presently it is not known whether raised skin temperatures

alone result in increased LV twist and untwisting or whether changes in LV twist and untwisting are related to the magnitude of heat stress. Furthermore, when exercise is performed whilst heat stressed the competing demands for blood flow to the working musculature and skin perfusion mean that the combined stress of heat and exercise represents an even larger challenge for the cardiovascular system than passive heat stress (González-Alonso et al., 2008a). Similar to heat stress at rest, it has been shown that the combination of small muscle mass exercise and heat stress also results in a maintained SV (Savard et al., 1988) despite the higher cardiovascular demand. It is possible that increases in systolic and diastolic LV twist mechanics beyond those shown at rest may facilitate the maintenance of SV during the combined challenge of exercise and heat stress.

To further explore the left ventricular response to heat stress at rest and during exercise, the aims of the present study were to assess whether 1) LV twist and untwisting increase progressively with enhanced skin and core temperatures at rest and 2) the higher cardiovascular demand during the combined challenge of exercise and heat stress would result in greater LV twist mechanics than at rest.

4.2 Methods

4.2.1 Study population

Following ethical approval from Brunel University's ethics committee, ten healthy recreationally active males (21 ± 2 years, 179 ± 7 cm, 76.5 ± 10.8 kg) provided verbal and written informed consent to take part in the study.

4.2.2 Habituation and heat acclimation

Participants attended the laboratory four times; twice for initial habituation and twice for the main investigation. During the first two visits participants cycled in a heat chamber (35°C) at 120 – 140 W for one hour. Sweat rate was calculated from the change in body weight (kg) and the amount of fluids (litres) ingested during cycling. Following habituation with knee-extensor exercise at the beginning of visit three, participants completed an incremental knee-extension exercise test with exercise intensity increasing by 10 W every minute until volitional failure. From the peak power achieved 50% was determined and used as the exercise intensity during the experimental trial.

4.2.3 Experimental procedures

On arrival, each participant was dressed in a tube-lined water-perfused suit and placed in a semi-recumbent position with their left foot strapped into the knee-extensor ergometer. The suit covered the whole body except the head, hands and feet and incorporated a movable panel to facilitate probe-to-skin contact for echocardiographic assessment. Thereafter, participants completed four conditions of progressively increasing heat stress: 1) *control* (mean body temperature ~36.2±0.3°C), 2) *mild heat stress* (~36.9±0.4°C, skin temperature was increased but core temperature was maintained at baseline levels), 3) *moderate heat stress* (~37.7±0.3°C) and 4) *severe heat stress* (~38.3±0.3°C). Each condition comprised a 20 min resting period followed by 12 min of sub-maximal constant load (21±2 W) unilateral knee-extensor exercise. Increases in body temperature were achieved by pumping (Julabo F34, Seelbach, Germany) hot water (42 – 48ºC) through the suit. Participants wore a woollen hat and their legs were wrapped in a thermo foil. Once an increase in mean body temperature

of ~0.6°C was achieved, the water circulator was switched off to prevent further increases in body temperatures whilst cardiovascular measurements were obtained. In order to keep participants euhydrated, carbohydrate electrolyte drinks were ingested regularly with the volume prescribed matching the sweat rates calculated during the acclimation sessions.

Throughout the main investigation mean body temperature was calculated using a combination of rectal temperature (Thermalert, Physitemp, Clifton, New Jersey, USA) and weighted mean skin temperature (Squirrel 1000 Series, Grant, Cambridge, United Kingdom), calculated from seven sites on the body (left foot, left calf, left thigh, left hand, left forearm, abdomen, forehead) (Hardy and Dubois, 1937, Hardy and Stolwijk, 1966). Hydration status was assessed via changes in body weight. Mean arterial pressure (MAP) was obtained either from pressure transducers (Pressure Monitoring Kit, Baxter) connected to a catheter (Leader Cath, Vygon, Ecouen, France) in the radial artery (n=6) or calculated from systolic (P_{sys}) and diastolic (P_{diast}) blood pressures obtained from an automated sphygmomanometer (Omron M5-I, Omron Healthcare, Hoofddorp, Netherlands) (n=4) and using the formula $P_{diast} + [(P_{sys} – P_{diast}) / 3]$. Heart rate (HR) was recorded via ECG (Vivid 7, GE Medical, Horton, Norway).

4.2.4 Echocardiography

Echocardiographic images for the assessment of systolic and diastolic LV volumes and LV mechanics were acquired and analysed as outlined in chapter 3 of this thesis.

4.2.5 Statistical analysis

Statistical analyses to determine the effect of heat stress were performed as presented in chapter 3. Additionally, interaction between responses at rest and during exercise was assessed using two-way repeated measures ANOVA.

4.3 Results

4.3.1 Haemodynamics and left ventricular function during heat stress at rest

At rest, mean body temperature increased progressively from *control* to *severe heat stress* (P<0.01) whilst hydration status, assessed by changes in body weight, was maintained (P>0.05, Table 1). In line with changes in temperature, HR increased by 86% (P<0.01) and cardiac output increased by 106% (P<0.01). These changes did not affect MAP, which remained at *control* levels throughout (P>0.05). Furthermore, progressive heat stress led to a 12% reduction in EDV and a 38% reduction in ESV (both P<0.01). This resulted in an 18% increase in EF (P<0.01) and a maintained SV throughout heat stress (P>0.05) (Figure 4-1).

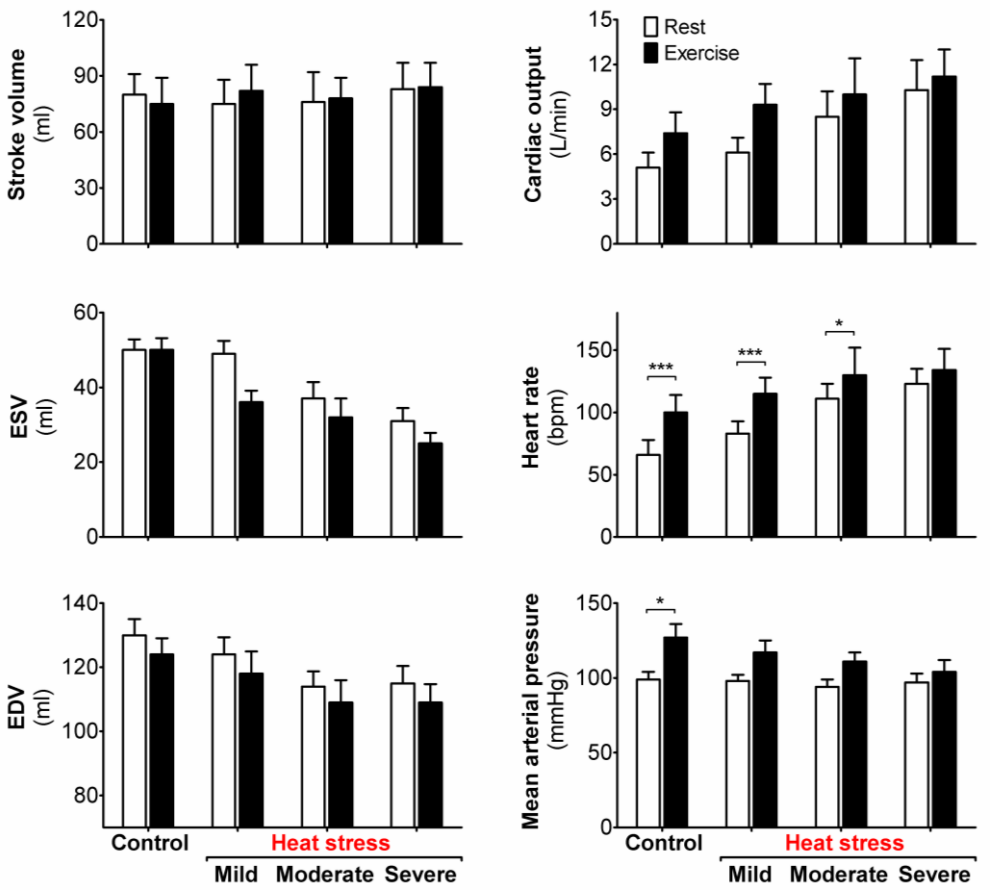

Figure 4-1. Comparison between cardiovascular responses at rest and during exercise with progressive heat stress (n=10). Although not statistically significant, cardiac output was ~2.5–3 $^{L \cdot min^{-1}}$ higher at control exercise and mild heat stress exercise compared with the same resting conditions, indicating an elevated metabolic demand. EDV: end-diastolic volume; ESV: end-systolic volume. Data represent mean ± SEM. *: P<0.05 and ***: P<0.001 compared with rest. The effect of heat stress is presented in table 4-1.

Table 4-1. Systemic and cardiac responses at control and three progressive levels of heat stress, at rest and during exercise.

	REST				EXERCISE			
	Control Rest	*Mild Heat Stress*	*Moderate Heat Stress*	*Severe Heat Stress*	*Control Exercise*	*Mild Heat Stress*	*Moderate Heat Stress*	*Severe Heat Stress*
Mean body temp. (°C)	36.2 ± 0.3	36.9 ± 0.4*	37.7 ± 0.3*†	38.3 ± 0.3*†‡	36.3 ± 0.4	37.2 ± 0.4*	37.8 ± 0.3*†	38.4 ± 0.3*†‡
Core temp. (°C)	37.1 ± 0.4	37.1 ± 0.5	37.9 ± 0.4*†	38.5 ± 0.4*†‡	37.1 ± 0.5	37.3 ± 0.5*	38.1 ± 0.4*†	38.7 ± 0.4*†‡
Skin temp. (°C)	32.6 ± 0.6	36.1 ± 0.4*	36.6 ± 0.6*†	37.3 ± 1.1*†	33.0 ± 0.6	36.5 ± 0.4*	36.8 ± 0.8*	37.2 ± 1.0*
Body mass (kg)	76.7 ± 10.6	—	—	—	—	—	—	76.8 ± 10.6
HR (beats.min^{-1})	66 ± 12	83 ± 10*	111 ± 12*†	123 ± 12*†	100 ± 14#	115 ± 13*#	130 ± 22*#	134 ± 17*†
IVRT (ms)	64 ± 9	56 ± 14	54 ± 11	49 ± 10*	62 ± 15	58 ± 9	56 ± 14	52 ± 10
Cardiac output (L.min^{-1})	5.0 ± 1.0	6.1 ± 1.0*	8.5 ± 1.7*†	10.3 ± 2.0*†‡	7.4 ± 1.4	9.3 ± 1.4	10.0 ± 2.4	11.2 ± 1.8*
EDV (ml)	130 ± 16	124 ± 17	114 ± 15	115 ± 17*	124 ± 16	118 ± 22	109 ± 22	109 ± 18*
ESV (ml)	50 ± 9	49 ± 11	37 ± 14*	31 ± 11*†	50 ± 10	36 ± 10*	32 ± 16*	25 ± 9*
SV (ml)	80 ± 11	75 ± 13	76 ± 16	83 ± 14	75 ± 14	82 ± 14	78 ± 11	84 ± 13
EF (%)	62 ± 4	61 ± 7	67 ± 11	73 ± 8*†	60 ± 7	70 ± 5*	72 ± 9*	78 ± 6*†
MAP (mmHg)	99 ± 15	98 ± 14	94 ± 16	97 ± 18	127 ± 27	117 ± 24	111 ± 19*†	104 ± 24*†

HR: Heart rate; EDV: End-diastolic volume; ESV: End-systolic volume; EF: Ejection fraction; SV: Stroke volume; MAP: Mean arterial pressure; SVR: Systemic vascular resistance. *: $P<0.01$ from *control*; †: $P<0.01$ from *mild heat stress*; ‡: $P<0.01$ from *moderate heat stress*; #: $P<0.01$ compared with the same condition at rest.

Peak systolic basal rotation, peak twist and peak twist velocity increased significantly by 52%, 46% and 70%, respectively (all P <0.01, Figure 4-2) from *control* to *severe heat stress* at rest. During diastole, peak untwisting velocity increased significantly by 82% (P <0.01). The increase in twist velocity and untwisting velocity was underpinned by significant improvements in both basal and apical rotation velocity (P<0.01, Table 4-2). IVRT was significantly shortened with *severe heat stress* (P<0.01), however, temporal analysis revealed that despite the shortening in IVRT and a reduction in the time to mitral valve opening, peak LV untwisting velocity attained its peak prior to mitral valve opening at all stages of heat stress (P<0.01). The change in LV twist and untwisting velocity was significantly related to the changes in body temperature, heart rate, ESV and EDV.

Peak basal radial and circumferential strain, apical radial and longitudinal strain were not different from *control* (all P>0.05) whereas peak apical circumferential strain was significantly enhanced with heat stress (P<0.01, Fig. 4-3). With the exception of basal radial strain rate, all systolic strain rates increased significantly (P<0.01). In contrast, all diastolic strain rates were maintained with progressive heat stress (P>0.05, Table 4-2).

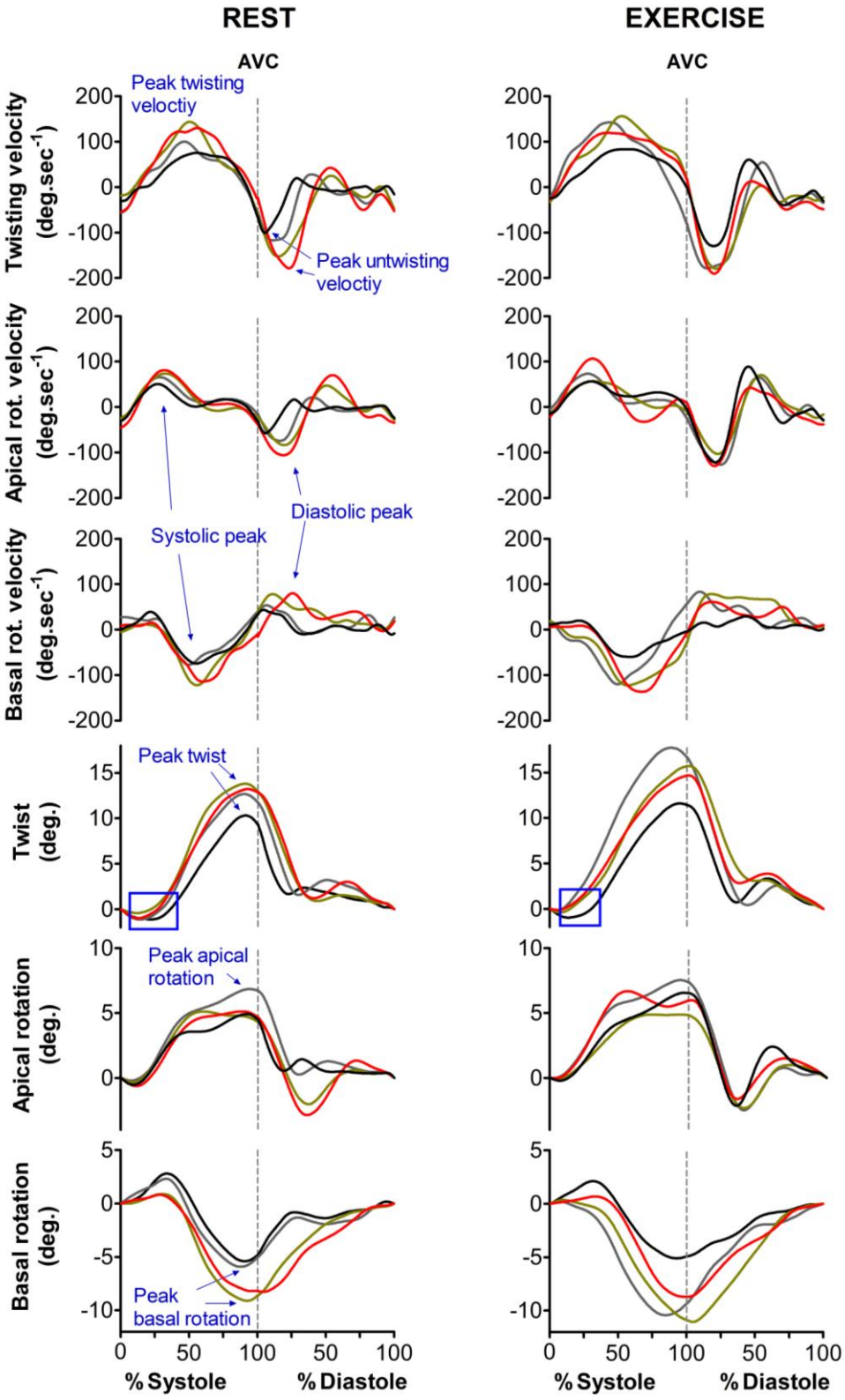

Figure 4-2. Graphical representation of mean left ventricular twist mechanics over the course of an entire cardiac cycle at control and three different levels of heat stress (n=10). At rest LV twist mechanics were enhanced with progressive heat stress whereas during exercise and heat stress peak LV twist and untwisting velocity were unaltered. Blue boxes highlight the decrease in early systolic clockwise twist at rest and during exercise. Vertical dashed line shows aortic valve closure (AVC). Rot.: rotation. For the purpose of clarity error bars have been omitted, values are provided in table 4-2. **- Control; - Mild heat stress; - Moderate heat stress; - Severe heat stress.**

Table 4-2. Peak systolic and diastolic LV strain and rotation parameters.

	REST				EXERCISE			
	Control Rest	*Mild Heat Stress*	*Moderate Heat Stress*	*Severe Heat Stress*	*Control Exercise*	*Mild Heat Stress*	*Moderate Heat Stress*	*Severe Heat Stress*
Peak (Systole)								
Basal rotation (deg.)	-5,9 ± 2,8	-6,7 ± 3,2	-10,0 ± 3.0*†	-11,0 ± 3.6*†	-6,9 ± 3,2	-10,9 ± 3.3*#	-13,2 ± 3.1*#	-10,0 ± 3,0
Apical rotation (deg.)	5,3 ± 1,3	7,4 ± 3,1	6,0 ± 4,7	5,8 ± 1,7	7,3 ± 3,8	8,6 ± 4,7	6,1 ± 2,4	8,2 ± 4,6
Twist (deg.)	10,6 ± 3,3	13,3 ± 3,6	14,8 ± 6,1	15,1 ± 5.2*	12,9 ± 5,8	18,2 ± 5,5	17,0 ± 3,5	16,2 ± 6,5
Longitudinal strain (%)	-15 ± 2	-12 ± 3	-14 ± 3	-12 ± 2	-14 ± 3	-16 ± 2	-13 ± 5	-12 ± 5
Radial strain (%)								
Basal Level	43 ± 16	40 ± 17	38 ± 21	42 ± 22	42 ± 19	39 ± 13	38 ± 22	44 ± 19
Apical Level	29 ± 17	28 ± 11	31 ± 19	33 ± 14	33 ± 20	34 ± 17	35 ± 19	36 ± 12
Circumf. strain (%)								
Basal Level	-16 ± 4	-18 ± 5	-17 ± 5	-17 ± 3	-16 ± 4	-17 ± 4	-15 ± 5	-15 ± 6
Apical Level	-22 ± 4	-26 ± 5*	-25 ± 7	-27 ± 6*	-26 ± 5	-28 ± 8	-25 ± 3	-28 ± 8
Basal rot. vel. (deg.sec.$^{-1}$)	-87 ± 25	-93 ± 23	-135 ± 52	-156 ± 61*†	-78 ± 38	-146 ± 40*#	-166 ± 52*#	-169 ± 47*
Apical rot. vel. (deg.sec.$^{-1}$)	55 ± 17	72 ± 28	82 ± 46	102 ± 38*	86 ± 44	95 ± 40	93 ± 44	128 ± 66
Twist vel. (deg.sec.$^{-1}$)	95 ± 32	120 ± 24*	154 ± 53	188 ± 62*†	121 ± 79	181 ± 81*	183 ± 45	210 ± 90*
Strain rate (deg.sec.$^{-1}$)								
Longitudinal	-0,83 ± 0,18	-0,95 ± 0,07	-1,18 ± 0.22*	-1,21 ± 0,35	-1,12 ± 0,21	-1,22 ± 0,24	-1,20 ± 0,47	-1,44 ± 0,34
Radial basal	2,07 ± 0,85	2,00 ± 0,29	2,76 ± 0,95	2,51 ± 0,77	1,96 ± 0,73	2,68 ± 0.47*#	2,60 ± 0,77	3,04 ± 0.44*
Radial apical	1,12 ± 0,55	1,29 ± 0,35	1,49 ± 0,60	1,90 ± 0.78*	1,41 ± 0,85	1,97 ± 0,83	2,46 ± 1.07*	2,90 ± 1.18*†
Circumf. basal	-1,12 ± 0,28	-1,32 ± 0,27	-1,77 ± 0.51*†	-1,93 ± 0.46*†	-1,25 ± 0,31	-1,58 ± 0,27	-1,84 ± 0.35*	-1,88 ± 0.58*
Circumf. apical	-1,51 ± 0,51	-1,89 ± 0,48	-2,50 ± 0.62*†	-2,89 ± 0.46*†‡	-2,03 ± 0,77	-2,61 ± 0,70	-2,66 ± 0,40	-3,17 ± 0.77*
Peak (Diastole)								
Basal rot. vel. (deg.sec.$^{-1}$)	75 ± 41	86 ± 36	118 ± 38†	133 ± 46*†	79 ± 42	117 ± 53	142 ± 48*	136 ± 38*
Apical rot. vel. (deg.sec.$^{-1}$)	-68 ± 26	-89 ± 33	-96 ± 70	-120 ± 43*	-137 ± 78	-151 ± 71	-114 ± 35	-152 ± 57
Untwisting vel. (deg.sec.$^{-1}$)	-123 ± 55	-150 ± 40	-188 ± 44	-210 ± 49*†	-180 ± 78	-207 ± 73	-204 ± 69	-228 ± 60
Strain rate (deg.sec.$^{-1}$)								
Longitudinal	1,21 ± 0,28	0,96 ± 0,24	1,13 ± 0,35	1,08 ± 0,38	1,31 ± 0.21#	1,74 ± 0.46#	1,45 ± 0,40	1,28 ± 0,51
Radial basal	-2,36 ± 0,60	-2,32 ± 0,62	-2,83 ± 0,61	-3,48 ± 1,39	-2,87 ± 0,67	-3,00 ± 0,85	-3,39 ± 0,97	-3,63 ± 1,22
Radial apical	-2,08 ± 0,56	-2,19 ± 0,62	-2,35 ± 0,68	-2,51 ± 0,53	-2,80 ± 1,11	-2,84 ± 0,72	-3,34 ± 1,48	-3,24 ± 0,96
Circumf. basal	1,44 ± 0,39	1,75 ± 0,62	1,79 ± 0,62	1,99 ± 0,59	1,69 ± 0,61	2,03 ± 0,51	1,92 ± 0,59	2,08 ± 0,67
Circumf. apical	1,92 ± 0,52	2,19 ± 0,77	2,27 ± 0,72	2,67 ± 0,99	2,36 ± 0,89	2,89 ± 1,59	2,58 ± 0,35	2,81 ± 1,12

Circumf.: circumferential; Deg.: degrees; Rot.: rotation; Vel.: velocity; *: *P*<0.01 from *control*; †: *P*<0.01 from *mild heat stress*.; ‡: *P*<0.01 from *moderate heat stress*. #: *P*<0.01 compared with the same condition at rest.

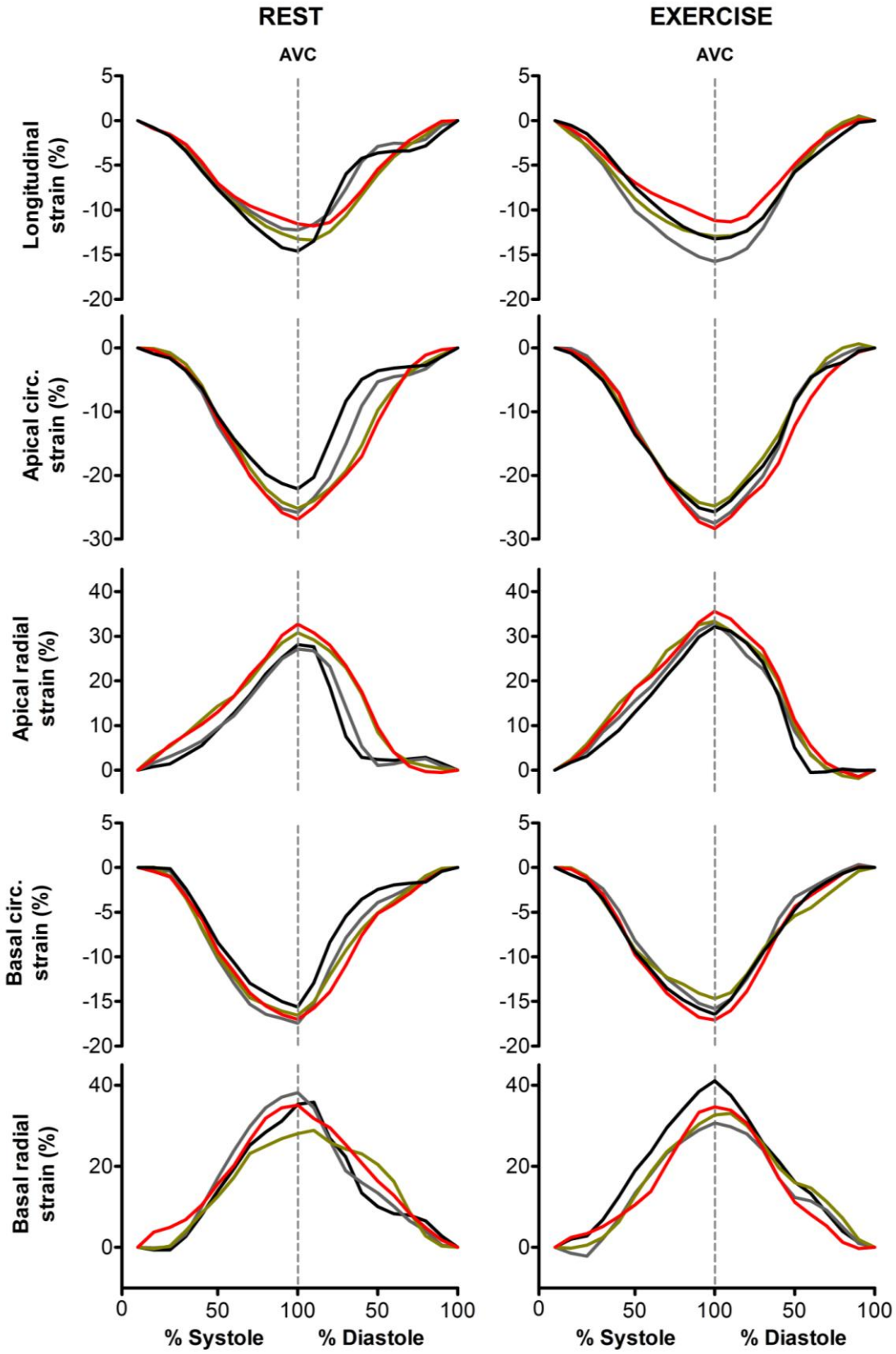

Figure 4-3. Graphical representation of mean left ventricular (LV) strain over the course of an entire cardiac cycle at control and three different levels of heat stress (n=10). At rest and during exercise LV strain was maintained. Vertical dashed line shows aortic valve closure (AVC). For the purpose of clarity error bars have been omitted, values are provided in table 4-2. **- Control; - Mild heat stress; - Moderate heat stress; - Severe heat stress.**

4.3.2 Haemodynamics and left ventricular function during exercise and heat stress

Whilst core temperatures and thus mean body temperature during the combined challenge of exercise and heat stress were statistically higher than at rest ($P<0.01$), the difference was only 0.2°C on average. HR and cardiac output increased over the four exercise stages by ~34 and 51%, respectively ($P<0.01$, Table 4-1), although HR did not increase significantly beyond *moderate heat stress* and the increase in cardiac output only reached statistical significance during the *severe heat stress* stage ($P<0.01$). In contrast to rest, MAP declined significantly with progressive heat stress during exercise ($P<0.01$). Similar to resting conditions, with progressive heating EDV was reduced by 12% and ESV was further reduced by 50% during exercise ($P<0.01$). Accordingly, EF increased by 30% ($P<0.01$) and SV were maintained across all stages of exercise ($P>0.05$).

Other than longitudinal strain rate, all peak systolic strain rates increased significantly from *control* to *severe heat stress* ($P<0.01$, Table 4-2). In contrast, there was no significant difference in diastolic strain rates during exercise and progressive heat stress ($P>0.05$). Similar to rest, peak basal rotation and peak twist velocity increased during exercise and heat stress (both $P<0.01$). However, from *control* exercise to *severe heat stress* exercise peak LV twist, untwisting velocity and IVRT were unaltered (all $P>0.05$, figure 4-3). This did not affect the time to peak LV untwisting which still occurred prior to mitral valve opening at every level of heat stress during exercise ($P<0.01$, figure 4-2). As a result of the increase in heart rate and cardiac output but an unaltered twist mechanics the relationships between these variables during exercise and heat stress were not significant (Figure 4-4).

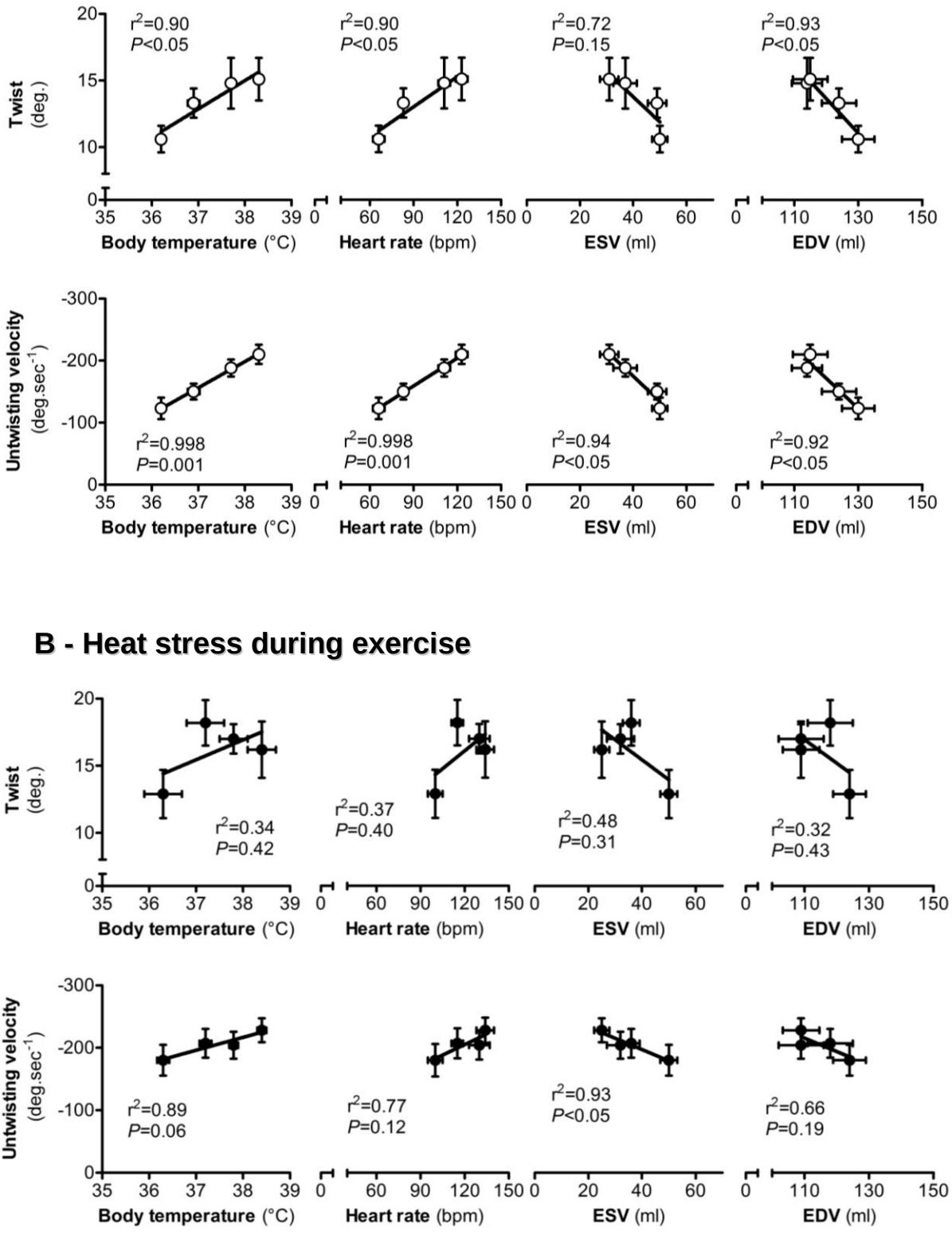

Figure 4-4. Correlations between left ventricular (LV) mechanics, body temperature and LV volumes with heat stress (A) at rest and (B) during exercise (n=10). LV twist mechanics correlated significantly with mean body temperature, heart rate, end-systolic volume (ESV) and end-diastolic volume (EDV) at rest with progressive heat stress. Data represent mean ± SEM.

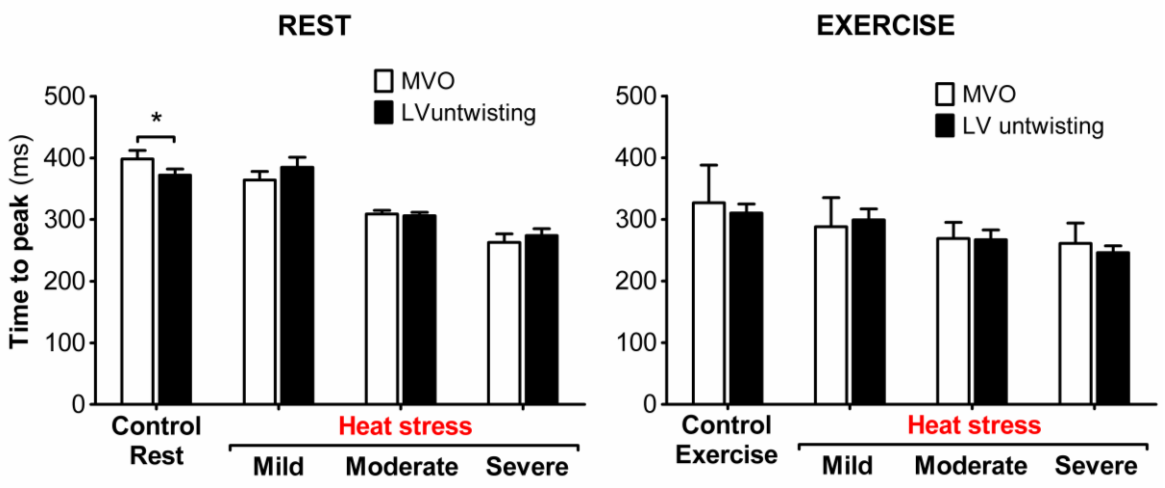

Figure 4-5. Time to peak left ventricular diastolic rotation velocity (n=10). Despite a significant reduction in the time to mitral valve opening (MVO) peak LV untwisting velocity was attained prior to or simultaneously with MVO in all conditions at rest and during exercise, suggesting that heat stress does not affect the normal chronological order of events within the cardiac cycle. *: $P<0.05$.

4.4 Discussion

The present study has for the first time examined the response of LV twist mechanics to progressively increasing skin and core body temperatures in healthy humans. We show that the increase in systolic and diastolic LV twist mechanics is closely related to the progressively increasing mean body temperature and heart rate at rest. Furthermore, this study is the first to provide a direct comparison between LV function during progressive heat stress at rest and in combination with exercise. The present findings demonstrate that LV twist mechanics do not increase during progressive heat stress and small muscle mass exercise, suggesting that the significantly greater reduction in ESV, and thus maintenance of stroke volume, may be facilitated by other factors such as the observed decline in afterload.

The haemodynamic response to passive heat stress in the present study was similar to that

reported previously showing an increase in cardiac output and EF (Crandall et al., 2008, Rowell et al., 1969a), a reduction in EDV (Wilson et al., 2009) and ESV and a maintained SV and MAP (Rowell et al., 1969a, Wilson et al., 2009). In contrast to Crandall *et al*. (2008), in the present investigation EDV decreased significantly with the highest level of heat stress. It is possible that the longer exposure to higher internal body temperature (~38.5 vs. ~38.2 °C) and higher heart rate (~123 vs. 93 beats.min^{-1}) in this study exacerbated the trend towards a decline in EDV observed by Crandall *et al*. (2008). Despite resulting in marked increases in heart rate and, thus, clear elevations in cardiac output above resting levels, knee-extensor exercise did not increase EDV or SV. This surprising finding is likely related to the exercise modality, the population studied (González-Alonso et al., 2008b, Mortensen et al., 2007) and the exercise intensity used (Savard et al., 1988) and therefore limits the application of the current findings to small muscle mass exercise.

Left ventricular systolic function

Whilst this study confirms the previous findings of an enhanced systolic function with heat stress at rest (Brothers et al., 2009, Nelson et al., 2010a) the present findings further demonstrate that LV systolic twist mechanics increase proportionally to the magnitude of passive heat stress, thereby contributing to an improved LV ejection. It has been previously suggested that an increased myocardial contractility may be involved in the improvement of LV systolic function with heat stress at rest (Rowell et al., 1969a). In addition to enhanced LV twist and twist velocity, our data show that despite reduced EDV, and thus less contribution from the Frank-Starling mechanism, LV strain was maintained and systolic strain rates, in particular circumferential strain rate, were significantly increased. These

findings suggest an enhanced intrinsic LV contractility (Teske et al., 2007) and support Rowell's description of heat stress as a "hyperadrenergic state" (Rowell, 1990). Previous authors have demonstrated significant increases in peak systolic mechanics with the administration of inotropic agents (Dong et al., 1999, Opdahl et al., 2008, Helle-Valle et al., 2005) showing that LV mechanics are closely linked to sympathetic tone. The progressive increase in LV twist and twist velocity in the present study may therefore be related to an increase in sympathetically mediated contractility, which will have contributed to the reductions in ESV observed during progressive heating at rest.

While heat stress appeared to enhance contractility it is interesting to note that strain, twist, SV and ejection fraction were uncoupled with heat stress. This finding disagrees with previous studies that have independently manipulated preload, afterload and contractility and that have shown LV strain and twist to be related to SV and ejection fraction (Morris et al., 1987, Dong et al., 1999, Weidemann et al., 2002). In the present study LV strain and SV were maintained whilst ejection fraction and LV twist increased. Based on the existing evidence we propose that uncoupling of strain/ejection fraction and twist/SV with heat stress may occur as a consequence of concomitant changes in preload, afterload and contractility. Future studies should specifically examine the interaction between physiological changes in loading status and contractility and their impact on coupling/uncoupling of LV strain, twist, SV and ejection fraction.

In contrast with previous work (Akagawa et al., 2007, Helle-Valle et al., 2005, Notomi et al., 2006, Opdahl et al., 2008) our findings demonstrate that the overall change in LV twist may be caused predominantly by enhanced LV basal rotation as opposed to increased LV

apical rotation. However, our study differs from existing reports in that it has assessed LV twist in the face of a reduced preload. Thus, we suggest that the reduction in preload may limit the positive effect of increased contractility on LV apical rotation typically seen with isolated inotropic stimulation or exercise (Akagawa et al., 2007, Helle-Valle et al., 2005, Notomi et al., 2006, Opdahl et al., 2008).

Similar to the response at rest, increased HR, maintained LV strain, increased systolic strain rates and the reduction in ESV during exercise and heat stress are indicative of an enhanced inotropic state expected with exercise. Although not statistically significant LV twist was elevated during *control exercise* and *mild heat stress exercise* compared with resting conditions despite significantly higher MAP. This may indicate a compensatory mechanism further showing that LV twist likely contributes to an improved LV ejection during exercise with *mild heat stress*. Notwithstanding, LV twist did not increase further with *moderate* and *severe heat stress exercise* despite significant reductions in MAP (Dong et al., 1999). Thus, it appears that systolic LV twist mechanics may not have contributed to an enhanced ejection during *moderate* and *severe heat stress* during exercise. Together with the non-linear relationship with heart rate the lack of a continuous increase in LV twist from *control exercise* up to *severe heat stress exercise* suggests that LV mechanics may have attained an upper limit during the combined stress of exercise and *mild heat stress*. Accordingly, it is possible that the maintenance of stroke volume is facilitated by other factors than those pertaining to LV twist mechanics, such as the progressive decline in afterload evidenced by the significant drop in MAP.

Left ventricular diastolic untwisting and filling

It has been shown that peak LV untwisting velocity plays an important role in early diastole by contributing to the intra-ventricular suction required for LV filling (Notomi et al., 2008, Dong et al., 2001, Popovic et al., 2006). Thus, the progressive increase in peak LV untwisting velocity during heat stress at rest likely facilitates a continuous improvement in LV filling. Since the kinetic energy required for diastolic untwisting or recoil is stored during ventricular systole (Ashikaga et al., 2004a, Helmes et al., 2003, Notomi et al., 2006, Granzier and Labeit, 2004), enhanced inotropic state and subsequent improvements in peak LV systolic twist mechanics (twist and twist velocity) during heat stress at rest are likely related to the increase in LV diastolic untwisting velocity. In addition to an enhanced inotropic state, the reduction in EDV *per se* may have contributed to an enhanced LV untwisting by changing the relative orientation of subendocardial and subepicardial fibres. Previous findings have demonstrated that altered subendocardial and subepicardial fibre orientation impacts LV twist (Taber et al., 1996). It has also been suggested that a decline in subendocardial function is reflected by a reduction in early systolic clockwise twist (Takeuchi et al., 2009, Ashikaga et al., 2007). Although it was not possible to statistically analyse each data point within the cardiac cycle, a reduction in the early systolic clockwise twist can be seen in the present study (blue boxes in figure 4-2). In accordance with Takeuchi *et al*. (2009) and Taber *et al*. (1996) this acute reduction in subendocardial function likely reflects a change in myocardial fibre orientation. Thus, altered fibre orientation subsequent to reduced EDV may contribute to enhanced systolic and diastolic LV twist mechanics during heat stress at rest. This is further supported by the presently observed significant correlations between LV twist mechanics and EDV.

Unlike the increase in LV diastolic untwisting velocity during heat stress at rest, there was no significant change in untwisting velocity from *control* exercise to *severe heat stress* exercise. However, peak untwisting velocity during *control* exercise was elevated to levels comparable to *severe heat stress* at rest. From the present findings it is not clear why LV twist and untwisting velocity did not increase further from *control* exercise to *severe heat stress* exercise. Although the use of small muscle mass exercise and the relatively low exercise intensity may have influenced the current findings the significant increase in heart rate and cardiac output from *control* exercise to *severe heat stress* exercise suggests that the lack of increase in LV twist mechanics cannot be entirely caused by an attenuated cardiovascular demand. Thus, similar to systolic twist mechanics during exercise and heat stress, diastolic untwisting appears to have reached an upper limit and it is possible that higher exercise intensities may have resulted in an actual decline in LV twist mechanics; a hypothesis that agrees with the previous reports of a reduced LV function during whole body exercise in the heat (Rowell et al., 1966, González-Alonso and Calbet, 2003, Lafrenz et al., 2008, Rowell et al., 1969b). In the present study, the lack of an increase in LV untwisting velocities with exercise and heat stress compared with heat stress at rest further suggests that additional factors such as the decline in MAP may have contributed to the maintenance of stroke volume. Future studies using whole-body exercise with higher exercise intensities or longer exercise durations may be able to demonstrate a link between a reduction in SV and a reduction in underpinning LV twist mechanics.

With regard to the timing of diastolic events, previous studies have suggested that cardiovascular disease may be associated with a delayed peak untwisting velocity (Takeuchi

and Lang, 2008, Wang et al., 2007b). Here, irrespective of the significant reduction in IVRT and the shortening of the time to mitral valve opening, heat stress does not alter the chronological sequence of diastolic events as peak untwisting velocity occurred prior to or with mitral valve opening at all levels of heat stress at rest and during exercise. This finding confirms that the ability of the LV to create suction appears to be improved during passive heat stress (Nelson et al., 2010a, Nelson et al., 2010b). As discussed above, the higher metabolic demand and the further altered haemodynamics during whole body exercise may alter this order of events and result in a delay in peak LV untwisting velocity until after mitral valve opening, presenting another potential source for the previously postulated reduction in LV function in these conditions (Rowell et al., 1966, González-Alonso and Calbet, 2003, Lafrenz et al., 2008, Rowell et al., 1969b).

4.5 Conclusion

In conclusion, this is the first study to show that increases in systolic and diastolic LV twist mechanics with passive heat stress are tightly related to the magnitude of heat stress. However, the increase in LV twist and untwisting velocity observed at rest with progressive heat stress is not seen during exercise and heat stress. We, therefore, suggest that the maintenance of stroke volume in the combined condition of heat stress and small muscle mass exercise may be facilitated by other factors such as the continuous decline in afterload.

CHAPTER 5

Study 2

Effect of progressive dehydration with hyperthermia on global left ventricular function and mechanics at rest and during exercise

5.1 Introduction

There is clear evidence that the combination of dehydration and increased body temperatures (hyperthermia) causes a reduction in cardiovascular function during exercise (Montain and Coyle, 1992, González-Alonso et al., 1997, González-Alonso et al., 2008a). The reduced cardiovascular function is characterised by a decline in cardiac output, muscle blood flow and systemic and cutaneous vascular conductance (González-Alonso et al., 1995, González-Alonso et al., 1998). Thus, in contrast to the increased cardiac output observed in study 1 with heat stress during exercise, the combined challenge of dehydration and hyperthermia during exercise represents a condition in which the stress imposed may exceed the capacity of the cardiovascular system (González-Alonso et al., 1995, Rowell et al., 1966). Central to these previously described changes in cardiovascular function is a large decline in SV. As discussed in detail in section 2.6, previous studies have shown that the reduction in blood volume, the redistribution of blood to the skin and the reduced filling time owing to ensuing tachycardia do not fully explain this large decrement in SV. One possible factor that has not yet been explored is a reduction in systolic and/or diastolic LV function with dehydration and hyperthermia during exercise.

Systolic LV twist and diastolic LV untwisting have been shown to contribute to LV ejection and filling at rest and during exercise (Vendelin et al., 2002, Burns et al., 2008a, Notomi et al., 2008, Rademakers et al., 1992, Notomi et al., 2006). Study 1 of this thesis revealed that LV mechanics were maintained with heat stress during exercise. It is possible that the combined challenge of dehydration and hyperthermia during exercise may result in a reduction in LV mechanics, possibly explaining the lower SV that has been observed in these conditions (González-Alonso et al., 1997). In relation to this, Nottin et al. (2009) have shown

that LV twist, untwisting velocity and strain are indeed significantly reduced following prolonged exercise that was accompanied by a reduction in body mass of approximately 4.5%. Although this twist and strain response may be the direct effect of prolonged exercise as suggested by the authors, it is probable that the post-exercise dehydration also influenced the reduction in LV mechanics. At present, however, the direct effect of dehydration and hyperthermia on LV mechanics during exercise is not known. The assessment of LV function including LV mechanics during dehydration and following rehydration would provide additional insight. Furthermore, study 1 of this thesis showed that there were differences in LV mechanics between isolated heat stress (i) at rest and (ii) during small muscle mass exercise. Assessing the influence of combined dehydration and hyperthermia on LV mechanics at rest will further help to determine whether dehydration *per se* is associated with a decline in LV mechanics.

In view of the existing evidence the aims of this study were 1) to examine the effect of combined dehydration and hyperthermia on LV mechanics at rest and 2) to ascertain the impact of dehydration and hyperthermia on LV mechanics during exercise. It was hypothesised that 1) dehydration and hyperthermia would reduce LV mechanics at rest and 2) dehydration and hyperthermia would also lower LV mechanics during exercise.

5.2 Methods

5.2.1 Study population

Following ethical approval from Brunel University's ethics committee, eight healthy active males (age 20±2 years, height 177±5 cm, body mass 72.7±9.6 kg, $\dot{V}O_{2peak}$ 58±7ml.kg^{-1}.min^{-1},

peak power 336±27 W) provided verbal and written informed consent to take part in the study.

5.2.2 Habituation and heat acclimation

Participants attended the laboratory a total of five times; for initial habituation and determination of their maximal oxygen consumption ($\dot{V}O_{2peak}$), heat acclimation (x3) and for the main investigation. During the first visit participants were familiarised with knee-extensor exercise. Participants then completed an incremental knee-extension exercise test with exercise intensity increasing by 5 W every minute until volitional failure. Following ten minutes of recovery all participants performed an incremental exercise test on an upright cycle ergometer (Lode, Excalibur, Groningen, Netherlands) to determine each individual's maximal power output and $\dot{V}O_{2max}$.

The second, third and fourth visits served as acclimation sessions, with participants cycling in a heat chamber (35°C, 55% humidity) for 60 minutes at 50% of the individual peak power achieved during the incremental test in the first visit. During exercise participants did not ingest any fluids. Throughout all acclimation sessions core temperature was monitored using a rectal thermistor and sweat rate was indexed as the loss of body weight (kg) following one hour of exercise in the heat. The three acclimation sessions were separated by at least 48 and at most 72 hours.

5.2.3 Experimental procedures

On arrival, participants were weighed and placed in a semi-recumbent position with their left foot strapped into a knee-extensor ergometer. Baseline body temperatures, blood pressure and echocardiographic images were recorded during ten minutes at rest according to the procedures previously outlined in chapter 3. Following resting measurements participants performed 16 minutes of one-legged knee-extensor exercise (23±2 W); body temperatures, blood pressure and cardiac function were recorded during the last ten minutes of exercise. After completion of these control measurements participants performed two one hour bouts of cycling exercise (50% peak power) in the heat (35°C, 55% humidity) without any fluid ingestion. The first bout of exercise in the heat resulted in approximately 2% dehydration as determined by reductions in body mass; total dehydration following the second bout of exercise in the heat was approximately 3.5% on average. Each one hour bout of cycling exercise in the heat was followed by a 10 minute semi-recumbent resting period after which the measurement of body temperatures, blood pressure and echocardiographic images was repeated, both at rest and during knee-extensor exercise. Following completion of 16 minutes of knee-extensor exercise at ~3.5% dehydration, participants rehydrated for one hour by ingesting a chilled 4.5% carbohydrate drink. The volume of fluid ingested in litres matched the body mass lost in kilograms. Twenty minutes following rehydration cardiovascular measurements were repeated at rest and during knee-extensor exercise.

Throughout the experiment, mean skin temperature and core temperature were calculated from the weighted mean of the six sites (Taylor et al., 1989) and using a rectal thermistor (Thermalert, Physitemp, Clifton, New Jersey, USA), respectively. Absolute blood volume was estimated at *control* based on the results from previous studies (Sawka et al., 1992).

Changes in blood volume consequent to dehydration were calculated from the haemoglobin and haematocrit concentration obtained from venous blood samples as previously described (Dill and Costill, 1974). Mean arterial pressure (MAP) was obtained either invasively from pressure transducers (Pressure Monitoring Kit, Baxter) connected to a catheter (Leader Cath, Vygon, Ecouen, France) in the radial artery (n=6) or calculated from systolic (P_{sys}) and diastolic (P_{diast}) blood pressures obtained from an automated sphygmomanometer (Omron M5-I, Omron Healthcare, Hoofddorp, Netherlands) (n=2) using the formula $P_{diast} + [(P_{sys} - P_{diast}) / 3]$. Heart rate (HR) was recorded via ECG (Vivid 7, GE Medical, Horton, Norway).

5.2.3 Echocardiography

Echocardiographic images for the assessment of systolic and diastolic LV volumes and LV mechanics were acquired and analysed as outlined in chapter 3 of this thesis.

5.2.4 Statistical analysis

Statistical analyses to determine effect of dehydration and differences between groups at rest and during exercise were performed as presented in chapter 3. All repeated measures ANOVA analyses included the rehydration condition.

5.3 Results

5.3.1 Haemodynamics and global left ventricular function at rest with dehydration and following rehydration

Core and skin temperature increased with the first level of dehydration (2% loss in body mass) and remained at this level during the following level of dehydration (3.5% loss in body mass) while blood volume declined ($P<0.01$). End-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV, all $P<0.01$) also decreased significantly whereas cardiac output and ejection fraction (EF) were maintained (both $P>0.05$). Dehydration did not significantly change mean arterial pressure (MAP) from *control rest* ($P>0.05$). Following rehydration, body mass, core temperature, EDV, ESV and SV were restored ($P>0.05$ versus *control rest*) whilst there was a small increase in blood volume and HR remained slightly elevated ($P<0.01$). The higher HR was associated with a small but significant increase in cardiac output following rehydration at rest ($P<0.01$, see figure 5-1). Data are summarised in table 5-1.

With regard to LV mechanics, there was a significant increase in LV twist with the highest level of dehydration at rest ($P=0.016$). This was the result of progressive increases in basal rotation with dehydration ($P<0.01$) whilst apical rotation was maintained ($P>0.05$, figure 5-2). Similarly, the increase in peak twist velocity ($P=0.017$) with dehydration at rest was underpinned by progressive increases in basal rotation velocity ($P<0.01$) whereas apical rotation velocity was maintained ($P>0.05$). Peak untwisting velocity was unaltered with progressive dehydration at rest ($P>0.05$). Furthermore, there was a small but significant reduction in peak longitudinal strain with *2% dehydration* ($P=0.018$), whereas peak radial and circumferential strain was maintained across all resting conditions ($P>0.05$). In line with this, peak diastolic longitudinal strain rate also decreased with both levels of dehydration at

rest and remained significantly lower following rehydration compared with *control rest* (all $P<0.01$). All other diastolic strain rates were maintained across all conditions ($P>0.05$). In systole, peak longitudinal, radial and basal circumferential strain rates were unaltered with dehydration ($P>0.05$). However, systolic apical circumferential strain rate increased with 3.5% dehydration ($P=0.014$) and returned to control levels when participants were rehydrated.

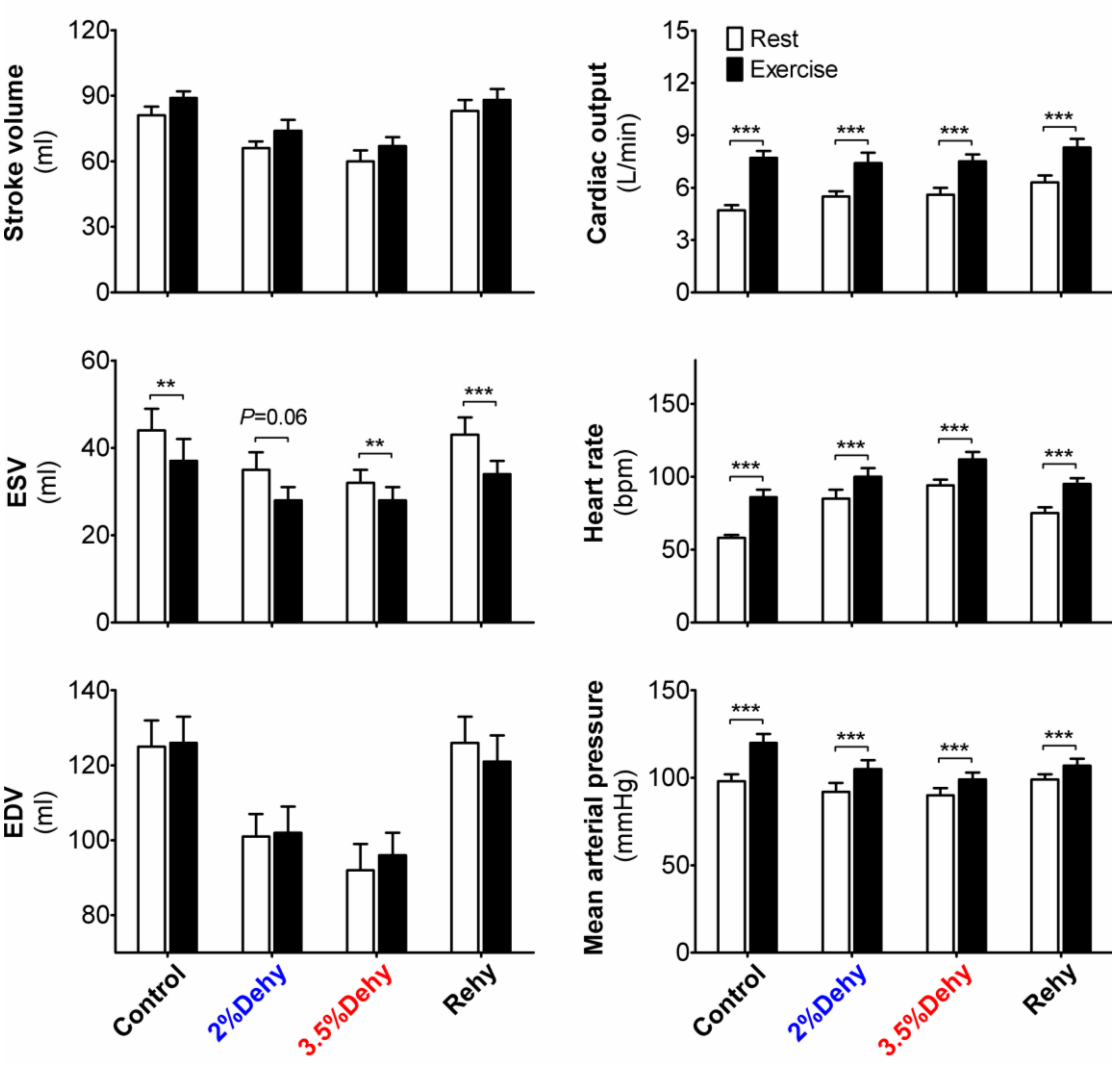

Figure 5-1. Comparison of the effect of dehydration and rehydration on cardiovascular responses at rest and during small muscle mass exercise (n=8). Data represent mean ± SEM. **: $P<0.01$ and ***: $P<0.001$ compared with rest. The effect of dehydration and rehydration is presented in table 5-1.

Table 5-1. Changes in body temperature and cardiac function at control, two levels of dehydration and following rehydration.

	REST				EXERCISE			
	Control Rest	2% DEHY	3.5% DEHY	REHY	Control Exercise	2% DEHY	3.5% DEHY	REHY
Body mass (kg)	72.7 ± 9.6	71.5 ± 9.5*	70.3 ± 9.4*†	72.7 ± 9.6†‡	72.7 ± 9.6	71.3 ± 9.5*	70.2 ± 9.4*†	72.6 ± 9.7†‡
Core Temp. (°C)	37.1 ± 0.1	38.0 ± 0.5*	38.1 ± 0.4*	37.1 ± 0.3†‡	37.1 ± 0.2	37.7 ± 0.3*	37.9 ± 0.3*†	37.2 ± 0.3†‡
Mean Skin Temp. (°C)	33.9 ± 0.5	34.7 ± 0.8	34.9 ± 0.7*	34.5 ± 0.7*	34.1 ± 0.7	34.8 ± 0.6	35.1 ± 0.7*	34.7 ± 0.8
Blood volume (ml)	5251 ± 594	5072 ± 626*	4989 ± 546*	5453 ± 583*†‡	5164 ± 708	4995 ± 544	4941 ± 543	5350 ± 620†‡
HR (beats·min^{-1})	58 ± 6	85 ± 16*	94 ± 11*†	75 ± 10*†‡	86 ± 13	100 ± 17*	112 ± 14*†	95 ± 10‡
IVRT (ms)	74 ± 16	73 ± 8	74 ± 10	72 ± 7	63 ± 10	60 ± 7	58 ± 8	61 ± 11
Cardiac output (L·min^{-1})	4.7 ± 0.8	5.5 ± 0.9	5.6 ± 1.1	6.3 ± 1.2*	7.7 ± 1.0	7.4 ± 1.8	7.5 ± 1.2	8.3 ± 1.5
EDV (ml)	125 ± 21	101 ± 17*	92 ± 21*	126 ± 21†‡	126 ± 19	102 ± 21*	96 ± 16*	121 ± 21†‡
ESV (ml)	44 ± 14	35 ± 11	32 ± 8*	43 ± 11‡	37 ± 15	28 ± 9*	28 ± 8	34 ± 9
SV (ml)	81 ± 10	66 ± 9*	60 ± 14*	83 ± 13†‡	89 ± 9	74 ± 14*	67 ± 11*	88 ± 13‡†
EF (%)	66 ± 7	66 ± 5	66 ± 4	66 ± 4	71 ± 8	74 ± 5	71 ± 6	72 ± 3
MAP (mmHg)	98 ± 12	92 ± 13	90 ± 12	99 ± 9	120 ± 13	105 ± 14*	99 ± 10*	107 ± 10*‡

DEHY: Dehydration; HR: Heart rate; IVRT: Iso-volumic relaxation time; EDV: End-diastolic volume; ESV: End-systolic volume; EF: Ejection fraction; SV: Stroke volume; MAP: Mean arterial pressure. *: P<0.01 from *control*; †: P<0.01 from 2% DEHY; ‡: P<0.01 from 3.5% DEHY; #: P<0.01 compared with the same condition at rest.

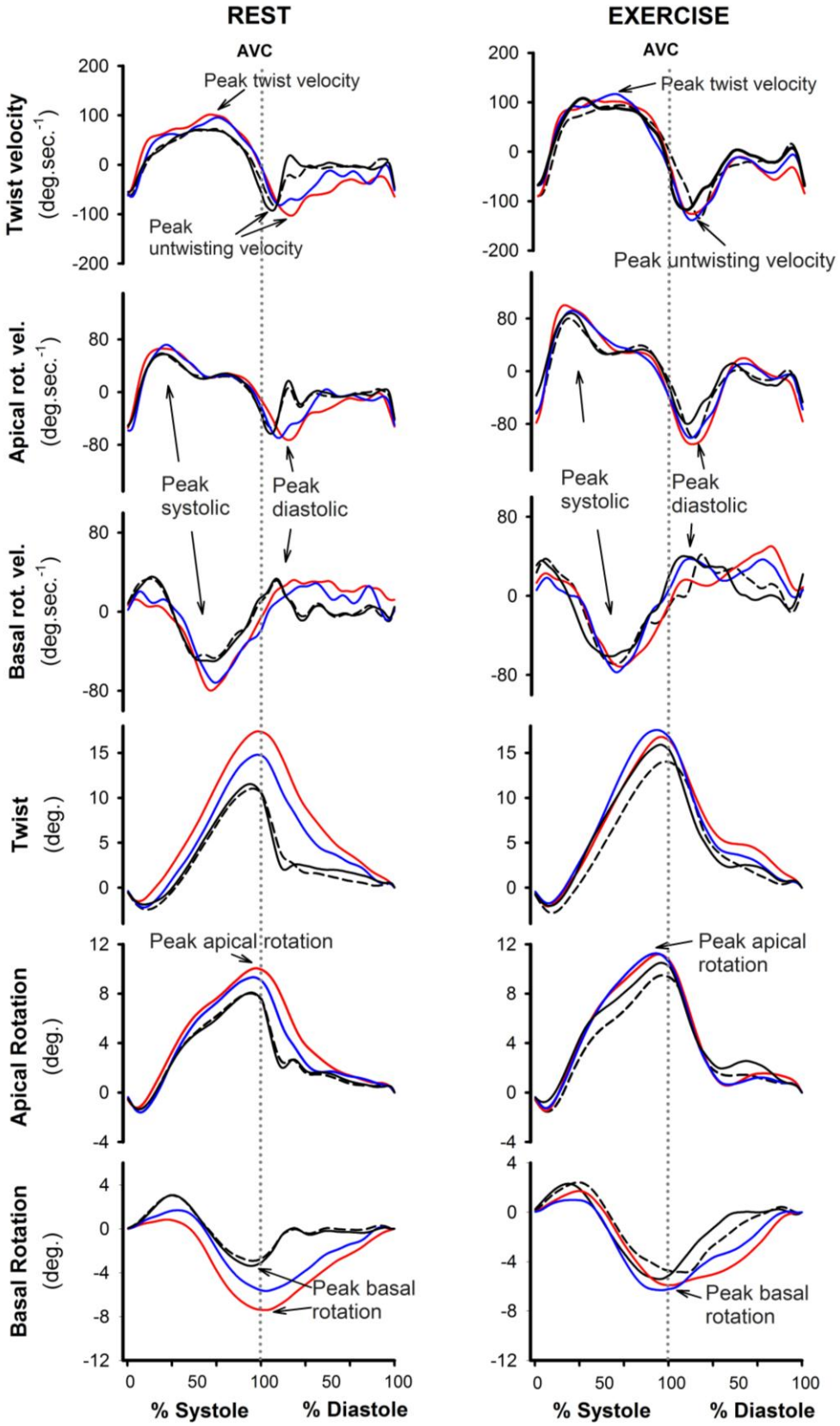

Figure 5-2. Graphical representation of mean left ventricular (LV) twist mechanics over the course of an entire cardiac cycle at control, dehydration and rehydration (n=8). Vertical dashed line shows aortic valve closure (AVC). Deg.: degrees; Rot.: rotation; Vel.: velocity. For the purpose of clarity error bars have been omitted, values are provided in table 5-2.

- Control; - 2.0% dehydration; - 3.5% dehydration; - - Rehydration.

5.3.2 Haemodynamics and global left ventricular function during exercise with dehydration and following rehydration

During exercise, dehydration and hyperthermia caused a reduction in end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV, all $P<0.01$). However, ESV was significantly lower than at rest (figure 5-1). Cardiac output and ejection fraction (EF) were maintained throughout exercise conditions (both $P>0.05$). The decrease in total blood volume during exercise was not significant ($P>0.05$). In contrast to resting conditions, MAP declined progressively with dehydration from *control exercise* ($P<0.01$) and remained lower than *control exercise* following rehydration ($P<0.01$).

Unlike rest, dehydration during exercise did not alter systolic or diastolic LV twist indices (all $P>0.05$). Whilst peak LV untwisting velocity occurred prior to mitral valve opening at *control rest* ($P>0.01$), with dehydration and following rehydration at rest and during knee-extensor exercise peak LV untwisting velocity tended to be delayed (figure 5-4). Dehydration and hyperthermia during exercise reduced peak longitudinal strain ($P<0.01$) whilst radial and circumferential strain were maintained ($P>0.05$). Furthermore, diastolic longitudinal strain rate decreased and remained lower following rehydration ($P<0.01$). All other systolic and diastolic strain rates were maintained ($P>0.05$).

Table 5-2. Peak systolic and diastolic rotation and strain parameters at control, two levels of dehydration and following rehydration.

	REST				EXERCISE			
	Control	2% DEHY	3.5% DEHY	REHY	Control	2% DEHY	3.5% DEHY	REHY
Peak (Systole)								
Basal Rotation (deg.)	-3,4 ± 2,4	-6,2 ± 2.5*	-7,6 ± 1.7*	-4,4 ± 2.7‡	-5,7 ± 2,5	-6,9 ± 2,7	-6,3 ± 2,9	-5,6 ± 1,6
Apical Rotation (deg.)	8,5 ± 3,4	9,8 ± 3,4	10,5 ± 2,9	6,5 ± 3,4	10,7 ± 2,7	11,5 ± 2,3	11,9 ± 3,6	9,7 ± 3,6
Twist (deg.)	11,7 ± 3,7	15,2 ± 3,9	17,6 ± 2.9*	10,4 ± 3.1‡	16,2 ± 4,1	18,0 ± 4,2	17,0 ± 3,3	14,4 ± 4,8
Longitudinal Strain (%)	-19 ± 2	-17 ± 3*	-17 ± 2	-17 ± 1	-21 ± 3	-17 ± 2*	-18 ± 2*	-18 ± 1*
Radial Strain (%)								
Basal Level	45 ± 15	34 ± 16	37 ± 14	42 ± 14	54 ± 14	41 ± 21	45 ± 19	41 ± 16
Apical Level	37 ± 11	35 ± 12	34 ± 20	40 ± 8	42 ± 15	36 ± 9	41 ± 13	42 ± 16
Circumferential Strain (%)								
Basal Level	-17 ± 3	-15 ± 3	-15 ± 2	-15 ± 1	20 ± 3	-16 ± 4	-15 ± 4	-15 ± 2‡
Apical Level	-24 ± 5	-23 ± 4	-25 ± 3	-19 ± 3‡	-27 ± 5	-27 ± 4	-27 ± 3	-25 ± 2
Apical rot. vel (deg.sec^{-1})	-78 ± 18	-92 ± 29	-87 ± 25	-74 ± 33	-107 ± 30	-128 ± 55	-132 ± 39	-117 ± 53
Basal rot. vel. (deg.sec^{-1})	-57 ± 22	-80 ± 34*	-84 ± 22*	-59 ± 21‡	-70 ± 25	-83 ± 22	-88 ± 14	-77 ± 18
Twist vel. (deg.sec^{-1})	82 ± 21	109 ± 39	126 ± 36*	84 ± 17‡	125 ± 46	131 ± 37	133 ± 40	110 ± 24
Strain Rate (sec^{-1})								
Longitudinal	-1,03 ± 0,19	-1,07 ± 0,24	-1,18 ± 0,15	-1,03 ± 0.16‡	-1,29 ± 0,21	-1,23 ± 0,26	-1,47 ± 0,28	-1,23 ± 0.16‡
Radial basal	1,66 ± 0,18	1,72 ± 0,49	1,78 ± 0,40	2,03 ± 0,55	1,96 ± 0,41	2,02 ± 0,57	2,20 ± 0,76	2,08 ± 0,57
Radial apical	1,45 ± 0,51	1,63 ± 0,61	1,55 ± 0,44	1,44 ± 0,22	1,57 ± 0,40	1,63 ± 0,75	1,81 ± 0,53	1,68 ± 0,31
Circumferential basal	-1,19 ± 0,28	-1,28 ± 0,39	-1,39 ± 0,25	-1,05 ± 0,14	-1,48 ± 0,30	-1,41 ± 0,23	-1,51 ± 0,39	-1,25 ± 0,16
Circumferential apical	-1,66 ± 0,43	-1,88 ± 0,64	-2,12 ± 0.30*	-1,49 ± 0.32‡	-2,00 ± 0,60	-2,26 ± 0,65	-2,47 ± 0,49	-2,00 ± 0.46‡
Peak (Diastole)								
Basal rot. vel. (deg.sec^{-1})	46 ± 16	55 ± 23	58 ± 16	54 ± 18	76 ± 28	62 ± 18	56 ± 20	62 ± 14
Apical rot. vel. (deg.sec^{-1})	70 ± 25	82 ± 23	89 ± 21	56 ± 13‡	104 ± 34	102 ± 24	114 ± 32	85 ± 28
Untwisting vel. (deg.sec^{-1})	-104 ± 19	-117 ± 43	-123 ± 38	-117 ± 31	-153 ± 48	-169 ± 62	-153 ± 45	-147 ± 47
Strain Rate (sec^{-1})								
Longitudinal	1,53 ± 0,17	1,12 ± 0.27*	1,46 ± 0.29†	1,32 ± 0.17*	1,81 ± 0,23	1,47 ± 0.21*	1,72 ± 0.20†	1,40 ± 0.31*
Radial basal	-1,88 ± 0,93	-2,11 ± 1,02	-2,08 ± 0,47	-2,00 ± 0,92	-2,27 ± 0,56	-2,53 ± 1,23	-2,64 ± 0,76	-2,48 ± 1,00
Radial apical	-2,34 ± 0,55	-2,25 ± 0,60	-2,50 ± 0,53	-2,69 ± 0,56	-2,24 ± 0,81	-2,39 ± 0,40	-2,37 ± 0,62	-2,62 ± 0,31
Circumferential basal	1,28 ± 0,24	1,39 ± 0,41	1,51 ± 0,43	1,17 ± 0,25	1,89 ± 0,58	1,57 ± 0,50	1,73 ± 0,60	1,34 ± 0,23
Circumferential apical	2,18 ± 0,69	2,06 ± 0,82	2,32 ± 0,26	2,02 ± 0,64	2,55 ± 0,83	2,45 ± 0,48	2,69 ± 0,47	2,59 ± 0,54

Deg.: degrees; DEHY: dehydration; REHY: rehydration; Rot.: rotation; Vel.: velocity;. *: *P*<0.01 from *control*; †: *P*<0.01 from 2% DEHY.; ‡: *P*<0.01 from 3.5% DEHY; #: *P*<0.01 compared with the same condition at rest.

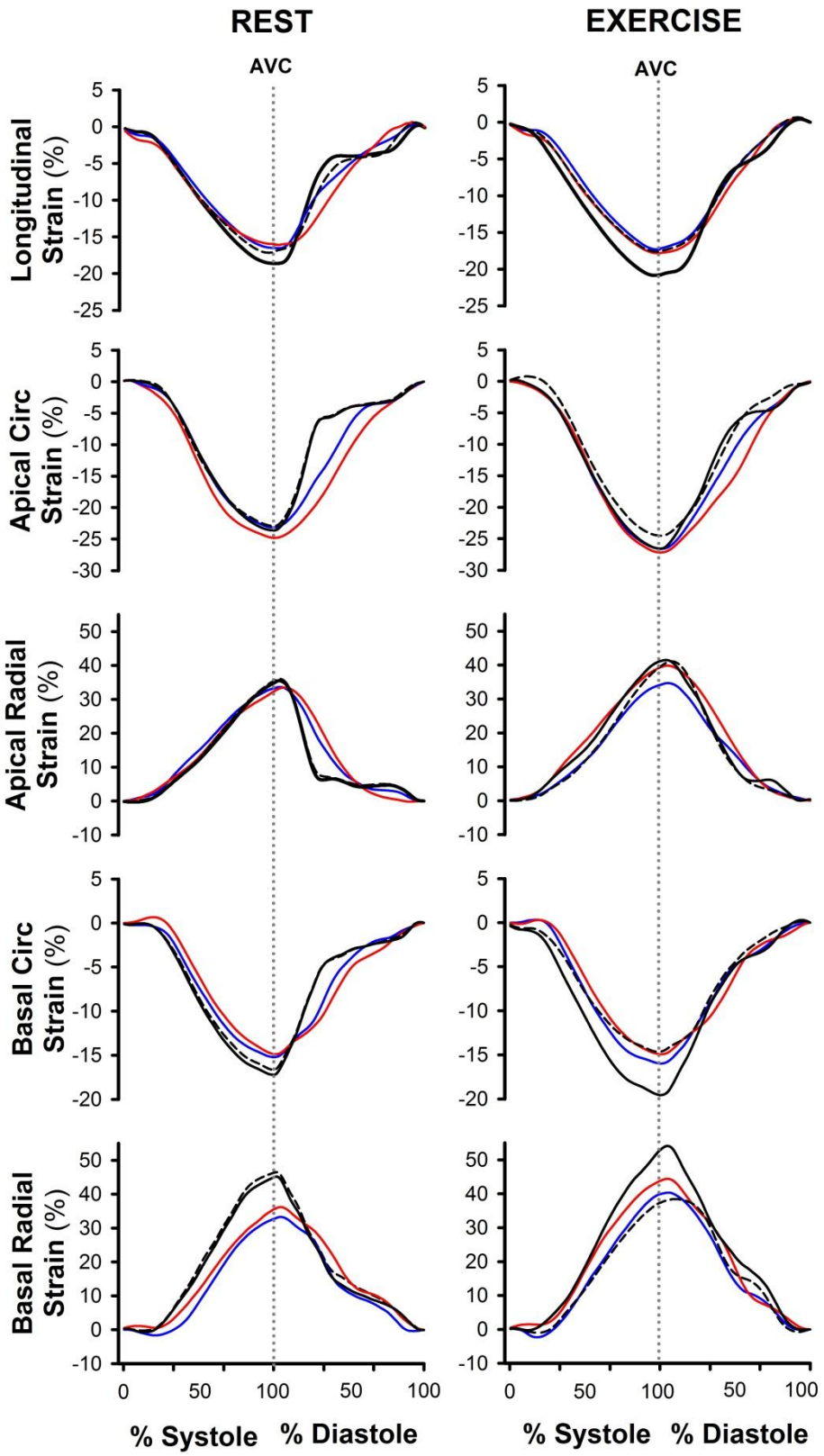

Figure 5-3. Graphical representation of left ventricular (LV) strain over the course of an entire cardiac cycle at control, dehydration and rehydration (n=8). With the exception of a small but significant reduction in longitudinal strain, LV strain was maintained throughout all conditions. Vertical dashed line shows aortic valve closure (AVC). Circ: Circumferential. For the purpose of clarity error bars have been omitted, values are provided in table 5-2.

- Control; - 2% dehydration; - 3.5% dehydration; - - Rehydration

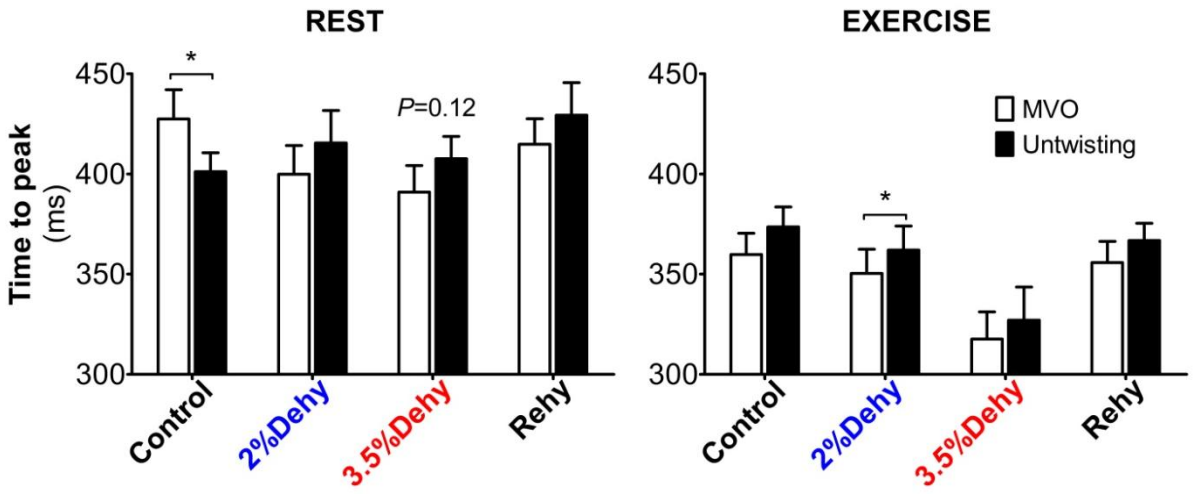

Figure 5-4. Time to peak left ventricular untwisting velocity in relation to mitral valve opening (MVO) (n=8). Although the effect was small, there was a general trend for peak LV untwisting velocity to occur after MVO. This was not fully reversed following rehydration. *: *P*<0.05.

5.4 Discussion

The main aim of this study was to examine whether the decline in SV caused by the combination of dehydration and hyperthermia during exercise would be in part underpinned by reduced LV mechanics. This study provides five novel findings: 1) Dehydration significantly reduces EDV and SV at rest and during knee-extensor exercise, 2) Systolic twist mechanics are slightly enhanced with dehydration at rest but not during exercise, 3) Diastolic twist mechanics are unaltered with dehydration at rest and during exercise, 4) Longitudinal strain and diastolic longitudinal strain rate are slightly reduced with dehydration at rest and during exercise and 5) Peak LV untwisting velocity tends to be delayed with dehydration at rest and during exercise. Together, the findings show that dehydration at rest and during small muscle mass exercise results in a large reduction in SV caused by a decreased EDV whilst LV mechanics are maintained or even slightly enhanced, suggesting that the decline in SV is likely caused by peripheral factors and not by a reduction in LV function *per se*.

Global left ventricular function

The present study shows for the first time that the combination of dehydration and hyperthermia at rest and during small muscle mass exercise results in a significant reduction in EDV which is not compensated for by the smaller decline in ESV, causing a decrease in SV of approximately 20 ml. These results indicate that the decline in SV is to a large extent the result of reduced LV filling. Both dehydration and hyperthermia have been shown to independently reduce venous return as indicated by lower cardiac filling pressures at rest (Kirsch et al., 1986, Wilson et al., 2007). Thus, it is likely that the large reduction in EDV, and thus SV, is related to a decrease in venous return. In accordance with the previously observed restoration of SV with fluid replacement during exercise (Hamilton et al., 1991, Montain and Coyle, 1992), rehydration in the present study fully restored body mass, core temperature, EDV, ESV and SV to baseline levels, showing that the observed effects of dehydration and hyperthermia were transient in nature and likely unrelated to the preceding exercise.

Whilst the maintenance of resting cardiac output following exercise-induced dehydration is in agreement with previous studies (Lynn et al., 2009), maintained cardiac output with small muscle mass exercise and dehydration differed from previous studies (González-Alonso et al., 1995, González-Alonso et al., 1997, Hamilton et al., 1991, Montain and Coyle, 1992). The differential response during exercise can likely be attributed to the relatively low exercise intensities in the present study which enabled a compensatory increase in heart rate. However, despite the lower exercise intensity and smaller muscle mass used in this study, the ~20 ml decline in SV observed at rest and during exercise in this study was comparable to that previously seen with dehydration during whole-body exercise (González-Alonso et al.,

1995, González-Alonso et al., 1997). Thus, similar levels of dehydration appear to result in comparable absolute reductions in SV irrespective of exercise modality and intensity.

Systolic left ventricular mechanics

Further to the observed decline in EDV, ESV and SV this study shows that except for a small but significant reduction in longitudinal strain, systolic LV mechanics are maintained or even significantly enhanced with progressive dehydration at rest and during knee-extensor exercise. Longitudinal strain has previously been shown to be sensitive to reductions in preload (Choi et al., 2008). The small reduction in longitudinal strain in the present study was, therefore, likely caused by the lower venous return rather than a reflection of impaired intrinsic myocardial function. Moreover, systolic radial and circumferential strain was maintained with dehydration at rest and during exercise. In the face of a large reduction in preload this maintenance of strain and systolic strain rates further suggests that intrinsic myocardial contractility was actually enhanced and overall contractile state was maintained with dehydration. Thus, reduced myocardial shortening expected from a decreased EDV appears to be compensated for by an increase in sympathetic activity induced by dehydration and hyperthermia as previously shown (González-Alonso et al., 1999a) and subsequent improvements in LV myocardial function.

The increase in systolic LV twist observed with progressive dehydration at rest was probably also mediated by an enhanced sympathetic activity. This finding is in contrast with the previous observation of a significant reduction in LV mechanics at rest following ultra-endurance exercise that was accompanied by ~4.5% dehydration (Nottin et al., 2009). In this

study, the lower SV and the higher heart rate compared with that reported by Nottin et al. (2009) indicate that the acute cardiovascular stress was higher, yet LV mechanics were maintained or even enhanced. The present results, therefore, suggest that the significant reduction in LV mechanics reported by Nottin et al. (2009) may well have been a consequence of the prolonged exercise rather than that of ensuing dehydration. Conversely, in the present setting the observed changes in systolic LV mechanics were likely caused by dehydration as rehydration fully restored global LV function and LV mechanics.

Whilst strain and strain rates were maintained with dehydration and hyperthermia during exercise (indicating a similar contractile state to that seen at rest), peak systolic basal rotation and twist did not increase from *Control* exercise to *3.5% dehydration* exercise. Given that MAP was significantly higher during *2%* and *3.5% dehydration exercise* maintained LV circumferential and radial strain and LV twist may indicate an important compensatory mechanism. However, as previously discussed in chapter 4, the significant decline in MAP with progressive dehydration would be expected to increase LV twist (Dong et al., 1999). Thus, the present data suggest that dehydration prevented an increase in LV twist during knee-extensor exercise. Considering that the magnitude of dehydration and the response in EDV was identical at rest and during exercise, it appears that the difference in MAP may have caused the variation in the LV twist response at rest and during exercise. Future studies may wish to explore the impact of altered blood pressure during exercise upon LV twist.

Diastolic left ventricular mechanics

LV untwisting velocity has been shown to be related to diastolic ‘suction’ (Notomi et al.,

2008, Firstenberg et al., 2001, Nelson et al., 2010a). In this study, LV untwisting was maintained with dehydration and rehydration at rest and during small muscle mass exercise. Thus, it can be assumed that LV suction was also maintained. Maintained suction concomitant with a reduced venous return (Kirsch et al., 1986), however, will result in a reduced LV filling as evidenced by the significant decline in EDV in this study. Whilst maintained LV untwisting indicates that the reduction in EDV and the resulting decline in SV with dehydration and hyperthermia is probably not caused by reduced LV function *per se*, it remains unknown why LV untwisting was unaltered at rest. Given that systolic twist increased at rest and the kinetic energy required for diastolic untwisting is thought to be stored in systole (Helmes et al., 2003, Notomi et al., 2006, Granzier and Labeit, 2004), it may seem surprising that LV untwisting did not also increase. However, it is important to note that the increase in systolic twist and twist velocity at rest was solely caused by enhanced basal rotation/rotation velocity. Conversely, previous studies have shown that LV untwisting is largely determined by diastolic apical function (Helle-Valle et al., 2005, Notomi et al., 2006, Opdahl et al., 2008). In this study, neither diastolic basal rotation velocity nor diastolic apical rotation velocity increased with dehydration at rest. Thus, it appears that the combination of dehydration and hyperthermia inhibits diastolic basal and apical function at rest and during small muscle mass exercise. A central characteristic of dehydration with hyperthermia that may possibly explain the absence of an increase in LV untwisting was the pronounced decline in EDV of approximately 30ml. Smaller reductions in EDV of 15 and 17 ml caused by heat stress (study 1) and lower body negative pressure (Esch et al., 2010), respectively, are accompanied by significantly enhanced LV untwisting velocities. Thus, it is possible that the actual reduction in blood volume consequent to dehydration, as opposed to a mere redistribution of blood induced by heat stress or lower body negative pressure, has prevented

an increase in LV untwisting velocity.

Similar to the response in diastolic LV mechanics at rest, diastolic LV mechanics during knee-extensor exercise were maintained at *control* levels throughout all conditions of dehydration and rehydration. This response differs from that of systolic LV mechanics, which showed an increase with dehydration at rest and maintenance during exercise. As discussed in the previous section the difference between resting and exercising systolic LV mechanics may be explained by the altered MAP response during exercise. In contrast, the similarity of diastolic LV mechanics during rest and exercise may be predominantly influenced by the prevailing preload as EDV was identical at rest and during exercise. The present study thereby provides novel insight into LV mechanics by demonstrating that systolic and diastolic LV mechanics can be uncoupled as a consequence of concomitant changes in preload and afterload. This finding warrants further investigation as it could also have important clinical implications for the understanding of cardiac dysfunction.

5.5 Conclusion

The present study shows that the marked reduction in SV caused by progressive dehydration at rest and during small muscle mass exercise is the result of reduced LV filling as indicated by the decline in EDV. Despite the large reduction in EDV, systolic and diastolic LV mechanics are maintained or even slightly enhanced. Thus, it is concluded that the reduction in SV with dehydration and hyperthermia at rest and during small muscle mass exercise is closely related to the decreased EDV and does not appear to be the result of reduced LV function but likely caused by peripheral factors such as the lower venous return.

CHAPTER 6

Study 3

Effect of continuous and discontinuous incremental exercise on systolic and diastolic left ventricular mechanics

6.1 Introduction

It is well-documented that in healthy individuals, stroke volume (SV) increases at the onset of incremental exercise when this is performed in normothermic and euhydrated conditions (Poliner et al., 1980, Higginbotham et al., 1986, Mortensen et al., 2008). This increase in SV is paralleled by a rise in heart rate, the product of both providing the higher cardiac output required to match an enhanced blood flow demand from the contracting skeletal muscles and other metabolically active tissues. Similarly, it has been shown that at low and moderate exercise intensities, systolic and diastolic LV mechanics are significantly enhanced in healthy individuals (Notomi et al., 2006, Doucende et al., 2010), whereas patients with hypertrophic cardiomyopathy are unable to improve LV twist or untwisting during mild effort (Notomi et al., 2006). Although these findings underline the importance of dynamic twist and untwisting for normal LV function during exercise in individuals without any known cardiac disease, the normal response of LV mechanics to exercise intensities exceeding ~40% maximal exercise capacity is not known.

In the first two studies of this thesis the combination of exercise with heat stress or dehydration resulted in maintained LV mechanics. It was concluded that the reduced venous return caused by heat stress and dehydration may have impacted on LV mechanics. The significant increase in LV mechanics during low and moderate exercise, the latter of which is also characterised by an increase in EDV as outlined in chapter 2, further supports the importance of preload as a determinant of LV mechanics during exercise. Although some investigators have contested the existences of a plateau in SV during incremental exercise (Gledhill et al., 1994, Warburton et al., 2002), clear evidence exists that SV plateaus at approximately 40 – 50% maximal exercise capacity (Higginbotham et al., 1986, Mortensen et

al., 2005, Mortensen et al., 2008, Rowland, 2009b). Furthermore, studies have shown that the SV response is accompanied by a plateau in EDV (Poliner et al., 1980), therefore suggesting that preload does not increase further above ~50% maximal exercise capacity. Conversely, inotropic state appears to increase continuously during incremental exercise to the point of volitional fatigue (Galbo et al., 1975). As discussed in previous chapters, progressively increasing inotropic state could be expected to continuously enhance LV mechanics. However, given the impact of altered preload on LV mechanics observed in the first two studies of this thesis, it is possible that in the healthy human heart LV twist mechanics and in particular diastolic untwisting may not increase further during incremental exercise at intensities exceeding 50% maximal capacity. Examining LV mechanics during incremental exercise in healthy individuals would, therefore, further the current knowledge on LV function during incremental exercise and provide important insight into the determinants of LV mechanics.

In view of the current findings the aim of the present study was to determine the normal response of LV twist mechanics during incremental exercise to near maximal levels. We hypothesised that the plateau in stroke volume would be associated with a concomitant plateau in LV twist and/or untwisting.

6.2 Methods

6.2.1 Study population

Following local ethical approval, nine healthy recreationally active males (age 26±4 years, height 175.1±4.9 cm, peak power 249±31 W, peak heart rate 173±14 bpm) provided verbal and written informed consent to take part in the study. To ensure optimal echocardiographic images, participants were examined for quality of images prior to enrolment.

6.2.2 Habituation and exercise testing

Participants attended the laboratory a total of four times with visits separated by at least 48 and at most 72 hours. On day one and two, participants were familiarised with supine cycling in the left lateral position tilted at a 45° angle (Lode, Angio 2003, Groningen, Netherlands). On day three, each participant performed an incremental exercise test to volitional fatigue from which individual peak power (Lode, Angio 2003, Groningen, Netherlands) and peak heart rate (Vivid 7, GE Medical, Horton, Norway) were determined. On the experimental day, following ten minutes of rest on the supine cycle ergometer, each participant performed incremental exercise to volitional fatigue. To have confidence that our results were reproducible and that the observation would be directly related to the exercise performed each participant completed both a continuous and discontinuous exercise protocol in a randomized order separated by one hour of rest. Exercise during the continuous and discontinuous trial was performed for four minutes at 10%, 30%, 50%, 70% and 90% of the peak power achieved at the end of the previous incremental exercise test. In addition to the counter balanced order of the two trials, the order of exercise stages in the discontinuous trial was also randomised. Following completion of each stage during the discontinuous trial, participants were given 5 minutes recovery in the supine position to allow HR to return to

baseline. To avoid changes in hydration status participants were provided with a 4.5% glucose solution to drink *ad libitum* between the continuous and discontinuous exercise protocols.

Throughout both trials, mean arterial blood pressure was assessed using a beat-by-beat arterial blood pressure monitoring system (FinometerPRO, FMS, Finapres Measurement Systems, Arnhem, Netherlands) and recorded continuously for off-line analysis (PowerLab, ADInstruments, Chalgrove, UK). Mean arterial blood pressure was calculated as the average blood pressure obtained from the beat-by-beat pressure waveforms during the last two minutes of each exercise stage (Chart Version 5.5.6, ADInstruments, Chalgrove, UK). Heart rate (HR) was recorded continuously via the ECG inherent to the ultrasound (Vivid 7, GE Medical, Horton, Norway).

6.2.3 Echocardiography

Echocardiographic images for the assessment of systolic and diastolic LV volumes and LV mechanics were acquired and analysed as outlined in chapter 3 of this thesis.

6.2.4 Statistical analysis

Analyses were performed as outlined in chapter 3. In addition, comparison between the responses during the continuous and discontinuous trial was performed using two-way repeated measures ANOVA. Stepwise forward multiple regression analysis was used to test the strength of relationships between heart rate, stroke volume and cardiac output as independent and LV twist, twist velocity and untwisting velocity as dependent variables.

6.3 Results

6.3.1 Left ventricular volumes and arterial blood pressure

During both continuous and discontinuous incremental exercise, EDV and SV were significantly increased whilst ESV was significantly decreased compared with rest (all $P<0.01$, fig. 1). Following the initial change at the onset of exercise, EDV reached a plateau at ~30% peak power while ESV and SV reached a plateau at ~50% peak power, respectively. However, as a result of the progressive rise in heart rate, cardiac output increased continuously (all $P<0.01$). Due to a significantly higher HR during the continuous trial the increase in cardiac output was also larger during the continuous protocol (both $P<0.01$). In contrast, the increase in MAP was significantly lower during the continuous trial ($P<0.01$). During both exercise modalities, iso-volumic relaxation time (IVRT) declined continuously and was not significantly different between the trials. ($P<0.01$, table 6-1 and figure 6-1)

Table 6-1. Systemic haemodynamics and global cardiac function at rest and during incremental exercise.

		Exercise intensity (% peak power)					
		Rest	**10%**	**30%**	**50%**	**70%**	**90%**
Cardiac output ($L \cdot min^{-1}$)	*cont.*	5,5 ± 0,8	9,8 ± 0.7*	13,2 ± 1.9*†	16,6 ± 2.0*†‡	20,1 ± 1.8*†‡$	21,1 ± 1.6*†‡$
	discont.	5,2 ± 0,6	10,7 ± 2.5*	12,7 ± 2.5*†	16,3 ± 2.7*†‡	17,1 ± 2.0*†‡	19,2 ± 1.5*†‡$
Heart rate ($beats \cdot min^{-1}$)	*cont.*	62 ± 9	95 ± 10*	112 ± 17*†	134 ± 19*†‡	159 ± 18*†‡$	171 ± 14*†‡$&
	discont.	63 ± 7	105 ± 14*	117 ± 21*†	138 ± 21*†‡	147 ± 16*†‡	158 ± 12*†‡$&
Stroke volume (ml)	*cont.*	89 ± 8	104 ± 11*	118 ± 13*†	125 ± 15*†	127 ± 11*†	124 ± 14*†
	discont.	84 ± 5	101 ± 12*	109 ± 11*	119 ± 13*	117 ± 8*	122 ± 9*†‡
EDV (ml)	*cont.*	135 ± 6	145 ± 13	153 ± 10*	153 ± 14*	153 ± 11*	148 ± 18
	discont.	128 ± 10	138 ± 10	139 ± 11	145 ± 13	145 ± 10*	150 ± 11*
ESV (ml)	*cont.*	46 ± 4	41 ± 6*	35 ± 8*	28 ± 8*†‡	26 ± 8*†‡	24 ± 10*†‡
	discont.	44 ± 6	37 ± 8	31 ± 8*	26 ± 7*†	28 ± 8*	28 ± 8*
IVRT (ms)	*cont.*	75 ± 10	69 ± 11*	58 ± 6*†	51 ± 10*†	44 ± 9*†‡	44 ± 11*†‡
	discont.	75 ± 16	67 ± 12	55 ± 10*	50 ± 12*†	45 ± 12*†	41 ± 14*†
MAP (mmHg)	*cont.*	79 ± 8	99 ± 5*	104 ± 4*	109 ± 6&	113 ± 9*†‡	121 ± 10&
	discont.	84 ± 9	98 ± 8*	97 ± 10*	110 ± 12*‡	120 ± 13*†‡	126 ± 12*†‡$

EDV: end-diastolic volume; ESV: end-systolic volume; IVRT: iso-volumic relaxation time; MAP: mean arterial pressure. *: $P < 0.01$ compared with rest †: $P < 0.01$ compared with 10%; ‡: $P < 0.01$ compared with 30%; $: $P < 0.01$ compared with 50% exercise; &: $P < 0.01$ compared with 70%.

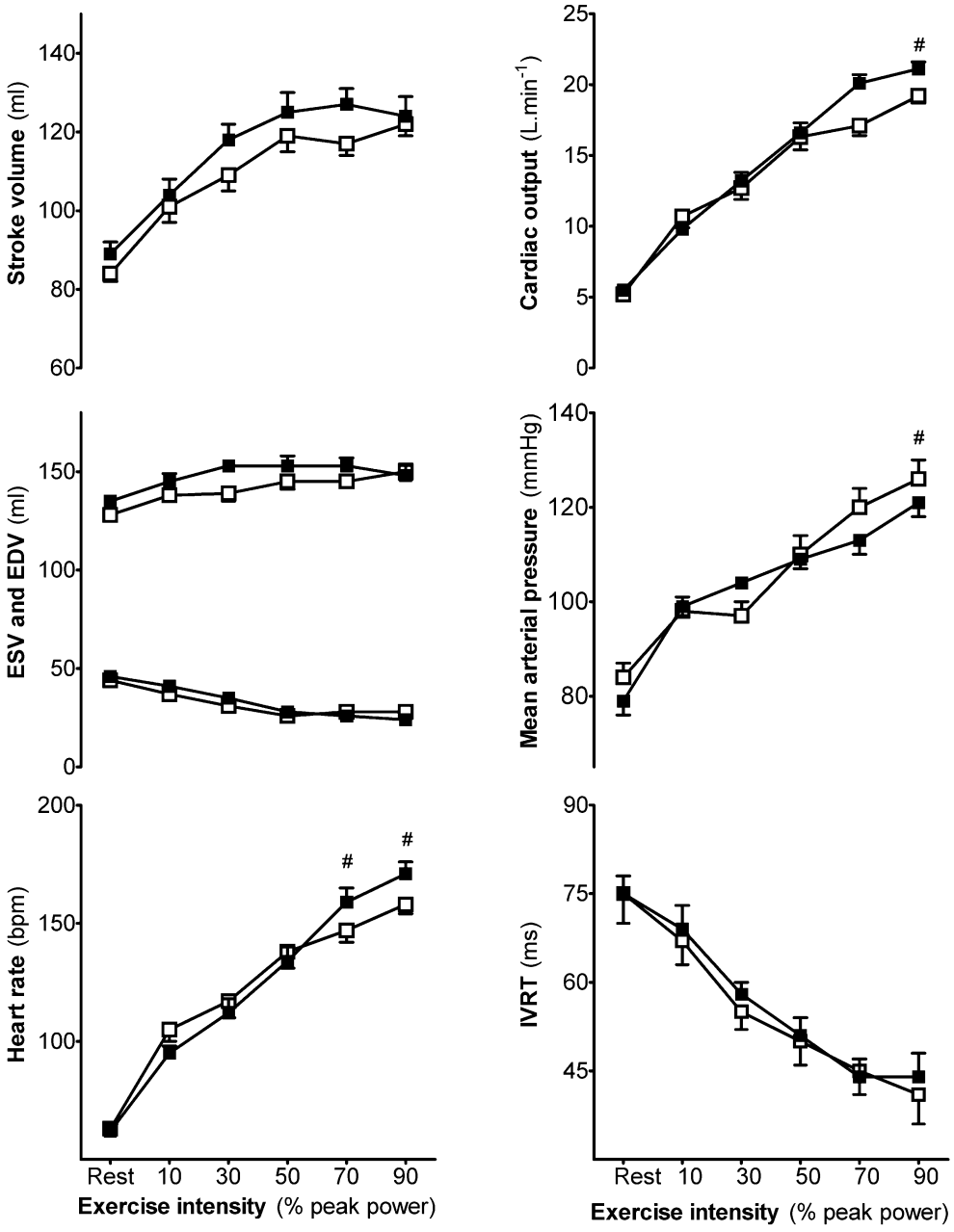

Figure 6-1. Systemic cardiovascular and global left ventricular function during continuous and discontinuous incremental exercise. Mean arterial pressure (MAP), heart rate (HR) and cardiac output increased continuously while end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV) reached a plateau at sub-maximal exercise intensities. Filled squares represent continuous and open squares discontinuous exercise. Data are mean ± SEM. #: $P<0.01$ between continuous and discontinuous trials (n=9). Differences between exercise intensities are presented in table 6-1.

6.3.2 Left ventricular twist mechanics

Peak LV systolic and diastolic basal rotation, apical rotation, twist and the respective velocities increased significantly from rest to exercise (all $P<0.01$, fig. 6-2, table 6-2). Similar to the stroke volume response, the increase in LV twist, twist velocity and untwisting velocity reached a plateau at ~50% peak power and remained at this level for all subsequent exercise stages. Compared with the discontinuous trial, twist and twisting velocity were significantly higher during the last two stages of continuous incremental exercise ($P<0.01$), however, all other LV twist indices did not differ between the two protocols ($P>0.05$).

Table 6-2. Peak systolic and diastolic LV twist indices at rest and during incremental exercise.

		Exercise intensity (% peak power)					
		Rest	**10%**	**30%**	**50%**	**70%**	**90%**
Peak (systole)							
Twist	*cont.*	13.9 ± 3.9	15.4 ± 2.4	19.8 ± 5.1*	23.8 ± 6.0*†	26.8 ± 4.2*†‡	24.7 ± 6.9*†
(deg.)	*discont.*	11.0 ± 4.2	17.2 ± 3.9	20.1 ± 6.3*	25.3 ± 7.0*	21.2 ± 3.6*	22.4 ± 3.1*
Apical rotation	*cont.*	10.0 ± 2.7	11.7 ± 2.2	15.4 ± 4.7	18.3 ± 5.6*†	19.5 ± 3.1*†‡	18.5 ± 5.2*†
(deg.)	*discont.*	8.0 ± 3.4	14.3 ± 3.4*	16.5 ± 4.3*	19.1 ± 5.4*	16.8 ± 3.3*	15.8 ± 2.8*
Basal rotation	*cont.*	-4.4 ± 2.3	-4.2 ± 2.0	-6.2 ± 2.3*†	-6.5 ± 2.6†	-8.0 ± 2.5†	-7.8 ± 3.3
(deg.)	*discont.*	-3.6 ± 2.3	-4.2 ± 2.1	-4.3 ± 2.8*†	-7.2 ± 3.7†	-5.6 ± 2.2†	-7.1 ± 2.5
Twist vel.	*cont.*	103 ± 21	105 ± 18	150 ± 31*†	200 ± 49*†‡	232 ± 35*†‡	216 ± 42*†‡
($deg.sec^{-1}$)	*discont.*	91 ± 22	130 ± 36	147 ± 42*	208 ± 43*†‡	193 ± 38*	206 ± 53*
Basal rot. vel.	*cont.*	-63 ± 15	-73 ± 19	-95 ± 30*	-114 ± 42*	-141 ± 32*†‡	-142 ± 36*†‡
($deg.sec^{-1}$)	*discont.*	-57 ± 24	-71 ± 29	-74 ± 29	-113 ± 43*†	-106 ± 32*	-113 ± 36*†
Apical rot. vel.	*cont.*	89 ± 22	107 ± 32*	149 ± 37*†	183 ± 43*†	199 ± 45*†	192 ± 44*†
($deg.sec^{-1}$)	*discont.*	75 ± 36	131 ± 28*	150 ± 30*	177 ± 48*	178 ± 53*	163 ± 52*
Peak (diastole)							
Basal rot. vel.	*cont.*	67 ± 22	70 ± 22	80 ± 22	93 ± 41	122 ± 44*†	103 ± 35
($deg.sec^{-1}$)	*discont.*	59 ± 19	65 ± 16	74 ± 37	89 ± 28	93 ± 34*	90 ± 32
Apical rot. vel.	*cont.*	-69 ± 18	-111 ± 35*	-165 ± 74*	-235 ± 76*†‡	-301 ± 118*†‡	-267 ± 106*†‡
($deg.sec^{-1}$)	*discont.*	-66 ± 35	-132 ± 57*	-180 ± 70*	-223 ± 99*	-235 ± 100*†	-254 ± 97*†
Untwisting vel.	*cont.*	-116 ± 28	-147 ± 39	-211 ± 61*†	-286 ± 88*†	-336 ± 110*†‡	-300 ± 101*†‡
($deg.sec^{-1}$)	*discont.*	-101 ± 38	-157 ± 59	-218 ± 96*	-264 ± 113*	-278 ± 93*†	-284 ± 94*†

Cont.: continuous incremental exercise; Deg.: degrees; Discont.: discontinuous incremental exercise; Rot.: rotation; Vel.: velocity; *: $P< 0.01$ compared with rest †: $P< 0.01$ compared with 10%; ‡: $P< 0.01$ compared with 30%; $: $P< 0.01$ compared with 50% exercise; &: $P< 0.01$ compared with 70%.

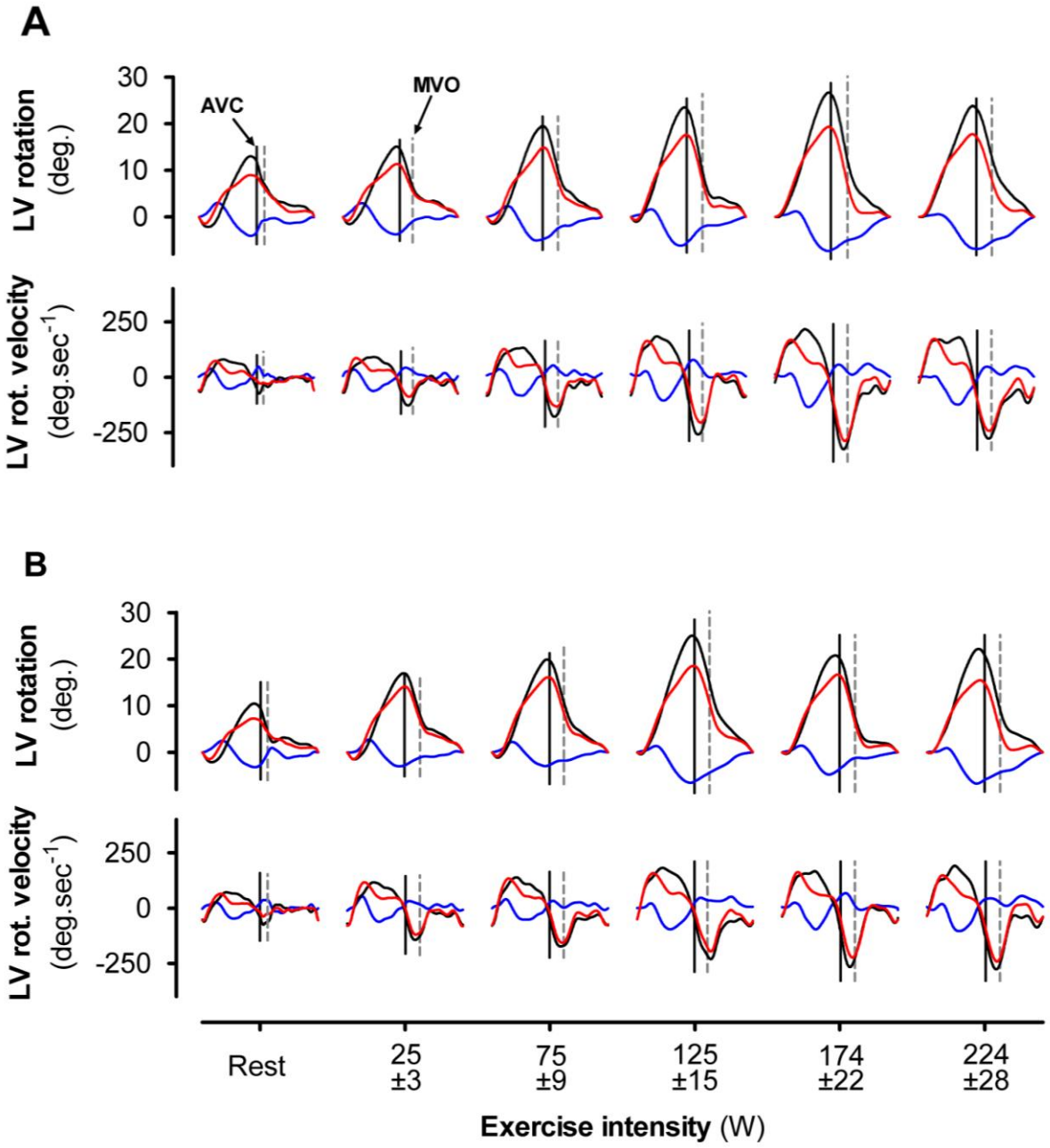

Figure 6-2. Graphical representation of the mean left ventricular (LV) twist mechanics over the course of an entire cardiac cycle during (A) continuous and (B) discontinuous incremental exercise (n=9). LV systolic and diastolic twist mechanics increased continuously up to 50% of maximal exercise capacity. Red, blue and black lines represent apical rotation, basal rotation and twist, respectively. Vertical lines show aortic valve closure (AVC, continuous line) and mitral valve opening (MVO, dashed line). Rot.: rotation. For the purpose of clarity error bars have been omitted; values and statistics are provided in table 6-2.

In both trials, the increase in systolic and diastolic apical rotation velocity from rest to 90% maximal exercise capacity was significantly higher than the increase in basal rotation velocity (P<0.01, fig. 6-3).

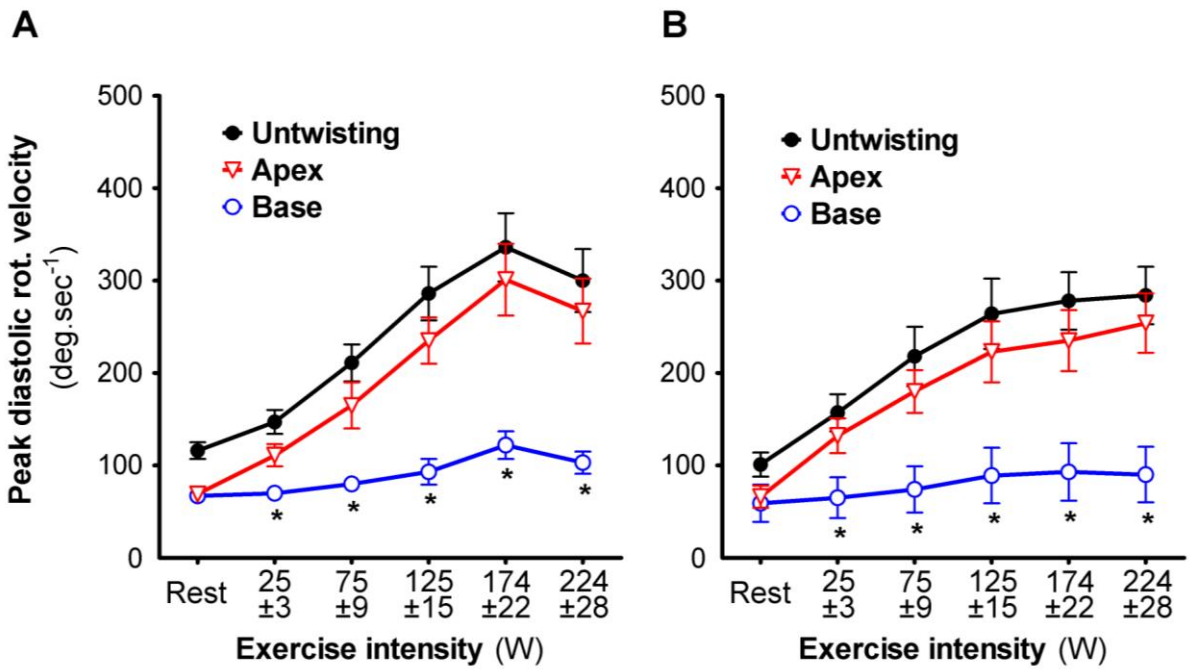

Figure 6-3. Peak diastolic rotation velocities at rest and during incremental exercise. Peak diastolic basal untwisting was significantly lower at all exercise intensities compared with peak diastolic apical untwisting. Thus, the increase in LV untwisting velocity is almost solely meditated by enhanced apical untwisting. Deg.: degrees; Rot.: rotation. Data are mean ± SEM. *: P<0.01 compared with apical diastolic velocity (n=9).

In addition to the significant change in peak values, temporal analysis showed that peak diastolic apical rotation and peak untwisting velocity reached their peak before mitral valve opening at all exercise stages (P<0.01) whereas peak LV diastolic basal rotation velocity occurred significantly after mitral valve opening at 70 and 90% maximal exercise capacity (all P<0.01, figure 6-4).

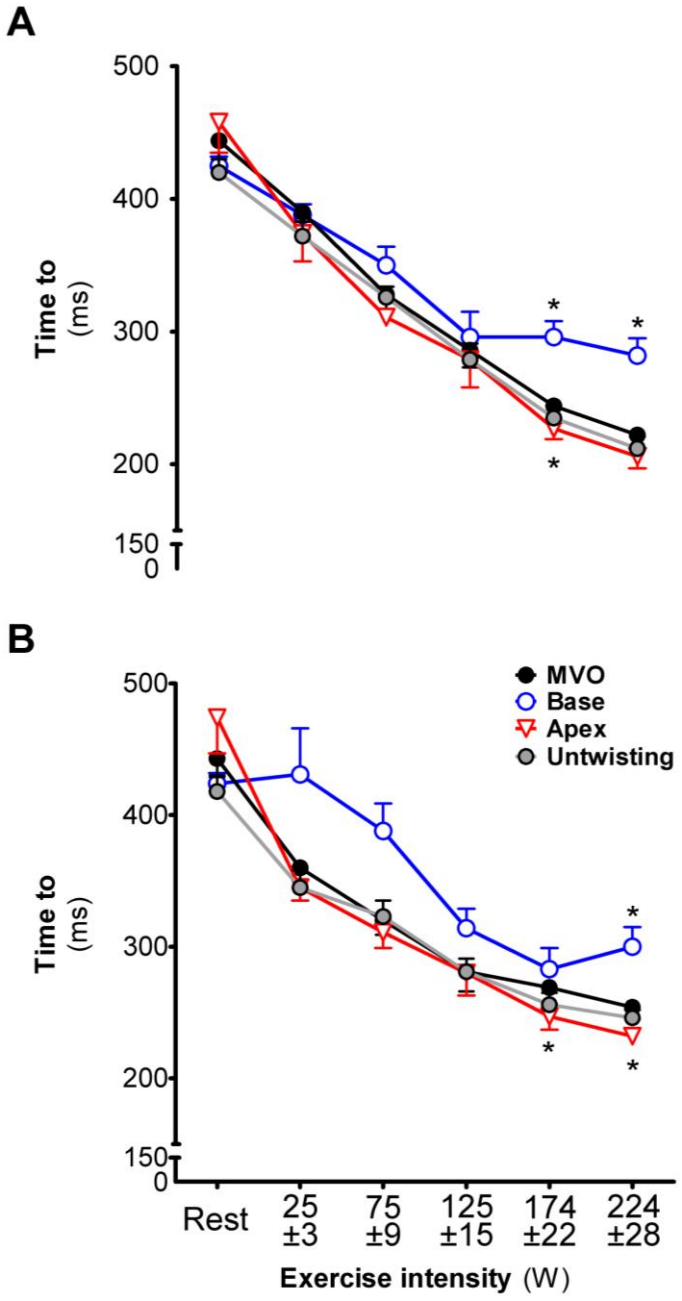

Figure 6-4. Time to peak diastolic velocity in relation to mitral valve opening (MVO). In parallel to the significant reduction in the time to mitral valve opening with increasing exercise intensity, time to peak LV diastolic apical rotation velocity and untwisting velocity was also shortened and their peaks were reached at the same time point as mitral valve opening. In contrast, time to peak diastolic basal rotation velocity did not shorten continuously and its peak occurred significantly later than MVO at 70 and 90% of maximal exercise capacity. Data are mean ± SEM. *: *P*<0.01 compared with time to mitral valve opening for base and apex, respectively (n=9).

Multiple regression analysis was used to identify the strongest relationship amongst heart rate, stroke volume and cardiac output as independent variables and LV twist, twist velocity and untwisting velocity as dependent variables. During both continuous and discontinuous exercise, cardiac output and LV untwisting showed the closest relationships (r^2=.93 and .96, respectively, $P \leq 0.001$, figure 6-5). LV untwisting also correlated with stroke volume (r^2=.91 and .97, $P \leq 0.001$) and heart rate (r^2=.91 and .96, $P \leq 0.001$). Furthermore, during continuous and discontinuous incremental exercise LV twist and untwisting velocity were strongly related (r^2=.99 and r^2=.85, respectively, $P \leq 0.01$).

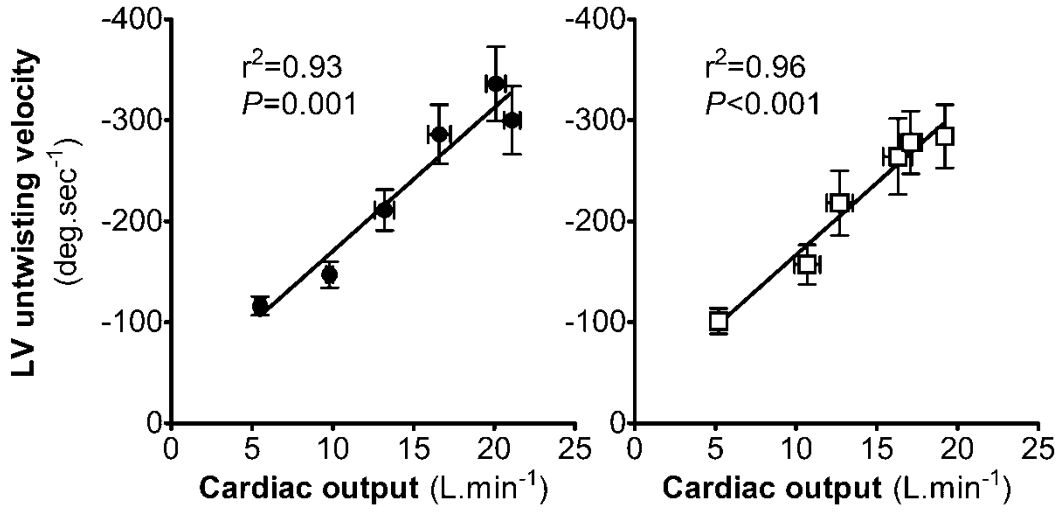

Figure 6-5. Relationships between left ventricular (LV) untwisting velocity and cardiac output during continuous and discontinuous incremental exercise (n=9). During both continuous and discontinuous exercise, cardiac output was the strongest significant predictor for LV untwisting velocity. Data are mean ± SEM.

6.4 Discussion

This is the first study to examine LV twist mechanics during continuous and discontinuous incremental cycling exercise up to intensities of ~90% peak power in healthy individuals.

There were four novel findings: 1) Similar to the observed plateau in stroke volume, LV systolic and diastolic twist mechanics also reached an upper limit at approximately 50% peak power and remained constant thereafter, 2) At all exercise intensities enhanced peak LV untwisting velocity was the result of higher apical rather than basal untwisting, 3) Peak LV untwisting velocity occurred prior to mitral valve opening at all exercise intensities 4) There were little differences in the response of LV twist mechanics between discontinuous and continuous incremental exercise.

Left ventricular twist mechanics and stroke volume

In the present study, the change in EDV, ESV and SV during continuous and discontinuous incremental exercise was similar to that reported by previous studies, demonstrating an initial increase up to exercise intensities of 30–50% maximal exercise capacity followed by a plateau which continued up to 90% peak power.(Higginbotham et al., 1986, Astrand et al., 1964, Poliner et al., 1980, Mortensen et al., 2005) Although some studies have proposed that LV volumes increase continuously up to the point of volitional fatigue,(Warburton et al., 2002, Gledhill et al., 1994) these findings have likely been influenced by the method used to assess LV volumes, the interpretation of the findings and the exercise protocols employed (Rowland, 2009a). The present data, however, strongly support the concept of a sub-maximal plateau in stroke volume during both continuous and discontinuous incremental exercise in healthy individuals by showing that the underpinning LV systolic and diastolic twist velocities also reach their peak at approximately 50% exercise capacity. Taking into account previous reports of a continuously increasing central venous pressure up to $\dot{V}O_2$max (Mortensen et al., 2005, Higginbotham et al., 1986), the present

findings suggest that the plateau in stroke volume during incremental exercise may be in part related to the inability of the LV to further improve diastolic filling at exercise intensities exceeding 50% maximal exercise capacity. Whilst the present data are the first to provide direct evidence of a limitation in the underpinning LV twist mechanics to facilitate further increases in SV beyond moderate exercise intensities the exact mechanisms for the plateau in LV twist and untwisting during incremental exercise are currently not clear. Previous studies have demonstrated that sympathetic stimulation enhances LV twist mechanics (Helle-Valle et al., 2005, Dong et al., 1999) and that during incremental exercise sympathetic activity increases progressively up to the point of volitional fatigue (Galbo et al., 1975). Accordingly, it might have been expected that LV twist and untwisting also increase in a similar fashion dependent upon exercise intensity. The lack of a continuous increase in LV mechanics in the present study may be indicative of a mechanical constraint during moderate to high levels of exercise. It has been shown that greater myocardial compliance, including the influence of the pericardium (Levine, 2008), plays an important role in the generation of a large EDV at a given filling pressure (Levine et al., 1991). During incremental exercise, the plateau in diastolic LV twist mechanics may, thus, be caused by the pericardium constraining end-diastolic distension. Whilst plausible, it has recently been shown that surgical removal of the pericardium does in fact decrease LV twist at rest while "LV systolic performance" is maintained (Chang et al., 2010). Although these data do not suggest a negative impact of the pericardium upon LV performance, the findings demonstrate that the pericardium does alter LV twist at rest. Furthermore, it is also possible that the present plateau in LV twist mechanics is related to the compression of tissue at end-systole. The plateau in peak systolic basal and apical rotation may reflect the maximum-compression of the myocardium. An inability to twist further during systole would in turn impact diastolic twist mechanics as the

energy required for untwisting is stored in systole (Helmes et al., 2003, Granzier and Labeit, 2004). Although theses hypotheses require further examination, it is plausible that in the intact human heart during moderate to high intensity exercise the pericardium and tissue compression properties would limit both; LV systolic and diastolic twist mechanics, which would consequently contribute to the plateau in LV ejection and filling.

Exercise-induced elevations in arterial blood pressure may further contribute to the plateau in LV twist mechanics during incremental exercise. In the present study, although absolute differences were small, MAP was significantly higher during the discontinuous protocol compared with the continuous trial. Conversely, LV twist was significantly lower during the discontinuous protocol. These data are in accordance with earlier findings showing that an increased afterload reduces peak LV systolic twist (Dong et al., 1999). Thus, enhanced arterial blood pressure may offset the positive inotropic effect caused by augmented sympathetic activity during exercise intensities exceeding 50% maximal capacity and thereby limit the increase in LV twist. Since the energy required for active diastolic untwisting is stored during systole (Helmes et al., 2003, Granzier and Labeit, 2004), the increase in arterial blood pressure may also indirectly attenuate the increase in LV untwisting velocity. Previous work has shown a negative impact of elevated afterload upon LV twist (Dong et al., 1999). Additionally, Takeuchi et al. (2008) have reported a reduced peak LV untwisting velocity in arterial hypertension at rest, further suggesting an impact of increased arterial blood pressure on LV twist mechanics. However, in contrast to the present data the authors also reported a delay in LV untwisting (Takeuchi and Lang, 2008). This study shows that during exercise, despite extensive shortening of the cardiac cycle, peak LV untwisting occurs prior to mitral valve opening at all exercise intensities. These findings strongly suggest that a delay in peak

LV untwisting velocity after mitral valve opening indicates impaired myocardial function. In the context of the hypothesis that an enhanced arterial blood pressure impacts negatively on LV twist mechanics during incremental exercise, it must be noted that the higher arterial pressure in the present study was accompanied by a significantly lower heart rate during discontinuous exercise, potentially also affecting the LV twist response. Notwithstanding, enhanced arterial pressure in particular during higher exercise intensities is likely to influence LV twist, yet the magnitude of its impact remains to be elucidated.

Differential response in basal and apical twist mechanics

To establish an intra-ventricular suction that maximises LV diastolic filling, pressure at the LV apex must be lower than at the base. Accordingly, during exercise greater apical untwisting compared with basal untwisting is beneficial for LV filling. Indeed, Doucende *et al*. (2010) showed that the increase in apical untwisting during incremental exercise up to ~40% maximal exercise capacity was significantly higher than the increase in basal untwisting. Our data further these findings by showing that this pattern is maintained up to near maximal exercise intensities, underlining the importance of an apical untwisting reserve in healthy individuals that may be absent in cardiac disease (Notomi et al., 2006). Further, the previous and present data indicate that during exercise in healthy individuals, a differential response between the LV base and the LV apex can be interpreted as a normal response. In line with this, Akagawa *et al*. (2007) observed a greater increase in apical subendocardial rotation than basal rotation following dobutamine infusion in healthy individuals at rest. Some authors have proposed that this response may be caused by region specific adrenoreceptor sensitivity (Nottin et al., 2008). Similarly, it has been shown that electrical

activation at the apex is longer than at the base (Sengupta et al., 2006a) which may potentially result in an enhanced Ca^{2+}-release and re-uptake in apical myocytes. In addition, the greater increase in apical rotation during incremental exercise could also be related to an alteration in the relative myofibre alignment between the subendocardial and subepicardial helix due to the increase in LV volumes. It has been shown that the LV wall is thinner at the apex than at the base and myofibre orientation is more circumferential (Greenbaum et al., 1981). Furthermore, a more spherical LV shape is mechanically more efficient and improves ejection (Adhyapak and Parachuri, 2009). Thus, enhanced venous return from the exercising muscles and a resultant larger EDV may have contributed to a change in myofibre alignment and increased LV twist and untwisting. These findings and the present data suggest that the LV apex, in contrast to the base, may have a greater reserve to respond to an enhanced preload. By implication, it also follows that the lower potential of the LV base to respond may represent a key factor in the limitation of overall LV twist mechanics to increase beyond 50% of maximal exercise capacity.

Similar to the observed difference in peak basal and apical rotation, the time for diastolic and rotation velocities to reach their peak also differed between the base and the apex at higher exercise intensities. Figure 6-4 shows that during exercise at 70 and 90% of peak power, peak LV diastolic basal rotation velocity occurred after mitral valve opening whereas peak apical untwisting still occurred prior to the start of early LV filling. Since both peak apical and basal diastolic untwisting plateau at these exercise intensities, it is possible that the higher peak basal untwisting later in diastole is more reflective of an enhanced atrial contraction rather than a reduction in basal function. This is in agreement with the previous observation of a good relationship between diastolic basal rotation velocity and early filling velocity (E) during moderate intensity exercise (Doucende et al., 2010). The functional significance of

this association during exercise and other conditions of altered haemodynamics and inotropic state is, however, currently not clear and requires further study.

Clinical implications

As reflected by the rapidly increasing number of clinical trials assessing LV twist and untwisting, the ability to non-invasively quantify LV twist mechanics has stimulated much interest in LV twist mechanics as a potential new marker for cardiovascular dysfunction (Russel et al., 2009, Sengupta et al., 2008a, Shaw et al., 2008). In the clinical environment, exercise tests are frequently used to uncover cardiovascular abnormalities. The present study provides insight into the normal response of LV twist mechanics during an incremental test to near maximal exercise capacity. Failure to match the presently shown exercise-induced changes in LV mechanics (namely progressive increase in LV twist mechanics up to ~50% maximal exercise capacity, peak LV untwisting velocity occurring before MVO at all exercise intensities and a greater increase in apical rotational indices compared with basal rotation parameters) may reflect underlying pathology. Furthermore, despite some small differences between trials, the present investigation also demonstrates that LV twist and untwisting are similar whether incremental exercise is performed continuously or discontinuously. The option to employ a discontinuous protocol which drastically reduces the time that individuals are exposed to acute cardiovascular stress may be preferable in some populations and conditions.

Limitations and technical considerations

At present the assessment of LV twist mechanics with 2-D speckle tracking ultrasound has

some inherent limitations pertaining to image quality and through-plane motion of myocardial tissue. While through-plane motion cannot be avoided it appears to have relatively little impact on tracking of myocardial tissue during incremental exercise as tracking in the present study was successful in >90% of myocardial segments, indicated by the tracking score provided by the software. This successful tracking was likely related to the assessment of echocardiographic image quality in participants prior to enrolment and the standardisation of image acquisition. To ensure accuracy and comparability of data, image acquisition must take into account frame rates (Helle-Valle et al., 2005), imaging depth (Sivesgaard et al., 2009) and the level of the apical short-axis view (van Dalen et al., 2008). In this study a constant frame rate of 97 frames per second between all participants and all conditions was applied. Furthermore, imaging depth within the basal and apical short-axis views was also standardised and the apical short-axis level was chosen as caudal as possible to estimate the largest "true" LV twist. Finally, as a consequence of the increasing heart rates during exercise the large changes observed in the present study are, if anything, underestimated. The much lower temporal resolution inherent to MRI and 3-D ultrasound modalities would likely result in a greater error in the assessment of LV twist indices whereas the present study demonstrates that the assessment of LV twist mechanics with 2-D speckle tracking echocardiography during incremental exercise up to near maximal peak power is feasible.

6.5 Conclusions

During incremental exercise in healthy individuals, LV systolic and diastolic twist mechanics plateau at approximately ~50% maximal exercise capacity and are closely related

to cardiac output and stroke volume. Furthermore, at all exercise intensities up to 90% of peak power, peak LV untwisting velocity occurs with or prior to mitral valve opening, underlining the importance of untwisting for early diastole and demonstrating that a delay in peak LV untwisting after mitral valve opening is likely an indication of impaired myocardial function and not the cause of a reduced diastolic duration.

CHAPTER 7

General discussion

7.1 Introduction

The main purpose of this thesis was to examine the impact of heat stress, dehydration and exercise on LV mechanics. In all three studies presented in this thesis healthy participants were repeatedly examined during progressively increasing cardiovascular challenges. Study 1 explored the effect of isolated heat stress, study 2 focused on the impact of the combined influence of dehydration and hyperthermia and study 3 examined the normal response of LV mechanics to incremental exercise in normothermic euhydrated individuals. This chapter first summarises the findings from these three studies and follows with a discussion of the similarities and differences between them. Finally, the significance of the findings and potential future directions are presented.

7.2 Summary of findings

As intended, the three studies completed for this thesis were characterised by distinctly different haemodynamics as evidenced by the maintenance of SV with heat stress, the decline in SV with dehydration and hyperthermia and the increase in SV during incremental exercise. Similar to previous studies (2010a, Brothers et al., 2009, Nelson et al., 2010b), study 1 showed that passive heat stress at rest causes a small but significant reduction in EDV that is fully offset by a decline in ESV and, thus, results in a maintained SV. Furthermore, the study provided evidence that LV twist mechanics are closely related to progressive elevations in body temperature and heart rate at rest. During the combined challenge of small muscle mass exercise and heat stress EDV, ESV and SV were identical to resting conditions; however, LV mechanics were unaltered from *control exercise* to *severe heat stress exercise*.

In contrast to the maintained SV in the first study, the combination of dehydration and hyperthermia in study 2 lead to a reduced SV at rest and during small muscle mass exercise. The study further revealed that the reductions in SV were caused by a marked decline in EDV. Despite this reduction in preload, LV twist was enhanced at rest and LV mechanics were maintained during exercise.

During incremental exercise in study 3, SV increased significantly from rest. This increase was initially achieved by an elevation in EDV and a concomitant reduction in ESV, as has been previously shown (Poliner et al., 1980, Higginbotham et al., 1986, Mortensen et al., 2005). The plateau in both, EDV and ESV, at approximately 50% peak power consequently resulted in a similar plateau in SV. Likewise, LV twist and untwisting also increased up to approximately 50% peak power and remained at this level thereafter. The strongest relationship between LV mechanics and systemic haemodynamics was observed between peak LV untwisting velocity and cardiac output.

7.3 Effect of heat stress, dehydration and incremental exercise on systolic left ventricular function

During heat stress and dehydration at rest as well as during incremental exercise at 30% peak power and above, systolic LV twist increased significantly, thereby likely improving LV ejection (Vendelin et al., 2002, Burns et al., 2008a). The increase in LV twist from *control rest* to either *severe heat stress* or *3.5% dehydration* at rest was 4.5-6 degrees. During whole-body exercise, the maximal rise in systolic LV twist from rest was approximately 13 degrees. The larger increase in LV twist during incremental exercise can be attributed to the combined

influence of enhanced preload and inotropic state (Helle-Valle et al., 2005, Opdahl et al., 2008, Rowell, 1990, Dong et al., 1999). Conversely, owing to the reduction in preload with heat stress and dehydration, the increase in LV twist at rest was probably achieved solely by enhanced contractility (Helle-Valle et al., 2005, Opdahl et al., 2008, Rowell, 1990).

Further to the already discussed influence of inotropic stimulation on systolic LV twist, previous studies have also shown that an increase in LV twist is typically caused by both, enhanced basal and apical rotation (Helle-Valle et al., 2005, Akagawa et al., 2007, Opdahl et al., 2008). The strain rate data during heat stress, dehydration and incremental exercise suggest that inotropic state was enhanced in all three studies. Thus, an increase in both, LV basal and apical rotation would be expected. Whilst the increase in LV twist during incremental exercise was indeed achieved by both, enhanced LV basal and apical rotation, the rise in LV twist during dehydration and heat stress at rest was accomplished only by increased basal rotation. One important difference between the present findings and previous studies was the altered preload. The positive effect of enhanced inotropic state on LV twist in previous studies was always accompanied by maintained or even enhanced preload (Helle-Valle et al., 2005, Akagawa et al., 2007, Opdahl et al., 2008). In contrast, within the present studies, during heat stress and dehydration at rest preload was reduced and apical rotation failed to increase. Unfortunately, there are no data available that resemble the present scenario. Those studies that have examined the influence of reduced preload on systolic LV twist have not reported the underpinning basal and apical response (Burns et al., 2010, Dong et al., 1999, Esch et al., 2010). The present data, however, strongly suggest that the decline in preload during dehydration and heat stress at rest may have prevented an increase in LV

apical rotation whilst LV basal rotation appeared to remain unaffected. This hypothesis is supported by the results from study 3 showing that incremental exercise, which increased preload, significantly enhanced both basal and apical rotation. Although uncoupling of LV basal and apical function has been previously discussed (Sengupta et al., 2006a, Sengupta et al., 2006b, Bogaert and Rademakers, 2001), to the best knowledge of the author this is the first time that studies have shown an increase in systolic basal rotation without concomitantly enhanced apical rotation. At present, the cause for this phenomenon remains unknown but may involve a change in the myocardial fibre alignment due to altered preload or altered β-adrenergic responsiveness (Lyon et al., 2008).

In studies 1 and 2 the level of heat stress or dehydration during knee-extensor exercise was the same as during resting conditions. However, in contrast to rest, peak LV twist did not significantly change from *control exercise* to *severe heat stress exercise* or *3.5% dehydration* exercise. Furthermore, MAP declined with progressive dehydration and heat stress during exercise and was only 7-8 mmHg higher with *3.5% dehydration* and *severe heat stress exercise* than the same resting conditions. Although maintained LV twist along with increased MAP or reduced preload likely represents an important compensatory mechanism during exercise, the ability of the LV to enhance LV twist appears to be limited. It must be noted, however, that although the change in LV twist with heat stress during exercise was not statistically significant, LV twist did increase by 4 degrees whereas LV twist during dehydration was truly maintained. Notwithstanding, studies 1 and 2 suggest that exercise in combination with enhanced thermoregulatory demand or altered hydration status attenuates the increase in LV twist that was seen with both heat stress and dehydration at rest. Similar to

whole-body exercise in normothermic and euhydrated conditions, elevated blood pressure may play an important role in the ability of LV twist mechanics to increase in the face of a reduced preload.

Further to the previously discussed dissociation between LV basal and apical rotation with all; progressive heat stress, dehydration and incremental exercise, a clear difference in basal rotation was observed between heat stress and dehydration during exercise. Whilst heat stress during exercise increased LV basal rotation by approximately 6 degrees, dehydration during exercise did not alter LV basal rotation. Thus, the data suggest that the combination of dehydration, hyperthermia and exercise limits both systolic LV basal and apical rotation. As outlined previously, limited apical function was likely related to a reduction in preload. However, since preload was the same with dehydration during exercise compared with dehydration at rest, the lack of an increase in basal rotation must be related to a factor other than preload. It is possible that the combination of large reductions in preload in conjunction with an increased MAP may inhibit an increase in LV basal rotation as well as apical rotation.

7.4 Effect of heat stress, dehydration and incremental exercise on diastolic left ventricular function

In the present studies, the lower venous return known to occur with heat stress (Wilson et al., 2007) and dehydration (Kirsch et al., 1986) resulted in a reduced EDV whilst enhanced venous return during incremental exercise (Poliner et al., 1980, Higginbotham et al., 1986) caused an increase in EDV. The review of literature in chapter two highlighted that LV

mechanics are sensitive to alterations in preload. Although previous studies have mostly examined the impact of altered preload on systolic LV mechanics, the present studies also show that altered preload impacts on diastolic LV mechanics.

There is increasing evidence that peak LV untwisting velocity is related to the ability of the LV to create suction and, thus, to facilitate early diastolic filling (Notomi et al., 2008, Dong et al., 2001, Esch and Warburton, 2009). Accordingly, enhanced LV untwisting velocity during passive heat stress (study 1, Nelson et al., 2010a) and during whole-body exercise (study 3, Notomi et al., 2006, Doucende et al., 2010) improves LV filling. However, the present studies also suggest that diastolic LV untwisting may have been affected by heat stress and dehydration as indicated by the dissociation with preceding systolic mechanics.

Thompson et al. (2010) recently showed that in healthy individuals at rest, systolic and diastolic shear strain rates (including twist and untwisting velocity) are closely related. A similar interaction was observed in this thesis during incremental exercise and heat stress at rest. In both conditions an increase in systolic LV twist was followed by an increase in diastolic LV untwisting. Conversely, systolic twist and diastolic untwisting velocity were dissociated during heat stress exercise and dehydration at rest. This is somewhat surprising as there is increasing evidence that the kinetic energy required for active diastolic relaxation is stored during the preceding systolic period (Granzier and Labeit, 2004, Helmes et al., 2003, Fuchs and Martyn, 2005). Consequently, a greater storage of energy during systolic contraction will result in enhanced diastolic ‘recoil’ (Notomi et al., 2006, Esch and

Warburton, 2009, Helmes et al., 2003, Thompson et al., 2010). Based on this principle the dissociation between systolic and diastolic LV mechanics seen in some of the present conditions is not clear. With progressive heat stress during exercise or dehydration at rest, LV twist increased while peak untwisting velocity was unaltered. Although it is likely that this response is caused by several factors, it appears that the reduction in preload may be of major influence.

As outlined in section 2.2.1, Esch et al. (2010) examined the influence of reduced preload on LV untwisting. The study showed that lower body negative pressure results in an increase in LV untwisting velocity in untrained individuals. This finding agrees with the results presented in study 1 at rest where preload was also reduced (Δ~15 ml) and a concomitant increase in LV untwisting velocity was seen. Conversely, the larger reduction in preload during dehydration (Δ~35 ml) was accompanied by maintained LV untwisting compared with control conditions. Although these two studies cannot be compared directly as sympathetic activity probably differed, the results suggest that a threshold may exist below which a further reduction in preload will not result in a compensatory increase in LV untwisting velocity. Furthermore, a clear link between diastolic LV untwisting velocity and apical untwisting was observed. Enhanced LV untwisting was consistently underpinned by enhanced apical untwisting whereas maintained LV untwisting was associated with maintained apical untwisting. These findings highlight the importance of LV apical function for diastole and further support the concept of an acute dissociation between LV basal and apical mechanics.

In the context of studies that have examined the mechanisms behind the lower SV with heat stress and dehydration during exercise (González-Alonso et al., 1995, González-Alonso et al., 1997, González-Alonso et al., 2000, Montain and Coyle, 1992, Hamilton et al., 1991), the present findings suggest that reduced SV is in part related to the inability of the LV to increase suction and, therefore, to compensate for the lower venous return. However, it is important to note that the present results do not show a reduction in LV function *per se* as heat stress and dehydration did not decrease LV mechanics below baseline. Although exercise intensity in the present studies during heat stress and dehydration was low, it is improbable that an improved preload during whole-body exercise would actually reduce LV mechanics. From the present results it is more likely that heat stress and dehydration during whole-body exercise will cause an attenuated increase in LV mechanics compared with that seen in normothermic and euhydrated conditions.

7.5 Comparison of knee-extensor exercise with whole-body exercise

In the first two studies of this thesis knee-extensor exercise was performed whilst in the third study whole-body exercise was chosen to maximise the increase in preload and heart rate. Owing to clear differences in the haemodynamics expected between the two different exercise modalities, this section briefly discusses the impact of both types of exercise and their impact on LV function. In contrast to whole-body exercise at the lowest intensity (25 W), constant load knee-extensor exercise (21–25 W) in *control* conditions did not cause an increase in SV. Whilst the present EDV data show that maintained SV was the result of a maintained preload, the exact cause for the lack of an increase in SV with knee-extensor exercise is not clear. As discussed in chapter 4, it is likely that higher exercise intensities

would have elicited an increase in SV as evidenced by a study in which knee-extensor exercise was performed at 30–42 W (Savard et al., 1988). Despite the clear differences in SV, LV twist remained at baseline levels during both whole-body exercise at 10% peak power and knee-extensor exercise during *control* conditions. Since the increase in SV during whole-body exercise was mostly achieved by an improved preload, the findings suggest that at low exercise intensities differences in preload may not significantly impact on systolic LV twist mechanics. The elevation in MAP does not explain this response either as it was comparable between whole-body exercise (Δ20 mmHg) and knee-extensor exercise (Δ 22-28 mmHg).

Similar to maintained LV twist, systolic strain rates remained at baseline levels during *control* knee-extensor exercise and whole-body exercise at 10% peak power, suggesting maintained contractile state. Additionally, it was noted that in all three exercise conditions heart rate was below 100 beats·min^{-1}. It is well-documented that up to heart rates of approximately 100 beats·min^{-1} vagal withdrawal predominates whereas at heart rates exceeding 100 beats·min^{-1} sympathetic activity is significantly enhanced and the vagal influence is minimal (Rowell, 1993). As outlined in chapter 2, previous studies have uniformly shown that enhanced inotropic state increase LV twist and strain rate (Helle-Valle et al., 2005, Opdahl et al., 2008, Dong et al., 1999, Akagawa et al., 2007). The maintenance of LV twist during whole-body exercise at 10% peak power, therefore, suggests that an elevation in the overall myocardial contractile state may be required to significantly enhance LV twist, irrespective of the prevailing SV and cardiac output. Augmented contractile state is possibly required to overcome the negative impact of increased afterload on LV twist as previously discussed (Dong et al., 1999). This may be an important area of future

investigation as the interaction between blood pressure and contractility is of interest in performance and health.

7.6 Significance of findings and future directions

Using an integrative approach, the findings from this thesis advance previous results by providing novel insight into LV mechanics during isolated heat stress, dehydration with hyperthermia and exercise. The findings contribute to the existing pool of knowledge related to cardiovascular function during heat stress and dehydration (Nybo, 2008, González-Alonso et al., 1999b, Rowell, 1974, González-Alonso et al., 2008a, Crandall and González-Alonso, 2010) and also further the existing knowledge on LV mechanics during exercise. The rapidly increasing number of publications investigating LV mechanics is reflective of the novel perspective that these indices offer. As seen in the present studies, LV mechanics respond specifically to acute cardiovascular challenges. Thus, the present data add to the currently limited number of interventional studies examining LV mechanics in healthy humans. The results from this thesis also highlight the need for further integrative studies as the 'healthy' behaviour of LV mechanics remains incompletely understood. Knowledge regarding normal LV mechanics may inform clinical personnel and researchers and enable the differentiation between physiological and pathological responses. Based on the findings of the present studies, some ideas for potential future projects are presented below.

In all three studies individual variability in the response in LV mechanics to progressively increasing cardiovascular stress was noticed. Some of this variability was probably caused by

differences in training status amongst individuals. Although all participants were recreationally active, the average weekly activity and the type of activity (endurance, strength, combined) differed slightly. Future studies may wish to explore the influence of training status on acute alterations in LV mechanics as it is likely that the trained heart will respond differently. Because athlete's heart is also characterised by changes in LV structure, studying individuals with athlete's heart during an acute bout of exercise will further improve our understanding of the factors that govern systolic and diastolic LV mechanics.

From a clinical perspective, many cardiovascular diseases are associated with a change in preload, afterload or both. The present studies have demonstrated that systolic and diastolic LV mechanics can be uncoupled in conditions of physiologically altered haemodynamics. In the case of systolic or diastolic heart failure in particular, the impact of altered load upon LV mechanics would be a valuable addition to the existing data. It is possible that some changes seen in LV mechanics are caused by altered load which in turn may impact predominantly on LV apical function and, thus, affect overall left ventricular performance.

In addition to examining the impact of training status and pathological changes of LV mechanics the present studies suggest that changes in arterial blood pressure may also impact on LV mechanics. Future studies may wish to explore acute and chronic alteration in blood pressure and individuals' responses to an exercise challenge. At present, it is not known whether alteration in blood pressure in healthy individuals performing exercise may be beneficial or detrimental for LV mechanics. In this context it would be important to

determine whether strength training, endurance training or a combination of both would alter LV mechanics differently.

7.7 Hypotheses

Study 1

Research hypothesis 1: LV mechanics will increase progressively with passive heat stress at rest. **ACCEPTED**

Research hypothesis 2: Heat stress during exercise will significantly increase LV mechanics compared with normothermic exercise. **REJECTED**

Study 2

Research hypothesis 1: The combination of dehydration and hyperthermia will significantly reduce LV mechanics at rest. **REJECTED**

Research hypothesis 2: Dehydration and hyperthermia during exercise will significantly reduce LV mechanics compared with exercise in a euhydrated and normothermic state. **REJECTED**

Study 3

Research hypothesis: During incremental exercise, LV mechanics will be closely related to stroke volume. **ACCEPTED**

7.8 Limitations

The methods employed in this thesis including study design, methods of data collection and data analysis have been chosen carefully and to the best knowledge of the principal investigator. Several echocardiographic methods are available for the calculation of LV volumes. The advantages and drawbacks of the chosen method are discussed below. Furthermore, in all three studies of this thesis LV mechanics were assessed using speckle tracking echocardiography. Despite the greatest effort to optimise the validity and reliability of data, some inherent limitations of this technique also deserve brief discussion.

7.8.1 Assessment of left ventricular volumes

As briefly alluded to in chapter 3.3.1, LV end-diastolic and end-systolic volumes were calculated from M-mode derived LV dimensions according to the method by Teichholz et al. (1976). Owing to the three-dimensional cardiac structure, currently the assessment of cardiac volumes using the biplane Simpson's method is favourable over the use of M-mode as it is less reliant on geometrical assumptions (Lang et al., 2005, Folland et al., 1979). However, despite its limitations relating to geometrical assumptions, M-mode derived LV volumes have been shown to be valid in "healthy individuals" with symmetrically contracting myocardium (Kronik et al., 1979), similar to the population studied in this thesis. Furthermore, the temporal resolution of M-mode (up to 2000 frames per second; Feigenbaum, 2010) far exceeds that of two- and three-dimensional echocardiography. This presents a great advantage during exercise when heart rates are high, resulting in fast myocardial movement. The inclusion of a rehydration condition in study 2 and discontinuous exercise in study 3 provided further internal validity. The similarity of results in these conditions compared with

their respective control conditions suggests that the Teichholz method reliably detected changes in LV volumes as a consequence of haemodynamic challenges in the present studies. In future studies, however, using the Simpson's biplane method or possibly 3-D echocardiography would be preferable to further reduce geometric assumptions whilst trying to maintain an appropriate temporal resolution.

7.8.2 Technical considerations regarding the assessment of left ventricular mechanics using speckle tracking ultrasound

One important determinant of the validity and reliability of LV twist and strain parameters using speckle tracking technology is the quality of 2-D images (Notomi et al., 2005a). Low image quality will result in the myocardium not being tracked properly by the software, thereby causing inaccurate LV twist and strain results. To ensure high quality of echocardiographic images in this thesis all participants underwent a pre-participation examination. Any participants who did not display optimal acoustic windows were excluded from the studies. Furthermore, image quality was maximised by standardisation of the acquisition procedure and appropriate adjustment of ultrasound settings during data collection. Particular attention was paid to maintaining the highest frame rates possible as this increases accuracy of tracking (Helle-Valle et al., 2005). In all studies, frame rates were kept constant within individuals and ranged between 77 – 97 frames per second.

Another aspect affecting the assessment of LV strain and twist by speckle tracking is related to the longitudinal displacement of the myocardium during ventricular contraction. In systole, the LV base descends towards the apex and, at least in part, leaves the imaging plane

of the ultrasound transducer. Previous authors have measured this longitudinal displacement and found it to be approximately 4mm (Helle-Valle et al., 2005). As the ultrasound slice is 2-3 mm thick (Helle-Valle et al., 2005) the displacement of the base will result in some of the speckles not being tracked accurately. Accordingly, poor tracking of LV segments in this thesis was observed more often at the base than at the apex. However, the vast majority of segments were tracked appropriately and the results from the reliability trials do not suggest a greater variability of LV rotation at the base compared with the apex. This robustness despite longitudinal displacement of the LV can likely be explained by the fact that "the STI method only requires a statistically meaningful proportion of speckles to be present on successive frames, expecting some randomness superimposed on the true motion" (Notomi et al., 2005a).

2-D derived assessment of LV twist is composed of the analysis of two separate images and, thus, different cardiac cycles. Although this limitation may affect the true evaluation of absolute LV twist there are several factors that can alleviate the impact of this issue. In all of the experimental studies of this thesis LV basal and apical short-axis images were acquired successively, thereby minimising changes in the physiological state. Moreover, images were acquired at rest or during steady state exercise, further reducing the influence of separate image acquisition. Currently, the alternative method that would avoid image acquisition from different cardiac cycles is 3-D speckle tracking ultrasound. Like MRI, however, frame rates of 3-D speckle tracking ultrasound are relatively low (below 30 frames per second, Crosby et al., 2009). Although low frame rates may not affect reliability and validity during data

acquisition at rest (Crosby et al., 2009), they would most certainly introduce a large error when used during exercise.

7.9 Summary

The present studies contribute to the existing knowledge on cardiovascular function during heat stress, dehydration and exercise as well as to the body of studies exploring LV mechanics. The findings from the first study presented in chapter 4 indicate that systolic and diastolic function is enhanced with heat stress at rest and that both, systolic and diastolic LV mechanics, are closely related to the magnitude of heat stress and ensuing heart rate. Conversely, progressive heat stress during exercise appears to attenuate the increase in systolic and diastolic LV mechanics. The second study demonstrated that systolic LV mechanics were mildly enhanced and diastolic LV mechanics were maintained despite extensive reductions in preload. However, similar to study 1 the results show that exercise may have attenuated the increase in systolic mechanics seen at rest. It appears that reductions in preload may have had a strong influence on LV mechanics in the first two studies and future investigations should further explore the effect of altered preload during heat stress and dehydration on LV mechanics. Finally, the third study described the normal response of LV mechanics to incremental exercise in healthy humans. The study showed that enhanced systolic and diastolic LV mechanics likely contribute to enhanced ejection and filling up to approximately 50% peak power. Thereafter, the plateau in LV mechanics suggests a cardiac limitation in continuously improving systolic and diastolic function up to the point of fatigue.

Future studies should aim to examine LV mechanics during incremental exercise in highly trained individuals to determine whether chronic training influences the response presented in chapter 6 of this thesis. Additionally, alteration in loading status and contractility during exercise may further improve the current understanding of systolic and diastolic LV mechanics. In healthy individuals as well as in patients with cardiovascular disease studies should focus especially on; the interaction between LV twist and strain; the potential uncoupling of systolic and diastolic mechanics and the dissociation of LV basal and apical mechanics.

7.10 Conclusions

The results from the three studies contained in this thesis show that enhanced systolic and diastolic LV mechanics contribute to an improved ejection and filling during heat stress and incremental exercise. Conversely, the combined stress of dehydration and hyperthermia appears to limit the ability of LV mechanics to increase, thus, in part explaining the large reduction in SV known to occur in these conditions. Together, the findings emphasise the importance of acute adjustments in LV mechanics during periods of increased cardiovascular demand.

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APPENDICES

Appendix I – Ethical approval

Study 1 – Heat stress

RE39-07.

Brunel UNIVERSITY WEST LONDON

University Research Ethics Committee

31 January 2008

Proposers: Mr James Pearson and Mr Eric Stöhr (submitted by Prof. Jose González-Alonso

Title: The effects of hyperthermia on the regulation of human skeletal muscle blood flow and cardiac function

Dear Professor González-Alonso,

The Research Ethics Committee has approved your application for research ethical approval for the above-named project, which is to be undertaken in February 2008.

Any changes to the protocol contained in your application, and any unforseen ethical issues which arise during the project, must be notified to the Committee.

Kind regards,

David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Study 2 – Dehydration and heat stress

Head of School of Sport & Education
Professor Susan Capel

Brunel UNIVERSITY WEST LONDON

Heinz Wolff Building,
Brunel University, Uxbridge,
Middlesex, UB8 3PH, UK
Telephone +44 (0)1895 266494
Fax +44 (0)1895 269769
Web www.brunel.ac.uk

Mr Eric Stohr
c/o School of Sport and Education
Brunel University

8th October 2008

Dear Eric

RE80-07 - The effects of graded dehydration and hyperthermia on the regulation of human skeletal muscle blood flow and cardiac function

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University Research Ethics requirements and guidelines. In view of the protocols you proposed, the School Research Ethics Committee referred your application to the University Research Ethics Committee for review. The University Research Ethics Committee granted approval to your study on 11th September 2008 – see attached letter.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and the University Research Ethics Committee and is pleased to confirm there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely,

Dr Simon Bradford
Chair of Research Ethics Committee
School Of Sport and Education

Brunel
UNIVERSITY
WEST LONDON

University Research Ethics Committee

11 September 2008

Proposer: Mr. James Pearson
Mr. Eric Stöhr
Centre for Sports Medicine & Human Performance
Heinz Wolff

Title: **The effects of graded dehydration and hyperthermia on the regulation of human skeletal muscle blood flow and cardiac function**

Dear . Pearson and Mr. Stöhr,

The University Research Ethics Committee has considered the amendments recently submitted by you in response to the Committee's earlier review of the above application.

The Chair, acting under delegated authority, is satisfied that the amendments accord with the decision of the Committee and has agreed that there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained in your application, and any unforseen ethical issues which arise during the project, must be notified to the Committee.

Kind regards,

David Anderson-Ford
Chair, Research Ethics Committee
Brunel University

Head of School of Sport & Education
Professor Susan Capel

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Mr Eric Stohr
PhD (Sport Science) Student
c/o School of Sport and Education
Brunel University

21st September 2009

Dear Eric

RE80-07 – The effects of graded hyperthermia and dehydration on the regulation of human skeletal muscle blood flow and cardiac function

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application to amend the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to you amending your study as proposed.

A copy of the approval granted to this extension by the University Research Ethics Committee has been provided to the School for our records.

Any further changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your revised study.

Yours sincerely

Dr Simon Bradford)
Chair of Research Ethics Committee
School Of Sport and Education

Study 3 – Incremental exercise

Head of School of Sport & Education
Professor Susan Capel

Brunel UNIVERSITY WEST LONDON

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Eric Stohr
PhD (Sport Science) Student
School of Sport and Education
Brunel University

16th December 2009

Dear Eric

RE02-09: Response of LV mechanics during incremental exercise in healthy individuals

I am writing to confirm the Research Ethics Committee of the School of Sport and Education received your application connected to the above mentioned research study. Your application has been independently reviewed to ensure it complies with the University Research Ethics requirements and guidelines.

The Chair, acting under delegated authority, is satisfied with the decision reached by the independent reviewers and is pleased to confirm there is no objection on ethical grounds to the proposed study.

Any changes to the protocol contained within your application and any unforeseen ethical issues which arise during the conduct of your study must be notified to the Research Ethics Committee for further consideration.

On behalf of the Research Ethics Committee for the School of Sport and Education, I wish you every success with your study.

Yours sincerely

Dr Simon Bradford
Chair of Research Ethics Committee
School Of Sport and Education

Appendix II – Pre-participation health questionnaire

PRE-PARTICIPATION HEALTH CHECK QUESTIONNAIRE

Health and safety within this investigation is of paramount importance. For this reason we need to be aware of your current health status before you begin any testing procedures. The questions below are designed to identify whether you are able to participate now or should obtain medial advice before undertaking this investigation, Whilst every care will be given to the best of the investigators ability, an individual must know his/her limitations.

Subject name:.....
Date of birth:.....
Doctors Surgery Address:.....
Emergency Contact Name:.....

Please answer the following questions:	YES	NO
1. Has your doctor ever diagnosed a heart condition or recommend only medically supervised exercise?	☐	☐
2. Do you suffer from chest pains, heart palpitations or tightness of the chest?	☐	☐
3. Do you have known high blood pressure? If yes, please give details (i.e. medication)	☐	☐
4. Do you have low blood pressure or often feel faint or have dizzy spells?	☐	☐
5. Do you have known hypercholesteremia?	☐	☐
6. Have you ever had any bone or joint problems, which could be aggravated by physical activity?	☐	☐
7. Do you suffer from diabetes? If yes, are you insulin dependent?	☐	☐
8. Do you suffer from any lung/chest problem, i.e. Asthma, bronchitis, emphysema?	☐	☐
9. Do you suffer from epilepsy? If yes, when was the last incident?	☐	☐
10. Are you taking any medication?	☐	☐
11. Have you had any injuries in the past? E.g. back problems or muscle, tendon or ligament strains, etc…	☐	☐
12. Are you currently enrolled in any other studies?	☐	☐
13. I have already participated in a blood donation program	☐	☐
14. Are you a smoker?	☐	☐
15. Do you exercise on a regular basis (at least 60 min a week)?	☐	☐

16. Describe your exercise routines (mode, frequency, intensity/speed, race times):

If you feel at all unwell because of a temporary illness such as a cold or fever please inform the investigator. Please note if your health status changes so that you would subsequently answer YES to any of the above questions, please notify the investigator immediately.

I have read and fully understand this questionnaire. I confirm that to the best of my knowledge, the answers are correct and accurate. I know of no reasons why I should not participate in physical activity and this investigation and I understand I will be taking part at my own risk.

Participant's name & signature:_____ Date:_____.

Investigator's name & signature:_____ Date:_____.

Appendix III – Consent form

CONSENT FORM

EFFECTS OF GRADED HYPERTHERMIA ON THE REGULATION OF HUMAN SKELETAL MUSCLE BLOOD FLOW AND CARDIAC FUNCTION

The participant should complete the whole of this sheet himself	*Please tick the appropriate box* YES	NO
Have you read the Research Participant Information Sheet?	☐	☐
Have you had an opportunity to ask questions and discuss this study?	☐	☐
Have you received satisfactory answers to all your questions?	☐	☐
Who have you spoken to?		
Do you understand that you will not be referred to by name in any report concerning the study?	☐	☐
Do you understand that you are free to withdraw from the study:		
- at any time	☐	☐
- without having to give a reason for withdrawing?	☐	☐
- *(where relevant)* without affecting your future employment as a member of staff of the University or your progression or assessment as a student of the University.	☐	☐
Do you agree to take part in this study?	☐	☐

Signature of Research Participant:
Date:
Name in capitals:
Witness statement
I am satisfied that the above-named has given informed consent.
Witnessed by:
Date:
Name in capitals:

Appendix IV – Conference abstracts and manuscripts in press

Proc Physiol Soc 11: C64, 2008.
Oral communication at the Annual Meeting of the Physiological Society, 2008, Cambridge, UK.

Effects of heat stress on left ventricular rotation and rotation rate in resting humans

Eric J. Stöhr[1], José González-Alonso[1], James Pearson[1], David A. Low[1], Leena Ali[2], Horace Barker[2], Rob Shave[1]

[1]*Centre for Sports Medicine and Human Performance, Brunel University, Uxbridge, UK,*
[2]*Department of Anaesthetics, Ealing Hospital, Southall, UK*

Introduction: Counter rotation in the left ventricular (LV) base and apex during systole and diastole plays an important role in the filling and emptying of the LV. Heat stress leads to increased cardiac work, evidenced by a rise in heart rate (HR) and cardiac output at rest. Whilst the influence of heat stress on LV dimensions has been assessed (Crandall et al 2008) the effects on cardiac twisting (systolic rotation) and untwisting (diastolic rotation) remain unknown. In order to further elaborate the influence of passive heating on LV function, the present study sought to evaluate the hypothesis that passive heat stress increases LV rotation and rotation rate. **Methods:** Six active male subjects (21±2yr) completed the study, remaining fully hydrated throughout. Measurements were made at 4 different thermal conditions: 1) Control (Tcore ~37°C, Tskin ~32°C), 2) skin hyperthermia (Tc ~37°C, Tsk ~36°C), 3) skin and mild core hyperthermia (Tc ~38°C, Tsk ~37°C), and 4) high skin and core hyperthermia (Tc ~39°C, Tsk ~38°C). Echocardiographic images were acquired at each stage of heat stress. Two-dimensional images were analysed for LV basal and apical peak

rotation (ROT_{bas}, ROT_{api}) and rotation rates ($ROTR_{bas}$, $ROTR_{api}$) and ejection fraction (EF). Mean arterial pressure (MAP) was measured online via a canula inserted in the radial artery. HR was assessed throughout the trial using a three lead ECG. A repeated measures ANOVA was used to detect effects over time and paired student t-test was applied post-hoc to ascertain differences between conditions. Alpha was set at 0.05, Bonferroni adjustment was made for repeated comparisons. **Results:** ROT_{bas} increased significantly between skin hyperthermia and high skin and core hyperthermia (-6.5±3.1 vs. -11.3±3.5°, $p<0.05$). ROT_{api} ($p>0.05$) was unaltered. Systolic and late diastolic $ROTR_{bas}$ increased significantly between control and high skin and core hyperthermia (-88±13 vs. -161±48 s^{-1} & 25±10 vs. 76±19 s^{-1}, $p<0.05$). Late diastolic $ROTR_{api}$ increased significantly between control and high skin and core hyperthermia (-6±12 vs. -47±31 s^{-1}, $p<0.05$). EF also increased between control and high core hyperthermia (61±4 vs. 76±7%, $p<0.05$). MAP remained constant throughout the experiment ($p>0.05$) and HR significantly increased between control and high core hyperthermia (62±14 vs. 119±14 beats·min^{-1}, $p<0.05$). **Conclusion:** Similar to previous studies we demonstrated an increase in systolic function with passive heating, as evidenced by an increase in EF. Our findings suggest that heat stress also increases LV systolic basal rotation, systolic and late diastolic $ROTR_{bas}$ and late diastolic $ROTR_{api}$. The enhanced twisting and untwisting rate of the LV may facilitate the pronounced increase in EF observed with increased core temperature.

Reference : Crandall CG *et al*. (2008). *J Physiol* **586**, 293-301.

Supported by the Gatorade Sports Science Institute

Medicine & Science in Sports & Exercise:
May 2010 - Volume 42 - Issue 5 - p 6.
Oral communication at the ACSM conference 2010, Baltimore, USA.

Challenging the heart – Effects of exercise, dehydration and hyperthermia

ERIC J. STÖHR[1], JOSÉ GONZÁLEZ-ALONSO[1], JAMES PEARSON[1], DAVID A. LOW[1], LEENA ALI[2], HORACE BARKER[2], ROB SHAVE[1]

[1]Centre for Sports Medicine and Human Performance, Brunel University, Uxbridge, UK,
[2]Department of Anaesthetics, Ealing Hospital, Southall, UK

The combined stress of prolonged exercise, dehydration and hyperthermia leads to a reduced stroke volume (SV) in humans, yet the precise left ventricular (LV) diastolic and systolic responses remain unclear. This reduction in SV may be underpinned by lower LV strain rates and/or twist rates (LV mechanics), which have been shown to link LV filling with ejection.

PURPOSE: To examine diastolic and systolic LV strain and twist rates at rest and during exercise with hyperthermia and progressive levels of dehydration. **METHODS:** Echocardiographic images were obtained, from seven healthy young men ($\dot{V}O_2$max 58±8 $ml.kg^{-1}.min^{-1}$) at rest and during sub-maximal one-legged knee-extensor exercise, to estimate end-diastolic volume (EDV), end-systolic volume (ESV), SV, strain rates, systolic twist rate, diastolic untwisting rate in four experimental conditions: 1) Control (euhydration, core temperature ~37°C), 2) ~2% Dehydration (~38°C), 3) ~3.5% Dehydration (~38°C), and 4) after Rehydration ~37°C). **RESULTS:** At rest, 3.5% Dehydration significantly reduced EDV and SV by 34±15 and 22±15 ml, respectively (P<0.01), whilst ESV, cardiac output and systolic strain rates were maintained (P>0.05). In contrast, peak systolic twist rate increased from 77±21 to 148±35 $deg.sec^{-1}$ (P<0.01) while peak diastolic untwisting rate tended to

increase (P=0.11) and was significantly delayed from 7±3% to 23±8% of diastole (P<0.01). Delayed peak untwisting rate at rest was paralleled by a delay in peak diastolic strain rates (P<0.01). Similar to rest, during exercise, EDV and SV were reduced (32±9 and 23±9 ml, respectively, P<0.01) and ESV, cardiac output and systolic strain rates were maintained (P>0.05). However, the significant change in twist rate observed at rest with dehydration was not apparent during exercise as LV twist rate was already enhanced during Control exercise and did not increase further with superimposed hyperthermia and dehydration. **CONCLUSION:** Maintained strain rates coupled with reduced EDV and enhanced twist rates suggest that the lowering of SV with dehydration and hyperthermia at rest and during small muscle mass exercise is associated with reductions in LV filling likely due to hypovolemia rather than reduced contractility or LV mechanics.

This study was supported by the Gatorade Sports Science Institute.

Abstract submitted for oral presentation at the Annual Meeting of the American College of Sports Medicine, 2011, Denver, USA.

Left ventricular twist mechanics during continuous and discontinuous incremental exercise in healthy individuals

ERIC J. STÖHR[1,2], JOSÉ GONZÁLEZ-ALONSO[1] and ROB SHAVE[1,2]

[1]*Centre for Sports Medicine and Human Performance, Brunel University, Uxbridge, Middlesex, UK.* [2]*Cardiff School of Sport, University of Wales Institute Cardiff, Wales.*

Purpose: In healthy individuals, stroke volume (SV) reaches a plateau during incremental exercise at ~50% peak power. Left ventricular (LV) twist and untwisting ('LV twist mechanics') contribute to the generation of SV at rest, however, their response during incremental exercise is not known. **Methods:** Nine healthy young males performed continuous and discontinuous incremental supine cycling exercise up to 90% peak power in a randomized order. **Results:** During both exercise protocols SV attained a plateau at ~50% peak power. Following an initial linear increase, 2-D speckle tracking derived LV twist mechanics also reached a plateau at approximately 50% peak power. Throughout both trials the increase in LV apical untwisting was significantly larger than that of basal untwisting (P<0.01), emphasizing the importance of dynamic apical function. Temporal analysis revealed that peak LV untwisting velocity, which contributes to early LV filling at rest, occurred simultaneously with mitral valve opening at all exercise intensities despite a significant shortening of the iso-volumic relaxation time (IVRT) (P<0.01). SV and LV twist mechanics correlated strongly during continuous and discontinuous incremental exercise, respectively (twist: r^2=.89 and .92; untwisting velocity: r^2=.91 and .97, P<0.01).

Table shows heart rates, SV and LV twist mechanics at rest and five stages of cycling exercise.

		Exercise intensity (% peak power)					
		Rest	**10%**	**30%**	**50%**	**70%**	**90%**
Heart rate (bpm)	*cont.*	62 ± 9	95 ± 10*	112 ± 17*†	134 ± 19*†‡	159 ± 18*†‡$	171 ± 14*†‡$&
	discont.	63 ± 7	105 ± 14*	117 ± 21*†	138 ± 21*†‡	147 ± 16*†‡	158 ± 12*†‡$&
Stroke volume (ml)	*cont.*	89 ± 8	104 ± 11*	118 ± 13*†	125 ± 15*†	127 ± 11*†	124 ± 14*†
	discont.	84 ± 5	101 ± 12*	109 ± 11*	119 ± 13*	117 ± 8*	122 ± 9*†‡
Twist (deg.)	*cont.*	13.9 ± 3.9	15.4 ± 2.4	19.8 ± 5.1*	23.8 ± 6.0*†	26.8 ± 4.2*†‡	24.7 ± 6.9*†
	discont.	11.0 ± 4.2	17.2 ± 3.9	20.1 ± 6.3*	25.3 ± 7.0*	21.2 ± 3.6*	22.4 ± 3.1*
Untwisting vel. ($deg.sec^{-1}$)	*cont.*	-116 ± 28	-147 ± 39	-211 ± 61*†	-286 ± 88*†	-336 ± 110*†‡	-300 ± 101*†‡
	discont.	-101 ± 38	-157 ± 59	-218 ± 96*	-264 ± 113*	-278 ± 93*†	-284 ± 94*†

Data are mean ± standard deviation. *: *P*< 0.01 compared with rest; †: *P*< 0.01 compared with 10%; ‡: *P*< 0.01 compared with 30%; $: *P*< 0.01 compared with 50% exercise; &: *P*< 0.01 compared with 70%.

Conclusion: In healthy individuals performing incremental cycling exercise, LV systolic and diastolic twist mechanics increase from rest and then plateau at approximately 50% peak power. The strong relationship between SV and LV twist mechanics suggests that a constraint in rotational mechanics during moderate to high intensity exercise may limit a further increase in SV above 50% peak power.

Manuscript accepted for publication.

Exp Physiol 00.00 pp 1–11

Experimental Physiology – *Research Paper*

Effects of graded heat stress on global left ventricular function and twist mechanics at rest and during exercise in healthy humans

Eric J. Stöhr[1], José González-Alonso[1], James Pearson[1], David A. Low[1], Leena Ali[2], Horace Barker[2] and Rob Shave[1]

[1] *Centre for Sports Medicine and Human Performance, Brunel University, Uxbridge, UK*
[2] *Department of Anaesthesia, Ealing Hospital, Southall, UK*

Increased left ventricular (LV) twist and untwisting (LV twist mechanics) contribute to the maintenance of stroke volume during passive heat stress. However, it remains unknown whether changes in LV twist mechanics are related to the magnitude of heat stress and whether performing exercise during heat stress alters this response. We examined global LV function and LV twist mechanics in 10 healthy men at baseline and three progressive levels of heat stress, at rest and during knee-extensor exercise. At rest, heat stress increased cardiac output and reduced end-diastolic volume and end-systolic volume, whilst stroke volume and mean arterial pressure (MAP) were maintained. Left ventricular twist and untwisting velocity also increased from baseline to severe heat stress (from 10.6 ± 3.3 to 15.1 ± 5.2 deg and from -123 ± 55 to -210 ± 49 deg s^{-1}, respectively, both $P < 0.01$) and correlated significantly with body temperature, heart rate and LV volumes ($P < 0.05$). Similar to resting conditions, progressive heat stress during exercise increased cardiac output and reduced end-diastolic volume and end-systolic volume with a maintained stroke volume. However, MAP declined ($P < 0.01$) and there was no significant change in LV twist and untwisting velocity, resulting in non-significant relationships between twist mechanics and systemic responses. In conclusion, LV twist mechanics increase proportionally with the magnitude of heat stress at rest. However, there is no increase in LV twist and untwisting velocity from control exercise to severe heat stress during exercise despite a significant increase in body temperatures and cardiac output. We, therefore, suggest that the maintenance of stroke volume in the combined conditions of heat stress and small muscle mass exercise may be further facilitated by other peripheral factors, such as the continuous decline in MAP.

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In heat-stressed humans, reductions in central venous pressure (Rowell, 1974; Wilson *et al.* 2007), central blood volume (Crandall *et al.* 2008) and right ventricular volume (Nelson *et al.* 2010*a*) indicate a lowered venous return to the heart. However, this reduction in venous return does not appear to compromise stroke volume (SV), as SV is largely maintained (Rowell *et al.* 1969*a*; Crandall *et al.* 2008). Accordingly, during passive heat stress systolic and/or diastolic left ventricular (LV) function must be enhanced to compensate for the reduction in venous return. Indeed, systolic and late diastolic tissue Doppler and transmitral inflow velocities have been shown to be increased during passive heat stress (Brothers *et al.* 2009). Recently, Nelson *et al.* (2010*a*) further showed that early diastolic function is also enhanced, as indicated by an increase in peak LV untwisting velocity. Left ventricular twist is the result of counter-directional rotation of the LV base and LV apex during ventricular contraction and has been shown to facilitate improved LV filling and ejection during exercise (Notomi *et al.* 2006). With the onset of myocardial relaxation twist is reversed, resulting in LV untwisting or recoil (Notomi *et al.* 2006), and the

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When the music's over,

Turn out the lights.

Jim Morrison