Challenging the Right Ventricle

from Elite Athletes to Patients with Pulmonary Hypertension

Geert Kleinnibbelink

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

This research programme was carried out in collaboration with the Radboud University Nijmegen, the Netherlands

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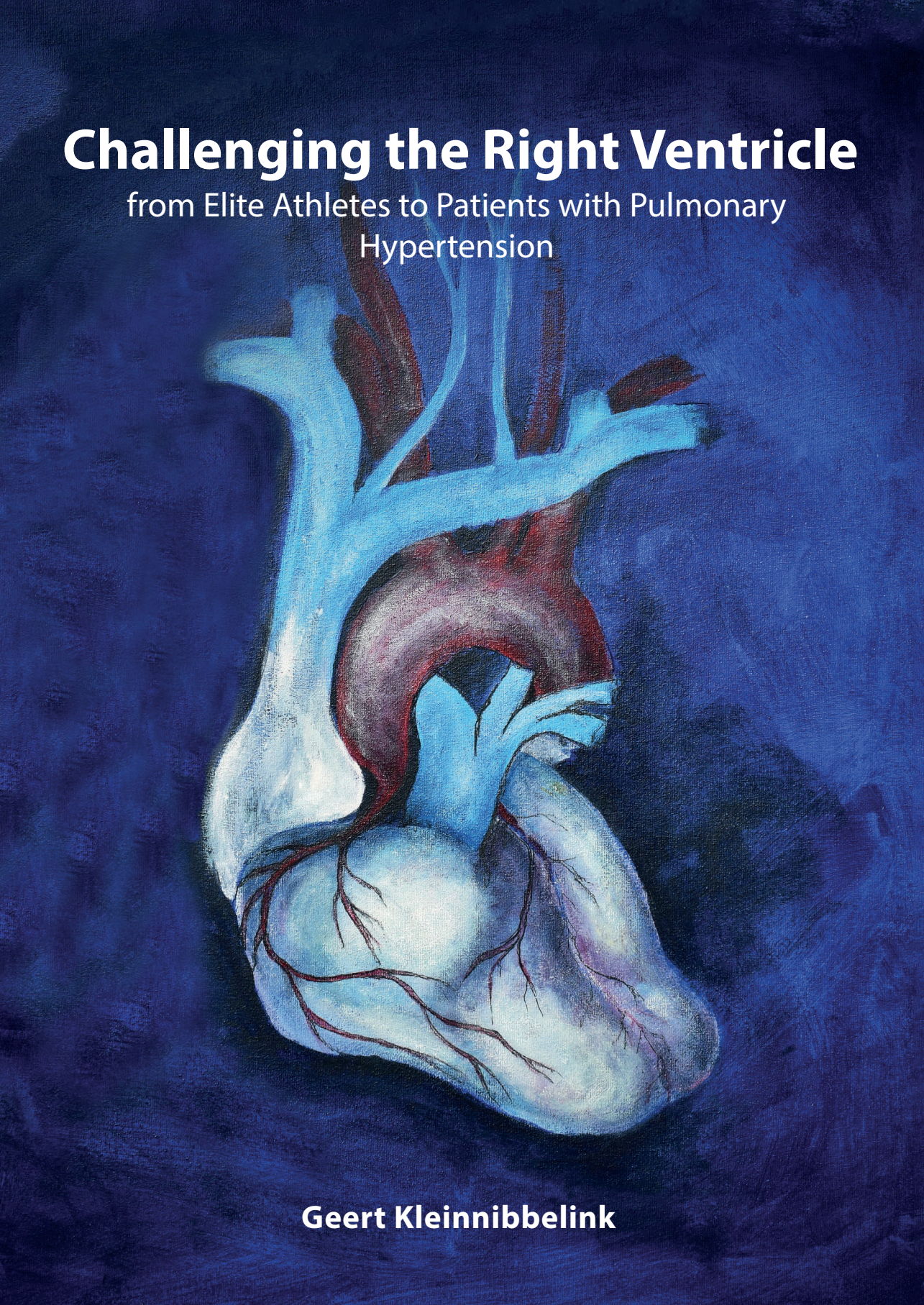

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COLOFON

Challenging the Right Ventricle: from Elite Athletes to Patients with Pulmonary Hypertension

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General Introduction and Outline of Thesis

CHAPTER 1

The cardiovascular system

The circulation of blood through the human body as we know it today, with the heart as a central pump that propels the blood through the body and ultimately returns in the heart, was described first by the English Physician Sir William Harvey (1578-1657). In 1628, Sir William Harvey published the book *exercitatio anatomica de motu cordis et sanguinis in animalibus* ("On the motion of the heart and blood in animals") where he described his research and experiments.[1] Before publication of this landmark book, the Ancient Greeks including Hippocrates and Galenus viewed the cardiovascular system as comprising two distinct networks of arteries and veins.[2] A long-adhered view was introduced by Claudius Galenus around 160 AC, who hypothesised that the liver produced blood that was transported through the body, whereas air was absorbed from the lung into the pulmonary veins and carried by arteries to the various tissues of the body (**Figure 1A**). The arteries also contained blood, whereas the blood passes from the venous side via invisible pores in the interventricular septum. Blood was not seen to circulate but rather to slowly ebb and flow. This hypothesis was acknowledged for over fifteen centuries until Sir William Harvey provided evidence for his hypothesis, using simple calculations and non-invasive experiments that only required a ligature.[1] His description of the "motion of the heart and blood" is still considered valid nowadays. Harvey described, based on his experiments, that blood circulates through a closed loop system consisting of a pulmonary and systemic circulation where the heart acts as mechanical force to foresee the movement of blood (**Figure 1B**).

As introduced by Harvey, the primary function of the cardiovascular system is to deliver nutrients and oxygen to tissues and removing carbon dioxide and other wastes.[2] The heart consists of a right and a left side, each consisting of an atrium and ventricle, which serves the pulmonary and systemic circulation of blood respectively.[3, 4] Once the nutrients and oxygen are delivered to peripheral tissues and organs, and when carbon dioxide and other wastes are taken up from the tissues via the capillaries, deoxygenated blood flows back to the heart via the venous system and enters the right atrium (RA) via the vena cava inferior and superior. During the diastolic phase, the right ventricle (RV) relaxes, causing the pressure in the RV to become lower than the RA, which leads to opening of the tricuspid valve and blood flows into the RV. During the systolic phase, the RV contracts

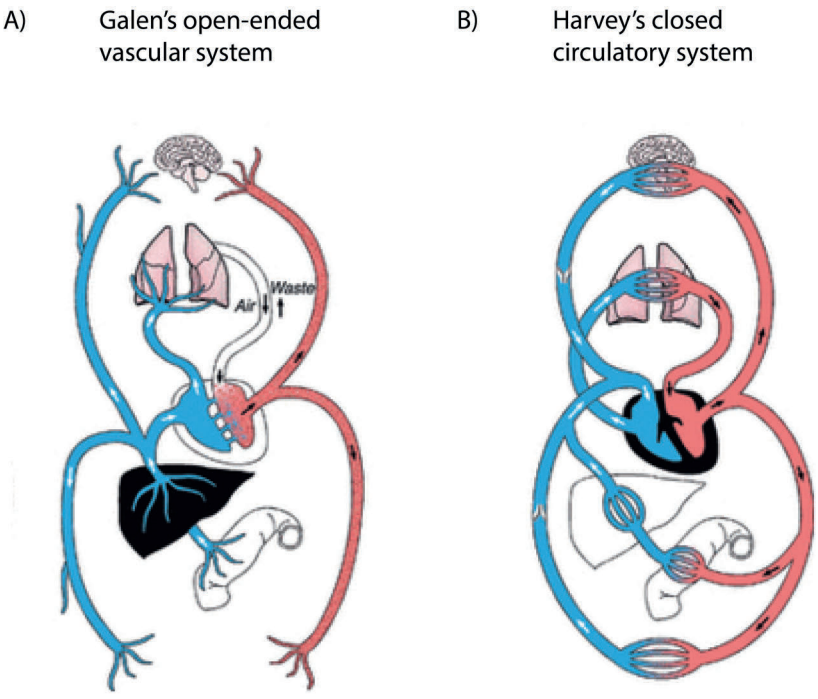

Figure 1. Schematic overview of the circulation hypothesis by Galenus (A) and Harvey (B) (source: Aird *et al.* (2011)[2])

causing the pressure to increase and exceed the pressure in the pulmonary artery, which leads to opening of the pulmonary valve and blood flows to the lung where it becomes oxygenated. The oxygenated blood is then carried to the left atrium (LA) by the pulmonary veins. During the diastolic phase, blood enters the left ventricle (LV) by passing through the mitral valve. During the systolic phase, the LV contracts to overcome the pressure in the systemic circulation to deliver the oxygenated blood to the tissues.[3, 4]

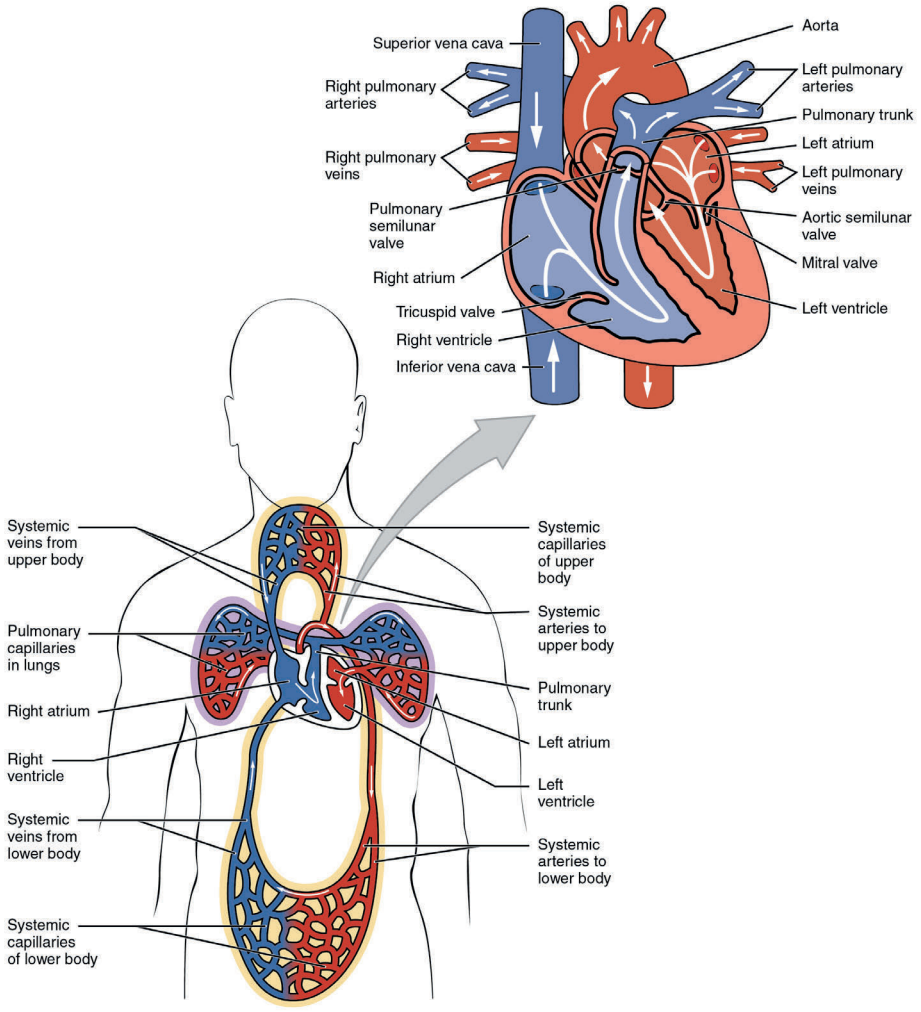

Figure 2. Schematic overview of the circulation as it is seen nowadays with the heart highlighted in the upper right panel (source: Betts *et al. (2013)*[3])

The heart: a tale of two sides

The heart is located within the thoracic cavity in the middle mediastinum with the dorsal surface near to the bodies of the vertebrae and the anterior surface close to the sternum and ribs.[3] The base of heart is located at the level of the third rib and the apex lies just to the left of the sternum between the fifth intercostal space. The right side of the heart is deflected anteriorly, and the left side is deflected posteriorly. The size of the heart is

about that of a fist and weighs approximately 250-350 grams (250-300 grams for females, 300-350 grams for males). The shape similar to a pinecone, broad at the base and tapered to the apex, and it is enclosed with the pericardium, a double-walled sac consisting of a serious and a fibrous layer.[3]

The heart consists of four compartments: the right and left atria and ventricles. For a long time, it was assumed that the RV was not a significant contributor to maintain the circulation and therefore its importance has been neglected for decades.[5] Studies have strongly focused on the LA and LV, and their ability to acutely and chronically adjust and adapt to physiological and pathological stimuli.[6-8] However, over time, increasing evidence supports a separate view and interest on the right side of the heart.[6] It became increasingly clear to scientists and clinicians that the RV is anatomically and functionally different from the LV.[5, 9] The direct consequence is that this insight rejected the concept to extrapolate physiological and pathophysiological knowledge of the LV to the RV, which was routinely done in the past. In this thesis, a strong focus is placed on the ability of the right ventricle to acutely and chronically adapt to (patho)physiological stimuli. For this reason, a short introduction into the RV is provided below.

The RV is crescent-shaped and is wrapped-around the LV.[10] The RV has a thinner wall (3-5 mm), is 1/3 to 1/6 smaller in mass, but the cavity has 10-15% larger volume than the LV (**Figure 3A**).[11, 12] The RV is uniformly trabeculated, has multiple papillary muscles, a moderator band and a full muscular outflow tract. Thereby is the myoarchitecture between both ventricles different.[10, 11] Where the LV has three distinct layers of aggregated cardiomyocytes, the RV only has two. The RV wall consists of longitudinal, transverse and oblique oriented muscle fibers.[10] The superficial layer of the RV free wall is composed of predominantly transverse fibers with the subendocardial layer composed of scanty longitudinal fibers, while the septal wall consists of oblique helical fibers.[10] In contrast, the whole LV (free wall and septal wall) consists of oblique helical fibers.[10] Coiling and shortening of the longitudinal and helical-shaped oblique fibers determine the shortening of the RV, producing 80% of RV systolic function. In contrast, contraction of the transverse fibers accounts for just 20% of RV systolic function. In a healthy RV, contraction is therefore predominantly driven by shortening of the RV in the longitudinal direction, highlighting the importance of examining longitudinal function in clinical and research

scenarios.[10] These anatomical differences between ventricles originate from embryological development and the substantially different haemodynamic loading conditions faced.

In addition to anatomical differences, function of the left and right side may differ. Function of the ventricles is defined as the ability of the heart to meet the metabolic demands of the body which is reflected by the cardiac output (CO). The CO is defined by the volume of blood which is pumped out per minute by the ventricles and can be calculated by multiplying stroke volume (SV) (the amount of blood ejected by each ventricle every contraction (end-diastolic volume [EDV] minus end-systolic volume [ESV])) by heart rate (HR). Mathematically is this represented by following equations:

$$\textit{Cardiac output (CO)} = \textit{Stroke volume (SV)} \times \textit{Heart rate (HR)}$$

$$\textit{Stroke volume (SV)} = \textit{End-diastolic volume (EDV)} - \textit{end-systolic volume (ESV)}$$

Both ventricles, left and right, serve as the primary pumping chambers of the heart serving both separate, but linked circulations. Under resting conditions, both the RV and LV pump out 5-6 L blood per minute. The RV serves as pump for the pulmonary circulation where it transports blood to and from the lungs to deliver carbon dioxide for exhalation and to pick up oxygen. The LV serves as a pump for the systemic circulation to deliver oxygenated blood to all tissues of the body. Despite the fact that pulmonary and systemic are separate circulations, both RV and LV pump the same amount of blood since both are serially linked. As mentioned above, the CO is the product of HR and SV. The major factors who are influencing SV, and so cardiac function, are preload, afterload and contractility.

Preload is defined as end-diastolic ventricular wall stress or stretch which is directly proportional to EDV. The higher EDV, the more cardiac muscle sarcomeres will stretch resulting in proportional increase in contractility until a certain optimum. The relationship between ventricular stretch and contraction is also known as the Frank-Starling mechanism. Preload is mainly driven by the venous return. Although both RV and LV receive the same amount of blood, as the pulmonary and systemic circulation are serially linked, their preload differs due to their different geometry and mass resulting in different end-diastolic wall stress per unit area myocardium.

Afterload is defined as ventricular wall stress during ventricular ejection which is proportional to the mean pressure that the ventricle must develop to eject blood during systole. The LV and RV need to generate the pressure to overcome the vascular resistance in the respective circulations. For the systemic circulation this is the mean arterial pressure (MAP) and for the pulmonary circulation the mean pulmonary arterial pressure (mPAP). Both are proportionate with vascular resistance and cardiac output.

$$systemic\ vascular\ resistance\ (SVR) =$$

$$\frac{80 \cdot (mean\ arterial\ pressure - mean\ pulmonary\ artery\ wedge\ pressure)}{cardiac\ output}$$

$$pulmonary\ vascular\ resistance\ (PVR) =$$

$$\frac{80 \cdot (mean\ pulmonary\ arterial\ pressure - mean\ right\ atrial\ pressure)}{cardiac\ output}$$

In general, any decrease (e.g. vasodilation) or increase (e.g. vasoconstriction) in vascular resistance will lead to a lower or higher afterload, respectively. A higher afterload will lead to a lower SV (due to an increase in ESV) and a lower afterload will lead to a higher SV (due to a decrease in ESV).

Contractility refers to the ability of the myocardium to contract. The more forceful the contraction, the smaller ESV and the greater the SV. Less forceful contractions result in smaller SVs and larger ESVs.

Where the pulmonary circulation is a low-pressure system, the systemic circulation is a high-pressure system. As these circumstances (i.e. afterload) differ in both circulations, it's understandable that the RV and LV behave functionally different as is outlined in **Figure 3B**. As is demonstrated, during the cardiac cycle the pressure variation in the RV is much lower, whereas both ventricles have the same SV. The above described haemodynamic differences results in different function between both ventricles to generate the same CO.

During exercise, CO can increase 4- to 5-fold up to 20-30 L/min. When exercise performed under demanding conditions, a temporary reduction in cardiac function post-exercise lasting for hours to days has been observed.[13-19] This transient decline in cardiac function is typically referred to as exercise-induced cardiac fatigue (EICF). Several hypotheses

have been proposed as possible mechanisms for EICF, including β-adrenergic receptor desensitization, oxidative stress and altered post-exercise loading.[13, 20-22] Interestingly, the RV appears to be affected to a greater magnitude than the LV.[19, 23] A possible explanation for this observation may be the disproportionately higher wall stress experienced by the RV relative to the LV during exercise.[24] However, no studies have directly examined this concept. Hypoxic exposure induces altered loading conditions for the RV and could therefore be an important substrate to investigate the direct relation between EICF and wall stress experienced during exercise. Specifically, acute exposure to hypoxia induces an increase in PVR and subsequently in PAP.[25] Accordingly, exercise under hypoxia may exaggerate RV wall stress and increase the workload of the RV to maintain CO. Therefore, exercise under hypoxia *versus* exercise under normoxia provides the possibility to examine the direct relationship between changes in RV afterload as a contributing factor to the magnitude of EICF. In this thesis, we will examine this potential relationship between RV afterload and EICF.

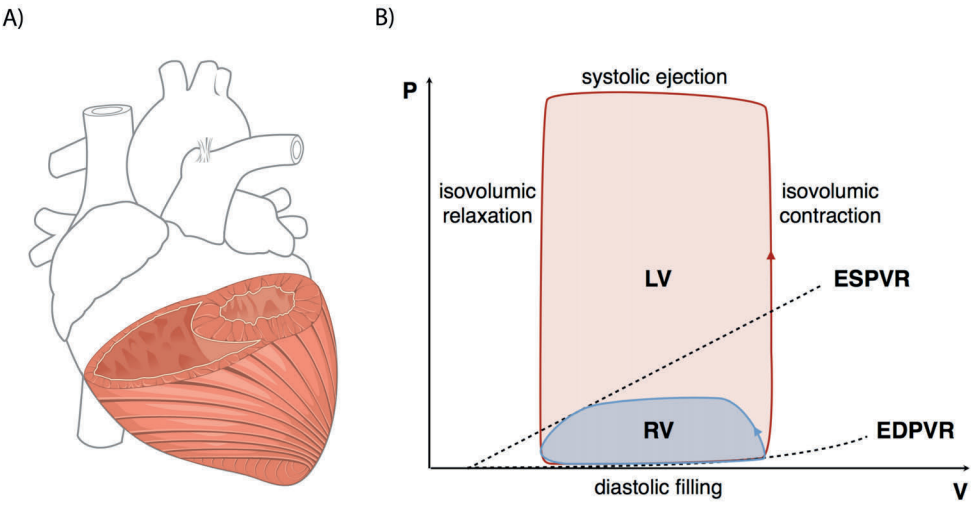

Figure 3. A) Differences between right and left ventricular structure (i.e. cavity size and wall thickness) (source: Betts *et al.*[3]) and B) function (source: Bellofiore *et al.*[9]).

Cardiac plasticity

The adult human heart has an exceptional ability to alter its phenotype to adapt to changes in environmental demand. This response involves metabolic, mechanical, electrical, and

structural alterations, and is known as cardiac plasticity.[8, 26, 27] The process of these changes is also referred to as cardiac remodelling or cardiac adaptation and can be distinguished into a physiological and pathophysiological variant. A variety or stimuli can induce this remodelling through alterations in preload, afterload and contractility.[5, 26]

Physiological remodelling

Cardiac remodelling to exercise is a form of physiological adaptation. During recreational or competitive exercise, the repetitive participation in vigorous physical exercise stimulates adaptive changes in cardiac structure and function.[6, 28] These changes vary among individuals and is based on a number of factors, including type (static *versus* dynamic), duration and intensity of exercise, but also differ between ethnicities, genetics and sex.[28] All these factors influence the haemodynamic challenges to the heart and the subsequent nature and magnitude of cardiac remodelling.

In the past, research examining exercise-induced cardiac remodelling was primarily focused on the LV. In 1975, the Morganroth hypothesis stated that resistance and endurance training cause divergent patterns of remodelling.[29, 30] The assumption was that resistance and endurance exercise would lead to a concentric (increase in LV mass, equal EDV) and eccentric (increase in LV mass and EDV) type of remodelling, respectively. Over years, more research has been undertaken and this hypothesis has become obsolete with the paradigm shifting towards a dose-dependent relation between the amount of haemodynamic stress exposure (time x intensity) and cardiac remodelling.[6] Importantly, the Morganroth hypothesis did not consider the RV. As a result, the RV has been neglected for many years regarding exercise-induce cardiac remodelling. For obvious reasons, the RV and LV are strongly coupled and are ultimately exposed to the same volume of cardiac output. As a result, traditionally it has been supposed that RV follows the LV adaption pathway, however, important differences exist between both ventricles for other haemo- and cardiodynamic stimuli. For example, the acute haemodynamic response during exercise appeared to induce a relative higher increase in wall stress compared to the LV, which possible underly a rationale for the side-specific cardiac remodelling hypothesis (**Figure 4**).[24] La Gerche *et al.* demonstrated that during rest the end-systolic wall stress is higher in the LV compared to the RV, but that during exercise the relative increase in wall stress is higher in the RV compared to the LV (**Figure 4**).[24] Therefore, a central hypothesis

in this thesis is that the RV remodels differently compared to the LV to physiological stimuli, including regular exercise. However, prospective longitudinal evaluation of this hypothesis is lacking. Also, the RV has been under increasing attention as previous studies have linked exercise-induced RV cardiomyopathy to high volumes of exercise training in elite athletes.[18, 31] Insight into the physiological remodelling of the RV might therefore be helpful to contribute in the challenging preparticipation screening for the detection of heart diseases associated with risk for sudden cardiac death.[32]

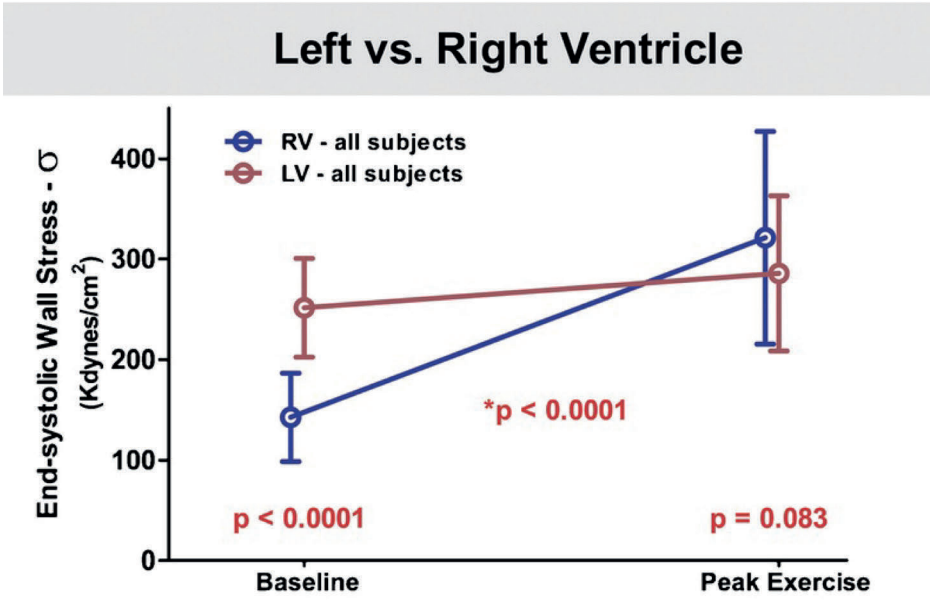

Figure 4. End-systolic wall stress during rest is lower in the RV compared to the LV but the relative increase is higher during exercise (source: La Gerche *et al.*[24]).

Pathophysiological remodelling

Hypertension in the systemic circulation could lead to a continuous pressure overload of the LV resulting in hypertrophic remodelling.[33] Hypertension can also occur in the pulmonary circulation which is referred to as pulmonary hypertension (PH).[34] PH is defined as an increase in mean pulmonary arterial pressure (mPAP) ≥25 mmHg at rest assessed by right heart catheterization (RHC).[34] PH can be classified into five groups according to their similar clinical presentation, pathological findings, haemodynamic characteristics and treatment strategy[35]:

- Group 1: Pulmonary arterial hypertension
- Group 2: Pulmonary hypertension due to left heart disease
- Group 3. Pulmonary hypertension due to lung diseases and/or hypoxia
- Group 4: Chronic thromboembolic pulmonary hypertension and other pulmonary artery obstructions
- Group 5: Pulmonary hypertension with unclear and/or multifactorial mechanisms

Pulmonary arterial hypertension (PAH) is a clinical condition characterized by the presence of pre-capillary PH and pulmonary vascular resistance >3 Wood units, in the absence of other causes of pre-capillary PH such as PH due to lung diseases, chronic thromboembolic PH, or other rare diseases.[34] In PAH, as a consequence of an increased resistance in the pulmonary artery there is a greater drive for the RV to produce an increased pressure to circulate the same amount of blood. Facing this continuous pressure-overload, the RV will remodel. As a consequence, the RV becomes hypertrophied and dilated, whilst in a later stage dysfunction occurs which might lead to RV heart failure.[5, 36]

The gold standard for diagnosis of PH is a RHC.[34] RHC is an invasive, time-consuming and expensive procedure with a relative risk of complications. Since the previous decade, vasodilators have been introduced into the treatment of PH and have improved life-expectancy.[37] Although RV function may be the most important factor with regards to prognosis, direct measures of RV function are not routinely used into the follow-up of the patients' condition and/or risk stratification.[34] Currently, risk assessment in PH is based on clinical signs of right heart failure, progression of symptoms, functional classification, 6-minute walk test, cardiopulmonary exercise testing, NT-proBNP and invasively obtained haemodynamic characteristics. These current guidelines utilise only right atrial size and the presence of pericardial effusion into account as determined by echocardiography.[34] Due to the complex RV geometry and load dependency of RV functional parameters, traditional echocardiographic indices such as RV fractional area change (RVFAC) and tricuspid annular plane systolic excursion (TAPSE) have limited prognostic power in patients with PAH.[38]

The introduction of speckle tracking has allowed for the measurement of ventricular longitudinal strain, a measure of ventricular deformation to assess specific local and global function.[39] Several studies have examined the prognostic value of RV longitudinal strain in patients with PH, however, these studies report a broad range of outcomes, ranging from no significant predictive capacity to a high predictive capacity.[40-46] These differences may relate to the heterogeneity between the studies such as study design, included study population, treatment used etcetera. Therefore, one of the aims of this thesis is to perform a meta-analysis to determine the independent prognostic value of RV longitudinal strain in patients with PH.

Echocardiography

Echocardiography represents the central technique in this thesis to better understand RV changes and remodelling to (patho)physiological stimuli. It is based on ultrasound technology and can be used to evaluate cardiac structure and function. Structures such as cavities can be quantified in linear measurements, areas or volumes whereas wall thickness can be quantified with linear measurements (**Figure 5**). RV wall thickness can be linearly measured in the subcostal view at end-diastole (**Figure 5A**). Linear dimensions of the RV cavity are obtained in the RV-focused view (**Figure 5B**) whilst RV outflow tract linear dimensions can be measured in the parasternal short-axis (**Figures 5C**) and the parasternal long-axis view (not shown).

Contemporary methods to assess RV function by echocardiography are the tricuspid annular plane systolic excursion (TAPSE), right ventricular fractional area change (RVFAC) and tissue doppler imaging (TDI) (**Figure 5**).[47] TAPSE represents the displacement of the annulus as a measure of RV longitudinal function and is obtained in the apical four-chamber view (**Figure 5D**). RVFAC provides a global estimation of the global RV function and is calculated by 100*(end-diastolic area – end-systolic area)/end-diastolic area with end-diastolic and -systolic areas obtained in the RV focused apical four-chamber view (**Figure 5E**). TDI measures velocity of the myocardium and represents a useful estimation of global RV function (**Figure 5F**). However, these measures of RV function have inherent limitations related to angle and load dependency secondary to the complex geometry, heterogenous morphology and function of the RV.[47, 48] Novel echocardiography such as

speckle tracking echocardiography may overcome some of these limitations (**Figure 5G**). Therefore, this novel technique will be applied in this thesis and further introduced below. The contemporary methods to assess RV function are limited to display peak values only whereas speckle tracking echocardiography has the ability to analyse RV function over time. In order to utilise these technical advances, our group has introduced the strain-area loop which combines structure and function into one echocardiographic measurement and provides the RV structure-function relationship which will be further introduced below (**Figure 5H**).[49]

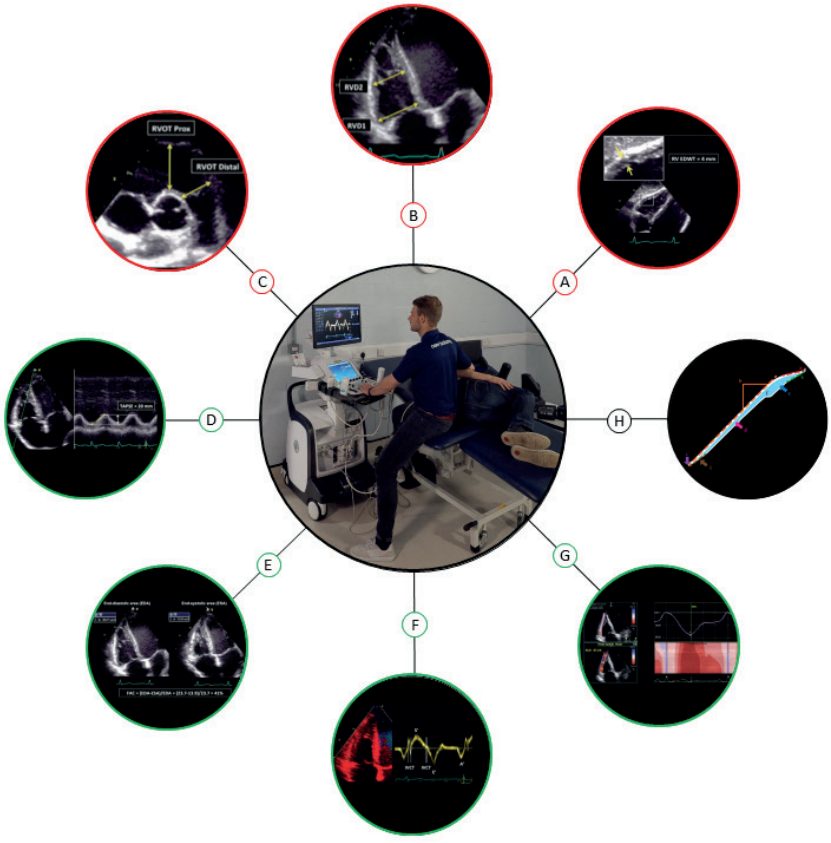

Figure 5. Diagram representing echocardiographic RV structure (red circles) and function (green) measurements. The black circle (H) represents the novel strain-area loop introduced by our research group. A) RV wall thickness; B) RV linear dimensions; C) RV outflow tract (RVOT) linear dimensions; D) tricuspid annular plane systolic excursion (TAPSE); E) RV fractional area change (RVFAC); F) tissue Doppler imaging (TDI); G) Speckling tracking echocardiography; H) Strain-area loop.

Speckle tracking echocardiography

Speckle tracking echocardiography (STE) is a relatively novel method to assess ventricular function and was first described by Kaluzynski *et al.*[50] and D'Hooge *et al.*[51] in 2001. Speckles are small areas of increased echogenicity caused by reflections, refraction and scattering of ultrasound waves. Each region of the myocardium has a unique speckle pattern that allows the region to be traced from one frame to the next, and therefore allows the calculation of deformation, also referred to as strain, of the myocardium. Deformation in the myocardial wall can be calculated in three directions; radial, circumferential and longitudinal. Because of the complex geometry and the alignment of muscle fibers in the RV, longitudinal lateral / free wall strain is commonly used to assess RV function using speckle tracking echocardiography.[52] Strain imaging has shown to be a valuable measurement of RV function and has the advantage that it is less angle and load dependent compared to the conventional indices.[48] More specifically, RV strain imaging provides both diagnostic and prognostic information on cardiac diseases including RV focused diseases as pulmonary hypertension.[53, 54] In line with a majority of work in the heart, most studies have strongly focused on the potential role of speckle tracking in the LV. Especially for the RV this measure may be of added value, supported by the relatively poor ability of currently used measures of RV function to detect abnormalities in the RV (such as with PAH). Therefore, one of the aims of this thesis is to explore the potential role for RV longitudinal strain in prediction disease progression of pulmonary artery hypertension.

Strain-area loop

Recent innovation in echocardiography provided novel and improved opportunities to assess RV function. In that speckle tracking echocardiography allows for evaluation of myocardial deformation, it also provides the ability to detect subclinical groups and hence greater prognostic value compared to conventional echocardiography.[54] Recently, a novel method, developed by our group at the Liverpool John Moores University, United Kingdom, was introduced to assess the interaction between deformation (i.e. related to pressure generation) and area (i.e. related to volume) across the cardiac cycle.[49] This novel method, the strain-area loop, provides simultaneous temporal relationships of the RV structure and longitudinal function. In other words, it elucidates, non-invasively, the structure function relationship throughout systole and diastole and provides estimates of

the relative contribution of longitudinal mechanics to area change, and so provides novel haemodynamic insights into RV function (**Figure 6**). The RV strain-area loop has been applied in cross-sectional studies to describe and demonstrate different characteristics among different type of sports[49], however, it has not been used in demonstrating RV mechanical adaption in training studies. Previously, our group has demonstrated that the is associated with changes in afterload in PAH patients.[55] In summary, the RV strain-area loop may provide additional insight into physiology as well as pathophysiology, which will both be further explored in this thesis.

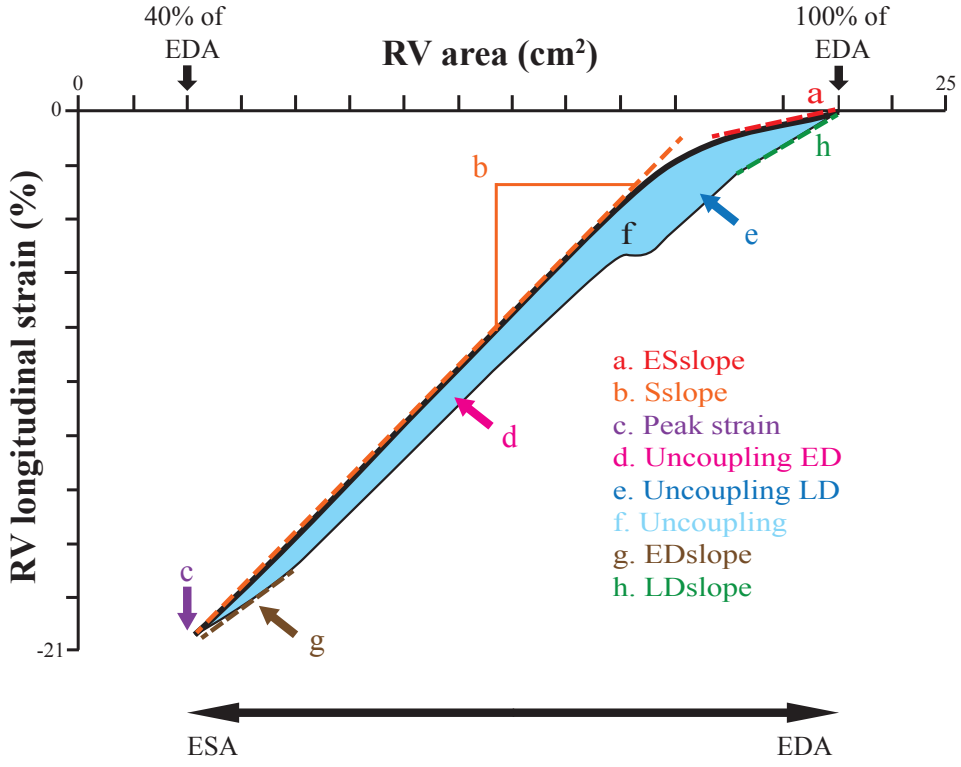

Figure 6. Example of a RV strain-area loop. The thick black line represents the systolic strain area relation whilst the thin black line represents the diastolic strain-volume relation. Several loop related parameters can be derived: (a) early linear slope during first 5% of volume ejection in systole (ESslope), (b) the overall linear slope during systole (Sslope) and (c) end-systolic peak longitudinal strain (peak strain). Furthermore, (d) early, (e) late diastolic and (f) overall (un)coupling which is defined as the relationship between systolic and diastolic strain (difference in strain) for any given area. Lastly, (g) the early linear slope during first 5% (EDslope) and (h) late linear slope (LDslope) during last 5% of volume increase in diastole.

General aims of this thesis

Using echocardiography and adopting novel techniques such as speckle tracking echocardiography and the strain-area loop, we aimed to investigate acute and chronic effects of load challenges on right ventricular structure, function and mechanics. In the first part, we focused on the physiological cardiovascular effects of acute and chronic exercise in healthy individuals and elite athletes. Thereby, we explored whether acute effects were related to chronic cardiovascular adaptations. In the second part, we focused on altered haemodynamics and exercise in patients with PH.

Outline of this thesis

Acute and long-term responses to exercise have traditionally focused on changes in the LV. To better understand adaptation of the RV, the focus of the first part of this thesis is the impact of acute exercise bouts and regular exercise training on the RV in healthy individuals and elite athletes. In **Chapter 2**, we first examined the effects of acute exercise on RV function. Using a randomized cross-over design, we tested the hypothesis whether exercise under hypoxia *vs.* normoxia induced EICF to a greater magnitude during and following relatively short duration, high-intensity exercise. As hypoxia induces pulmonary vasoconstriction resulting in a higher afterload and therefore higher workload of the RV, the influence of afterload on EICF could be investigated. Secondly, these observations in relation to acute exercise were extended to regular exercise training. In **Chapter 3**, we examined the impact of a 12-week hypoxic endurance exercise training program on right- and left-sided cardiac structure, function and mechanics in healthy individuals. In addition, we explored if pre-training changes in cardiac responses to acute exercise are related to structural adaptation after 12 weeks of hypoxic endurance exercise training. In **Chapter 4**, we examined the impact of an increase in training volume across 9-months in Olympic rowers on left- and right-sided cardiac structure, function and mechanics, and explored potential sex differences. In addition to the direct effect of (acute) exercise on cardiac remodelling, we also linked (acute) exercise to blood pressure. In **Chapter 5**, we examined the acute and chronic effects of high-intensity exercise in hypoxia on blood pressure and post-exercise hypotension.

In the second part of this thesis, the focus is on the impact of altered haemodynamics and exercise on the RV in patients with PH. In **Chapter 6**, we performed a systematic review and meta-analysis to determine the independent prognostic value of RV global longitudinal strain for a combined endpoint of mortality and PH-related events or all-cause mortality in patients with pulmonary hypertension. In **Chapter 7,** we elaborated on previous observations from our group, as we have demonstrated that the strain-area loop is able to detect afterload changes in PH patients. To better understand this observation, we assessed whether the strain-area loop is able to detect changes in preload and whether these lead to comparable changes in the invasive pressure-area loop. Furthermore, we investigated whether the strain-area loop would be valuable in the follow-up of PH patients. In **Chapter 8**, we examined the impact of Selexipag, a drug prescribed to lower pulmonary vascular resistance, on the RV strain-area loop in PAH patients and how these effects translate to clinical benefits (at group level and within individuals). In **Chapter 9**, we compared 1-year mortality rates between patients with patients with primary PH who were prescribed cardiac rehabilitation or exercise programmes *versus* a propensity-matched control group of primary PH without prescription for CR or exercise programmes.

In the third part of this thesis, in **Chapter 10**, we will integrate findings of this thesis with insight from other studies and we will discuss potential future directions for research.

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NIJMEGEN LIVERPOOL

Right Ventricular Responses to Acute and Chronic Exercise

PART I

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Exercise-Induced Cardiac Fatigue after a 45-minute Bout of High-Intensity Running Exercise is not Altered under Hypoxia

CHAPTER 2

ABSTRACT

Background. Acute exercise promotes transient exercise-induced cardiac fatigue (EICF), which affects the right ventricle (RV) and to a lesser extent the left ventricle (LV). Hypoxic exposure induces an additional increase in RV afterload. Therefore, exercise in hypoxia may differently affect both ventricles. The aim of this study was to investigate the acute effects of a bout of high-intensity exercise under hypoxia *versus* normoxia in healthy individuals on right- and left-sided cardiac function and mechanics.

Methods. 21 healthy individuals (22.2±0.6 years, fourteen men) performed a 45-minute high-intensity running exercise, under hypoxia (fraction of inspired oxygen [FiO_2] 14.5%) and normoxia (FiO_2 20.9%) in a randomized order. Pre- and post-exercise echocardiography, at rest and during low-to-moderate intensity recumbent exercise ('stress'), was performed to assess RV and LV cardiac function and mechanics. RV structure, function and mechanics were assessed using conventional 2D, Doppler, tissue Doppler, speckle tracking echocardiography and novel strain-area loops.

Results. Indices for RV systolic function (RVFAC, TAPSE, RVS', RV free wall strain) as well as LV function (LV ejection fraction, LV global longitudinal strain)) significantly reduced after high-intensity running exercise ($p<0.01$). These exercise-induced changes were more pronounced when echocardiography was examined during stress compared to baseline. These responses in RV or LV were not altered under hypoxia ($p>0.05$).

Conclusion. There was no impact of hypoxia on the magnitude of EICF in the RV and LV after a bout of 45-minute high-intensity exercise. This finding suggests that any potential increase in loading conditions does not automatically exacerbate EICF in this setting.

INTRODUCTION

It is well established that exercise is associated with potent cardioprotective effects[1-3], but acute exercise can lead to a paradoxical short-term increase in cardiac events.[4-6] One potential explanation is that exercise performed under demanding conditions (i.e. exercise at high-intensity and/or during prolonged duration) may lead to an acute reduction in cardiac function.[7-13] This transient decline in cardiac function after strenuous exercise is typically referred to as exercise-induced cardiac fatigue (EICF). EICF may affect both left (LV) and right ventricles (RV), with possibly a larger impact on the RV due to the disproportionately higher wall stress experienced by the RV relative to the LV during exercise.[11, 14, 15]

Previous studies have demonstrated that hypoxia increases the demands on the cardiovascular system.[16] Specifically, acute exposure to hypoxia induces a decrease in systemic vascular resistance at rest, which may contribute to a decrease in LV afterload.[17, 18] In contrast, hypoxia leads to a resting increase in pulmonary artery resistance, and subsequently to an increase in pulmonary vascular resistance (PVR) and pulmonary artery pressure (PAP).[19] Exercise in normoxic conditions results in additional load challenges and an increased PAP secondary to the mismatch of elevated stroke volume to inadequate pulmonary vascular distension.[20] This is exacerbated when exercising in hypoxic conditions, leading to an even greater PAP and RV wall stress and potentially further increasing the risk of RV EICF.[19-23]

To non-invasively examine right heart haemodynamics, studies have examined conventional and Doppler based echocardiographic indices at rest and during exercise.[24-26] Recently, the strain-area loop has been introduced assessing simultaneous structure and strain across the cardiac cycle.[5] Previously, we found that RV loop characteristics relate to PVR in patients with pulmonary arterial hypertension (PAH) whilst also demonstrating value in the assessment of EICF.[27, 28] Therefore, these non-invasive characteristics may provide additional insight in understanding exercise-induced changes in hypoxia.

In view of this, the aim of this study, was to investigate the acute effects of a bout of high-intensity exercise under hypoxia *versus* normoxia in healthy individuals on right- and left-sided cardiac function and mechanics (i.e. longitudinal strain and strain-area

loops). Based on the presumed higher workload of the RV during hypoxic *versus* normoxic exercise, we hypothesize that exercise under hypoxia exaggerates RV to a greater extent than LV compared to exercise under normoxia. To investigate EICF, we examined pre- and post-exercise echocardiography at rest, but also during a standardized low-to-moderate-intensity recumbent exercise challenge ('stress'). As the post-exercise recovery period is associated with persistent sympathoexcitation and peripheral vasodilation[17, 18], evaluation of EICF could be confounded when evaluated solely at rest. Therefore, evaluation during stress echocardiography may better reflect cardiac function *during* exercise and offsets the key limitation of (para)sympathetic imbalance associated with echocardiographic assessment in recovery.[14]

METHODS

Study population

Twenty-one participants (mean age , 22.2±0.6 years; 14 men; mean body mass index, 24.0±0.6 kg/m^2; mean maximal oxygen consumption [VO_2max] per kilogram, 52.4±1.8 mL/min/kg) completed the study. Baseline characteristics are shown in **Table 1**. Participants were eligible to take part in this study if they were able to run on a treadmill and if they trained <2 hours a week at moderate to high intensity for the last six months. Exclusion criteria were a body mass index (BMI) <18 or >30 kg/m^2, active smoker, any possibility of pregnancy, personal history of cardiovascular disease, positive family history of cardiovascular death (<55y), exercise-limiting respiratory disease, physical (i.e. musculoskeletal) complaints making completion of a bout of high-intensity running exercise impossible, abnormal resting 12-lead electrocardiogram (ECG) and abnormalities observed on resting transthoracic echocardiography. The procedures were in accordance with institutional guidelines and conformed to the declaration of Helsinki. The study was approved by the ethics research committee of Liverpool John Moores University (18/SPS/065). Participants gave full written and verbal informed consent before participation.

Table 1. Subject characteristics

Sex (m/f)	14/7
Age (yr)	22.2±0.6
Height (cm)	170±2
Body Mass (kg)	70±2
BMI (kg/m2)	24.0±0.6
BSA (m2)	1.8±0.04
Resting HR (bpm)	65±2
Resting SBP (mmHg)	119±1
Resting DBP (mmHg)	69±2
Resting MAP (mmHg)	85±1
resting SpO2 (%)	98.4±0.3
VO_2max (L/min)	3.6±0.1
VO_2max/kg (mL/min/kg)	52±2
VE (L/min)	138±6
HRmax (bpm)	199±2

Data are expressed as means±SEM. m, male. f, female. BMI, body mass index. BSA, body surface area. HR, heart rate. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. SpO2, oxygen saturation. VO_2max, maximal oxygen uptake. VE, ventilation.

Study design

In this randomized crossover trial, participants attended the laboratory on three separate occasions (**Figure 1**). During the first visit, a medical screening was performed to determine eligibility of the potential participants. After signing informed consent, baseline measurements were performed. Visits two and three included performance of a bout of 45-minute high-intensity running exercise under normobaric hypoxia or normoxia, which were performed in a randomized order. Participants were blinded for the order of test days and abstained from exercise for a minimum of 48 hours, and from alcohol and caffeine consumption 24 hours before the test days.

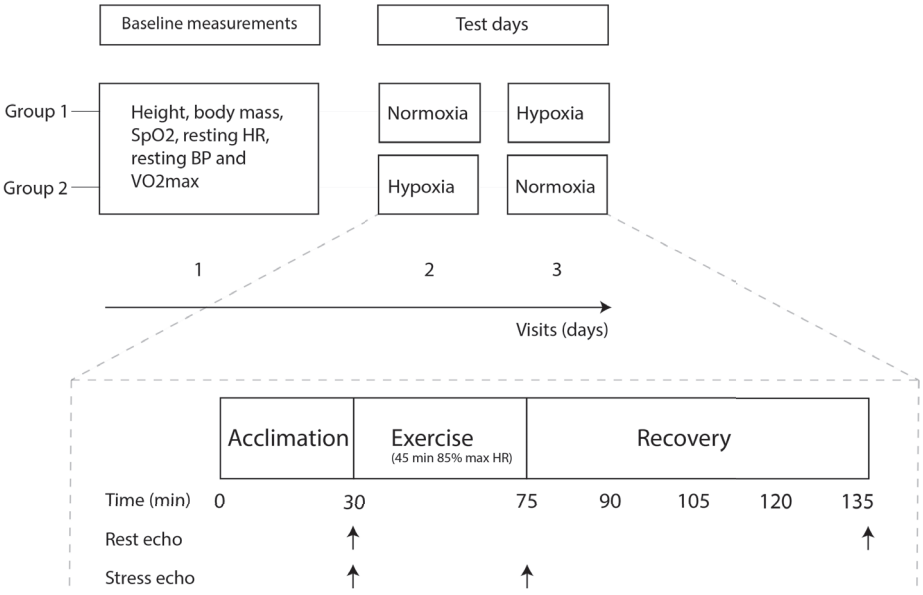

Figure 1. Overview of study design, where the dotted panel is highlighting visit 2 and 3 (test days).

Baseline measurements. Participants were examined for height (SECA stadiometer, SECA GmbH, Germany), weight (SECA scale, SECA GmbH, Germany), oxygen saturation (SpO_2, pulse oximetry; Ana Pulse 100, Ana Wiz Ltd., UK), 12-lead ECG (Cardiovit MS-2010, Schiller, Switzerland) and maximal oxygen consumption (VO_2max). Resting heart rate (HR, Polar, Kempele, Finland) and resting blood pressure (BP, Dinamap V100, GE Medical, Norway) were determined at the end of ten minutes of quiet rest in a supine position. A standardized maximal cardiopulmonary exercise test (CPET, Oxycon pro, CareFusion, VS) for VO_2max assessment was conducted on a motorized treadmill (HP Cosmos, Nussdorf, Germany) after a 10-min warm-up and familiarization. VO_2max was defined as the highest value of a 30-s average[29], and attainment was verified according to previous recommended criteria.[30]

Test days. **Figure 1** outlines the details of a single test day. One of the test days was performed at normoxia (sea level, equivalent to fraction of inspired oxygen [FiO_2] 20.9%) and the other at normobaric hypoxia (FiO_2 14.5%; equivalent to a simulated altitude of 3,000m), separated by at least 48 hours of rest. Participants were subjected to 30 minutes of acclimation in a seated position followed by 45-minute of high-intensity (85% of maximum achieved HR during CPET) endurance running exercise on a motorized treadmill

(HP Cosmos, Nussdorf, Germany) and 60 minutes of recovery in seated position. HR was measured continuously throughout (Polar, Kempele, Finland), and rate of perceived exertion (RPE) was monitored during the 45-minutes high-intensity running exercise.[31]

In total four echocardiographic assessments were performed per test day. After acclimation and prior to the 45-minute exercise, echocardiography was performed under resting conditions ('rest') and during recumbent cycling to elevate heart rate to directly assess cardiac function during exercise ('stress', target HR 110-120 bpm). The 'stress' echocardiogram was repeated directly after the 45-minute exercise, to prevent sympathetic withdrawal (i.e. a drop in BP and HR).[32] Finally, images were obtained at the end of the 60 minutes of recovery in a resting state. During every echocardiography assessment, BP measurements were performed. Measurements were performed at the same time on both days to control for diurnal variation. Fluid intake was controlled by providing the same amount of water to participants during both testing days.

Environmental chamber and safety. All exercise tests were conducted in an environmental chamber (TISS, Alton, UK; Sportingedge, Basingstoke, UK). Normobaric hypoxia was achieved by a nitrogen dilution technique. Ambient temperature, carbon dioxide (CO_2) and oxygen (O_2) levels were controlled in all sessions (20°C; FiO_2 14.5%; CO_2 0.03%), whilst a Servomex gas analysis system (Servomex MiniMP 5200, Servomex Group Ltd., UK) was used inside the chamber to provide the researcher continuous information regarding O_2 and CO_2 levels. Acute mountain sickness symptoms (AMS, measured by Lake Louise Score[33] (LLS)) were monitored during testing and training sessions every 20 minutes. The subject was removed from the environmental chamber if oxygen saturation levels dropped below 80% or severe AMS was suspected (LLS≥6).

Echocardiographic measurements

Rest and stress echocardiography were performed in the left lateral decubitus position on a supine cycle ergometer (Lode B.V.; Groningen, The Netherlands) by one highly experienced sonographer (DO) using a Vivid E95 ultrasound machine (GE Medical, Horton, Norway), equipped with a 1.5-4.5 MHz transducer. Images were stored in raw digital imaging and communication in medicine (DICOM) format and were exported to an offline workstation (EchoPac, version 203, GE Medical, Horton, Norway). Data-analysis, from three

stored cycles, was performed by a single observer with experience in echocardiography (GK) using commercially available software (EchoPac, version 203, GE Medical, Horton, Norway). The observer was blinded for the timing (pre *vs*. post) and condition (normoxia *vs*. hypoxia) under which echocardiography was performed. For stress echocardiography, low-to-moderate-intensity (target HR 110-120 bpm) exercise consisted of recumbent cycling at a cadence of ~60 revolutions per minute with watts manually adjusted to stabilise at target HR.

Conventional measurements. Cardiac structural and functional measurements were made according to the current guidelines for cardiac chamber quantification.[34] Regarding the right heart, we examined the following structural and functional indices: basal and mid-cavity end-diastolic diameters, RV end-diastolic area (RVEDA), RV end-systolic area (RVESA), RV outflow tract (RVOT) diameter at the proximal level in the parasternal long-axis (PLAX) and parasternal short-axis (PSAX) view, right atrial (RA) area, RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE), tissue Doppler imaging (TDI) of the tricuspid annulus (RV 's, e', a') and pulmonary artery Doppler acceleration time (PAT). Tricuspid regurgitation velocity was not obtainable in the major part of the participants and therefore was unable to be utilized in this study.

Regarding the left heart, the following structural and functional indices were determined: LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), LA diameter, LA volume, modified Simpson's left ventricular ejection fraction (LVEF), tissue Doppler imaging (TDI) of the mitral annulus (LV ', e' and a') and trans-mitral Doppler (E, A and E/A ratio). Doppler A and RV and LV TDI a' were not measurable on account of e'/a' and E/A fusion during stress echocardiography at higher heart rates.

Mechanics. Images were acquired and optimized for STE. This involved maintaining frame rates between 40 and 90 frames s^{-1}, depth to ensure adequate imaging of the chamber of interest and compression and reject to ensure endocardial delineation. The RV focused and the apical two-chamber, four-chamber and long-axis view were utilized for the RV and LV global longitudinal strain, respectively. Pulmonary and aortic valve closure times were determined from the respective pulsed wave Doppler signals. For both the RV and LV views the myocardium was manually traced to include the septum and adjusted so

that the region of interest (ROI) incorporated all of the wall thickness while avoiding the pericardium.[35, 36] The region of interest was divided into six myocardial segments, providing segmental strain curves and a longitudinal strain curve as an average of all six segments for the LV views and as an average of the 3 segments of the RV free wall. LV global longitudinal strain (LVGLS) was obtained by averaging the single strain measurements of the three separate apical LV views. If inappropriate tracking of segments was observed visually or detected by the system, retracing was performed until all segments were considered acceptable.

RV strain-area loops. The longitudinal strain-area relationship (detailed methods of derivation see, Supplemental 1, Oxborough *et al.*[5] and Hulshof *et al.*[37]) was assessed using the following parameters (**Figure 2**): (I) the linear strain-area slope (Sslope) and early strain-area slope during first 5% of volume ejection in systole (ESslope); (II) end-systolic peak longitudinal strain (peak strain); (III) the early linear strain-area slope during first 5% (EDslope) and late linear strain-area slope (LDslope) during last 5% of volume increase in diastole; and (IV) diastolic uncoupling (i.e. difference in strain between systole and diastole at any given area), divided into uncoupling during early (Uncoupling ED) and late diastole (Uncoupling LD).[5, 28] Based on previous work from our laboratory, we found that PAH patients with higher PVR have a lower Sslope and a decreased Uncoupling LD. Therefore, these may serve as markers of an increased PVR and consequently PAP.[28]

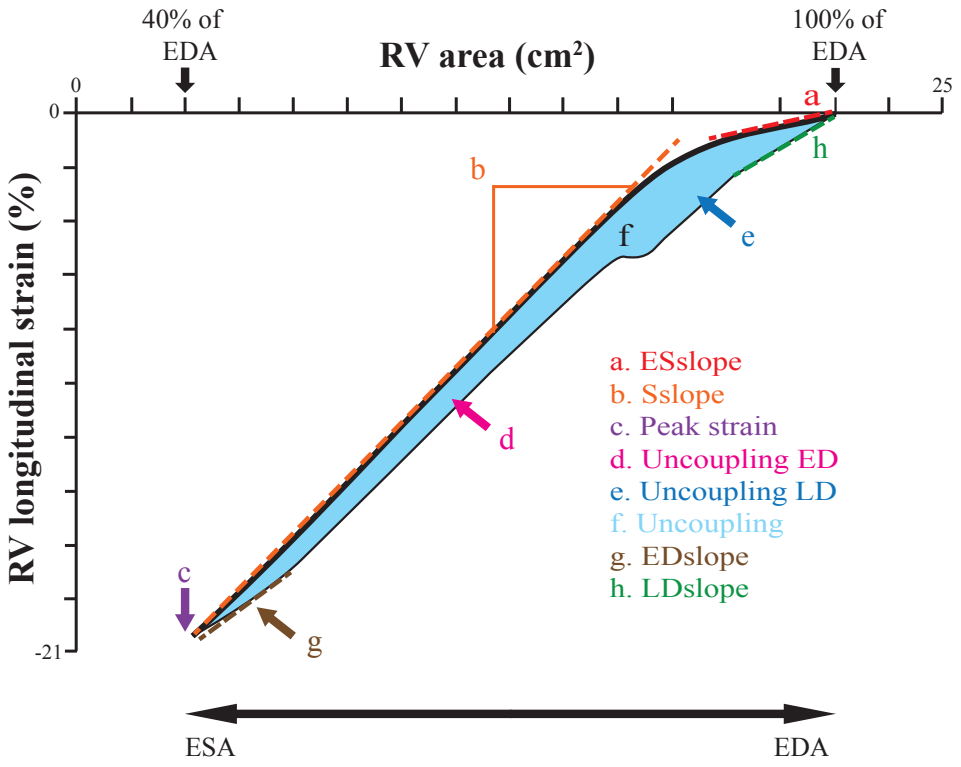

Figure 2. Schematic overview of the RV strain-area loop and the derived characteristics. The black line represents the strain-area loop, the thick part represents the systolic phase and the thin line the systolic phase. EDA, end-diastolic area. ESA, end-systolic area. ESslope, early systolic slope. Sslope, systolic slope. Uncoupling ED, uncoupling end-diastolic. Uncoupling LD, uncoupling late diastolic.

In order to obtain intra-observer variability, strain-area loops were re-analysed in 20 randomly selected echocardiograms (n=10 rest, n=10 stress). For all strain-area loop characteristics intra-class correlation coefficient (ICC) and Bland-Altman limits of agreement (LOA) analysis were performed.[38]

Statistical analysis

Statistical analysis was performed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, VS). All parameters were visually inspected for normality and tested with Shapiro-Wilk normality tests. Continuous variables were reported as mean ± standard error of the mean (SEM) and categorical variables were presented as proportions. Linear mixed models analysis for

repeated measurements were performed to test the acute effects of a bout of 45-minutes high-intensity exercise on cardiac function and mechanics (Exercise), and whether this effect was influenced when echocardiography was performed at rest or during stress (Exercise*Stress). Furthermore, linear mixed models were used to test the effect of hypoxia *versus* normoxia (Hypoxia) and the effect of rest versus stress echocardiography (Stress) on cardiac structure and function. To examine our primary objective, linear mixed models analysis was used to examine whether hypoxia impacted the effect of exercise on cardiac function (Exercise*Hypoxia), and how this was affected by testing condition rest *versus* stress (Exercise*Hypoxia*Stress). For all tests, we assumed statistical significance at $p<0.05$.

RESULTS

Both the right and left heart had normal geometry and all structural measurements were within normal ranges (**Table 2**). There were no abnormal 12-lead ECG findings.

Exercise characteristics. HR during exercise was matched between exercise under hypoxia and normoxia (172±1 bpm, 173±2 bpm respectively, p=0.23). Body mass loss (hypoxia -410±70g *vs*. normoxia -410±43g p=0.99) and water intake (hypoxia 373±60ml *vs*. normoxia 336±44ml, p=0.24) during exercise did not differ between testing sessions. Mean distance covered during exercise was significantly higher in normoxia (6,655±351m) compared to hypoxia (5,797±308m, $p<0.001$), whilst there was no significant difference in subjective ratings of perceived exertion (RPE normoxia 12.5±0.3, RPE hypoxia 13.3±0.3; p=0.07). SpO_2 during exercise was significantly lower in hypoxia (82±0.8) compared to normoxia (95±0.4).

Right ventricular structure, function and mechanics

All RV structural, functional and mechanicals indices pre- and post- 45-minute high-intensity running exercise are displayed in **Table 2**. Indices of RV systolic function (RVFAC, TAPSE, RVS', RV free wall strain (**Figure 3A**)) significantly reduced following 45-minute high-intensity exercise (Exercise: $p<0.01$). The decline in indices of RV function and mechanics after exercise were not different between rest and stress echocardiography, except for a more pronounced reduction in RV free wall strain during stress (Exercise*Stress: p=0.01, **Table 2**, **Figure 3A**)). Related to the strain-area loop, following 45-minute high-intensity

exercise there was a reduction in RV longitudinal strain, uncoupling and uncoupling LD (Exercise: $p<0.05$) without a rightward shift (RVEDA Exercise: $p>0.05$) (**Table 2 Figure 4A,B**).

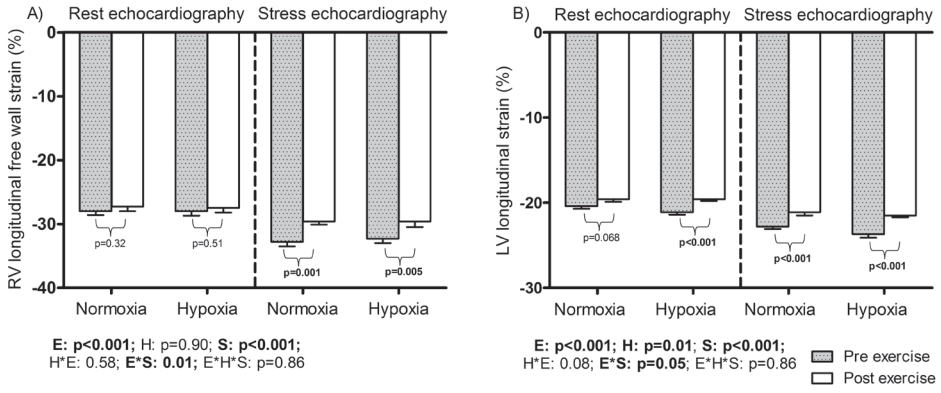

Figure 3. Right ventricular longitudinal strain (A) and left ventricular longitudinal strain (B) prior to and post 45-minutes high intensity running exercise. Error bars reflect the standard error of the mean.
Linear mixed models factors: E, Exercise: Comparison between all echocardiographic measurements performed pre *vs.* post 45-minutes high intensity exercise. H, Hypoxia: Comparison between all echocardiographic measurements performed under hypoxic *vs.* normoxic conditions. S, Stress: Comparison between all echocardiographic measurements performed during rest *vs.* during stress. H*E, Hypoxia*Exercise: Comparison whether the change pre- *vs.* post-exercise (EICF) is different during hypoxic *vs.* normoxic conditions. E*S, Exercise*Stress: Comparison whether the change pre- *vs.* post-exercise is different measured during rest *vs.* stress echocardiography. E*H*S, Exercise*Hypoxia*Stress: comparison whether the change pre- *vs.* post-exercise under hypoxic *vs.* normoxic conditions was different when observed using rest *vs.* stress echocardiography.

Exercise under hypoxia. Under hypoxia, PAT was significantly shorter, RA size significantly larger, late diastolic uncoupling (Uncoupling LD) significantly lower, and a trend was found for a lower systolic slope (Sslope) compared to normoxic conditions (Hypoxia: $p=0.04$, $p=0.04$, $p<0.001$, $p=0.07$, respectively, **Table 2, Figure 4A,B**). Importantly, hypoxia did not alter the impact of exercise and/or stress on indices of RV function (Hypoxia*Exercise and Exercise*Hypoxia*Stress: all $p>0.05$, **Table 2**).

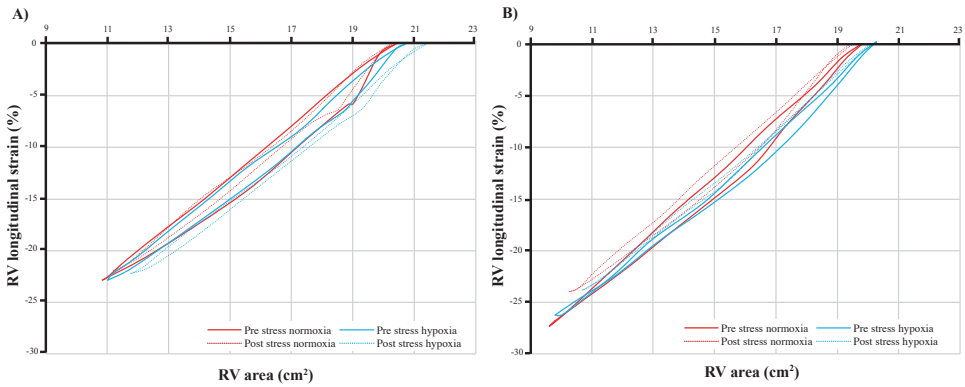

Figure 4. Right ventricular strain-area loops prior to and post 45-minute high intensity running exercise during rest (A) and stress (B). Red and blue lines indicating normoxic and hypoxic exercise, respectively. Solid and dotted lines reflecting pre- and post-exercise, respectively.

Intra-observer variability. ICC and LOA for RV strain-area loop characteristics were as follows: RV free wall strain ICC 0.95 (0.89-0.98), LOA 0.33 (-1.55, 2.21); Sslope ICC 0.91 (0.80-0.97), LOA -0.05 (-0.30, 0.20); ESslope ICC 0.60 (0.23-0.82), LOA 0.60 -0.17 (-1.20, 0.86); EDslope ICC 0.93 (0.84-0.97), LOA 0.19 (-0.37, 0.75); LDslope ICC 0.95 (0.87-0.98), LOA -0.30 (-0.93, 0.32); Uncoupling ICC 0.88 (0.73-0.95), LOA -0.27 (-2.36, 1.81); Uncoupling_ED ICC 0.86 (0.68-0.94), LOA -0.31 (-2.63, 2.01); Uncoupling_LD ICC 0.88 (0.72-0.95), LOA -0.20 (-2.25, 1.86).

Table 2. Right ventricular function and mechanics during rest and stress pre- and post-exercise under normoxia and hypoxia

	Rest echocardiography				Stress echocardiography				p-values					
	Normoxia		Hypoxia		Normoxia		Hypoxia							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	H	E	S	H*E	E*S	E*H*S
Structure														
RV basal diameter (mm)	36.8±0.9	37.4±0.7	36.9±0.7	37.8±0.7	36.4±0.7	35.6±0.8	36.9±.7	37.1±0.6	0.13	0.45	**0.03**	0.25	0.10	0.36
RV mid-cavity diameter (cm)	28.9±0.8	29.4±0.6	29.4±0.9	29.9±0.7	28.8±0.7	28.2±0.8	28.9±.7	28.8±0.8	0.19	0.9	0.07	0.61	0.22	0.65
RVEDA (cm2)	20.4±0.7	20.3±0.6	20.7±0.7	21.4±0.7	19.9±0.6	19.7±0.8	20.3±0.6	20.3±0.7	**0.01**	0.56	**0.01**	0.14	0.38	0.46
RVESA (cm2)	10.8±0.5	11.2±0.4	11.0±0.4	11.8±0.4	9.8±0.3	10.3±0.5	9.9±0.4	10.8±0.5	0.14	**0.001**	**<0.001**	0.14	0.65	0.99
RVOTplax (mm)	24.0±0.7	24.4±0.8	25.5±0.6	25.6±0.5	24.2±0.7	23.0±0.7	24.6±0.7	24.1±0.7	0.07	0.22	**0.006**	0.55	**0.04**	0.21
RVOT1psax (mm)	24.8±0.8	25.6±0.7	26.1±0.7	25.9±0.6	25.2±0.6	24.2±0.6	25.9±0.9	25.1±0.7	0.15	0.34	0.15	0.36	0.08	0.11
RVOT2psax (mm)	16.6±0.4	16.7±0.4	17.3±0.4	16.8±0.4	17.0±0.5	16.6±0.5	17.1±0.4	17.0±0.5	0.17	0.28	0.62	0.75	0.82	0.21
RA area (cm2)	14.5±0.5	13.8±0.5	14.8±0.6	14.4±0.6	13.4±0.4	13.1±0.5	14.0±0.5	13.4±0.4	**0.04**	**0.001**	**0.001**	0.98	0.8	0.28
Function and mechanics														
RVFAC (%)	47±1	45±1	47±1	45±1	50±1	48±1	51±1	47±1	0.93	**0.007**	**<0.001**	0.49	0.43	0.36
TAPSE (cm)	27±1	26±1	28±1	26±1	30±1	28±1	30±1	28±1	0.89	**<0.001**	**<0.001**	0.52	0.20	0.9
TDI s' (cm/sec)	15±1	14±1	15±1	15±1	19±1	17±1	20±1	18±1	**0.02**	**0.002**	**<0.001**	0.55	0.11	0.73
TDI e' (cm/sec)	17±1	16±1	18±1	17±1	28±1	28±1	29±1	27±1	0.20	**0.02**	**<0.001**	0.45	0.61	0.45
TDI a' (cm/sec)	13±1	12±1	13±1	13±1	-	-	-	-	0.19	0.33	-	0.24	-	-
RV free wall strain (%)	-28.0±1	-27±1	-28±1	-28±1	-33±1	-30±1	-32±1	-30±1	0.90	**<0.001**	**<0.001**	0.58	**0.01**	0.86

Table 2. (Continued)

	Rest echocardiography				Stress echocardiography				p-values					
	Normoxia		Hypoxia		Normoxia		Hypoxia							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	H	E	S	H*E	E*S	E*H*S
RV time of peak (sec)	0.36±0.01	0.37±0.01	0.37±0.01	0.36±0.01	0.28±0.01	0.32±0.01	0.29±0.01	0.31±0.01	0.51	**<0.001**	**<0.001**	0.09	**0.004**	0.57
PAT (ms)	152±3	151±3	139±4	134±3	122±4	120±3	106±4	105±3	**<0.001**	**0.008**	**<0.001**	0.60	0.36	0.30
Strain-area loop characteristics														
Uncoupling (%)	2.0±0.2	1.0±0.4	1.4±0.3	0.6±0.4	1.4±0.5	1.2±0.5	0.7±0.4	0.3±0.5	0.07	0.05	0.32	0.99	0.23	0.66
Uncoupling ED (%)	2.0±0.3	1.0±0.4	1.4±0.3	0.6±0.4	1.4±0.5	1.3±0.5	0.7±0.5	0.4±0.6	0.10	0.10	0.40	0.92	0.14	0.7
Uncoupling LD (%)	2.0±0.2	1.1±0.3	1.3±0.3	0.6±0.4	1.5±0.4	1.0±0.5	0.8±0.4	0.1±0.5	**0.04**	**0.01**	0.22	0.96	0.67	0.62
Sslope (%/cm2)	2.5±0.1	2.5±0.1	2.4±0.1	2.4±0.1	2.8±0.1	2.7±0.1	2.6±0.1	2.6±0.1	0.07	0.53	**0.003**	0.8	0.35	0.33
Esslope (%/cm2)	2.4±0.2	2.6±0.1	2.4±0.1	2.6±0.1	2.9±0.2	2.7±0.2	2.9±0.2	2.7±0.2	0.85	0.97	**0.05**	0.87	**0.04**	0.88
Edslope (%/cm2)	1.4±0.1	1.9±0.2	1.7±0.1	1.8±0.2	1.8±0.2	1.8±0.2	1.7±0.2	2.2±0.3	0.41	0.08	0.29	0.73	0.82	**0.03**
Ldslope (%/cm2)	3.3±0.2	3.0±0.2	3.1±0.2	2.8±0.2	3.6±0.3	3.5±0.3	3.4±0.3	3.1±0.3	0.11	0.18	**0.02**	0.77	0.73	0.34

Data are expressed as means±SEM. ED, Early diastole. ES, Early systole. LD, Late Diastole. PAT, pulmonary acceleration time. PLAX, Parasternal long axis. PSAX, parasternal short axis. RA, Right atrium. RV, Right ventricle. RVFAC, RV fractional area change. RVEDA, Right ventricular end-diastolic area. RVESA, Right ventricular end-systolic area. RVOT, Right ventricular outflow tract. TAPSE, Tricuspid annular plane systolic excursion. TDI, Tissue Doppler imaging. Linear mixed models factors: H, Hypoxia: Comparison between all echocardiographic measurements performed under hypoxic *vs.* normoxic conditions. E, Exercise: Comparison between all echocardiographic measurements performed pre *vs.* post 45-minutes high intensity exercise. S, Stress: Comparison between all echocardiographic measurements performed during rest *vs.* during stress. E*S, Exercise*Stress: Comparison whether the change pre- *vs.* post-exercise is different measured during rest *vs.* stress echocardiography. H*E, Hypoxia*Exercise: Comparison whether the change pre- *vs.* post-exercise (EICF) is different during hypoxic *vs.* normoxic conditions. E*H*S, Exercise*Hypoxia*Stress: comparison whether the change pre- *vs.* post-exercise under hypoxic *vs.* normoxic conditions was different when observed using rest *vs.* stress echocardiography.

Left ventricular structure, function and mechanics

All LV structural, functional and mechanicals indices pre- and post- 45-minute high-intensity running exercise are displayed in **Table 3**. With the exception of LVS' (Exercise: p=0.78), indices of LV systolic function (LVEF, LVGLS) significantly reduced following high-intensity exercise (Exercise: $p<0.001$). The reduction in LVEF and LVGLS was more pronounced in stress *versus* rest echocardiography (Exercise*Stress: both $p<0.05$, **Figure 3B**).

Exercise under hypoxia. Changes in LV indices in response to exercise, either examined at rest and/or during stress, were not different when performed under hypoxic conditions (Hypoxia*Exercise and Exercise*Hypoxia*Stress: $p>0.05$, **Table 3**). Blood pressure response patterns did not significantly differ between hypoxic and normoxic conditions (Hypoxia and Hypoxia*Exercise: all $p>0.05$, **Table 3**).

Table 3. Haemodynamics and left ventricular function and mechanics during rest and stress pre- and post-exercise under normoxia and hypoxia

	Rest echocardiography				Stress echocardiography				p-values					
	Normoxia		Hypoxia		Normoxia		Hypoxia							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	H	E	S	H*E	E*S	E*H*S
Haemodynamics														
Heart rate (bpm)	68±2	71±3	74±2	79±3	113±1	112±1	111±1	113±1	**0.03**	0.07	**<0.001**	0.18	0.10	0.41
Systolic blood pressure (mmHg)	121±2	118±2	124±2	117±2	143±3	120±3	141±4	125±3	0.38	**<0.001**	**<0.001**	0.56	**<0.001**	**0.03**
Diastolic blood pressure (mmHg)	70±2	69±2	70±2	67±2	75±2	60±1	73±2	60±1	0.47	**<0.001**	0.16	0.98	**<0.001**	0.19
Mean arterial pressure (mmHg)	87±1	85±2	88±2	84±2	98±2	80±2	96±2	82±1	0.99	**<0.001**	0.08	0.75	**<0.001**	**0.04**
SpO2 (%)	98±0.2	98±0.3	90±0.5	90±0.7	94±0.8	95±0.6	82±1.0	83±0.9	**<0.001**	0.17	**<0.001**	0.36	0.86	0.68
Structure														
LVEDV (ml)	120±7	113±7	123±6	113±6	114±6	109±6	117±7	110±6	0.30	**<0.001**	**0.004**	0.46	0.55	1.0
LVESV (ml)	50±2	52±3	50±3	49±3	42±2	45±2	42±2	43±2	0.24	0.06	**<0.001**	0.17	0.12	0.76
LA diameter (mm)	30±1	29±1	30±1	28±1	31±1	27±1	30±1	27±1	0.79	**<0.001**	0.34	0.72	0.06	0.25
LA volume (ml)	38±2	34±0.3	39±2	34±2	38±1	35±1	39±1	36±1	0.21	**<0.001**	0.18	0.13	0.12	0.50
Function and mechanics														
LVEFbip (%)	58±1	56±1	59±1	56±1	63±1	58±1	65±1	60±1	**0.008**	**<0.001**	**<0.001**	0.69	**0.005**	0.44
TDI s' (cm/sec)	10±0.4	11±0.4	11±0.3	11±0.4	14±0.5	13±0.5	14±0.4	14±0.4	**0.04**	0.78	**<0.001**	0.98	0.24	0.19
TDI e' (cm/sec)	18±0.5	17±0.6	19±0.4	16±0.4	22±1.1	19±0.7	21±0.6	20±0.5	0.89	**<0.001**	**<0.001**	0.45	0.52	0.09
TDI a' (cm/sec)	8±0.4	9±0.3	9±0.3	10±0.3	-	-	-	-	**0.001**	0.054	-	0.60	-	-

Table 3. (Continued)

	Rest echocardiography				Stress echocardiography				p-values					
	Normoxia		Hypoxia		Normoxia		Hypoxia							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	H	E	S	H*E	E*S	E*H*S
E (cm/sec)	1.02±0.03	0.85±0.04	1.06±0.03	0.86±0.03	1.28±0.05	1.14±0.03	1.32±0.04	1.16±0.04	0.09	**<0.001**	**<0.001**	0.30	0.37	0.80
A (cm/sec)	0.56±0.02	0.57±0.02	0.55±0.02	0.62±0.03	-	-	-	-	**0.004**	0.39	-	0.18	-	-
E/A ratio	1.86±0.06	1.6±0.10	1.83±0.07	1.41±0.06	-	-	-	-	**0.048**	**<0.001**	-	0.15	-	-
LV longitudinal strain (%)	-20±0.3	-20±0.3	-21±0.3	-20±0.2	-23±0.3	-21±0.4	-24±0.4	-22±0.2	0.01	**<0.001**	**<0.001**	0.08	**0.05**	0.86
LV time of peak (sec)	0.36±0.01	0.36±0.01	0.35±.01	0.34±0.01	0.28±0.01	0.29±0.01	0.28±0.01	0.29±0.01	0.14	**0.03**	**<0.001**	0.22	**<0.001**	0.88

Data are expressed as means±SEM. ES, Early systole. LA, Left atrium. LVEDV, Left ventricular end-diastolic volume. LVESA, Left ventricular end-systolic volume. S, Stress. TDI, Tissue Doppler imaging.
Linear mixed models factors: H, Hypoxia: Comparison between all echocardiographic measurements performed under hypoxic vs. normoxic conditions. E, Exercise: Comparison between all echocardiographic measurements performed pre vs. post 45-minutes high intensity exercise. S, Stress: Comparison between all echocardiographic measurements performed during rest vs. during stress. E*S, Exercise*Stress: Comparison whether the change pre- vs. post-exercise is different measured during rest vs. stress echocardiography. H*E, Hypoxia*Exercise: Comparison whether the change pre- vs. post-exercise (EICF) is different during hypoxic vs. normoxic conditions. E*H*S, Exercise*Hypoxia*Stress: comparison whether the change pre- vs. post-exercise under hypoxic vs. normoxic conditions was different when observed using rest vs. stress echocardiography.

DISCUSSION

The aim of our study was to investigate the impact of a bout of high-intensity exercise under hypoxia *versus* normoxia on EICF on both ventricles. The main findings were 1) a bout of 45-minute high-intensity exercise induced a reduction in functional indices of right- and left-sided cardiac function and mechanics in healthy individuals, 2) the reduction in right- and left-sided cardiac function was more pronounced when echocardiography was performed during a standardized low-to-moderate-intensity recumbent exercise challenge and 3) there was no impact of hypoxia on exercise-induced reduction in right- or left-sided cardiac function and mechanics, either under rest or under stress. Taken together, these data indicate that EICF after short-term high-intensity exercise is not exaggerated under hypoxia, suggesting that an additional cardiac load (induced by hypoxia) on the RV does not necessarily relate to an exaggerated EICF in this setting.

High-intensity exercise-induced cardiac fatigue

A bout of 45-minute high-intensity running exercise induced a reduction of both RV and LV function indicative for EICF, which was mainly expressed during a low-to-moderate-intensity exercise challenge ('stress') compared to resting conditions. Earlier studies primarily investigated EICF after prolonged exercise (>180minutes)[4, 27], however, recent research has revealed a dose-response relationship between EICF and the duration and intensity of exercise.[14, 39] Our study adds the novel knowledge that EICF also occurs after relatively short periods of high-intensity exercise in both the RV and LV. Interestingly, in contrast to other short-term high-intensity EICF studies[10, 14, 39], we showed also marked reductions in LV function which may be due to the different type of exercise (running *vs.* cycling). An explanation for our ability to detect EICF after a relatively short duration of exercise may relate to the post-exercise assessment of cardiac function during 'stress', i.e. low-to-moderate-intensity exercise-Indeed, some of the indices for systolic function were primarily/only reduced when echocardiography was performed during the low-to-moderate-intensity exercise challenge. For example, a reduction in RVLS post-exercise was only apparent during the low-to-moderate-intensity exercise challenge (**Figure 4A**). We believe the echocardiography assessment under low-to-moderate-intensity exercise is more likely to detect EICF. The recovery phase post-exercise is associated with a change

in autonomic tone and vasodilation, which may result in post-exercise tachycardia and hypotension, respectively. These (para)sympathetic imbalance factors likely influence cardiac function measurements such as strain, and therefore potentially mask the presence of EICF. Evaluation of cardiac function *during* the high-intensity exercise, therefore, is preferred. However, one should consider the practical aspects (e.g. echocardiography is impossible during running) and that reliable speckle tracking is extremely challenging with higher heart rates (i.e. 70% of maximum HR).[40] Low-to-moderate intensity cycling exercise at a semi-recumbent bike is both feasible and reliable, and allows to examine cardiac function during exercise. Utilising this approach, our data indicates that, with short durations of high-intensity exercise, EICF occurs when assessment of cardiac function is performed during an exercise challenge.

Impact of exercise under hypoxia

Under hypoxic conditions, less oxygen is bound to haemoglobin, and will, therefore, increase the demand on the cardiovascular system. In our population, this was reflected by a higher resting HR under hypoxia *versus* normoxia and the less distance covered under hypoxia *versus* normoxia during the exercise despite it being matched for relative intensity. More importantly, hypoxia has been shown to induce vasoconstriction of the pulmonary vasculature, leading to higher relative PVR resulting in a higher PAP, and consequently a higher RV wall stress. Elevated PAP has been previously demonstrated at conditions at 3000m altitude.[23] Although we were unable to directly measure PAP, we demonstrated shorter PAT and a larger RA size which indirectly supports the presence of an increase in PAP and, therefore potentially wall stress. Also, the strain-area loop showed less uncoupling in late diastole and a trend for a less steep systolic slope under hypoxia. In line with a previous study in PAH patients, these changes are associated with a higher PVR at rest.[28] Although we adopted a non-invasive approach and one should consider alternative explanations (i.e. related to the assessment), these findings support the presence of an elevated wall stress in our study under hypoxia. That aside, our hypothesis was rejected as the 45-minute high-intensity running exercise under hypoxia did not exaggerate RV EICF compared to exercise under normoxia. This suggests that changing cardiac workload does not necessarily change the magnitude of RV EICF and may not be the principle mechanism for RV EICF. One potential explanation for the lack of an impact

of hypoxia on EICF may be that the exaggerated loading conditions under hypoxia were not sufficient enough at 3000m of simulated altitude, and/or the exposure time to the raised afterload of the RV was not long enough to contribute to the EICF magnitude. There are also indications that hypoxia itself may induce cardiac dysfunction due to sustained low oxygen availability, however, this seems mainly during prolonged exposure.[41]

Our hypothesis originated from the accepted phenomenon of disproportionately higher relative wall stress in the RV compared to the LV during exercise, but also based on observations suggesting a larger magnitude of EICF in the RV compared to the LV.[11, 14, 15] For example, Stewart *et al.* examined the influence of high-intensity exercise on RV free wall and segmental LV strain EICF following 90 minutes cycling[10], and found that the reduction in strain was more profound in the RV than in the LV. In their study they demonstrated a relative reduction in RV strain of -17.5% compared to -9.8% in our study, which supports a dose-response relationship. Our study is the first to our knowledge to directly compare normoxic and hypoxic conditions on EICF, and demonstrated similar changes in both RV and the LV. Although mechanical changes in the RV and LV are independent of each other[27], and likely differ during exercise, our work suggests that (after)load dependency may be a less contributing factor to EICF as previously suggested. Alternatively, intrinsic myocardial factors such as β-adrenergic receptor desensitization[7, 42] and oxidative stress[43] may play a more substantial role. Our study, however, is unable to provide further insight into these other possible mechanisms.

It is also of interest that following the 45-minute high-intensity exercise, this study showed a lack of any RV dilation (no rightward shift strain-area loop, **Figure 4**) as previously demonstrated following prolonged exercise.[27] Previous studies have demonstrated a serial and parallel impact from ventricular interdependence on LV filling secondary to RV volume / pressure overload.[27, 44] This finding is consistent with other studies of high-intensity exercise of relative short durations rather than is seen in EICF studies of prolonged exercise highlighting a possible dose response related to both intensity and duration.[10, 14, 27] In the shorter duration exercise intervention studies, the reduction in LV size occurs irrespective of changes in RV size which provides additional support for an intrinsic mechanism independent to both the right and left side of the heart. Moreover, the decreased uncoupling in the strain-area loop (**Figure 4**), indicating less longitudinal

contribution to area change, in combination with a lack of RV dilatation, supports that the reduction in peak longitudinal strain post-exercise (i.e. EICF) is more likely representative of intrinsic dysfunction.

Perspectives

The mechanisms underlying EICF are likely multifactorial, and importantly may differ between the RV and LV. Previous research has proposed several influencing factors varying from β-adrenergic receptor desensitization, oxidative stress, impaired calcium metabolism to altered post-exercise loading. The influence of afterload conditions on RV EICF have rarely been explored. This study demonstrated that, under hypoxic conditions at 3000m altitude (FiO_2 14.5%), the magnitude of EICF is not augmented and thus it may be less likely that a role for elevated RV wall stress is relevant. Although knowledge about the clinical long-term consequences of these temporary post-exercise reductions in cardiac function is lacking, it has been hypothesized that this may be associated with myocardial damage and worse clinical outcome. The absence of an effect in EICF between exercising at sea level (normoxia) and 3000m altitude (hypoxia) is interesting, but long-term studies that link these findings to prolonged follow-up is needed to better understand these findings. The novel strain-area loop, introduced to assess haemodynamics non-invasively, provided substantial added value in this study where it was sensitive enough to detect changes due to hypoxia. This novel technique seems promising in providing physiological and pathophysiological insight and might be of added value in clinical practice.[5, 27, 28, 37, 45-48]

Limitations

This study implemented a standardized exercise challenge to prevent a pre- and post-exercise (para)sympathetic imbalance during echocardiographic evaluation. Instead of the methodology of Stewart *et al.*[14] (aiming at 100 bpm), we set our target HR at 110-120 bpm during the exercise challenge, to better mimic cardiac function during exercise. This higher HR may impede speckle tracking quality. With current frame rates used, we experienced that tracking was still good to excellent for LV global longitudinal strain and RV free wall strain. A further limitation is that we did not obtain direct measures of RV wall stress as this would require invasive procedures. Alternatively, we used only non-invasive echocardiographic, indirect measures to estimate any potential difference in

RV wall stress under hypoxia *versus* normoxia. When considering these indirect indices, some studies have demonstrated value of PAT during stress to estimate PAP in PAH patients whilst others have questioned the outside of the normal heart rate range (<60 or >100 bpm).[24, 26] It is clear that a more robust assessment of PAP would provide added support to the well-established physiological concepts and understanding of hypoxia and pulmonary haemodynamics. Previous studies have applied strain-area loops to PAH patients and demonstrated an association between PVR and the late diastolic uncoupling and the Sslope during rest only.[28] Further work should aim to validate the strain-area loops during stress. Finally, for technical reasons we only evaluated right heart function and haemodynamics during low-to-moderate stress echocardiography rather than during the high-intensity running exercise.

CONCLUSION

There was no impact of hypoxia on the magnitude of EICF in the RV and LV after a bout of 45-minute high-intensity exercise. This finding suggests that any potential increase in loading conditions does not automatically exacerbate EICF in this setting.

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APPENDICES

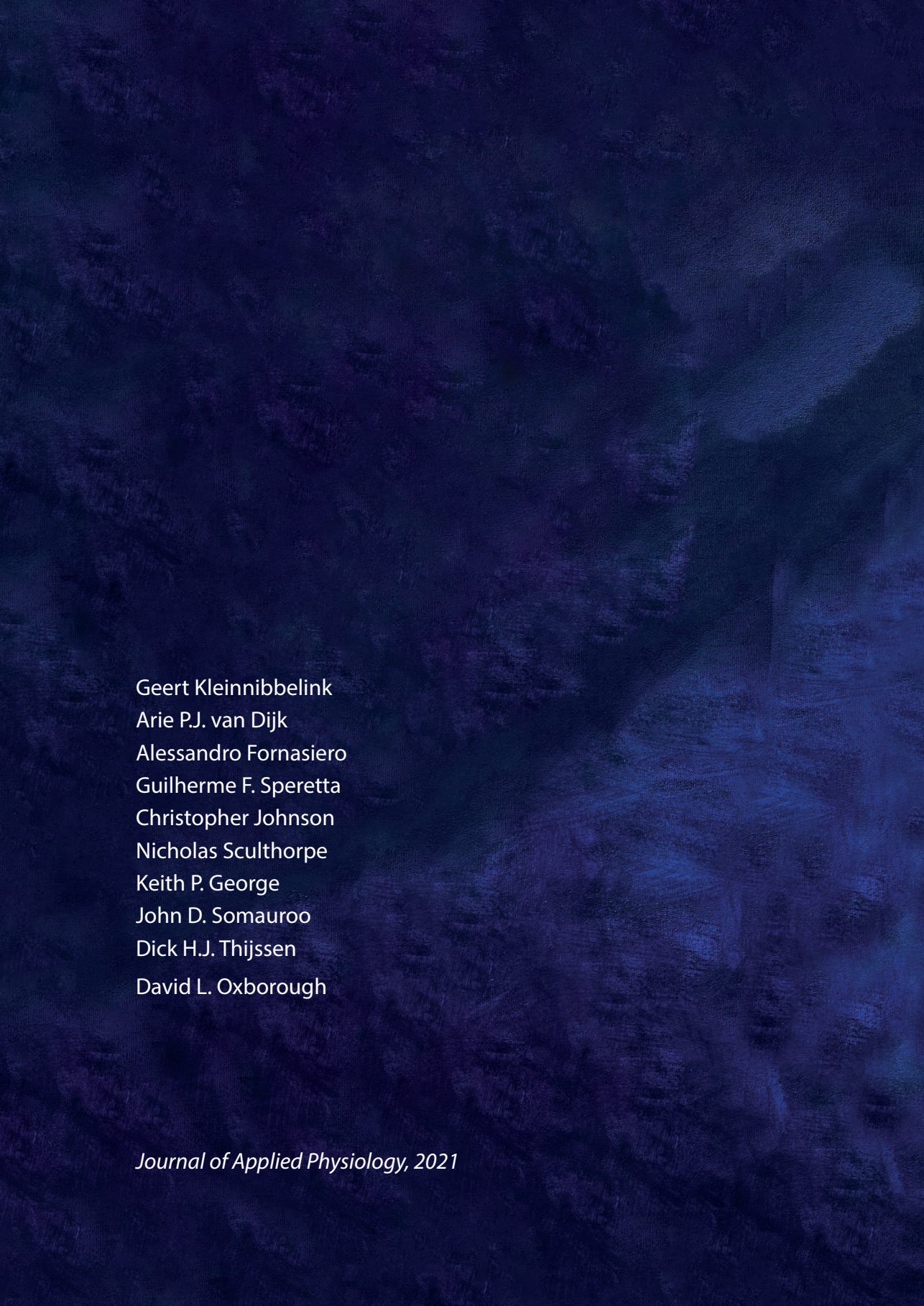
Supplemental 1. Strain-Area Loop – methods of derivation

To calculate right ventricular (RV) strain-area loops the following steps have been taken. Temporal longitudinal strain values were exported to a spreadsheet (Excel; Microsoft Corp., Redmond, WA, USA). Using cubic spline interpolation, the global temporal longitudinal strain values were divided into 300 points for systole and 300 points for diastole in order to correct for variable heart rates. For both systole and diastole, the 300 strain values were then split into 5% increments of the cardiac cycle, providing longitudinal strain values at 10 time points in systole and 10 time points in diastole. Concomitant time points for the strain values were used in the same image and cardiac cycle to trace RV monoplane areas to provide simultaneous strain and area values.

Using the individual strain–area loop, a linear regression line and a polynomial of two orders was applied to both diastolic and systolic parts of the loop. This derived polynomial equation allowed the derivation of strain at percentage increments of RV end-diastolic area (RVEDA). The longitudinal strain–area loop was assessed using the following parameters (**Figure 2 manuscript**): (a) early linear slope during first 5% of volume ejection in systole (ESslope), (b) the overall linear slope during systole (Sslope) and (c) end-systolic peak longitudinal strain (peak strain). In addition (un)coupling was termed to describe the relationship between systolic and diastolic strain for any given area/volume and was assessed during (d) early (Uncoupling ED), (e) late diastole (Uncoupling LD) and (f) overall. Furthermore, (g) the early linear slope during first 5% (EDslope) and (h) late linear slope (LDslope) during last 5% of volume increase in diastole.

The Sslope was derived as the gradient of the linear regression line over the systolic phase of the strain–area loop. Longitudinal peak strain was derived as the raw peak strain value from the longitudinal strain data. The Uncoupling ED and Uncoupling LD were calculated across the area between the systolic and diastolic polynomial curves. Using the equations of the polynomial regression lines, strain at % increments of RVEDA were calculated. By subtracting diastolic from systolic strain, the difference at each point was calculated. Based on individual RV fractional area change (RVFAC), the working range of the heart

was determined, after which Uncoupling ED was calculated as the sum of the differences at the lowest two-thirds of increments of EDA in the working range of the heart, and Uncoupling LD was calculated as the sum of the differences at the highest one-third of increments of EDA in the working range of the heart.`



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Acute Exercise-Induced Changes in Cardiac Function Relates to Right Ventricular Remodelling Following 12-weeks Hypoxic Exercise Training

CHAPTER 3

ABSTRACT

Repeated ventricular exposure to alterations in workload may relate to subsequent cardiac remodelling. We examined whether baseline acute changes in right (RV) and left ventricular (LV) function relate to chronic cardiac adaptation to 12-week exercise training. Twenty-one healthy individuals performed 12-week high-intensity endurance running training under hypoxia (fraction of inspired oxygen: 14.5%). Resting transthoracic echocardiography was performed before and after the training programme to assess ventricular structure, function and mechanics (including strain-area/volume loops). In addition, we examined systolic cardiac function during recumbent exercise under hypoxia at baseline (heart rate of 110-120 bpm, 'stress echocardiography'). Fifteen individuals completed training (22.0±2.4y, 10 male). Hypoxic exercise training increased RV size, including diameter and area (all $p<0.05$). With exception of an increase in RV fractional area change ($p=0.03$), RV function did not change post-training (all $p>0.05$). Regarding the RV strain-area loop, lower systolic and diastolic slopes were found post-training ($p<0.05$). No adaptation in LV structure, function or mechanics were observed (all $p>0.05$). To answer our primary aim, we found that a greater increase in RV fractional area change during baseline stress echocardiography ($r=-0.67$, $P=0.01$) inversely correlated with adaptation in RV basal diameter following 12-week training. In conclusion, 12-week high-intensity running hypoxic exercise training induced right-sided structural remodelling, which was, in part, related to baseline increase in RV fractional area change to acute exercise. These data suggest that acute cardiac responses to exercise may relate to subsequent RV remodelling after exercise training in healthy individuals.

INTRODUCTION

Exercise training results in remodelling of the heart, including chamber enlargement and hypertrophy.[1] Studies examining the impact of exercise training on cardiac remodelling have predominantly focused on left ventricular (LV) adaptation, with few studies revealing right ventricle (RV) changes to training.[2-4] To better understand the effects of exercise on RV and LV function, recent studies suggest a relative larger increase in wall stress for the RV *versus* LV during exercise.[5] These acute effects of exercise on cardiac function may be of importance. Indeed, cardiac remodelling seems mechanistically related to the repeated exposure to acute changes in wall stress. Therefore, in-exercise echocardiographic indices of cardiac function may (partly) relate to the presence of subsequent cardiac remodelling. However, no study directly examined this hypothesis in relation to exercise training and remodelling in humans.

Recently, the strain-area/volume loop has been introduced to allow for the assessment of simultaneous structure and strain across the cardiac cycle providing mechanical insight into cardiac function.[6] We found that post-surgery changes in LV strain-volume loop characteristics relate to subsequent cardiac remodelling in patients with aortic stenosis.[7] Therefore, these changes may serve as a proxy of changes in wall stress. Furthermore, we observed different RV loop characteristics in the 'four cornerstones' of the Mitchell classification of sports potentially due to their difference in cardiac structure and function.[6] Possibly, these differences in strain-area/volume loops may relate to cardiac remodelling to exercise training. Therefore, the strain-area loop, in conjunction with other measures of cardiac function, may provide insight into cardiac adaptation to exercise training.

The aim of this study was to relate pre-training changes in cardiac function during low-to-moderate-intensity exercise to subsequent adaptations to a 12-week hypoxic endurance exercise training program on cardiac structure, function and mechanics (i.e. longitudinal strain and strain-area/volume loops) in healthy individuals. We specifically choose hypoxic exercise since, due to a smaller reduction in pulmonary vascular resistance compared to normoxic exercise[8], this type of exercise causes a higher RV afterload.[9-12] Indeed, we showed that 45 minutes high-intensity running exercise under hypoxia lowers pulmonary acceleration time, increases right atrial size and lowers the late diastolic uncoupling of

the RV strain-area loop compared to exercise under normoxia.[13] These echocardiographic markers support indirectly the presence of an increase in pulmonary artery pressure, and therefore, RV afterload. Accordingly, hypoxic exercise may exaggerate the disproportionate elevation in wall stress for the RV *versus* LV during exercise and may therefore lead to more rapid adaptations in the RV to exercise training allowing us to further explore our hypothesis.

METHODS

Study population

Twenty-one healthy individuals (fourteen males) were recruited for the study. Participants were eligible to take part in this study if they were able to run on a treadmill and that they did not engage in sport-related exercise for more than two hours a week at moderate-to-high intensity for the last six months. Exclusion criteria were a body mass index (BMI) <18 or >30 kg/m^2, any possibility of pregnancy, personal history of cardiovascular disease, a family history of cardiovascular death (<55y), exercise-limiting respiratory disease, physical (i.e. musculoskeletal) complaints making completion of the 12-week training program impossible, abnormal resting 12-lead electrocardiogram (ECG) and abnormalities observed on resting transthoracic echocardiography. The procedures were performed in accordance with institutional guidelines and conformed to the declaration of Helsinki. The study was approved by the Ethics Research Committee of the Liverpool John Moores University (18/SPS/065). Participants gave full written and verbal informed consent before participation.

Study design

In this prospective study, participants attended the laboratory on 35 separate occasions, see **Figure 1**. During the first visit, a medical screening was performed to determine eligibility of the potential participants. After signing informed consent, baseline measurements including echocardiographic assessment at rest were performed under normoxic conditions (FiO_2 20.9%). During visit 2, after 30 minutes of acclimation echocardiographic assessments at rest and during stress under hypoxic conditions (FiO_2 14.5%) were performed. These assessments were obtained in order to relate acute

RV functional responses to exercise to chronic RV adaptation after 12 weeks of hypoxic training. Visit 3 to 34 comprised the individual sessions of the hypoxic training program. Visit 35 comprised follow-up measurements, including echocardiographic assessment at rest and were performed under normoxic conditions.

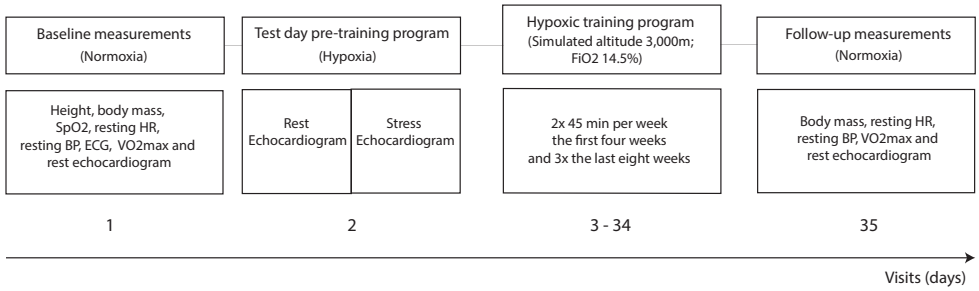

Figure 1. Overview of study design. Longitudinal data assessment (baseline and follow-up measurements including echocardiography) were performed under normoxic conditions whereas the training program was performed under hypoxic conditions. Additionally, during visit 2, an echocardiographic assessment was performed (after 30 minutes of acclimation) to obtain acute exercise induced changes in cardiac function to relate to chronic structural remodelling to hypoxic training.

Baseline and follow-up measurements. Participants were examined for height (SECA stadiometer, SECA GmbH, Germany), weight (SECA scale, SECA GmbH, Germany), oxygen saturation (SpO_2, pulse oximetry; Ana Pulse 100, Ana Wiz Ltd., UK), 12-lead ECG (Cardiovit MS-2010, Schiller, Switzerland) and maximal oxygen consumption (VO_2max). Resting heart rate (HR, Polar, Kempele, Finland) and resting blood pressure (BP, Dinamap V100, GE Medical, Norway) were determined at the end of ten minutes of quiet rest in supine position. A standardized maximal cardiopulmonary exercise test (CPET, Oxycon pro, CareFusion, VS) for VO_2max assessment was conducted on a motorized treadmill (HP Cosmos, Nussdorf, Germany) after familiarization and a 10-min warm-up. VO_2max was defined as the highest value of a 30-s average[14], and attainment was verified according to previous recommend criteria.[15]

Training program. Participants took part in a 12-week normobaric hypoxic endurance exercise training program consisting of 2x45 minute sessions a week in the first four weeks and 3x45 minute sessions in the last eight weeks. This running exercise was performed on

a motorized treadmill at 3,000m simulated altitude (equivalent to FiO_2 14.5%) at high-intensity (85% of maximal heart rate).

Environmental chamber and safety. All training sessions were conducted in an environmental chamber (TISS, Alton, UK; Sportingedge, Bastingstoke, UK), which was set-up by a qualified technician. Normobaric hypoxia was achieved by a nitrogen dilution technique. Ambient temperature, carbon dioxide (CO_2) and oxygen (O_2) levels were controlled in all sessions (20°C; FiO_2 14.5%; CO_2 0.03%), whilst a Servomex gas analysis system (Servomex MiniMP 5200, Servomex Group Ltd., UK) was used inside the chamber to provide the researcher continuous information regarding O_2 and CO_2 levels. Acute mountain sickness symptoms (AMS, measured by Lake Louise Score (LLS)[16]) were monitored during testing and training sessions every 20 minutes. Subjects were removed from the environmental chamber if oxygen saturation levels dropped below 75% or severe AMS was suspected (LLS≥6).

Echocardiographic measurements

Echocardiographic assessments, prior to and post training program, were performed at rest ('rest') and during recumbent cycling to elevate heart rate allowing direct assessment of cardiac function during exercise ('stress', target HR 110-120 bpm). Rest and stress echocardiography were performed in the left lateral decubitus position on a supine cycle ergometer (Lode B.V.; Groningen, The Netherlands). For stress echocardiography, low-to-moderate-intensity exercise consisted of recumbent cycling at a cadence of ~60 revolutions per minute. All examinations were performed by one highly experienced sonographer (DO) using a Vivid E95 ultrasound machine (GE Medical, Horton, Norway), equipped with a 1.5-4.5 MHz transducer. Images were stored in raw digital imaging and communication in medicine (DICOM) format and were exported to an offline workstation (EchoPAC, version 203, GE Medical, Horton, Norway). Data-analysis was performed by a single observer with experience in echocardiography (GK) using three consecutive stored cycles with exception of strain-volume loops which were analyzed from a single cardiac cycle. The observer was blinded for the timing (pre *vs*. post) under which echocardiography was performed.

Conventional measurements. Cardiac structural and functional measurements at rest and during low-to-moderate exercise were made according to the current guidelines for cardiac

chamber quantification.[17] Regarding the right heart, we examined the following structural and functional indices: basal and mid-cavity end-diastolic diameters, RV end-diastolic area (RVEDA), RV end-systolic area (RVESA), RV outflow tract (RVOT) diameter at the proximal level in the parasternal long-axis (RVOT PLAX) and the proximal and distal portion in the parasternal short-axis (PSAX) view (RVOT1 and RVOT2, respectively), right atrial (RA) area, RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE) and tissue doppler imaging (TDI) of the tricuspid annulus ('s, e', a'). Regarding the left heart, the following structural and functional indices were determined: biplane LV end-diastolic volume (LVEDV), biplane LV end-systolic volume (LVESV), LV mass, relative wall thickness (RWT), LV wall thickness (IVSd, septal; PWd, posterior), LV internal diameter (LVIDd), LA diameter, LA volume, modified Simpson's left ventricular ejection fraction (LVEF), tissue Doppler imaging (TDI) of the mitral annulus (s', e' and a'), trans-mitral Doppler (E, A and E/A ratio). All RV and LV structural indices were allometrically scaled to body surface area (BSA) according to the laws of geometric similarity.[18]

Mechanics. Images were acquired specifically for offline speckle tracking analysis. This involved the optimization of frame rates between 40 and 90 frames s^{-1}, depth to ensure adequate imaging of the chamber of interest and compression and reject to ensure endocardial delineation. The RV focused and the apical two-chamber, four-chamber and long-axis view were utilized for the RV free wall (RVFWS) and LV global longitudinal strain (LVGLS), respectively. Valve closure times were determined from the respective pulsed wave Doppler signals. For both the RV and LV the myocardium was manually traced to include the septum and adjusted so that the region of interest (ROI) incorporated all of the wall thickness, while avoiding the pericardium.[19, 20] The region of interest was divided into six myocardial segments, providing segmental strain curves. LV global longitudinal strain was obtained by averaging the 18 segments of the three separate apical LV views and global RV strain from three segments of the RV free wall. Where inappropriate tracking of segments was observed visually or detected by the system, retracing was performed until all segments were considered acceptable.

RV strain-area and LV strain-volume loops. The longitudinal strain-area/volume relationship (for methodology of derivation, see Supplemental 1, Oxborough *et al.*[6] and Hulshof *et al.*[21]) was assessed using the following parameters (**Figure 2**): (a) early linear slope during

first 5% of volume ejection in systole (ESslope), (b) the overall linear slope during systole (Sslope) and (c) end-systolic peak global longitudinal strain (peak strain). In addition (un) coupling was termed to describe the relationship between systolic and diastolic strain for any given area/volume. By subtracting diastolic from systolic strain, the difference at any given area/volume was calculated. Uncoupling was assessed as the mean of the differences during (d) early diastole (early 2/3 of diastole [Uncoupling ED]), (e) late diastole (late 1/3 of diastole [Uncoupling LD]) and (f) overall (complete cardiac cycle). Furthermore, (g) the early linear slope during first 5% (EDslope) and (h) late linear slope (LDslope) during last 5% of volume increase in diastole.

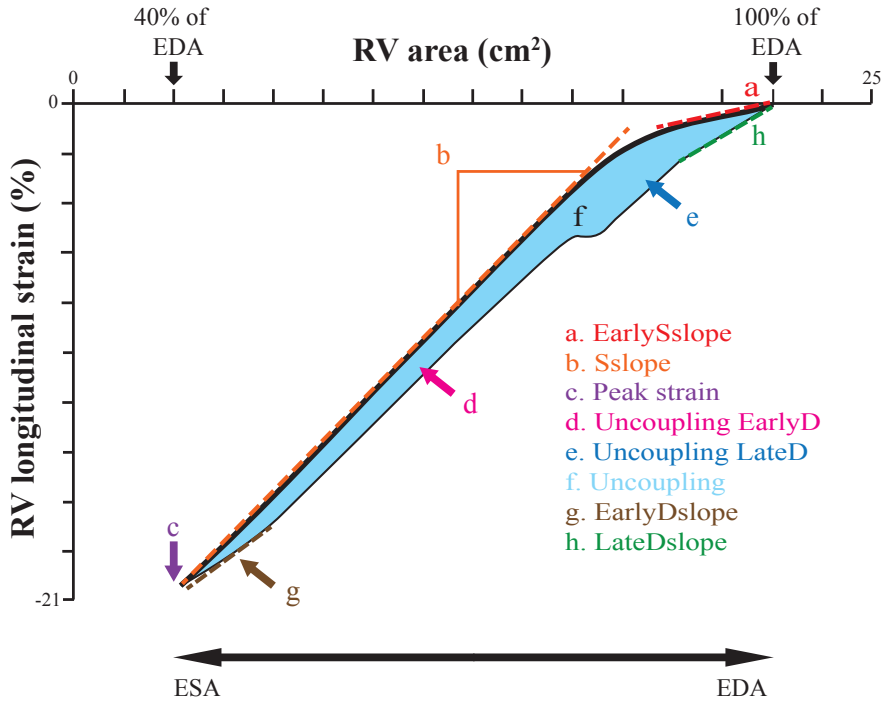

Figure 2. Schematic overview of the RV strain-area loop and the derived characteristics. The black line represents the strain-area loop; the thick part represents the systolic phase and the thin part the systolic phase. ED, End-diastolic, EDA, end-diastolic area; ESA, end-systolic area; LD, late diastolic.

In order to obtain intra-observer variability, 10 randomly selected echocardiograms were re-analyzed. Intra-class correlation coefficient (ICC) analysis was performed for the following measures: RV strain-area loop characteristics, RVEDA, RVESA, RVFAC, RV basal diameter, RV mid-cavity diameter, RVOT PLAX, RA area, IVSd, PWd, LVIDd.

Statistical measurements

Statistical analysis was performed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, VS). All parameters were visually inspected for normality and tested with Shapiro-Wilk normality tests. Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were presented as proportions. Paired-sampled T-tests were used to compare baseline and follow-up measurements, including echocardiographic indices, and to determine acute RV functional responses to exercise (augmentation in cardiac function between stress and rest echocardiography). Associations between acute RV functional responses to exercise (TDI s', RVFWS, TAPSE, RVFAC) and chronic RV adaptation (RV basal diameter, RV mid-cavity diameter, RVEDA) were analysed by Pearson's correlation coefficient, in which 'acute' is defined as the change in RV function from rest to exercise and 'chronic' as change in structure pre- versus post-training program. For all tests, we assumed statistical significance at $p<0.05$.

RESULTS

Twenty-one participants were initially included in the study, of which six dropped-out (motivational issues n=4; health problems unrelated to the study n=2). Participants completed on average 30±2 training sessions (94% adherence) at an average 83.5% of their maximum HR. The fifteen participants who completed the study (22.0±2.4 years, ten men, 24.0±3.0 kg/m^2) showed a significant increase in VO_2max/kg (52±7 to 56±7 mL/min/kg, $p<0.001$) (**Table 1**). BMI and BSA did not significantly change ($p>0.05$) (**Table 1**). Mean SpO_2 during the individual 45 minutes high-intensity running exercise sessions of the hypoxic training program was 81±4%. At baseline, both right and left heart had normal geometry and all structural measurements were within normal ranges (**Table 2)**. There were no abnormal 12-lead ECG findings.

Table 1. Subject characteristics: prior to and post-training program

	Pre	Post	p-value
Sex (m/f)	10/5		
Age (yr)	22.0±2.4		
Height (cm)	172±11		
Body Mass (kg)	71.2±11.7	70.3±12.3	0.17
BMI (kg/m2)	24.0±3.0	23.6±2.7	0.14
BSA (kg)	1.84±0.19	1.83±0.20	0.18
Resting HR (bpm)	77±10	66±6	**<0.001**
Resting SBP (mmHg)	118±4	113±9	**0.02**
Resting DBP (mmHg)	67±8	63±5	0.07
Resting MAP (mmHg)	84±6	80±6	**0.03**
VO2max (L/min)	3.7±0.7	3.9±0.8	**<0.001**
VO2max/kg (mL/min/kg)	52±7	56±7	**<0.001**
VE (L/min)	138±29	145±34	**0.002**
HRmax (bpm)	199±8	195±7	**0.008**

Data are expressed as means±SD. m, male. f, female. BMI, body mass index. BSA, body surface area. HR, heart rate. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. VO_2max, maximal oxygen uptake. VE, ventilation.

Cardiac adaptations to hypoxic exercise training

There was a significant increase in RV and RA size following the training intervention (all $p<0.05$) (**Table 2**). Exercise training caused an increase in RVFAC ($p=0.03$), whilst no other significant changes in RV function were observed (all $p>0.05$) (**Table 2**). In addition to a rightward shift of the strain-area loop (increased RVEDA), exercise training significantly decreased uncoupling and slopes of the RV strain-area loop (**Table 2, Figure 3A**). In contrast to the structural adaptation of the RV, exercise training did not alter LV structure (**Table 2**). Systolic LV function and mechanics, including LV loops, did not change following training (all $p>0.05$) (**Figure 3B**). Regarding diastolic function, A velocity decreased ($p=0.002$), resulting in an increased E/A ratio ($p=0.005$).

Table 2. Structural and functional cardiac adaptation after 12-week hypoxic high-intensity endurance exercise training program

	Right heart		Pre	Post	p-value
Structure	RV basal diameter (mm)		37.9±4.2	42.4±2.2	**<0.001**
		Index	28.0±2.5	31.4±1.8	**<0.001**
	RV mid-cavity diameter (mm)		29.0±3.9	32.3±3.8	**<0.001**
		Index	21.4±2.5	23.9±2.5	**<0.001**
	RVEDA (cm^2)		20.5±2.9	24.3±3.1	**<0.001**
		Index	11.3±1.2	13.6±1.6	**<0.001**
	RVESA (cm^2)		10.9±2.0	11.9±2.0	0.09
		Index	6.0±0.9	6.6±0.9	0.07
	RVOT PLAX (mm)		24.0±3.2	25.5±2.7	0.052
		Index	17.8±2.6	18.9±2.4	**0.045**
	RVOT1 PSAX (mm)		24.5±4.1	26.2±3.9	0.12
		Index	18.14±3.1	19.4±2.8	0.11
	RVOT2 PSAX (mm)		16.4±2.0	17.6±1.6	0.057
		Index	12.1±1.7	13.1±1.2	0.051
	RA Area (cm^2)		15.0±2.6	16.5±2.9	**0.038**
		Index	8.2±1.5	9.0±1.3	**0.02**

	Left heart		Pre	Post	p-value
Structure	LVEDV (ml)		124±30	128±30	0.25
		Index	49±8	51±7	0.11
	LVESV (ml)		51±11	51±12	0.93
		Index	20±3	21±4	0.73
	LV mass (g)		125±24	125±29	0.97
		Index	68±10	68±12	0.97
	RWT		0.35±0.05	0.35±0.04	0.94
	IVSd (mm)		7.8±0.8	7.6±1.2	0.42
		Index	5.7±0.4	5.6±0.8	0.47
	PWd (mm)		8.3±0.9	8.3±1.0	0.77
		Index	6.1±0.7	6.2±0.7	0.7
	LVIDd (mm)		47.2±4.0	47.3±3.3	0.83
		Index	34.9±2.7	35.1±2.0	0.63
	LA diameter (mm)		29.3±3.2	31.1±3.8	0.11
		Index	21.7±2.3	23.1±3.1	0.095
	LA volume (ml)		36.9±7.5	41.9±10.7	**0.04**
		Index	14.9±3.1	16.9±3.1	**0.014**

Table 2. (Continued)

	Right heart	Pre	Post	p-value
Function	RV free wall strain (%)	-28.3±2.3	-27.7±2.1	0.50
	RVFAC (%)	47.2±4.5	51.1±3.9	**0.03**
	TAPSE (mm)	27.5±2.1	26.7±2.5	0.32
	TDI s' (cm/s)	15.2±1.8	15.1±1.3	0.88
	TDI e' (cm/s)	17.5±1.9	16.5±2.3	0.17
	TDI a' (cm/s)	13.7±2.2	13.7±2.6	0.99
Strain-area loop	Uncoupling (%)	2.3±0.9	1.2±1.0	**0.02**
	Uncoupling ED (%)	2.3±1.1	1.1±1.1	**0.02**
	Uncoupling LD (%)	2.2±0.8	1.4±0.9	**0.04**
	Sslope (%/cm^2)	2.5±0.3	2.0±0.3	**<0.001**
	ESslope (%/cm^2)	2.3±0.6	1.6±0.4	**0.009**
	EDslope (%/cm^2)	1.4±0.6	1.7±0.7	0.12
	LDslope (%/cm^2)	3.4±0.9	2.0±0.5	**<0.001**
	RV global longitudinal strain (%)	-24.4±2.4	-23.2±.4	0.18

	Left heart	Pre	Post	p-value
Function	LV longitudinal peak strain (%)	-20.8±1.2	-20.3±0.9	0.13
	LVEF (%)	59±3	60±3	0.36
	TDI s' (cm/s)	10.3±1.8	10.9±1.6	0.07
	TDI e' (cm/s)	18.0±2.3	17.7±1.7	0.62
	TDI a' (cm/s)	8.8±1.8	9.2±1.2	0.16
	E (m/s)	1.02±0.12	1.01±0.15	0.34
	A (m/s)	0.57±0.11	0.51±0.13	**0.002**
	E/A ratio	1.82±0.28	2.13±0.47	**0.005**
Strain-volume loop	E/e' ratio	5.8±0.8	5.8±1.0	0.96
	Uncoupling (%)	-0.8±1.1	-0.8±1.0	0.99
	Uncoupling ED (%)	-1.0±1.2	-1.0±1.2	0.93
	Uncoupling LD (%)	-0.5±0.8	-0.6±0.9	0.87
	Sslope (%/cm^2)	0.4±0.1	0.4±0.1	0.78
	ESslope (%/cm^2)	0.3±0.1	0.3±0.1	0.47
	EDslope (%/cm2)	0.5±0.1	0.6±0.1	0.45
	LDslope (%/cm2)	0.2±0.2	0.2±0.1	0.55
	LV longitudinal strain A4C (%)	-20.0±1.7	-19.2±1.6	0.18

Data are expressed as means±SD. RVEDA, RV end-diastolic area. RVESA, RV end-systolic area. RVOT, RV outflow tract. PLAX, parasternal long axis. RVFAC, RV fractional area change. TAPSE, tricuspid annular plane systolic excursion. TDI, tissue doppler imaging. Uncoupling ED, Uncoupling early diastole. Uncoupling LD, Uncoupling late diastole. LVEDV, LV end-diastolic volume. LVESV, LV end-systolic volume. RWT, relative wall thickness. IVSd, septal wall thickness. PWd, posterior wall thickness. LVIDd, LV internal *diameter in diastole. LVEF, LV ejection fraction.*

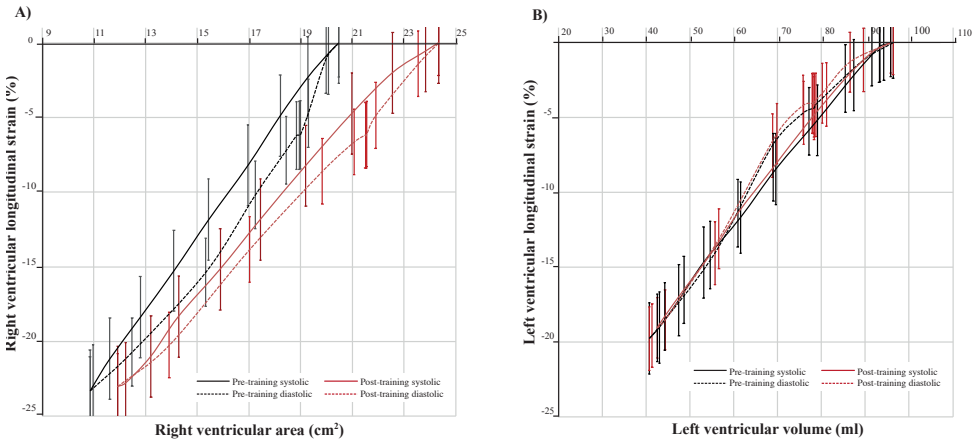

Figure 3. A) mean RV strain-area loops and B) mean LV strain-volume loops prior to ('Pre Systolic': black lines, 'Pre Diastolic': black dotted lines) and post ('Post Systolic': red lines, 'Post Diastolic': red dotted lines) 12-week hypoxic high-intensity running exercise training program. Error bars represent means±SE.

Acute exercise-induced changes in cardiac responses *versus* structural adaptation

Prior to training, all systolic indices for RV function (RVFWS, TDI s', RVFAC, TAPSE) significantly increased with acute exercise (all $p<0.05$) (**Table 3**). The RV strain-area loop characteristics did not significantly change with acute exercise (all $p>0.05$) (**Table 3**). The change in RVFAC with acute exercise showed a significant inverse correlation with changes in basal diameter post-training ($r=-0.66$, $p=0.01$) (**Figure 4**). The inverse relation indicates that a lesser increase in RVFAC with acute exercise is associated with greater RV structural adaptation to training. Changes in RVFWS, TDI s' and TAPSE with acute exercise did not correlate with RV structural indices (data in Supplemental 2). As strain-area loop characteristics did not significantly change with acute exercise, we did not perform correlations analysis on these data.

Intra-observer variability. ICC were as follows: RV free wall strain 0.96 (0.84-0.99), Sslope 0.92 (0.70-0.98), ESslope 0.84 (0.48-0.96), EDslope 0.94 (0.79-0.99), LDslope 0.95 (0.80-0.99), Uncoupling 0.87 (0.56-0.97), Uncoupling_ED 0.86 (0.52-0.96), Uncoupling_LD 0.88 (0.58-0.97), RVEDA 0.96 (0.87-0.99), RVESA 0.94 (0.78-0.99), RVFAC 0.92 (0.72-0.98), RV basal diameter 0.91 (0.68-0.98), RV mid-cavity diameter 0.80 (0.38-0.95), RVOT PLAX 0.75 (0.27-0.93), RA area 0.99 (0.97-0.99), IVSd 0.67 (0.12-91), PWd 0.74 (0.25-0.93), LVIDd 0.79 (0.35-0.94).

Table 3. Right ventricular acute functional responses to exercise pre-training program

		Rest	Stress	delta	p-value
Function	HR (bpm)	75±9	113±5	38±9	**<0.001**
	RVFAC (%)	46.9±2.3	52.0±4.9	5.1±4.7	**0.002**
	TAPSE (mm)	27.3±2.6	30.7±3.4	3.4±3.4	**0.002**
	TDI s' (cm/s)	15.8±2.2	20.1±2.6	4.3±2.9	**<0.001**
	RV free wall strain (%)	-27.5±2.5	-32.0±3.1	-4.5±3.8	**0.001**
Strain-area loop	Uncoupling (%)	1.5±1.2	0.8±1.8	-0.7±2.1	0.25
	Uncoupling ED (%)	1.6±1.3	0.7±2.0	-0.8±2.4	0.25
	Uncoupling LD (%)	1.4±0.9	0.9±1.6	-0.5±1.8	0.30
	Sslope (%/cm2)	2.4±0.4	2.5±0.4	0.1±0.4	0.20
	ESslope (%/cm2)	2.4±0.6	3.0±0.7	0.6±1.1	0.07
	EDslope (%/cm2)	1.6±0.5	1.5±0.5	-0.1±0.5	0.38
	LDslope (%/cm2)	3.1±0.8	3.4±1.1	0.4±0.7	0.11

Data are expressed as means±SD. HR, Heart rate. RVFAC, RV fractional area change. TAPSE, tricuspid annular plane systolic excursion. TDI, tissue doppler imaging. Uncoupling ED, Uncoupling early diastole. Uncoupling LD, Uncoupling late diastole.

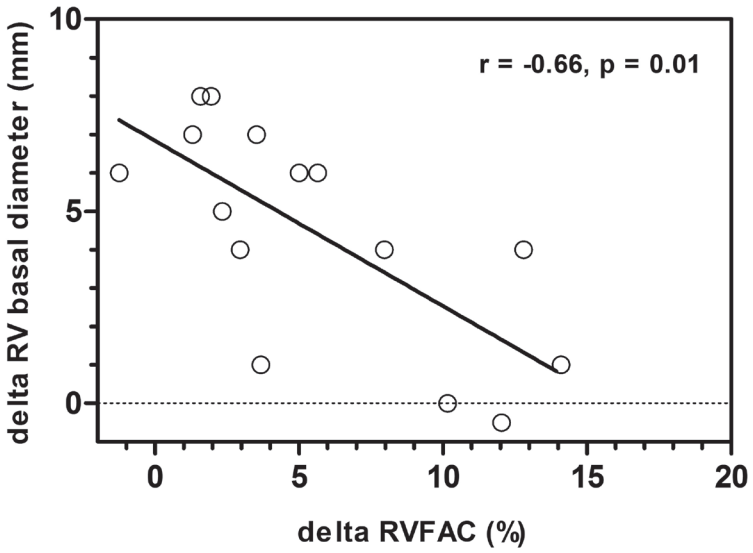

Figure 4. Correlation between acute increase in RV fractional area change during first exercise session under hypoxia (visit 2) and increase in resting RV basal diameter at completion of the training protocol

DISCUSSION

The aim of our study was to relate pre-training changes in cardiac function during acute hypoxic exercise to subsequent adaptations to a 12-week hypoxic endurance exercise training program on RV cardiac structure, function and mechanics in healthy individuals. We present the following findings. First, hypoxic exercise training increased RV size, including diameter and area. Whereas measures of RV function remained largely unchanged, exercise training resulted in adaptations in RV mechanics, with less uncoupling and lessening of the systolic and diastolic slopes of the RV strain-area loop. Second, no adaptation in LV structure, function or mechanics were observed. Third, pre-training augmentation in RV fractional area change to acute hypoxic exercise was inversely related to cardiac remodelling of the RV following 12 weeks of hypoxic endurance training in healthy individuals. Taken together, our results demonstrate that acute cardiac responses of the RV to hypoxic exercise are related to subsequent RV remodelling upon 12-weeks of hypoxic exercise training in healthy, relatively untrained individuals.

Acute exercise-induced changes in cardiac responses *versus* structural adaptation

In this study, we tested the assumption that any potential disproportionate ventricular wall stress contributes to RV remodelling. Since assessment of cardiac wall stress during exercise is highly challenging and invasive, we examined cardiac (systolic) function during hypoxic exercise and explored whether these changes related to structural adaptation post-training. We found that augmentation in RV fractional area change to acute exercise is inversely related to RV size following exercise training. In other words, small-to-modest (but not moderate-to-large) increases in RV systolic function during acute exercise relate to subsequent increases in RV structure post-training. One potential explanation for this observation may be that those individuals who had a blunted exercise-induced increase in RV fractional area change, were working at a higher afterload and hence received a greater stimulus for cardiac adaptation. Another potential explanation for this observation may relate to the structure of the RV. A smaller sized RV is less able to elevate measures of systolic RV function during exercise, and are therefore more susceptible for subsequent adaptation. Somewhat in line with this assumption, additional analysis revealed a positive relation between exercise-induced increases in RV fractional area change and RV size at

baseline (r=0.52, p=0.03), indicating that individuals with smaller RV cavity size show a smaller elevations in RV systolic function during exercise. In contrast to RVFAC, other measures did not significantly correlate with adaptation to exercise training. A possible explanation for this may be that RVFWS, TAPSE and TDI s' respond differently to alterations in load compared to RVFAC.[22] These elevations in load may be central as a stimulus for subsequent cardiac adaptation to exercise. Moreover, RVFAC takes into account both radial and longitudinal functional whereas the other systolic functional indices only take the latter into account. The stress received by the RV may therefore better reflected by the augmentation in RVFAC to acute exercise compared to RVFWS, TAPSE and TDI s'.

Right ventricular adaptations to hypoxic exercise training

After 12 weeks of hypoxic exercise training, the right side of the heart showed structural adaptation concomitant with altered mechanics in the strain-area loop. Our observation of RV remodelling contrasts with others, who report the absence of RV adaption after an increase in training volume.[23, 24] Importantly, the lack of structural RV remodelling observed in these previous studies is mainly observed when examining elite athlete populations, who already had a high level of training at baseline evaluation (e.g. they were not detrained for example during pre-season evaluation). Interestingly, the LV showed no evidence for adaptation after training. This agrees with a study by Arbab-Zadeh *et al.*[25] where they showed that after 12 months progressive and intensive marathon training in 12 previously sedentary subjects (mean age, 29±6 years), that RV size increased during the initial 3-month training period, but the LV only started to remodel after 6 months of training. The hypoxic exercise stimulus mainly effects RV afterload, and to a lesser extent LV afterload.[8-12] Moreover, it may be that LV afterload is reduced during hypoxic exercise as a result of hypoxic induced peripheral vasodilation.[26, 27] This may have amplified the disproportionate RV remodelling. However, due to the lack of a control group this remains speculative. Based on the lack of structural adaptation in the LV in this study, this may suggest that RV remodelling precedes LV remodelling in relatively untrained individuals. Future work, however, is required to better understand this phenomenon.

Previously, we have demonstrated changes in the strain-area loop in acute exercise settings[6, 13] but also marked differences in pulmonary hypertension populations[28-30] likely

due to variation in loading conditions. We also demonstrated that 24-weeks of endurance exercise induced a modest rightward shift with a somewhat stronger coupling of the LV strain-volume loop.[31] This is the first study, to our knowledge, that assessed RV strain-area loops following an exercise training in humans. We showed that training induced changes in RV mechanics concomitant to right-side structural adaptions. Specifically, lessening of the systolic and diastolic slope of the RV strain-area loop fits with the change in geometry of the RV, where the cavity size became larger. This is challenging to interpret but may be explained by the larger RV having greater unit area of myocardium requiring less deformation/contractility to facilitate the same stroke volume. Furthermore, we observed stronger coupling following training, potentially suggesting the presence of a more dominant longitudinal contribution to area change in diastole compared to systole. This adaptation fits with previous cross-sectional findings, in that we previously observed that athletes with a sports discipline with low-static and high-dynamic components (IIIA Mitchell classification[32]; e.g. high-intensity exercise as adopted in our study), showed more coupling in RV strain-area loops compared to other Mitchell classifications sports.[6] This could be suggestive for a sport discipline specific adaptation and the significant influence of variable loading conditions across disciplines on RV physiology. Moreover, the resemblance between the improved systolic-diastolic coupling following endurance training in the RV (this study) and LV (study by Oxborough *et al*.[31]) with increasing cavity sizes may indicate that a change in cardiac mechanics is not an isolated process but merely a consequence of cardiac structural remodelling due to exercise training. Future work, in larger cohorts assessing both RV and LV, is required to better understand this topic.

Perspectives

Challenging the cardiac system, e.g. through exercise, may be relevant in better understanding (patho)physiology. Indeed, exercise-induced troponin I elevation, independent from resting troponin I, predicts mortality and cardiovascular morbidity.[33, 34] In the present study, we found that exercise-induced changes in RV function relate to chronic RV adaptation. This concept, i.e. exploring cardiac responses to exercise, may be a potential strategy for future studies aiming to better understand cardiac (patho) physiology.

Limitations. We did not include a control group(s) who either; did not perform exercise or performed exercise under normoxic conditions. Whilst this may have provided additional insight into the role of hypoxia in mediating cardiovascular adaptations, we believe this does not impact the primary finding of our study, that exercise training may lead to RV structural adaptation, which seems to relate, at least partly, to acute baseline exercise-induced changes in cardiac function. A further limitation is that we did not collect blood samples to assess haematocrit and haemoglobin. Although, the participants were exposed to very short durations of intermittent hypoxic exercise training session (maximum of 1 hour including acclimation), this may have led to a change in haematocrit and haemoglobin.[35] In addition, the RV loop is based on area while volume would be more suitable given the complex RV geometry. However, the technique to derive the RV volume loops is not yet validated and will require 3D echocardiography. Finally, LV strain-volume loops were only constructed from an A4C view and not in the A2C and APLAX views.

CONCLUSION

12-week high-intensity running hypoxic exercise training induced right-sided structural remodelling, which was, in part, related to baseline cardiac increase in RV fractional area change to acute exercise. These data suggest that acute RV responses to exercise are related to subsequent right ventricular remodelling in healthy individuals upon hypoxic training.

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APPENDICES

Supplemental 1. Strain-Area Loop – methods of derivation

To calculate right ventricular (RV) strain-area loops the following steps have been taken. Temporal longitudinal strain values were exported to a spreadsheet (Excel; Microsoft Corp., Redmond, WA, USA). Using cubic spline interpolation, the global temporal longitudinal strain values were divided into 300 points for systole and 300 points for diastole in order to correct for variable heart rates. For both systole and diastole, the 300 strain values were then split into 5% increments of the cardiac cycle, providing longitudinal strain values at 10 time points in systole and 10 time points in diastole. Concomitant time points for the strain values were used in the same image and cardiac cycle to trace RV monoplane areas to provide simultaneous strain and area values.

Using the individual strain–area loop, a linear regression line and a polynomial of two orders was applied to both diastolic and systolic parts of the loop. This derived polynomial equation allowed the derivation of strain at percentage increments of RV end-diastolic area (RVEDA). The longitudinal strain–area loop was assessed using the following parameters (**Figure 2**): (a) early linear slope during first 5% of volume ejection in systole (ESslope), (b) the overall linear slope during systole (Sslope) and (c) end-systolic peak longitudinal strain (peak strain). In addition (un)coupling was termed to describe the relationship between systolic and diastolic strain for any given area/volume and was assessed during (d) early (Uncoupling ED), (e) late diastole (Uncoupling LD) and (f) overall. Furthermore, (g) the early linear slope during first 5% (EDslope) and (h) late linear slope (LDslope) during last 5% of volume increase in diastole.

The Sslope was derived as the gradient of the linear regression line over the systolic phase of the strain–area loop. Longitudinal peak strain was derived as the raw peak strain value from the longitudinal strain data. The Uncoupling ED and Uncoupling LD were calculated across the area between the systolic and diastolic polynomial curves. Using the equations of the polynomial regression lines, strain at % increments of RVEDA were calculated. By subtracting diastolic from systolic strain, the difference at each point was calculated. Based on individual RV fractional area change (RVFAC), the working range of the heart was determined, after which Uncoupling ED was calculated as the sum of the differences

at the lowest two-thirds of increments of EDA in the working range of the heart, and Uncoupling LD was calculated as the sum of the differences at the highest one-third of increments of EDA in the working range of the heart.

Supplemental 2. Table 1 - Associations between acute functional responses to exercise and chronic RV adaption

		Delta RVFAC (%)	Delta TAPSE (mm)	Delta RV s' (cm/s)	Delta RVFWS (%)
Delta RV basal diameter (mm)	Pearson Correlation	-0.66	-0.36	-0.27	-0.38
	Sig. (2-tailed)	0.01	0.18	0.32	0.18
Delta RV mid-cavity diameter (mm)	Pearson Correlation	-0.12	-0.24	-0.30	-0.28
	Sig. (2-tailed)	0.70	0.40	0.28	0.33
Delta RV end-diastolic area (mm)	Pearson Correlation	-0.09	-0.47	-0.45	-0.19
	Sig. (2-tailed)	0.77	0.09	0.11	0.52

RV, Right ventricle. RVFAC, RV fractional area change. TAPSE, tricuspid annular plane systolic excursion. TDI, tissue doppler imaging. RVFWS, RV free wall strain.

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Exercise Training Induces Left- but not Right-Sided Cardiac Remodelling in Olympic Rowers

CHAPTER 4

ABSTRACT

Whilst the athlete's heart has been extensively described, less work has focused on the potential for elite athletes to demonstrate further cardiac remodelling upon an increase in training volume. Moreover, little work explored potential side-specific cardiac remodelling. Therefore, we examined the impact of an increase in training volume across 9-months in elite rowers on left- and right-sided cardiac structure, function and mechanics (i.e. longitudinal, radial and circumferential strain, twist and strain-volume loops). As part of the preparations to the 2012 Olympic Games, twenty-seven elite rowers (26.4±3.7years, 19 male) underwent echocardiography prior to and post (9-months) an increase in training volume (24 to 30-35h weekly). Training increased left ventricular structure, including wall thickness, diameter, volume, mass and LV twist (all $p<0.05$). Female rowers demonstrated larger adaptation in left ventricular diameter and mass compared to male rowers (both $p<0.05$). No changes were observed in other measures of left ventricular function in both sexes (all $p>0.05$). The 9-month intervention showed no change in right ventricular/atrial structure, function or mechanics (all $p>0.05$). In conclusion, our data revealed that 9-month increased training volume in elite rowers induced left-sided (but not right-sided) structural remodelling, concomitant with an increase in left ventricular twist, with some changes larger in women.

INTRODUCTION

Exercise training represents a potent stimulus for remodelling of the heart. Recent prospective and long-term intervention studies support the presence of predominant eccentric ventricular adaptation in response to exercise training.[1, 2] In other words, regular exercise training leads to a balanced increase in both volume and mass, whilst function seems largely preserved.[1] Previous work largely focused on either adaptation of the left side or adaptation of the right side of the heart in response to exercise training. As such, there is an important gap in the literature pertaining to the lack of knowledge whether exercise training differentially affects the left *versus* right ventricle in elite athletes.

Both ventricles receive a similar amount of blood. Due to their distinct geometry and mass, with the right ventricle (RV) being larger in volume but smaller in wall thickness than the left ventricle (LV), both ventricles may be exposed to distinct hemodynamic stimuli, potentially leading to different patterns of adaptation in both structure and function.[3] Indeed, exercise leads to distinctly different changes in afterload for both ventricles, with relatively larger increases in afterload for the RV.[4] This supports the potential for different adaptation between ventricles. Better insight into differences in remodelling between ventricles is highly relevant, especially since previous studies have linked exercise-induced RV cardiomyopathy to high volumes of exercise training in elite athletes.[5, 6] Insight into the potential presence of side-specific physiological remodelling of the heart will also contribute to improved interpretation of pre-participation screening for high-risk cardiovascular conditions associated with sudden cardiac arrest in athletes.

The aim of this study, therefore, was to examine the impact of an increase in volume (across 9-months) in elite rowers on left- and right-sided cardiac structure, function and mechanics (i.e. longitudinal, radial and circumferential strain, twist and strain-volume/area loops). Based on the higher relative workload for the RV[4], we expect larger structural cardiac adaptation in the RV (whilst preserving function) compared to the LV in elite rowers.

Previous work suggested that sex may differently affect cardiovascular function during physiological stimuli.[7, 8] Based on cross-sectional comparisons, similar patterns of cardiac remodelling have been observed with static and mixed exercise between men and

women, whilst greater LV adaptation may be present in women during dynamic exercise.[9, 10] Therefore, as an explorative aim, we evaluate the impact of sex on the impact of exercise in elite rowers on left- and right-sided cardiac structure, function and mechanics.

METHODS

Study population and study design

In this prospective, longitudinal study, as part of the work-up to the 2012 Olympic Games, twenty-seven elite level rowers (male and female, all Caucasian) underwent baseline echocardiography prior to and post (9-months) a planned increase in training volume. Baseline echocardiograms were performed immediately after the 2011 World Rowing Championships (i.e. when all athletes were in a highly trained status), and 3 months before the 2012 Olympic Games. After the baseline echocardiograms, the rowers, both male and female, increased their training volume gradually from 24 hours to 30-35 hours per week (20% strength, 80% rowing training consisting of high-intensity interval and endurance training). Height and weight were obtained before echocardiography was performed (SECA scale and stadiometer, SECA GmbH, Hamburg, Germany). This study was conducted in accordance to with the ethical standards in sport and exercise science research and approved by the Radboud University Medical Center ethics committee.[11]

Echocardiographic measurements

The echocardiographic examinations were performed in the left lateral decubitus position by one highly experienced cardiologist (AvD) using a Vivid-Q ultrasound machine (GE Medical, Horton, Norway), equipped with a 1.5-4 MHz phased array transducer. Heart rate was calculated from a single lead ECG inherent to the ultrasound system. Images were stored in raw digital imaging and communication in medicine (DICOM) format and were exported to an offline workstation (EchoPac, version 113, GE Medical, Horton, Norway). Data-analysis, from three stored cycles, was performed by a single observer with experience in echocardiography (GK) using commercially available software (EchoPac, version 113, GE Medical, Horton, Norway). The echocardiograms were all coded so the observer was blinded for the timing (pre *vs.* post) and for sex (male *vs.* female).

Conventional measurements. Cardiac structural and functional measurements were made according to the current guidelines for cardiac chamber quantification.[12] Regarding the left heart, we examined the following structural and functional indices: wall thickness of the septum (IVSd) and posterior wall (PWd), internal cavity diameter at end-diastole (LVIDd), LV mass (LVM), anteroposterior diameter of the left atrium (LA), LA volume by the disk summations technique in apical 4-chamber (A4C) and apical 2-chamber (A2C) view, modified Simpson's left ventricular ejection fraction (LVEF), tissue Doppler imaging (TDI) of the mitral annulus (s', e' and a') and trans-mitral Doppler (E, A and E/A ratio). Regarding the right heart, following structural and functional indices were determined: basal and mid-cavity end-diastolic diameters, RV end-diastolic area (RVEDA), RV end-systolic area (RVESA), RV outflow tract (RVOT) diameter at the proximal level in the parasternal long-axis (PLAX) and parasternal short-axis (PSAX) view, right atrial (RA) area, RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE), TDI of the tricuspid annulus. All LV and RV structural indices were allometrically scaled to body surface area (BSA) according to the laws of geometric similarity.[13]

Mechanics. Images were acquired specifically for speckle tracking. This involved the optimization of frame rates between 40 and 90 frames s^{-1}, depth to ensure adequate imaging of the chamber of interest, a focal zone at mid-cavity to reduce the impact of beam divergence and gain, compression and reject to ensure endocardial delineation.

Ventricular and atrial mechanics. The A4C view was utilized for LV, LA and RA global longitudinal strain and the RV focused view for the RV longitudinal strain. The LV short-axis (SAX) views (basal, mid and apical) were utilized for radial, circumferential strain and twist. Valve closure times were determined from the respective pulsed wave Doppler signals.

For all compartments (LV, LA, RV, RA), the myocardium was manually traced and adjusted so that the region of interest (ROI) incorporated all of the wall thickness, while avoiding the pericardium. The region of interest was divided into six myocardial segments, providing segmental strain curves and a global longitudinal strain curve as an average of all six segments. In order to obtain peak LV circumferential strain, peak LV radial strain and peak apical and basal rotation, a full-thickness ROI of the mid-, basal- and apical-SAX views, which was divided into six segments, was selected. In addition, raw strain values

were exported and a cubic spline was applied to normalize for heart rate. This allowed the presentation of temporal strain and rotation across the cardiac cycle.

Strain-volume/area loops. The longitudinal strain-volume/area relationship (for methodology of derivation, see Supplemental 1 and Oxborough *et al.*[14]) was assessed using the following parameters: (I) the linear strain-area slope (Sslope) and early strain-area slope during first 5% of volume ejection in systole (ESslope); (II) end-systolic peak longitudinal strain (peak strain); (III) the early linear strain-area slope during first 5% (EDslope) and late linear strain-area slope (LDslope) during last 5% of volume increase in diastole; and (IV) diastolic uncoupling (i.e. difference in strain between systole and diastole at any given area), divided into uncoupling during early (Uncoupling ED) and late diastole (Uncoupling LD).[14, 15]

Statistical analysis

Statistical analyses were performed using SPSS Statistics 24 (SPSS, Inc., Chicago, Illinois). All parameters were visually inspected for normality and tested with Shapiro-Wilk normality tests. Continuous variables were reported as mean±SD and categorical variables were presented as proportions. Paired-Samples T-tests were used to compare echocardiographic continuous variables between the baseline and follow-up evaluation. Comparison of sex differences was performed using repeated measurements ANOVA with Bonferroni *post hoc* correction for multiple comparisons.

Consistency of intra-observer measurements of selected measurements were verified through the intra-class correlation coefficient (ICC). Therefore, both echocardiographs of 15 randomly chosen subjects were analysed by the same operator blinded from earlier results. ICC coefficients were as follows: RVOT-PLAX 0.96 (0.91-0.98), LVISd 0.91 (0.81-0.96), LVPWd 0.90 (0.80-0.95), LVIDd 0.97 (0.93-0.98), RV basal diameter 0.98 (0.96-0.99), RVEDA 0.98 (0.95-0.99), RA area 0.99 (0.97-0.99), LA volume 0.98 (0.95-0.99). In previous studies, we showed that strain measurements and both right and left ventricular loops have a good to excellent inter-observer variability.[14-17]

RESULTS

Baseline characteristics

All 27 rowers participated in the 2012 Olympic games. Mean age of the study population was 26.4±3.7 years, consisting of 19 males (70%, 26.3±4.3y) and eight females (30%, 26.6 ±1.9y). All rowers were Caucasian. Male rowers were significantly taller (193.7±6.6 *versus* 181.5±8.8 cm, p=0.001), heavier (88.0±12.0 *versus* 72.9±8.6 kg, p=0.003) and had greater BSA (2.2±0.2 *versus* 1.9±0.2 m^2, p=0.002), but had a similar BMI (23.3±2.1 *versus* 22.2±1.0, p=0.08) compared to female rowers. Weight, body surface area (BSA) and body mass index (BMI) did not significantly change over time (83.9±13.0 to 84.3±13.0 kg, p=0.10; 2.1±0.2 to 2.1±0.2 m^2, p=0.10; 23.0±1.9 to 23.1±1.9 kg/m^2, p=0.11, respectively). Resting heart rate was higher at follow-up compared to baseline 54±7 to 58±8 bpm (p=0.02).

Exercise training and cardiac remodelling: comparison between sides

Left ventricle and atrium. There was a significant increase in LV wall thickness, diameter, volume and mass (all p<0.01), which remained significant after correction for BSA (all p<0.05) (**Table 1, Figure 1**). Similarly, there was a significant increase in LA diameter and volume (both p<0.01), which remained significant after correction for BSA (both p<0.01). Exercise training increased LV twist, whilst no other changes in functional or mechanical indices were found (**Table 1, Figure 1, Figure 2**).

Right ventricle and atrium. We found no significant changes in right ventricular and atrial structure, function and mechanics (**Table 2, Figure 1, Figure 2**).

Exercise training and cardiac remodelling: comparison between sexes

Baseline characteristics. At baseline, female rowers had smaller LV and RV cardiac dimensions compared to male rowers (all p<0.05, **Table 1-2**), which was not present after correcting for BSA (all p>0.05). Absolute RVOT dimensions did not differ between sexes (**Table 2**). Female rowers had a smaller LV mass compared to male rowers (p<0.01), which remained significant after correction for BSA (p<0.05, **Table 1**). Except for a lower TAPSE and a higher E velocity in female rowers (both p=0.02), no significant differences were found in conventional measurements of left- or right-sided cardiac function (**Table 1-2**). Female rowers demonstrated significantly higher LV apical circumferential strain, lower

peak systolic apical rotation (both $p<0.05$) and steeper slopes of the left- and right-sided strain-volume/area loop compared to male rowers (LV – Sslope, LDslope, both $p<0.05$; RV – Sslope, ESlope, LDslope, all $p<0.05$) (**Table 1-2, Figure 2**).

Training-induced remodelling. Females demonstrated a significantly larger increase in absolute and scaled LV diameter and LV mass compared with male rowers (**Table 1, Figure 3**). No differences were found between sexes in the right ventricle or atrium (**Table 2**).

Table 1 – Left heart structural, functional and mechanical echocardiographic parameters observed in male and female elite rowers prior to and post a 9-month increase in training volume

		All rowers (n=27)		T-test	Male (n=19)		Female (n=8)		ANOVA		
		Pre	Post		Pre	Post	Pre	Post			
		Mean±SD	Mean±SD	P-value	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Time	Gender	Time* Gender
Left Ventricle	IVSd, mm	9.2±1.2	9.7±1.1	**<0.001**	9.5±1.2	10.5±1.2	8.4±0.9	9.0±0.8	**0.001**	**0.02**	0.62
	index	6.3±0.7	6.7±0.7	**<0.001**	6.4±0.7	6.8±0.7	6.1±0.6	6.6±0.6	**0.001**	0.36	0.34
	PWd, mm	8.3±1.3	8.7±1.4	**0.01**	8.6±1.3	9.0±1.3	7.4±0.5	8.1±1.5	**0.01**	**0.04**	0.34
	index	5.7±0.7	6.0±0.9	**0.02**	5.8±0.8	6.1±0.8	5.4±0.4	5.9±1.1	**0.02**	0.32	0.39
	LVIDd, mm	57±5	58±4	**0.001**	58±4	59±4	52±3	55±2	**<0.001**	**0.002**	**0.05**
	index	39±2	40±2	**0.002**	39±2	40±3	38±2	40±2	**<0.001**	0.41	**0.02**
	LVM, g	191±51	212±48	**<0.001**	210±47	228±46	144±22	175±31	**<0.001**	**0.002**	0.11
	LVMi, g/m2	90±18	101±17	**<0.001**	95±17	104±17	76±10	94±17	**<0.001**	**0.04**	**0.03**
	RWT, ratio	0.29±0.04	0.30±0.05	0.19	0.30±0.04	0.31±0.05	0.28±0.02	0.30±0.05	0.20	0.49	0.8
	LVEDV, ml	147±34	152±28	**0.04**	155±34	159±28	124±22	133±15	**0.03**	**0.03**	0.32
	index	47±7	49±5	**0.04**	48±7	49±5.2	46±6	50±5	**0.02**	0.99	0.17
	LVESV, ml	62±19	64±13	0.29	65±20	67±14	54±13	57±6	0.32	0.11	0.9
	index	20±5	21±3	0.20	20±5	21±3	20±4	22±3	0.20	0.71	0.73
	LVEF, %	58±6	58±4	0.66	57±5	59±6	58±4	57±5	0.77	0.99	0.32
	AoV Vmax, m/s	1.3±0.2	1.4±0.2	0.16	1.4±0.2	1.4±0.2	1.3±0.2	1.4±0.2	0.22	0.56	0.98
	E, m/s	0.8±0.1	0.8±0.1	0.48	0.8±0.1	0.8±0.1	0.9±0.1	0.9±0.1	0.54	**0.02**	0.92
	A, m/s	0.4±0.1	0.5±0.1	0.53	0.4±0.1	0.4±0.1	0.5±0.1	0.5±0.1	0.44	0.44	0.56
	E/A ratio	1.9±0.4	1.8±0.3	0.33	1.9±0.4	1.8±0.4	1.9±0.4	1.8±0.3	0.33	0.68	0.83

Table 1. (Continued)

		All rowers (n=27)		T-test	Male (n=19)		Female (n=8)		ANOVA		
		Pre	Post		Pre	Post	Pre	Post			
		Mean±SD	Mean±SD	P-value	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Time	Gender	Time* Gender
Left Ventricle	TDI E', cm/s	17±2	17±2	0.70	16±2	17±1	17±2	16±3	0.93	0.81	0.15
	TDI A', cm/s	7±1	8±1	0.20	8±2	7±1	8±2	8±1	0.24	0.62	0.86
	TDI S', cm/s	11±1	12±2	0.48	11±1	12±2	12±1	12±1	0.16	0.39	0.35
	LV global longitudinal strain, %	-19±2	-19±2	0.98	-18±2	-19±2	-20±1	-19±1	0.49	0.10	0.14
	LV Basal Circumferential Peak Strain, %	-18±4	-18±4	0.36	-18±4	-17±4	-21±4	-20±3	0.66	0.21	0.73
	LV Apical Circumferential Strain, %	-17±4	-18±4	0.55	-17±5	-18±4	-18±1	-18±4	0.77	**0.01**	0.58
	LV Basal Radial Peak Strain, %	33±16	30±10	0.42	32±18	29±10	36±2	30±12	0.43	0.72	0.75
	LV Apical Radial Peak Strain, %	55±22	57±17	0.83	50±14	56±14	69±33	59±23	0.73	0.05	0.23
	Peak Systolic Basal Rotation, ◦	-4±3	-5±3	0.84	-4±3	-4±4	-6±1	-6±2	0.89	0.36	0.67
	Peak Systolic Apical Rotation, ◦	6±3	7±4	**0.01**	6±3	8±3	3±1	2±1	0.36	**0.02**	0.07
	Peak Twist, ◦	9±5	11±5	**0.04**	10±5	12±5	7±1	8±2	0.29	0.20	0.46
	Uncoupling ED, %	-0.4±1.9	0.3±1.5	0.21	-0.8±2.0	0.3±1.6	0.7±1.1	0.1±1.2	0.62	0.18	0.13
	Uncoupling LD, %	0.1±1.5	0.6±1.0	0.23	-0.2±1.7	0.6±1.0	0.8±0.7	0.4±1.1	0.67	0.38	0.12
	Uncoupling, %	-0.2±1.7	0.4±1.2	0.22	-0.6±1.9	0.4±1.3	0.8±0.8	0.2±1.2	0.65	0.23	0.12
	Sslope, %/ml	0.2±0.1	0.2±0.04	0.73	0.2±0.1	0.2±0.04	0.3±0.04	0.2±0.03	0.23	**0.004**	0.05
	ESslope, %/ml	0.2±0.1	0.2±0.04	0.23	0.2±0.06	0.2±0.04	0.2±0.1	0.2±0.03	0.11	0.05	0.21
	EDslope, %/ml	0.3±0.1	0.2±0.1	0.07	0.3±0.1	0.2±0.1	0.3±0.1	0.3±0.1	0.20	0.07	0.42
	LDslope, %/ml	0.2±0.1	0.2±0.1	0.56	0.1±0.1	0.2±0.1	0.3±0.1	0.2±0.1	0.69	**0.04**	0.06

Table 1. (Continued)

			All rowers (n=27)		T-test	Male (n=19)		Female (n=8)		ANOVA		
			Pre	Post		Pre	Post	Pre	Post			
			Mean±SD	Mean±SD	P-value	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Time	Gender	Time* Gender
Left Atrium	LA diameter, mm		35±5	37±5	**0.001**	37±5	38±5	31±4	35±3	**<0.001**	**0.02**	0.07
		index	24±3	26±3	**0.003**	25±3	22±3	26±3	25±2	**0.001**	0.13	0.08
	LA volume, ml		59±15	65±18	**0.01**	60±17	68±20	55±9	59±10	**0.03**	0.34	0.61
		index	19±4	21±5	**0.01**	18±4	21±5	21±3	22±4	**0.03**	0.26	0.85
	LA longitudinal strain, %		57±19	53±12	0.19	56±22	52±13	61±12	57±11	0.23	0.41	0.92

Data are expressed as means±SD. AoV, aortic valve; ED, Early diastole; EDslope, Early linear slope during first 5% of volume increase in diastole; ESslope, Early linear slope during first 5% of volume ejection in systole; IVSd, Interventricular septum thickness at end-diastole; LA, Left atrium; LD, Late Diastole; LDslope, Late linear slope during last 5% of volume increase in diastole; LV, Left ventricle; LVIDd, Left ventricle internal diameter at end-diastole; LVM, Left ventricle mass; PLAX, Parasternal long axis; PSAX, Parasternal short axis; PWd, Posterior wall thickness at end-diastole; Sslope, Linear slope of the strain-volume loop during systole; TDI, Tissue doppler imaging. Level of significance P-value<0.05.

Table 2. Right heart structural, functional and mechanical echocardiographic parameters observed in male and female elite rowers prior to and post a 9-month increase in training volume

		All rowers (n=27)		T-test	Male (n=19)		Female (n=8)		ANOVA		
		Pre	Post		Pre	Post	Pre	Post			
		Mean±SD	Mean±SD	P-value	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Time	Gender	Time* Gender
Right Ventricle	RVOT PLAX, mm	34±4	34±4	0.42	34±4	34±3	33±5	33±5	0.66	0.53	0.49
	index	23±3	24±2	0.38	23±3	23±2	25±3	25±3	0.58	0.17	0.65
	RVOT PSAX, mm	34±5	35±4	0.15	34±5	35±3	33±6	35±5	0.13	0.85	0.56
	index	23±3	24±2	0.15	23±3	23±2	24±3	26±3	0.12	0.06	0.49
	RV Basal Diameter, mm	44±5	43±6	0.28	45±5	44±4	41±4	40±8	0.33	0.05	0.99
	index	30±3	30±3	0.44	30±3	30±3	30±2	30±4	0.66	0.91	0.62
	RV Mid-Cavity Diameter, mm	33±5	32±6	0.32	35±5	33±5	30±3	31±6	0.74	0.07	0.14
	index	22±3	23±4	0.44	23±4	22±4	22±2	23±3	0.87	0.65	0.06
	RVEDA, cm2	29±5	29±5	0.33	31±3	31±3	25±4	25±5	0.39	**0.001**	0.89
	index	14±1	14±1	0.36	14±1	14±1	13±1	13±1	0.49	0.26	0.67
	RVESA, cm2	17±3	17±3	0.82	18±2	18±2	15±3	15±4	0.98	**0.01**	0.54
	index	8±1	8±1	0.80	8±1	8±1	8±1	8±1	0.95	0.42	0.42
	RVFAC, %	42±4	42±4	0.47	42±4	42±4	43±5	41±4	0.32	0.94	0.31
	TAPSE, cm	30±4	31±5	0.09	31±5	33±5	27±2	28±2	0.23	**0.02**	0.30
	TDI E', cm/s	17±2	17±3	0.24	17±3	17±4	17±2	16±2	0.14	0.70	0.28
	TDI A', cm/s	12±3	12±3	0.59	12±4	11±3	13±3	12±3	0.60	0.40	0.90
	TDI S', cm/s	16±2	16±2	0.82	16±2	16±2	16±2	16±2	0.97	0.91	0.53
	RV Longitudinal Strain, %	-23±2	-23±2	0.09	-23±2	-22±2	-25±2	-23±1	**0.03**	0.09	0.10

Table 2. (Continued)

		All rowers (n=27)		T-test	Male (n=19)		Female (n=8)		ANOVA		
		Pre	Post		Pre	Post	Pre	Post			
		Mean±SD	Mean±SD	P-value	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Time	Gender	Time* Gender
	Uncoupling ED, %	1.3±1.9	1.4±1.3	0.92	1.3±1.8	1.2±1.4	1.4±2.3	1.6±1.2	0.86	0.59	0.78
	Uncoupling LD, %	2.0±1.8	2.2±1.3	0.65	2.2±1.6	1.9±1.1	1.7±2.2	2.9±1.6	0.37	0.60	0.14
	Uncoupling, %	1.6±1.8	1.6±1.2	0.88	1.6±1.7	1.5±1.2	1.5±2.2	2.0±1.1	0.73	0.64	0.50
	Sslope, %/cm²	1.9±0.4	1.9±0.5	0.69	1.7±0.3	1.7±0.3	2.2±0.5	2.3±0.5	0.50	**0.001**	0.35
	ESlope, %/cm²	2.0±0.7	2.1±0.7	0.47	1.8±0.7	1.9±0.5	2.3±0.6	2.4±0.8	0.46	**0.04**	0.85
	EDslope, %/cm²	1.4±0.6	1.3±0.5	0.65	1.2±0.6	1.2±0.5	1.6±0.5	1.4±0.6	0.59	0.09	0.73
	LDslope, %/cm²	2.1±0.9	2.2±0.6	0.91	1.9±0.7	1.9±0.5	2.6±1.0	2.5±0.6	0.99	**0.003**	0.68
Right Atrium	RA Area, cm2	19±4	19±4	0.10	20±4	21±4	16±4	16±3	0.24	**<0.001**	0.41
	index	9±1	9±2	0.09	9±1	10±2	8±1	8±1	0.22	0.06	0.54
	RA Longitudinal Strain, %	57±11	57±12	0.97	54±10	55±12	62±14	60±13	0.83	0.13	0.62

Data are expressed as means±SD. ED, Early diastole; EDslope, Early linear slope during first 5% of volume increase in diastole; ESslope, Early linear slope during first 5% of volume ejection in systole; Late Diastole; LDslope, Late linear slope during last 5% of volume increase in diastole; PLAX, Parasternal long axis; PSAX, Parasternal short axis; RA, Right atrium; RV, Right ventricle; RVEDA, Right ventricular end-diastolic area; RVESA, Right ventricular end-systolic area; RVFAC, Right ventricular fractional area change; RVOT, Right ventricular outflow tract; Sslope, Linear slope of the strain-volume loop during systole; TAPSE, Tricuspid annular plane systolic excursion; TDI, Tissue doppler imaging. Level of significance P-value<0.05.

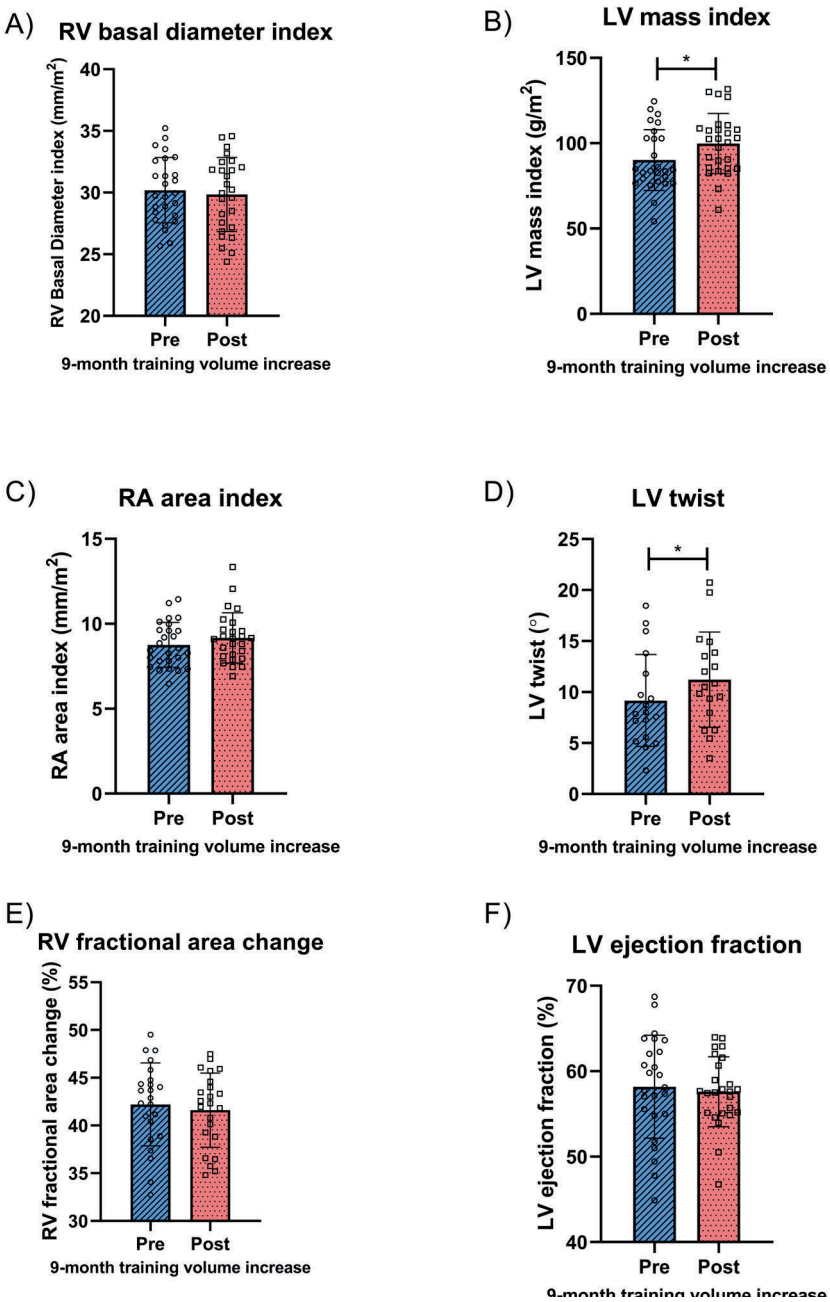

Figure 1. Change in right (A-C-E) and left (B-D-F) sided cardiac structure and function in elite rowers (n=27) before ('Pre': blue bars with striped lines) and after ('Post': red bars with dots) a 9-month training volume increase. Error bars represent SD. * significantly different from pre ($p<0.05$).

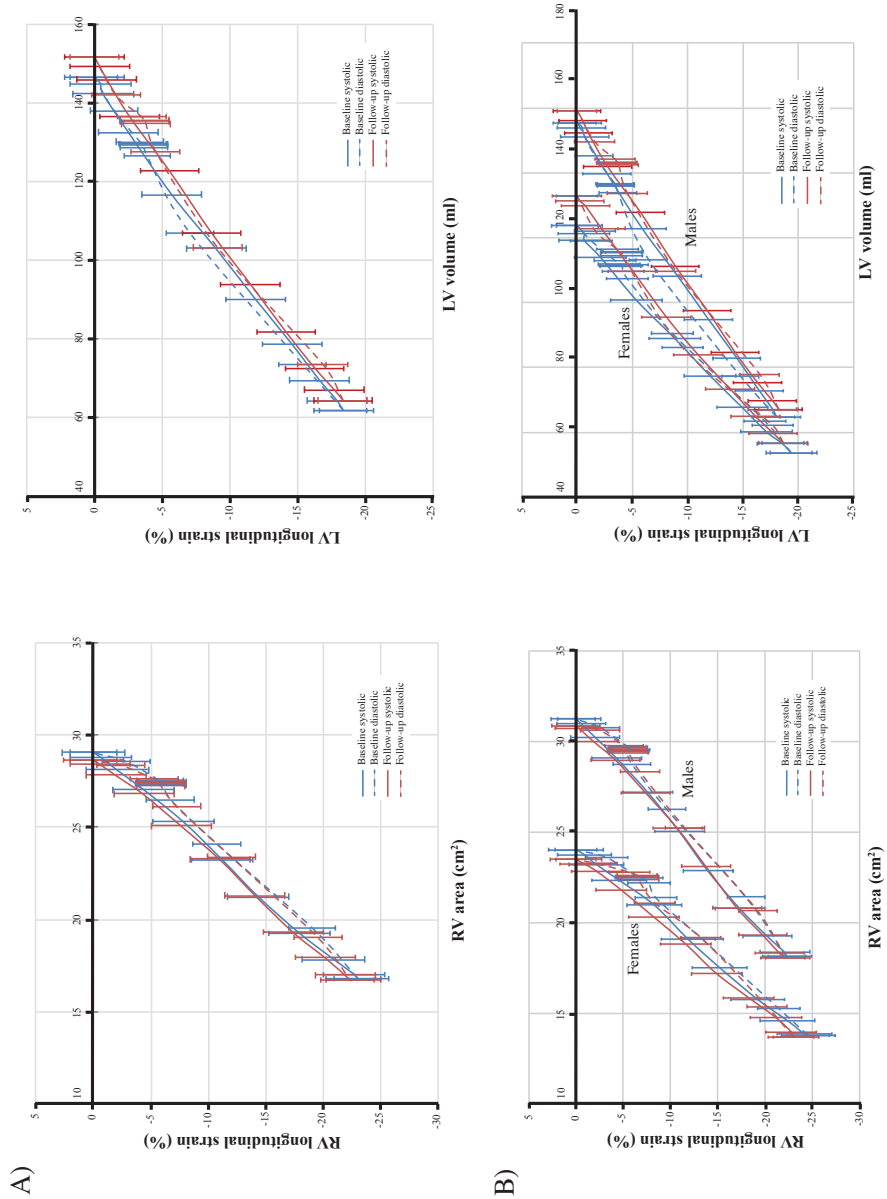

Figure 2. A) Right and left ventricular strain-area/volume loop in elite rowers before ('Pre Systolic': blue lines, 'Pre Diastolic': blue dotted lines) and after ('Post Systolic': red lines, 'Post Diastolic': red dotted lines) a 9-month training period. B) Strain-volume/area loops distributed to sex.

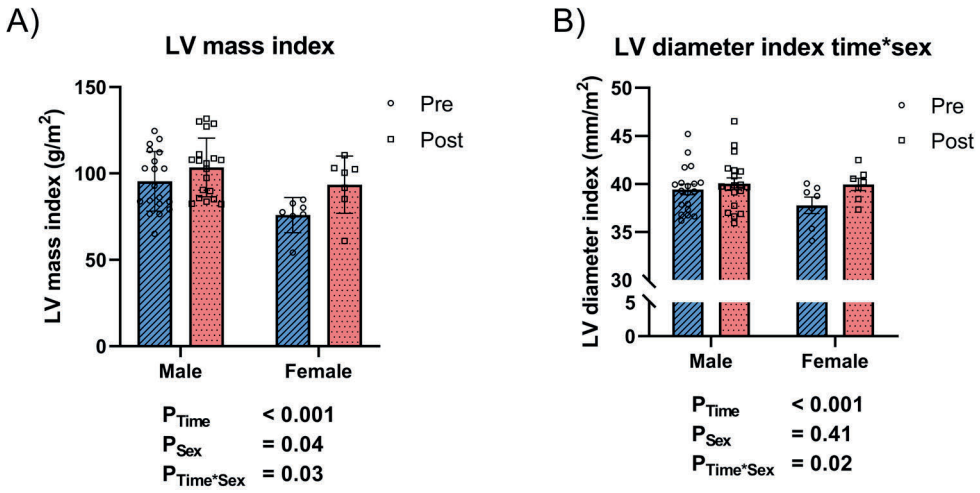

Figure 3. Change in (A) LV mass index and (B) LV diameter index in elite rowers before ('Pre': blue bars with striped lines) and after ('Post': red bars with dots) a 9-month training period distributed to sex. Error bars represent SD.

DISCUSSION

The aim of our study was to examine the impact of an increase in volume (across 9-months) in elite rowers on left- and right-sided cardiac structure, function and mechanics. We present the following findings. First, an increased training volume in elite rowers across 9-months resulted in significant structural adaptation of the left ventricle and atrium, with no adaptations observed on the right side. Second, left-sided structural cardiac adaptation was accompanied by an increase in LV twist, but no other left- or right-sided functional adaptations. This highlights the plasticity of the heart for remodelling in response to exercise training, even in elite athletes. Taken together, our results demonstrate cardiac side-, and possibly also sex-specific adaptation, which is relevant for future studies that should acknowledge that cardiac remodelling does not simply follow the same path between and within individuals.

After an increase in training volume across 9-months, the left heart of this cohort of elite rowers showed mild structural (eccentric) adaptation with an increased LV twist, whilst there was no significant remodelling in the right heart. This left-sided structural adaptation is in line with several previous longitudinal training studies including

sedentary, moderately- and highly-trained individuals.[2, 18-21] Interestingly, concomitant to left-sided structural adaptation, elite rowers also demonstrated augmented LV twist after the increase in training volume. The higher heart rate post-training may partially explain the increase in twist. However, this seems unlikely since no correlation was found between heart rate and twist (r=0.02, p=0.94). Moreover, other functional parameters (also susceptible for differences in heart rate) did not change over time. Although we found adaptation in functional and structural characteristics, both may demonstrate a distinct pattern and are not similarly present. Indeed, the increase in twist was not correlated with changes in LV cardiac morphology (data not shown). Moreover, *Weiner et al.* observed that exercise training may initially (i.e. 90 days) lead to increases in LV twist, which subsequently disappeared during the chronic training phase (i.e. 39 months).[22] Our finding provides some support for this concept, in that an increase in volume of exercise initially resulted in both functional and structural adaptations, where functional changes may ultimately normalize during the chronic phase when volume of exercise remains the same. Future work is required to better understand these time-dependent adaptations in cardiac remodelling.

Despite the disproportionate load on the right *versus* left ventricle during exercise[4], we found no adaptation of the right ventricle or atrium. This finding contrasts with our hypothesis, but also with others who addressed right-sided cardiac remodelling in elite athletes.[23, 24] D'Ascenzi *et al.* reported seasonal variation in RV size in a cohort of top-level basketball and volleyball players.[23] Across three consecutive Olympic Games, Aengevaeren *et al.* noted that RV remodelling occurred between the first two Olympics Games, followed by a plateau during the subsequent 4 years in a heterogeneous group of athletes (n=50, 17 different sports).[24] These studies, however, are limited by the impact of ageing (i.e. 8-year cycle), large variations in training status across the season, and/or the heterogeneous group of athletes included. The lack of RV remodelling in our study may be explained by achieving a physiological limit for further adaptation prior to the start of the increase in training volume in highly trained rowers due to pericardial constraint. At least, our observations support the presence of distinct remodelling between the left and right side of the athlete's heart. Future work is required to better understand these differences, specifically focusing on the distinct load placed on both ventricles during exercise, possibly underlying distinct remodelling to (high) volumes of exercise training.

Following the explorative analysis, this study examined the impact of sex on cardiac adaptation to training using a longitudinal design. This design markedly differs from most previous studies that have adopted a cross-sectional design, including a heterogeneous groups of athletes, and generally not using allometric scaling.[10, 25-29] Our data showed larger LV structural adaptation in female rowers, which remained present upon allometric scaling. These distinct adaptations cannot relate to differences in lifetime exposure to elite athlete level training, since both groups do not differ in age (males 26.3±4.3y *versus* females 26.6±1.9y, p=0.84). Alternative explanations for the distinct remodelling might be hormonal, molecular and/or genetic mechanisms. However, these mechanisms are not fully understood yet and represent topics for future research.[9] An important limitation is the relatively low sample size for female rowers within this analysis. Nonetheless, our study was sufficiently powered to detect a significant effect between sexes in adaptation. We performed *post hoc* calculations and found that our study has a statistical power of 0.51-0.64 to detect sex differences in LV mass and LV diameter. At least, our findings highlight the importance for future research to better understand and establish potential sex differences in cardiac adaptation in response to exercise training.

Clinical relevance. The observation of no further adjustment in RV remodelling seems relevant as RV enlargement may overlap with the pathological dilation of the RV in patients with an arrhythmogenic RV cardiomyopathy. Previous studies have related RV remodelling in the already highly trained athlete to potential clinical problems. Our work suggests that even high volumes of exercise does not automatically lead to further remodelling of the RV, despite structural changes in the LV. However, a potential limitation is that subjects followed an individually determined exercise training protocol to increase training volume, which makes it difficult to relate cardiac remodelling to specific determinants of the exercise training protocol and at a cohort level. Also, we did not perform cardiopulmonary exercise testing to examine the level of fitness following exercise training. However, all individuals significantly increased their training volume, highlighting that additional cardiac remodelling is possible upon increases in training volume. Our study may have further clinical relevance, since we specifically explored remodelling in female elite athletes. Participation of females in elite sports has increased significantly over the past decades. Current work on the athlete's heart, leading to insight

into (ab)normal levels of adaptation, largely originate from studies performed in males. Our work supports performing specific studies in women, examining the geometry and potential pathological relevance of the female athlete's heart.

CONCLUSION

In conclusion, our data suggest that an increased exercise training volume in elite rowers across 9-months induced side-specific cardiac remodelling. Specifically, we found left-sided (but not right-sided) structural adaptations, with concomitant increase in LV twist in already highly trained rowers. Interestingly, these adaptations were significantly larger in women compared to men, a finding that warrants further exploration in future work. Taken together, our work suggests that examining the athlete's heart should go beyond the single-sided approach most previous studies adopted, and should explore both left and right-sided adaptation.

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APPENDICES

Supplemental 1. Strain-Area Loop – methods of derivation

To calculate right ventricular (RV) strain-area loops the following steps have been taken. Temporal longitudinal strain values were exported to a spreadsheet (Excel; Microsoft Corp., Redmond, WA, USA). Using cubic spline interpolation, the global temporal longitudinal strain values were divided into 300 points for systole and 300 points for diastole in order to correct for variable heart rates. For both systole and diastole, the 300 strain values were then split into 5% increments of the cardiac cycle, providing longitudinal strain values at 10 time points in systole and 10 time points in diastole. Concomitant time points for the strain values were used in the same image and cardiac cycle to trace RV monoplane areas to provide simultaneous strain and area values.

Using the individual strain–area loop, a linear regression line and a polynomial of two orders was applied to both diastolic and systolic parts of the loop. This derived polynomial equation allowed the derivation of strain at percentage increments of RV end-diastolic area (RVEDA). The longitudinal strain–area loop was assessed using the following parameters (**Figure 1**): (a) early linear slope during first 5% of volume ejection in systole (ESslope), (b) the overall linear slope during systole (Sslope) and (c) end-systolic peak longitudinal strain (peak strain). In addition (un)coupling was termed to describe the relationship between systolic and diastolic strain for any given area/volume and was assessed during (d) early (Uncoupling ED), (e) late diastole (Uncoupling LD) and (f) overall. Furthermore, (g) the early linear slope during first 5% (EDslope) and (h) late linear slope (LDslope) during last 5% of volume increase in diastole.

The Sslope was derived as the gradient of the linear regression line over the systolic phase of the strain–area loop. Longitudinal peak strain was derived as the raw peak strain value from the longitudinal strain data. The Uncoupling ED and Uncoupling LD were calculated across the area between the systolic and diastolic polynomial curves. Using the equations of the polynomial regression lines, strain at % increments of RVEDA were calculated. By subtracting diastolic from systolic strain, the difference at each point was calculated. Based on individual RV fractional area change (RVFAC), the working range of the heart

was determined, after which Uncoupling ED was calculated as the sum of the differences at the lowest two-thirds of increments of EDA in the working range of the heart, and Uncoupling LD was calculated as the sum of the differences at the highest one-third of increments of EDA in the working range of the heart.

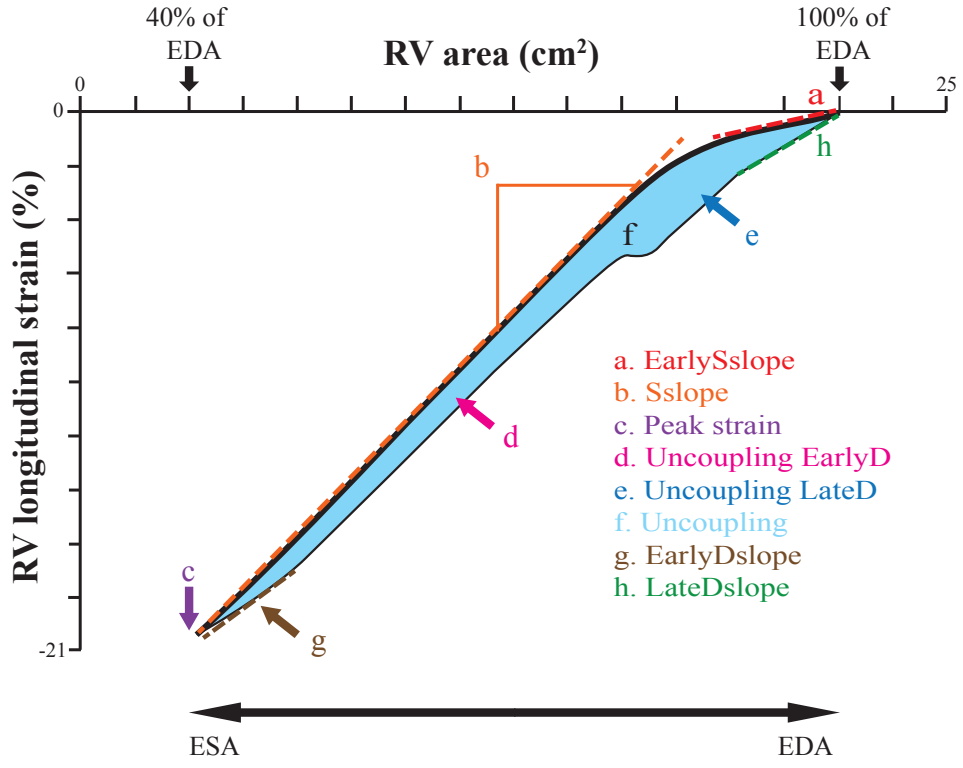

Figure 1. Strain-area loop. The linear strain-area slope (b. Sslope) and early strain-area slope during first 5% of volume ejection in systole (a. ESslope); (II) end-systolic peak longitudinal strain (c. peak strain); (III) the early linear strain-area slope during first 5% (g. EDslope) and late linear strain-area slope (h. LDslope) during last 5% of volume increase in diastole; and (IV) diastolic uncoupling (f.) (i.e. difference in strain between systole and diastole at any given area), divided into uncoupling during early (d. Uncoupling ED) and late diastole (e. Uncoupling LD).

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The Acute and Chronic Effects of High-Intensity Exercise in Hypoxia on Blood Pressure and Post-Exercise Hypotension

CHAPTER 5

ABSTRACT

Background and objectives. Acute exercise leads to an immediate drop in blood pressure (BP), also called post-exercise hypotension (PEH). Exercise in hypoxia is related to additional vasodilation, potentially contributing to more profound PEH. Therefore, we investigated the impact of hypoxia *versus* normoxia on the magnitude of PEH. Secondly, we examined whether these changes in PEH relate to the BP-lowering effects of 12-week exercise training under hypoxia.

Methods. In this prospective study, twenty-one healthy individuals (age 22.2±3.0 years, fourteen male) performed a 45-minute high-intensity running exercise on two different days in a random order, under hypoxia (fraction of inspired oxygen (FiO_2) 14.5%) and normoxia (FiO_2 20.9%). BP was examined pre-exercise (t=0) and at t=15, t=30, t=45 and t=60min post-exercise. Afterwards, subjects took part in a 12-week hypoxic running exercise training program. Resting BP was measured before and after the 12-week training program.

Results. Acute exercise induced a significant decrease in systolic BP (SBP, P=0.001), but not in diastolic BP (DBP, P=0.113). No significant differences were observed in post-exercise BP between hypoxic and normoxic conditions (SBP, P=0.324 and DBP, P=0.204). Post-exercise changes in SBP, DBP and MAP significantly correlated to the 12-week exercise training-induced changes in SBP (r=0.557, P=0.001), DBP (r=0.615, P<0.001), and MAP (r=0.458, P=0.011).

Conclusion. Our findings show that hypoxia does not alter the magnitude of PEH in healthy individuals, whilst PEH relates to the BP-lowering effects of exercise. These data highlight the strong link between acute and chronic changes in BP.

INTRODUCTION

Post-exercise hypotension (PEH) is a reduction in systolic and/or diastolic arterial blood pressure (BP) below resting BP levels following a single bout of exercise and is usually observed minutes to hours after exercise.[1-3] The decline in BP after exercise relates to a marked decrease in total peripheral resistance (TPR)[3, 4], due to sustained post-exercise local vasodilator mechanisms[5, 6], with unmatched elevations in cardiac output.[7] The potential clinical relevance of PEH is that the magnitude of PEH relates to the BP-lowering effect of exercise training.[8-10] Enhancing the magnitude of PEH may, therefore, have the potential to increase the anti-hypertensive effects of exercise training.

Previous research revealed that several factors, including exercise intensity[11-13], duration[13, 14], mode (interval or continuous[15]), time of day[16] and body position[17], may influence the magnitude of PEH. Relatively little work has examined the impact of hypoxia on PEH. This is relevant since previous work revealed that hypoxia contributes to a higher decrease in TPR post-exercise[18-20] potentially contributing to a larger PEH. Indeed, one previous study found a more profound PEH in response to resistance exercise under hypoxia *versus* normoxia.[21] Therefore, hypoxia may elicit a larger magnitude of PEH compared to normoxia. This is potentially relevant, since acute changes in BP with (hypoxic) exercise may relate to long-term changes in resting BP after regular exercise training.[8-10] A larger PEH in hypoxia may therefore translate into a larger decrease in resting BP, as previously suggested for normoxic exercise.[9]

The aim of this study was 1) to investigate the influence of hypoxia *versus* normoxia on the PEH magnitude of post-endurance exercise (high-intensity) in healthy individuals, and 2) whether the magnitude of PEH relates to the reduction in BP after a 12-week hypoxic endurance exercise-training program. According to previous research, we hypothesized that 1) high-intensity endurance exercise under hypoxia would elicit greater reductions in post-exercise BP compared to normoxia, and that 2) the magnitude of PEH would relate to the training-induced BP reduction.

METHODS

Study Population

Twenty-one healthy normotensive individuals (fourteen males) were recruited for the study. Participants were eligible to take part in this study if they were able to run on a treadmill and that they did not train for more than two hours a week at moderate-to-high intensity for the last six months. Exclusion criteria were a body mass index (BMI) <18 or >30 kg/m^2, a possibility of pregnancy, personal history of cardiovascular disease, positive family history of cardiovascular death (<55y), exercise-limiting respiratory disease and physical (i.e. musculoskeletal) complaints making completion of the 12-week training program impossible.

The procedures were in accordance with institutional guidelines and conformed to the declaration of Helsinki. The study was approved by the Ethics Research Committee of the Liverpool John Moores University (18/SPS/065). Participants gave full written and verbal informed consent before participation.

Study design

In this prospective randomized cross-over study, participants attended the laboratory on 36 separated occasions divided into four parts, see **Figure 1**. During the first visit, baseline measurements were performed. Visits 2 and 3 included the actual test days to study the acute effects of hypoxia *versus* normoxia on PEH. Visits 4 to 35 (training program) and visit 36 (follow-up measurements) comprised the chronic part to study; the relationship between PEH during the first visit versus the long-term changes in BP.

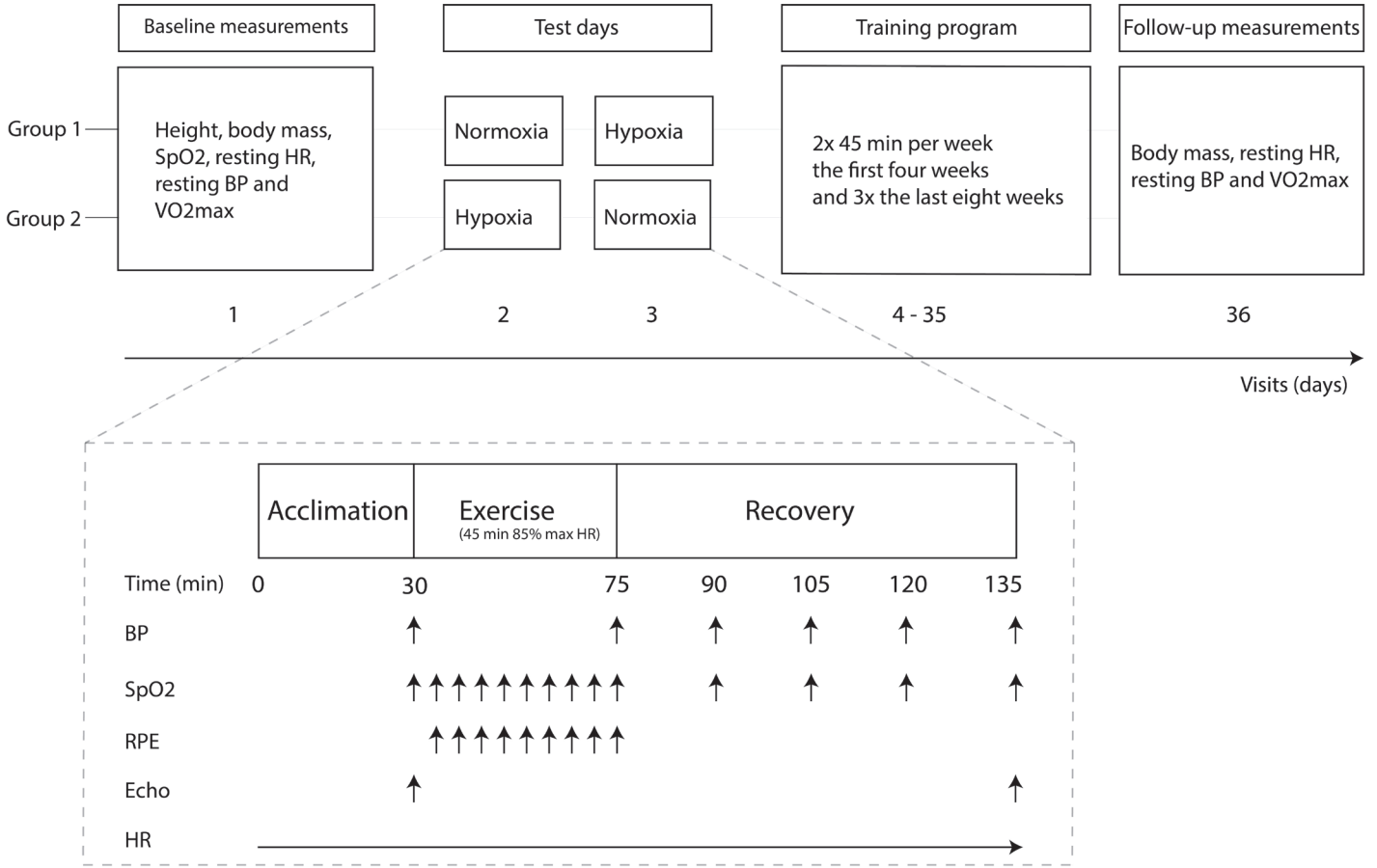

Figure 1. Overview of study design, where the dotted panel is highlighting visit 2 and 3 (test days).

Baseline and follow-up measurements. The measurements included determination of height (SECA stadiometer, SECA GmbH, Germany), weight (SECA scale, SECA GmbH, Germany), oxygen saturation (SpO_2, pulse oximetry; Ana Pulse 100, Ana Wiz Ltd., UK) and maximal oxygen consumption (VO_2max). Resting heart rate (HR, Polar, Kempele, Finland) and resting BP (Dinamap V100, GE Medical, Norway) were determined at the end of ten minutes of quiet rest in a supine position. Resting HR was averaged over 1 minute of continuous recording. Resting BP determination involved three serial measurements from the right arm taken 30 seconds apart. Cuff size was adjusted to arm circumference. A standardized maximal cardiopulmonary exercise test (CPET) for VO_2max assessment was conducted on a motorized treadmill (HP Cosmos, Nussdorf, Germany) after a 10-min warm-up and familiarisation. The test started at a speed of 7 km/h for 3 minutes followed by speed increments of 1 km/h every minute until subjects' volitional exhaustion. Careful calibration of flow sensors and gas analysers was performed before each measurement according to the manufacturer's instructions (Oxycon pro, CareFusion, VS). VO_2max was defined as the highest value of a 30-s average[22], and attainment was verified according to previous recommend criteria.[23]

Test days. **Figure 1** gives an overview of the test days described below. Participants were randomly allocated to one of two groups in a counterbalanced design and blinded for the order of testing days. One test day was performed at normoxia (sea level, equivalent to FiO_2 20.9%)) and the other test day at normobaric hypoxia (3,000m simulated altitude, equivalent to FiO_2 14.5%), separated by at least 48 hours and maximal 72 hours of rest. Participants were subjected to 30 minutes of acclimation in seated position followed by 45-minute of high-intensity endurance running exercise on a motorized treadmill (HP Cosmos, Nussdorf, Germany) and 60 minutes of recovery in seated position. Exercise intensity was set by using 85% of maximal heart rate for both hypoxia and normoxia sessions. HR, SpO_2 and BP measurements were performed at the end of acclimation (baseline) and at 15, 30, 45 and 60 minutes during post-exercise recovery in the seated position. HR was averaged over 1 minute of continuous recording. BP determination involved three serial measurements from the right arm taken 30 seconds apart. To assess PEH, post-exercise BP measurements were averaged to calculate the decline in BP from baseline. Participants remained in a seated upright position with back support and BP

measurements were obtained using an appropriately sized cuff. HR was measured continuously throughout (Polar, Kempele, Finland), and rate of perceived exertion (RPE) was monitored during exercise.[24] Echocardiography (Vivid E9; GE Medical; Horten, Norway) was performed at baseline and at 60 min of recovery to obtain cardiac haemodynamic parameters (stroke volume (SV), cardiac output (CO)). Estimated TPR (TPR_{est}) was calculated from the echocardiography-derived estimate of CO and mean arterial pressure (MAP) at baseline and at 60 min of recovery (TPR_{est}=MAP/CO). Measurements were performed at the same time at both days to control for diurnal variation, and fluid intake was controlled by providing the same amount of water to participants during both testing days.

Training program. Following the test days, subjects took part in a 12-week normobaric hypoxic exercise endurance-training program consisting of 2x45 minute sessions a week in the first four weeks and 3x45 minute sessions in the last eight weeks. This running exercise was performed on a motorized treadmill at 3,000m simulated altitude (equivalent to FiO_2 14.5%) at high-intensity (85% of maximal heart rate).

Environmental chamber and safety. All exercise tests and training sessions were conducted in an environmental chamber (TISS, Alton, UK; Sportingedge, Bastingstoke, UK), which was set-up by a qualified technician. Normobaric hypoxia was achieved by a nitrogen dilution technique. Ambient temperature, carbon dioxide (CO_2) and oxygen (O_2) levels were controlled in all sessions (20°C; FiO_2 14.5%; CO_2 0.03%), whilst a Servomex gas analysis system (Servomex MiniMP 5200, Servomex Group Ltd., UK) was used inside the chamber to provide the researcher continuous information regarding O_2 and CO_2 levels. Acute mountain sickness symptoms (AMS, measured by Lake Louise Score (LLS)[25]) were monitored during testing and training sessions every 20 minutes. The subject was removed from the environmental chamber if oxygen saturation levels dropped below 80% or severe AMS was suspected (LLS≥6).

Statistical analysis

Statistical analysis was performed using SPSS Statistics 24 (SPSS Inc., VS). All parameters were visually inspected for normality and tested with Shapiro-Wilk normality tests. Categorical variables were presented as proportions and continuous variables were reported as mean±SD, unless indicated otherwise. A two-way repeated measures ANOVA

was conducted to compare 1) pre- and post-exercise training data and 2) conditions. A Greenhouse-Geisser correction was used for estimating P-values if the sphericity assumption was violated ($P>0.05$, tested with Mauchly's test). A Sidak post-hoc correction was used to account for multiple testing.

Associations between acute PEH and chronic BP lowering effects were analysed by Pearson's correlation coefficient and compared using Fisher Z-transform, in which acute is defined as the BP response to a single bout of high intensity hypoxic exercise and chronic as the change in post-acclimated resting BP following a 12-week training program. An alpha level of $p\leq0.05$ was accepted a priori for significance.

RESULTS

Participants were aged 22.2±3.0 years, had a body mass of 69.5±10.7 kg, a VO_2max/kg of 52.4±8.1 mL/min/kg and were all normotensive (<140/90mmHg). All participants were non-smokers. Baseline characteristics are shown in **Table 1**. Fifteen of the 21 included participants completed the chronic part of the study (**Table 2**).

Table 1. Subject characteristics: baseline

Sex (m/f)	14/7
Age (yr)	22.2±3.0
Height (cm)	170.3±10.4
Body Mass (kg)	69.5±10.7
BMI (kg/m2)	24.0±2.7
BSA (kg)	1.81±0.18
Resting HR (bpm)	65±8
Resting SBP (mmHg)	119±5
Resting DBP (mmHg)	69±8
Resting MAP (mmHg)	85±6
SpO2 (%)	98.4±1.2
VO_2max (L/min)	3.6±0.7
VO2max/kg (mL/min/kg)	52.4±8.1
VE (L/min)	138±28
Hrmax (bpm)	199±8

Data are expressed as means±SD. m, male. f, female. BMI, body mass index. BSA, body surface area. HR, heart rate. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. SpO2, oxygen saturation. VO_2max, maximal oxygen uptake. VE, ventilation.

Table 2. Subject characteristics: baseline and post-training program

	Pre	Post	p-value
Sex (m/f)	10/5		
Age (yr)	22.0±2.4		
Height (cm)	172±11		
Body Mass (kg)	71.2±11.7	70.3±12.3	0.17
BMI (kg/m2)	24.0±3.0	23.6±2.7	0.14
BSA (kg)	1.84±0.19	1.83±0.20	0.18
Resting HR (bpm)	77±10	66±6	**<0.001**
Resting SBP (mmHg)	118±4	113±9	**0.02**
Resting DBP (mmHg)	67±8	63±5	0.07
Resting MAP (mmHg)	84±6	80±6	**0.03**
VO2max (L/min)	3.7±0.7	3.9±0.8	**<0.001**
VO2max/kg (mL/min/kg)	52.1±7.1	55.7±7.3	**<0.001**
VE (L/min)	138±29	145±34	**0.002**
Hrmax (bpm)	199±8	195±6	**0.008**

Data are expressed as means±SD. m, male. f, female. BMI, body mass index. BSA, body surface area. HR, heart rate. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. VO_2max, maximal oxygen uptake. VE, ventilation.

Post-exercise blood pressure response in normoxia and hypoxia (acute study)

HR during exercise was matched in exercise sessions in normoxia and in hypoxia (173±7 bpm, 172±7 bpm respectively, p=0.23). Body mass loss (hypoxia -410±320g vs. normoxia -410±199g, p=0.99) and water intake (hypoxia 373±228ml vs. normoxia 336±196ml, p=0.24) during exercise did not differ between both testing sessions. Mean distance covered during exercise was significantly higher in normoxia (6,655±1,266m) compared to hypoxia (5,797±1,112m, p<0.001), whilst there was no significant difference in subjective ratings of perceived exertion (RPE normoxia 12.5±1.3, RPE hypoxia 13.3±1.5; p=0.07). SV was significantly decreased during recovery (p<0.01), whilst this decline did not differ between hypoxia and normoxia (p=0.54) (**Table 3**)). Echocardiography showed a significantly higher CO during hypoxia compared to normoxia (P<0.01), whilst no differences were found between rest and post-exercise (p=0.09) (**Table 3**). TPR_{est} was significantly lower during hypoxia compared to normoxia (P<0.01), whilst no difference was found between baseline and recovery (P=0.83).

SBP and MAP significantly decreased over time during recovery ($p<0.01$), while DBP did not change ($p=0.11$) (**Figure 2, Table 3**). The mean PEH response for SBP, DBP and MAP in normoxia were -2.6±8.5, -2.5±5.0 and -2.6±4.9 mmHg respectively, and in hypoxia -6.2±8.4, -1.9±5.4 and -3.4±5.4 respectively. SBP, DBP and MAP did not differ between conditions at any time point (all $p>0.05$) (**Figure 2, Table 3**). For all BP responses, there were no significant interactions between condition and time (all $p>0.05$). Similar findings were observed when post-exercise BP responses were presented as relative changes (data not shown).

Table 3. Pre- and post-exercise values of physiological parameters

	Pre		15 min		30 min		45 min		60 min		p-value		
	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	Normoxia	Hypoxia	C	T	C*T
HR (bpm)	65±8	69±6	98±12	101±9	90±113	95±9	86±12	92±9	80±12	87±9	**0.002**	**<0.001**	0.08
SpO2 (%)	98±1	89±2	97±1	89±3	97±1	89±3	97±1	90±3	98±2	90±3	**<0.001**	**0.001**	0.55
SBP (mmHg)	121±10	124±10	121±10	120±9	119±9	118±9	117±9	117±8	118±9	118±10	0.93	**<0.001**	0.32
DBP (mmHg)	70±8	70±8	66±8	68±8	67±9	69±7	67±9	68±7	69±9	67±7	0.53	0.11	0.20
MAP (mmHg)	87±6	88±7	85±8	85±7	85±8	85±7	84±8	85±7	85±8	84±7	0.61	**0.004**	0.41
LVSV (mL)	70±20	73±18							64±18	64±18	0.33	**<0.001**	0.54
LVCO (L/min)	4.6±1.5	5.1±1.3							4.4±1.3	4.8±1.4	**0.002**	0.09	0.89
TPR_{est}	20.9±6.7	18.3±4.5							21.0±6.7	18.5±4.7	**0.003**	0.83	0.96

Data are expressed as means±SD. HR, heart rate. SpO2, oxygen saturation. SBP, systolic blood pressure. DBP, diastolic blood pressure. MAP, mean arterial pressure. SV, stroke volume. CO, cardiac output. TPRest, estimated total peripheral resistance. C, condition. T, time.

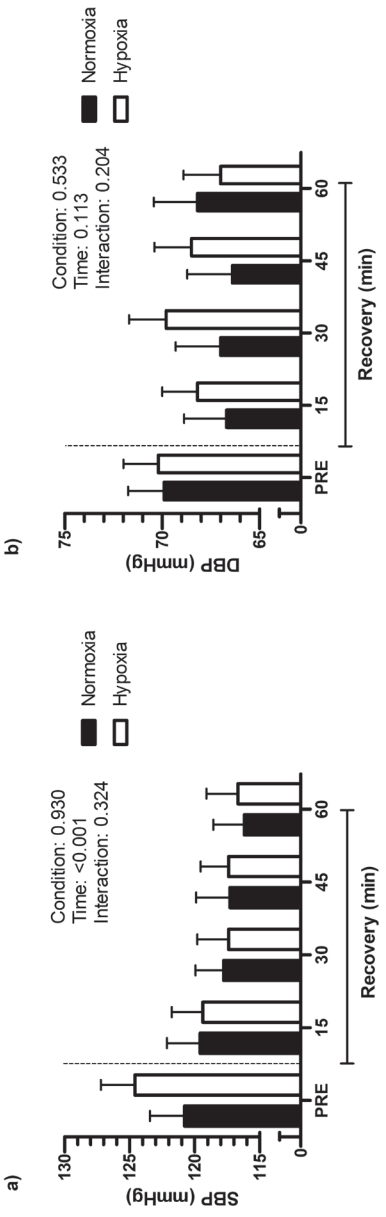

Figure 2. Post-exercise systolic blood pressure (panel a), diastolic blood pressure (panel b) and mean arterial pressure (panel c) response under normoxia (black bars) and hypoxia (white bars). The dotted line indicates 45 minutes' high-intensity exercise and the error bars reflect the standard error of the mean.

Correlation of acute and chronic BP response (chronic study)

During the prospective intervention part of our study, 6 participants dropped-out (motivational issues n=4; health problems unrelated to the study n=2). Participants completed on average 30±2 training sessions (94% adherence) at an average 83.5% of their maximum HR. These fifteen participants showed a significant increase in VO2max/kg (52.1 to 55.7 mL/min/kg, $p<0.001$) (Table 2). Resting SBP, MAP and resting HR significantly decreased (118 to 113 mmHg, 84 to 80 mmHg and 78 to 66 bpm, respectively, $p<0.05$) (Table 2). Resting DBP did not significantly change (67 to 63 mmHg, $p=0.067$) (Table 2). Pooled data derived from the experiments under normoxia and hypoxia indicate that the magnitude of PEH significantly correlated with the decrease in BP after 12-week of exercise training for DBP, SBP and MAP (Figure 3). When comparing data derived under normoxia versus hypoxia, no significant differences were observed in the correlation between PEH and resting BP (Fisher Z: SBP, $p=0.22$; DBP, $p=0.35$; MAP, $p=0.65$.

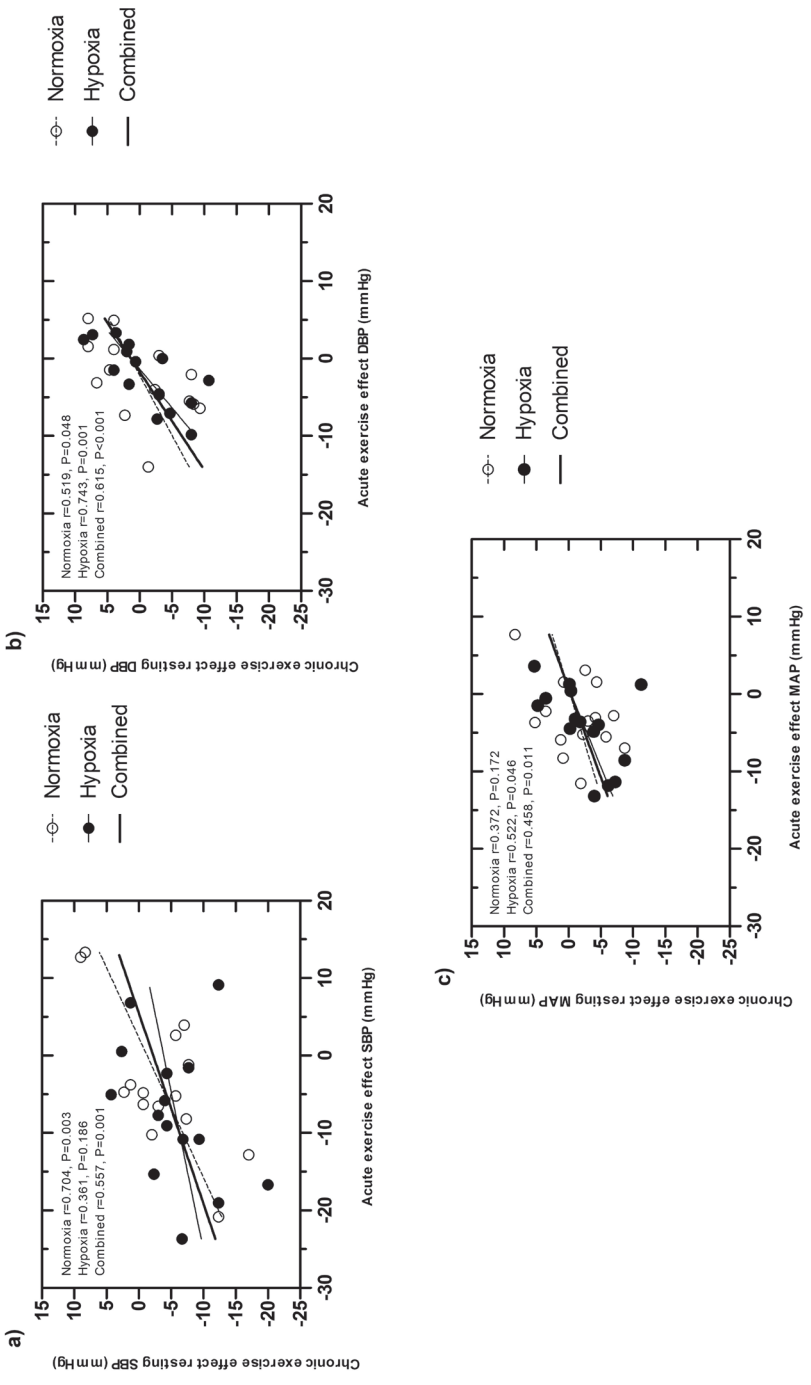

Figure 3. Correlations between acute exercise effect and chronic blood pressure lowering effect of 12-week hypoxic training intervention (SBP, panel a), diastolic blood pressure (DBP, panel b) and mean arterial pressure (MAP, panel c). Error bars are omitted for clarity.

DISCUSSION

The aim of this study was to investigate the impact of hypoxia on PEH, and whether the magnitude of PEH relates to the BP-lowering effect of 12-week hypoxic endurance exercise-training. We present the following findings. First, the magnitude of PEH does not differ when exercise, matched at relative intensity, is performed under hypoxia or normoxia. Second, the magnitude of PEH during the first exercise bout was positively related to the magnitude of the BP-lowering effect of 12-weeks high-intensity running exercise training under hypoxia. Taken together, our results demonstrate that hypoxia does not alter the PEH response, whilst we reveal the close relationship between acute and chronic changes in BP in response to high-intensity running exercise in healthy individuals.

Post-exercise hypotension in normoxia and hypoxia

Our study showed that a 45-minute high intensity running exercise bout leads to a decrease in mean arterial blood pressure of ~3 mmHg after exercise in healthy individuals, supporting the presence of PEH. This observation confirms findings from several previous studies that demonstrated the presence of PEH after a variety of types, durations and intensities of endurance exercise.[11, 12, 15, 26] However, in contrast to our hypothesis, the magnitude of PEH was not altered by hypoxia (FiO_2 14.5%). Under physiological conditions, changes in CO and TPR lead to alterations in BP.[27] After exercise, PEH seems to be largely explained by a decrease in TPR, likely due to a combination of centrally (i.e. arterial baroreflex resetting with inhibition of sympathetic outflow) and locally mediated vasodilator mechanisms, which is not compensated by adequate elevations in CO.[3] Several previous studies have shown that hypoxia represents a powerful vasodilator signal for cerebral and peripheral arteries, subsequently leading to a decrease in TPR.[18-20] Despite the decrease in TPR under hypoxia, BP and PEH did not differ between normoxia and hypoxia, possibly because of a compensatory increase in HR and CO under hypoxia. The elevated HR and CO under hypoxia may be explained by a preserved and well-functioning baroreflex sensitivity in healthy young individuals under hypoxia[28], or the hypoxia-induced chemoreceptors stimulation promoting greater sympathetic activation.[29, 30] Interestingly, HR recovery tend to be slower under hypoxia (Table 3), while total work done was lower under hypoxia. These differences in HR recovery and total work may also have contributed to the preserved PEH response under normoxia versus hypoxia.[13, 31, 32]

Our finding contrasts with a previous study that investigated PEH in hypoxia following resistance exercise.[21] In this study, healthy young males showed significantly lower SBP and DBP levels 10, 20 and 30 minutes post-resistance exercise in hypoxia (FiO2 13.0%) compared to normoxia.[21] A key difference with our study is that they examined resistance exercise, compared to endurance exercise in our study. Whilst this difference in exercise mode may explain cross-study findings, former within-subject comparisons support the hypothesis that the mode of exercise (resistance vs endurance) does not alter the magnitude of PEH.[33, 34] However, none of these previous comparisons have taken hypoxia into account. In addition, in the study of Horiuchi *et al.* post-exercise recovery was performed under normoxia, making any comparisons with the previous investigations difficult, due to the persistent influence of hypoxic stress on autonomic and haemodynamic post-exercise responses. Future work is required to better understand the potential difference in effect size of PEH between the different modes of exercise under hypoxia and recovery modalities.

Correlation post-exercise hypotension versus BP changes to training

The anti-hypertensive effects of regular exercise training for the general population are well known. This study further explored the relation between PEH and the long-term benefits of regular exercise training. The decrease of ~5mmHg in mean BP after 12-weeks of exercise training may seem marginal, but actually exceeds that of most previous studies examining the benefits of exercise training on BP in healthy individuals.[35] Within this context, it is important to realize that larger anti-hypertensive effects may be observed in those with (borderline) hypertension.[35] Importantly, we were able to link PEH, observed during the first session of high-intensity running exercise, to changes in resting BP after 12-weeks of exercise training. This observation provides further support that acute changes in BP after exercise ultimately relate to long-term changes.[8-10] An important addition to this knowledge, is that the correlation disappeared when we related PEH (taken after hypoxic exercise) to post-training BP assessed under normoxia. This suggests that the BP responses to acute and chronic exercise training, despite the similar magnitude of PEH, are related through distinct pathways. From a personalised exercise perspective, this observation means that those with the largest decline in PEH under normoxia, even when exercise training is performed under hypoxia, can expect the largest decline in resting BP (under normoxia). This may contribute to further personalise strategies to lower BP.

Limitations. A limitation is that we did not include a control group who either did not perform exercise or performed exercise under normoxic conditions across a 12-week period. Whilst this may have provided additional insight, this does not impact our primary finding of our study, in that PEH is strongly related to long-term declines in resting BP. In addition, our findings on PEH in hypoxia relate to a group of healthy young normotensive individuals and cannot be directly extrapolated to pre-hypertensive and hypertensive individuals, where PEH magnitude may be different.[36]

Perspectives

Hypoxia represents a relatively common stimulus that importantly alters the physiological demands of the cardiovascular system during exercise compared to normoxia. Nonetheless, we found that acute, high-intensity exercise under normoxia and hypoxia leads to a comparable post-exercise decline in BP in healthy volunteers (i.e. post-exercise hypotension), whilst the magnitude of post-exercise hypotension strongly relates to the anti-hypertensive effects of exercise training. Whilst this provides novel insight into the acute and chronic regulation of BP, the comparable effects of hypoxic and normoxic exercise may have potential clinical relevance. Whilst both types of exercise are linked to a similar subjective level of effort, absolute workloads with hypoxic endurance exercise are significantly lower. This makes hypoxic exercise a suitable alternative for sedentary and frail individuals as a non-drug antihypertensive treatment, since lower workloads will be linked to fewer injuries and risks of exercise.[31, 37, 38] Hypoxic exercise has already been used effectively to enhance vascular structure and function[39, 40], adaptive responses in metabolic capacity[41] and glucose tolerance[42], highlighting the potential of hypoxic exercise for health improvement.

CONCLUSION

Our findings show that hypoxia does not alter the magnitude of PEH in healthy individuals, whilst PEH relates to the BP-lowering effects of exercise. These data highlight the strong link between acute and chronic changes in BP.

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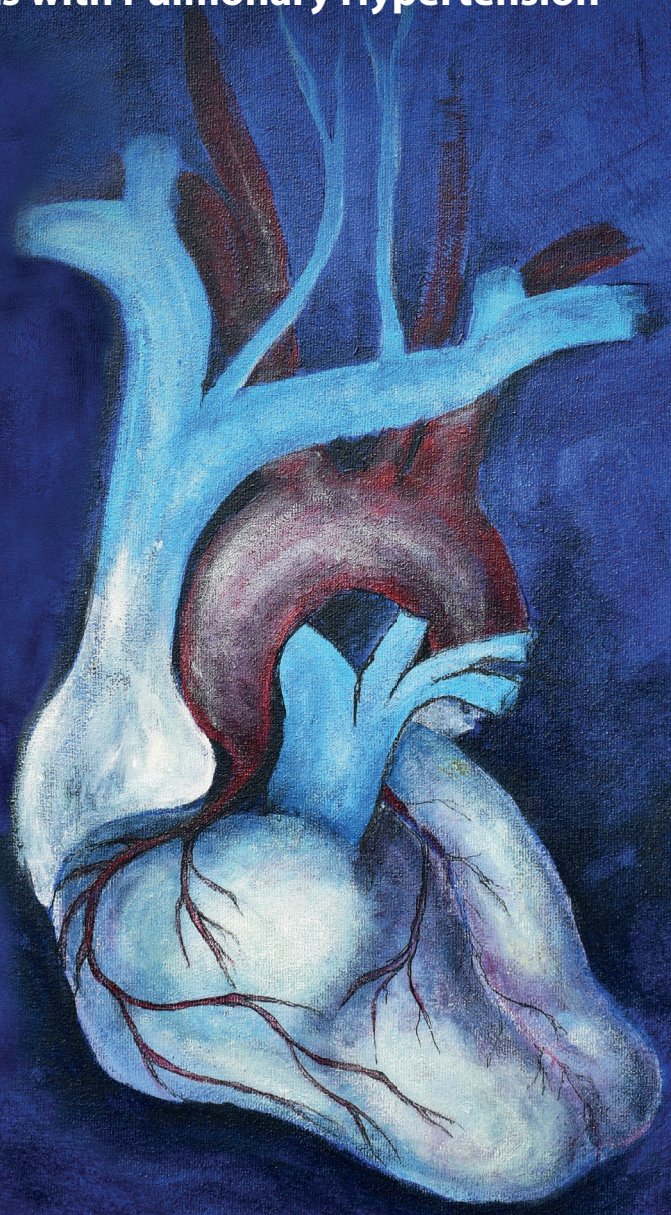
Altered Haemodynamics and Exercise in Patients with Pulmonary Hypertension



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Prognostic Value of Right Ventricular Longitudinal Strain in Patients with Pulmonary Hypertension: a Systematic Review and Meta-Analysis

CHAPTER 6

ABSTRACT

Background. Pulmonary hypertension (PH) is associated with high morbidity and mortality and the predictive capacity of traditional functional echocardiographic measures is poor. Recent studies assessed the predictive capacity of right ventricular longitudinal strain (RVLS). Diversity in methods between these studies resulted in conflicting outcomes. The purpose of this systematic review and meta-analysis was to determine the independent prognostic value of RVLS for PH-related events and all-cause mortality.

Methods. A systematic search in Pubmed (MEDLINE), Embase, the Cochrane Library and Web of Science was performed to identify studies that examined the prognostic value of RVLS in patients with PH. Studies reporting Cox regression based Hazard Ratios (HR) for a combined endpoint of mortality and PH-related events or all-cause mortality for echocardiographic derived RVLS were included. A weighted mean of the multivariate HR was used to determine the independent predictive value of RVLS.

Results. Eleven studies met our criteria, including 1,169 patients with PH (67% female, 0.6-3.8 years follow-up). PH patients with a relative reduction of RVLS of 19% had a significantly higher risk for the combined endpoint (HR: 1.22, 95%CI: 1.07-1.40), while patients with a relative reduction of RVLS of 22% had a significantly higher risk for all-cause mortality (HR: 2.96, 95%CI: 2.00-4.38).

Conclusion. This systematic review and meta-analysis showed that RVLS has independent prognostic value for a combined endpoint and all-cause mortality in patients with PH. Collectively, these findings emphasize that RVLS may have value for optimizing current predictive models for clinical events or mortality in patients with PH.

INTRODUCTION

Pulmonary hypertension (PH) is a progressive disease with a 5-year survival rate of approximately 50%, depending on aetiology and disease severity.[1] Although the aetiology of PH relates to an increased pulmonary artery resistance, the primary cause of death relates to right ventricular (RV) failure since the RV has to overcome the increased pulmonary resistance in order to maintain cardiac output.[2] Consequently, echocardiographic measurements of RV structure and function are routinely performed during follow-up of patients with PH.[3, 4] Due to complex RV geometry and load dependency of the RV functional parameters, traditional echocardiographic indices such as RV fractional area change (RVFAC) and tricuspid annular plane systolic excursion (TAPSE), have limited prognostic power in patients with PH.[3]

The introduction of speckle tracking echocardiography has allowed for the measurement of ventricular longitudinal strain, a measure of ventricular deformation to assess specific local and global function.[5] In heart failure, valvular heart disease, cardiomyopathy and ischaemic heart disease, left ventricular longitudinal strain independently predicts future events.[6] Patients with PH demonstrate a reduced RV longitudinal strain (RVLS) compared to healthy controls, whilst several studies have examined the prognostic value of RVLS in patients with PH.[7-30] These studies report a broad range of outcomes, ranging from no significant predictive capacity to a high predictive capacity. These differences in outcome may relate to differences in methodology between studies, such as variation in aetiology (PH *vs* pulmonary arterial hypertension (PAH)), included population for HR calculation (inclusion of healthy controls or non PH patient *vs* just PH patients), patient management at time of inclusion (treatment naive vs. single or combined therapy), follow-up duration (0.6-5.0 years), outcome parameters (morbidity *vs* all-cause mortality), group size (n=17 up to n=406) and methods in which the HRs were determined (percentile change (continuous parameter) *vs* a predefined cut-off point (dichotomous parameter)).[7-11, 14-17, 21, 23, 24, 28, 29] The heterogeneity in study designs and outcomes provide a challenge when attempting to establish the potential prognostic value of RVLS in patients with PH. Combining these studies in a systematic review and meta-analysis will provide clarity on the prognostic value of RVLS in patients with PH.

The purpose of this systematic review and meta-analysis was to determine the independent prognostic value of RVLS in patients with PH on PH-related events and all-cause mortality. We hypothesize that RVLS will have independent prognostic value in PH patients for PH-related events and all-cause mortality.

METHODS

Search strategy

A systematic search was performed with the use of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement 2015 (PRISMA).[31] Pubmed (MEDLINE), Embase, the Cochrane Library and Web of Science were systematically searched for articles published before February 1th, 2018. The following search strategy was used, with adaptation for each database: (((("Hypertension, Pulmonary"[Mesh]) OR ((Pulmonary hypertension[tiab] OR Pulmonary artery hypertension[tiab] OR Pulmonary arterial hypertension[tiab] OR PAH[tiab] OR lung arterial hypertension[tiab] OR lung artery hypertension[tiab] OR lung hypertension[tiab]))))) AND ((strain[tiab] OR deformation[tiab]))) AND ((("Prognosis"[Mesh] OR "Survival Analysis"[Mesh] OR "Mortality"[Mesh] OR "mortality"[Subheading] OR "Hospitalization"[Mesh])) OR (Prognos*[tiab] OR Predict*[tiab] OR Surviv*[tiab] OR Mortalit*[tiab] OR Hazard ratio*[tiab] OR Hospitalization[tiab] OR Hospitalisation[tiab])). References of included articles were manually checked for possible eligible studies that were missed during the literature search.

Study selection

After the initial search, duplicates were eliminated from the database. Two authors (H.H., G.K.) independently screened the remaining study titles and abstracts for eligibility using the predefined inclusion and exclusion criteria (**Table 1**), resulting in 42 articles from which full text was assessed (**Figure 1**).

Table 1. Inclusion and exclusion criteria

Inclusion	Exclusion
Population	
Pulmonary hypertension	Animal studies Paediatric studies
Outcome Echocardiography	
Right ventricular strain	
Outcome measures	
Hazard ratio's based on multivariate cox-regression analysis	Receiver operating curves Model based prediction
Other	
English language Full papers	Language other than English Abstract only Conference proceedings

We included studies in which either RV free wall longitudinal strain (RVFWS) or RV global longitudinal strain (RVGLS) was evaluated as a predictor for a combined endpoint of mortality and PH-related events or (all-cause) mortality. We excluded those studies, which did not perform Cox proportional hazard ratio analysis, or if the (independent) prognostic value of RVLS in PH patients was not reported. Additionally in order to ensure we determine the independent prognostic value of RVLS in patients with PH only, we excluded studies which performed Cox proportional hazard ratio analysis in a population which included non PH patients (i.e. healthy controls or suspected patients).

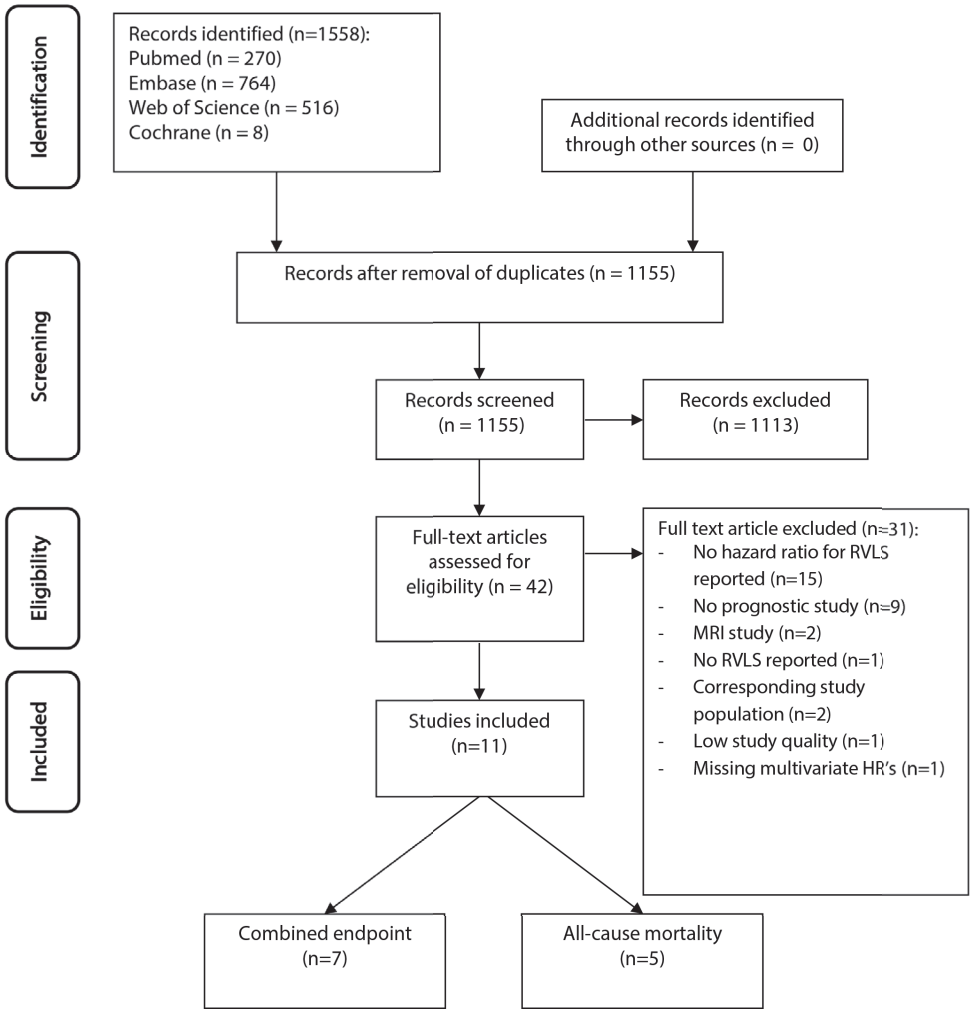

Figure 1. Flow chart of study selection

Data extraction

Data was independently extracted by two authors (H.H. and G.K.) using a predetermined data extraction file. Differences in data extraction were resolved by consensus and if necessary a third author was consulted (T.E.). Since all selected studies included strain, but only one study stain and strain rate, we focused on the prognostic value of strain only. Univariate and multivariate HR (95%-CI), the mean RVLS for the study population and the RVLS cutoff value for calculation of the HR were extracted from the individual studies (**Table 2**). The included studies reported HRs on either a continuous scale (i.e. change in risk per % RVLS) and/or a dichotomous scale (i.e. below/above a cut-off point). In case of a dichotomous scale the HR should increase with a higher absolute value (due to the negative nature of RVLS), but as some studies investigated the beneficial effect of a RVLS value below a certain cut-off point, we calculated the inverse HR (1/HR – [1/95%-CI]) to ensure homogeneous presentation of the data. Additional information gathered consisted of: sample size, age, sex, World Health Organisation (WHO) class, New York Heart Association (NYHA) class, the follow-up period and the clinical endpoint of the individual studies (**Table 3**). For assessment of study quality, data regarding the echocardiographic assessment was gathered consisting of manufacturer, assessment software, echocardiographic window / image, included segments, methods of optimization and usage of the guidelines. When viable data was missing, an attempt was made to request missing data from the authors by email (n=4 studies). Three out of four studies with missing data provided the requested information and were included in our meta-analysis (**Figure 1**).

Table 2. Values of right ventricular longitudinal strain and hazard ratios extracted from the included studies

First author	Absolute values of RVLS (mean±SD)				Cut-off		Relative reduction of RVLS (%)	HR ratio [95% CI]	Log (HR)	SE
	Healthy controls	PH-patients	PH-patients above cut-off value	PH-patients below cut-off value	Dichotomous	Continuous				
Combined endpoint										
da Costa et al. [7]	-27.5±2.4%	-16.1±6.8%			< -14%		13%	4.66 (1.25-7.37)	1.53902	0.67132
Fine et al. [8]	-25.0±5.2%	-19.6±6.6%				-6.7%	26%	1.27 (1.04-1.56)	0.23902	0.10343
Giusca et al. [11]		-17.3±7.2%				-1%	6%	1.22 (0.92-1.62)	0.19885	0.14434
Moceri et al. [29†]	-14.1±3.6	-8.4±3.6%				-1%	12%	1.15 (0.95-1.40)	0.13976	0.09892
Murata et al. [21]		-19.9±6.4%				-1%	5%	1.05 (0.97-1.15)	0.04879	0.04342
Park et al. [10]		-16.1±5.0%				-5%	31%	2.09 (1.05-4.15)	0.75142	0.26639
Unlu et al. [26*]		-16.6%[#]				-1%	5%	1.23 (1.04-1.45)	0.20701	0.08515
All-cause mortality										
Haeck et al. [15]			-23.5±3.7%	-14.0±3.5	< -19%		19%	3.40 (1.19-9.72)	1.22378	0.53577
van Kessel et al. [9]			-24.8±4.0%	-15.9±2.9	< -20%		19%	4.30 (1.11-16.61)	1.45861	0.69022
Park et al. [10]		-16.1±5.0%				-5%	31%	2.08 (1.13-3.80)	0.73716	0.35059
Sachdev et al. [23]		-15±5.0%				-5%	33%	2.00 (1.11-3.96)	0.69315	0.32446
Vitarelli et al. [28†]	-23.8±5.8	-18.8%[#]			< -17%		10%	4.60 (2.79-8.38)	1.52606	0.28056

Symbols denote *=Inverse HR with respect to original article, †=3D strain analysis and #=mean value calculated from multiple groups. (RVLS=Right Ventricular Longitudinal Strain; HR=Hazard Ratio; SE=Standard Error)

Table 3. Population data extracted from the included studies

First author	Study design	Study population	WHO group	NYHA class	PH specific therapy at inclusion	Follow-up (y)	Endpoint
Combined endpoint							
da Costa et al.[7]	NR	N: 66 Age: 45±15 Female sex: 83%	1 (n=66)	I-II (67%) III (33%)	Bosentan and ambrisentan (n=16) Sildenafil (n=31) Calcium channel blockers (n=2) Combined therapy (n=17)	3.3y	Cardiovascular mortality and hospitalization for worsening of PH
Fine et al.[8]	Prospective	N: 406 Age: 59±16 Female sex: 65%	1 (n=300) 3 (n=58) 4 (n=48)	I (20%) II (34%) III (38%) IV (8%)	Prostacyclin (n=50) Endothelin receptor antagonist (n=82) Phosphodiesterase-5 inhibitor (n=89)	1.5y	Cardiopulmonary death and cardiopulmonary events
Giusca et al.[11]	NR	N: 32 Age: 39±15 Female sex: 69%	1 (n=29) 4 (n=3)	II (40.6%) III (56.2%) IV (3.2%)	Bosentan (n=11) Sildenafil (n=16) Combined (n=5)	1.2y	All-cause mortality and treatment failure
Moceri et al.[29†]	Prospective	N: 104 Age: 66±4 Female sex: 56%	1 (n=65) 3 (n=26) 4 (n=11) 5 (n=2)	II (36.5%) III (44.2%) IV (19.3%)	Advanced targeted PAH therapy (n=87)	0.6y	PH related mortality
Murata et al.[21]	Retrospective	N: 100 Age: 51±17 Female Sex:74%	1 (n=72) 4 (n=28)	I (22%) II (46%) III (32%)	Phosphodiesterase-5 inhibitor (n=69) Endothelin receptor antagonist (n=56) Prostacyclins (n=26) Calcium channel blockers (n=11) Vitamin K antagonist (n=28)	1.2y	All-cause mortality, hospitalization and intervention for deterioration right-sided heart-failure
Park et al.[10]	Retrospective	N: 51 Age: 48±14 Female sex: 78%	1 (n=51)	I (4%) II (61%) III (35%)	Phosphodiesterase-5 inhibitor (n=29) Endothelin receptor antagonist (n=26) Prostacyclins (n=32) Calcium channel blockers (n=9)	3.8y	Clinical events
Unlu et al.[26*]	Retrospective	N: 62 Age: 61±15 Female sex: 68%	1 (n=33) 4 (n=29)	I (6.5%) II (25.8%) III (58%) IV (9.7%)	Treatment naïve	3.8y	All-cause mortality and heart or lung t ransplantation

Table 3. (Continued)

First author	Study design	Study population	WHO group	NYHA class	PH specific therapy at inclusion	Follow-up (y)	Endpoint
All-cause mortality							
Haeck et al.[15]	Retrospective	N: 142 Age: 59±15 Female sex: 63%	1 (n=53) 2 (n=46) 3 (n=32) 4 (n=7) 5 (n=4)	NR	Endothelin receptor antagonist (n=37) Phosphodiesterase-5 inhibitor (n=19) B-blocker (n=44) Angiotensin-converting-enzyme inhibitor/ angiotensin II receptor antagonist (n=58) Diuretics (n=91) Anticoagulation (n=64)	2.6y	All-cause mortality
van Kessel et al.[9]	Retrospective	N: 53 Age: 56±9 (n=25); 54±17 (n=28) Female sex: 66%	Mixed (n=53)	II (41.5%) III (41.5%) IV (9.4%) NR (7.%)	Mono therapy (n=27) Double therapy (n=16) Triple therapy (n=8)	2.3y	All-cause mortality
Park et al.[10]	Retrospective	N: 51 Age: 48±14 Female sex: 78%	1 (n=51)	I (4%) II (61%) III (35%)	Phosphodiesterase-5 inhibitor (n=29 Endothelin receptor antagonist (n=26) Prostacyclins (n=32) Calcium channel blockers (n=9)	3.8y	All-cause mortality
Sachdev et al.[23]	NR	N: 80 Age: 56±14 Female sex: 76%	1 (n=80)	I-II (28%) III (63%) IV (9%)	Treatment naïve	2.0y	All-cause mortality
Vitarelli et al.[28 †]	NR	N: 73 Age: 53±13 Female sex: 56%	1 (n=25) 2 (n=25) 4 (n=23)	I-II (71%) III-IV (29%)	NR	2.0y	All-cause mortality

Symbols denote †=3D strain analysis. (WHO=World Health Organisation; NYHA=New York Heart Association; PH=Pulmonary Hypertension; NR=Not Reported)

Study quality

All studies included in our meta-analysis were assessed for quality using the Quality In Prognosis Studies (QUIPS) checklist for measuring study quality by two authors (H.H. and G.K.).[32] The QUIPS checklist exists of 31 items divided over six domains; study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding and statistical analysis and reporting. For each domain, several items were evaluated after which the domain was scored for the presence of low, moderate or high risk of bias. As recommended, a predefined overall rating was applied.[32] Studies with a high risk of bias score in a single domain or ≥3 scores of moderate risk of bias in different domains were rated as high risk of bias and excluded from this review (**Supplementary Table 1**).

Echocardiographic assessment

To ensure high quality and consistency of the RVLS measurement we only included studies which reported adherence to the ASE guidelines for echocardiographic assessment of the right heart[33] and/or chamber quantification[34], used a (focused) RV apical 4 chamber view and traced the endocardial border for RVLS determination.

Statistical analysis

Review Manager 5.3 (Cochrane Community) was used to perform a meta-analysis of the reported multivariate HRs. The reported HRs [95%-CI] were converted to a log (HR) and the complementing standard error (SE) using the formula:

$$SE = \frac{\ln\big(upper\ boundary\ (95\% - CI)\big) - \ln\big(lower\ boundary\ (95\% - CI)\big)}{(2 * 1.96)}$$

The resulting values were inserted in the inverse variance method for calculation of HRs using a random effects analysis to calculate the mean weighted HR [95% CI] for all studies. Separate analysis were performed for 1) a combined endpoint of mortality and PH-related events and 2) all-cause mortality. To provide further insight in the relation between RVLS and the risk for the combined endpoint or all-cause mortality, we calculated the relative reduction of RVLS (in %) for which the HR was determined. For this purpose, we defined the relative reduction of RVLS as: the difference between the mean RVLS of the

PH patients above the cut-off point and the cut-off point (for dichotomous scales) or between the mean RVLS of the PH patients and the chosen amount of change in % strain (for continuous scales). The weighted mean relative reduction in RVLS and follow-up time was calculated by multiplying the relative % reduction of RVLS or months of follow-up with the number of included patients per study, after which the cumulative value was divided by the total number of patients included in each analysis.

RESULTS

Study selection

During our search we identified 1,558 potential articles for inclusion. After removal of duplicates, 1,155 articles remained, from which title and abstract were screened for potential inclusion. Finally, a total of 42 studies were considered to be eligible for inclusion (**Figure 1**). After carefully reading through the full-texts, we identified 12 studies that met our inclusion criteria.[7-11, 14, 15, 21, 23, 26, 28, 29] From these 12 studies, six provided data on all-cause mortality[9, 10, 14, 15, 23, 28], from which one study did not report nor provide the results of multivariate analysis.[14] This study was therefore excluded from our meta-analysis. Seven studies reported data for the combined endpoint.[7, 8, 10, 11, 21, 26, 29] One study reported separate data for all-cause mortality and combined endpoint and was included for both analysis.[10] The remaining 11 studies included a total number of 1,169 patients with PH. Studies included predominantly female patients (range: 56-83%), with a mean age varying from 39 to 66 years. Details about the patient population, WHO class, NYHA class and study design of studies that were included are summarized in **Table 3**.

Study endpoints

Studies that examined the combined endpoint included 821 patients with PH, with a follow-up time ranging from 0.6-3.8 years. PH-related events varied from hospitalizations for worsening of PH[7, 8, 10, 21], lung transplantation[8, 10, 26], atrial septostomy[8], pulmonary endarterectomy[21], balloon pulmonary angioplasty[21] and intensified PH medical therapy.[8, 11] Studies that explored all-cause mortality as the primary endpoint included a total of 399 patients with PH, with a follow-up time ranging from 2.0-3.8 years.

Echocardiographic assessment

All studies reported that strain was calculated from 2D or 3D grey scale apical 4-chamber orientation, whilst one study performed both 2D and 3D-strain imaging.[28] Strain was calculated with a variety of software packages (EchoPAC, GE Medical Systems, n=8; Syngo vector velocity imaging, Siemens, n=2; 2D cardiac performance analysis, TomTec, n=1). 10 out of 11 studies determined a multivariate HR for RVFWS, while 4 out of 11 studies determined the multivariate HR for RVGLS. Half of the studies (6 out of 11) reported the methods applied for image optimization (i.e. adjustment of image sector width, gain and greyscale), while 9 out of 11 studies reported a frame-rate of >40 frames/s for strain analysis.

Combined endpoint

Seven studies adopted a combined endpoint of mortality and PH-related events and had a mean follow-up time of 26±17 months.[7, 8, 10, 11, 21, 26, 29] All but one[26] study revealed a significant HR after univariate analysis. After multivariate analysis, four studies revealed a significant HR for mortality and PH-related events[7, 8, 10, 26], while HR did not achieve statistical significance in three studies.[11, 21, 29] Combining all multivariate HRs in our meta-analysis revealed that a relative reduction of 19% (range -5 to -31%) of RVLS significantly increased the risk (HR: 1.22, 95%CI: 1.07-1.40) for the combined endpoint of mortality and PH-related events (**Figure 2**). Studies with a relative reduction below 10% of RVLS tended to be insignificant after multivariate analysis while studies with a relative reduction larger than 10% of RVLS did present significantly higher HR's after multivariate analysis (**Figure 2**).

All-cause mortality

Using data from univariate analysis, all five studies revealed a significant increased HR for RVLS in the prediction for future all-cause mortality after a mean follow-up time of 30±9 months. Multivariate analysis revealed that a lower RVLS was associated with a significantly higher HR for all-cause mortality in all studies.[9, 10, 15, 23, 28] Combining all multivariate HRs, our meta-analysis revealed that a relative reduction of 22% (range -10 to -33%) of RVLS was associated with an increased risk (HR: 2.96, 95%CI: 2.00-4.38) for all-cause mortality (**Figure 3**). No clear relation between a larger relative reduction in % of RVLS and HR was present (**Figure 3**).

Author	Relative reduction of RVLS (%)	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Murata et al. 2016	5%	0.04879	0.04342	25.8%	1.05 [0.96, 1.14]
Giusca et al. 2012	6%	0.19885	0.14434	12.6%	1.22 [0.92, 1.62]
Unlu et al. 2016	6%	0.20701	0.08515	19.9%	1.23 [1.04, 1.45]
Moceri et al. 2015	12%	0.13976	0.09892	18.0%	1.15 [0.95, 1.40]
da Costa et al. 2011	13%	1.53902	0.67132	1.0%	4.66 [1.25, 17.37]
Fine et al. 2011	26%	0.23902	0.10343	17.4%	1.27 [1.04, 1.56]
Park et al. 2011	31%	0.75142	0.26639	5.4%	2.12 [1.26, 3.57]
Total (95% CI)				**100%**	**1.22 [1.07, 1.40]**

Heterogenity: $Tau^2 = 0.02$; $Chi^2 = 15.47$, df = 6 (P = 0.02); $I^2 = 61\%$
Test for overall effect: Z = 2.94 (P = 0.003)

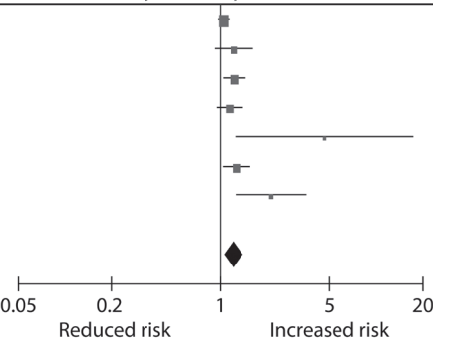

Figure 2. Forrest plot summarising the effect of a (relative) reduction of RVLS on a combined endpoint of mortality and PH-related events in PH patients. The red squares present the weighted effect size and the black lines the 95%-CIs. The size of the red squares indicate the weight of the study. The black diamond presents the mean weighted HR.

Author	Relative reduction of RVLS (%)	log[Hazard Ratio]	SE	Weight	Hazard Ratio IV, Random, 95% CI
Vitarelli et al. 2015	10%	1.52606	0.28056	31.4%	4.60 [2.65, 7.97]
Haeck et al. 2012	19%	1.22378	0.53577	11.9%	3.40 [1.19, 9.72]
Kessel, van et al. 2016	19%	1.45861	0.69022	7.6%	4.30 [1.11, 16.63]
Park et al. 2015	31%	0.73716	0.35059	23.3%	2.09 [1.05, 4.15]
Sachdev et al. 2011	33%	0.69315	0.32446	25.9%	2.00 [1.06, 3.78]
Total (95% CI)				**100%**	**2.96 [2.00, 4.38]**

Heterogenity: $Tau^2 = 0.05$; $Chi^2 = 5.27$, df = 4 (P = 0.26); $I^2 = 24\%$
Test for overall effect: Z = 5.45 (P < 0.00001)

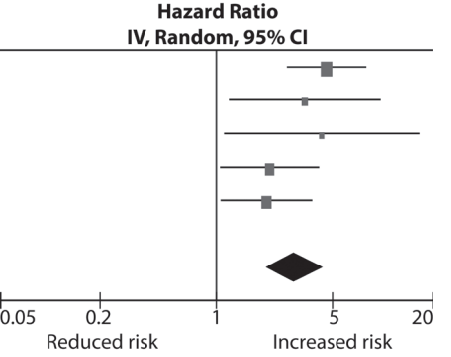

Figure 3. Forrest plot summarising the effect of a (relative) reduction of RVLS on all-cause mortality in PH patients. The red squares present the weighted effect size and the black lines the 95%-CIs. The size of the red squares indicate the weight of the study. The black diamond presents the mean weighted HR.

DISCUSSION

The purpose of this systematic review and meta-analysis was to examine whether RVLS has prognostic value for future events in patients with PH. The key finding was that RVLS has independent prognostic value for all-cause mortality (**Figure 3**). To a lesser extent, RVLS also demonstrated independent predictive capacity for the combined endpoint of mortality and PH-related events (**Figure 2**). Collectively, these findings emphasize that RVLS is a valuable tool with independent prognostic value for all-cause mortality in PH patients.

Impact of PH on RVLS

The thin RV walls consist of longitudinal, circumferential and oblique oriented muscle fibers.[35] The free wall predominantly consists of transverse fibers with scanty subendocardial longitudinal oriented fibers, while in the septal wall the oblique fibers are in a helical shape.[35] Coiling and shortening of the helical-shaped oblique fibers determine the shortening of the RV, producing 80% of RV systolic function. In contrast, contraction of the transverse fibers accounts for just 20% of RV systolic function.[35] In a healthy RV, contraction is therefore predominantly driven by shortening of the RV in the longitudinal direction[35, 36], highlighting the importance of examining RVLS[35] in clinical and research scenarios. In PH, an increase in afterload influences the mechanical function of the RV, which subsequently leads to a decrease in longitudinal shortening[37], indicating insufficient contraction and leading to a reduction of RV stroke volume. The increased afterload forces the RV to adapt, causing either hypertrophy and/or increased contractility to preserve function and stroke volume.[38] Ultimately, however, these processes may lead to maladaptive remodelling, which causes dilation of the chamber and altering of the helical orientation of the oblique fibers, leading to (progressive) attenuation of function.[35] This maladaptive process ultimately contributes to clinical progression and/or mortality. The strong relation between an increase in afterload and/or ventricular maladaptation alongside a decrease in RVLS likely explains the strong and independent prognostic value for RVLS for all-cause mortality in PH patients.

All-cause mortality vs. combined endpoint

Our meta-analysis revealed a lower predictive capacity for combined endpoints *versus* all-cause mortality. This difference may be explained by the fact that clinical events included in the analysis for the combined endpoint are heterogeneous and, therefore, not all events may directly relate to strain (hence, the lower predictive capacity). Other factors than cardiac strain (e.g. gas transfer in the lungs[39]) may contribute to the occurrence of these clinical events. In addition, several studies included intensified PH medical therapy as a combined endpoint, whilst this unlikely relates to cardiac strain. Therefore, the diversity in clinical events included in the combined endpoint, but also the weak link between some of these factors and cardiac strain, lowers the discriminating capacity of RVLS to predict a combined endpoint *versus* all-cause mortality.

Predictive capacity vs. a relative reduction in % of RVLS

As shown in **Figure 3** there is no clear relation between the relative reduction in % of RVLS and the HR for all-cause mortality. This may be explained by the differences across study designs. In contrast to our expectations, the three studies with the lowest relative reduction in RVLS presented the highest HRs in the analysis for all-cause mortality. These three studies all used a dichotomous cut-off value (between -17% and -20%) for RVLS[9, 15, 28], which was higher than the mean RVLS value for the PH patients in the two remaining studies (i.e. -16.1% and -15%).[10, 23] The latter two studies calculated the HR per SD-unit change in RVLS, which resulted in a lower absolute cut-off (approximately -11.1 and -10%) value and in a higher incidence of mortality in the group above the cut-off value. In contrast to the cut-off values in the latter two studies, additional analysis to identify the ideal cut-off value in 4 out of these 5 studies showed that an absolute cut-off between -12.5% and -19.1% had the highest sensitivity and specificity to detect all-cause mortality in PH patients.[9, 10, 23, 28] This indicates that the calculated HR per SD-unit change underestimates the predictive value of RVLS in the latter two studies.

Future direction and clinical implications

Outcomes of the present meta-analysis supports the use of RVLS in patients with PH. Although RVLS has independent predictive value, recent strategies for predicting mortality and events in PH patients consists of constructing multi-parameter predictive

models[40] including TAPSE and/or RFVAC to increase the predictive value in PH patients.[3, 41] Several studies included in our meta-analysis revealed RVLS to has superior predictive value over RVFAC and TAPSE, indicating that RVLS may be a more sensitive predictor for RV dysfunction.[8, 10, 15] Implementing RVLS in these multi-parameter predictive models therefore may increase their predictive value for future events. In addition to predicting future events, a relative reduction in RVLS might be indicative for (adjustment of) pharmacological therapy and/or surgery. Improvement of RVLS after pharmacological therapy and/or surgery has shown to be related to lower risks for mortality and PH-related events.[16, 24] These data further support the use of RVLS in clinical practice, as RVLS changes across time are associated to clinically relevant outcomes in PH patients. Future studies determining reference values and confirming clinically-relevant cut-off values are warranted to improve clinical decision-making and implementation of RVLS in practice.

Limitations

The studies within this meta-analysis were non-uniform in design and varied in the inclusion criteria, methods to measure RVLS (intervendor and technique variabilities), follow-up periods and endpoints. We corrected for these between-study variation using a random effects model in our meta-analysis. Additionally to minimize the impact of intervendor and technique variability we reported the relative reduction of % of RVLS rather than absolute values. We also included studies which used RVFWS (n=7) and RVGLS (n=1) or both (n=3) to determine the predictive value of RVLS in PH patients. Unfortunately, the small amount of studies investigating RVGLS did not allow for a comparison between the predictive value of RVGLS and RVFWS. Similarly, we were not able to compare data obtained with 2D vs. 3D echocardiography and/or machines from different vendors. Due to differences in methodology and statistical approach, not all relevant studies could be included in our analysis. Studies using ROC-analysis[18, 20, 22, 25, 30], Kaplan Meier survival curves[18, 19, 22, 25], odds ratios[20] or predictive models[12, 13, 19] reported outcomes that align with the findings of the present meta-analysis.

CONCLUSION

This systematic review and meta-analysis showed that RVLS possess independent prognostic value for a combined endpoint (HR: 1.22, 95%CI: 1.07-1.40) and all-cause mortality (HR: 2.96, 95%CI: 2.00-4.38) in patients with PH. Collectively, these findings emphasize that RVLS might be useful for optimizing current predictive models for morality or clinical events in PH patients.

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APPENDICES

Supplementary Table 1. Results Quality In Prognosis Studies checklist

	Da Costa et al.	Fine et al.	Giusca et al.	Goda et al. *	Hardergree et al. †	Heack et al.	Henein et al.#	Van Kessel et al.	Moceri et al.	Murata et al.	Murata et al.†	Park et al.	Sachdev et al.	Unlu et al.	Vitarelli et al.
Study participation	L	L	L	L	M	L	M	M	L	L	L	L	L	L	L
Study Attrition	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Prognostic Factor Measurement	L	L	L	L	M	L	M	L	L	L	L	L	M	L	L
Outcome Measurement	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
Study Confounding	L	L	M	L	L	L	H	L	L	M	M	L	L	L	L
Statistical Analysis and Reporting	M	L	M	L	L	L	M	L	L	M	M	L	L	L	L

Symbols denote: *=excluded due to absent multivariate HRs, †=excluded due to using a corresponding study population which was already included through another study, #=excluded based on study quality. L=Low risk of bias, M=Medium risk of bias, H=High risk of bias.

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Effects of Preload Manipulation on Right Ventricular Contractility: Invasive Pressure-Area Loop versus Non-Invasive Strain-Area Loop

CHAPTER 7

Invasive right ventricular (RV) pressure-volume loop provides the gold-standard to evaluate cardiac contractility, but also provides insight into cardiac function as increases in preload cause a rightward shift of the loop and elevates stroke volume (and *vice versa*). Echocardiography has relevance in evaluating cardiac function but also in mechanics, specifically regarding the dynamic relationship between RV longitudinal strain and RV area; strain-area loop.[1] RV strain-area loop characteristics relate to afterload, whilst characteristics hold independent predictive capacity for morbidity/mortality in pulmonary arterial hypertension.[2, 3] Changes in preload alter cardiac dynamics that may induce shifts in the non-invasive RV strain-area loop (similar to shifts in RV pressure-area loops). To better understand the potential of RV strain-area loops in assessing RV function, we compared the impact of preload manipulation on RV strain-area loop *versus* pressure-area loop, and subsequently compared invasive and non-invasive assessment of cardiac contractility.

We recruited 7 individuals (age 54±14 year, 71% female) undergoing right heart catheterisation (to diagnose pulmonary arterial hypertension). Participants provided informed consent prior to procedures. Study procedures were approved by local ethics committee (Radboudumc). During catheterisation a 24-mm AMPLATZER™ Sizing Balloon II (AGA Medical Corporation, Plymouth, USA) was introduced into the inferior vena cava for manipulation in preload. For direct time-point comparison between pressure, strain and area, we simultaneously recorded invasive RV pressure and 2D-echocardiographic images: 1) at baseline, 2) after intravenous infusion of 500ml saline (to increase preload), and 3) after intra-balloon inflation (to reduce preload). Echocardiographic data were analysed using QLAB V10.8 (Philips, Andover, USA) to measure RVLS and area (as previously described)[3, 4], whilst RV pressure data were retrieved from Mac-Lab (GE Medical, Horton, Norway). After preload manipulation data were recorded within 1-minute after stabilization of the signal. Mean strain-area loops and characteristics across the time-points were compared using one-way ANOVA.

The increase in preload caused a rightward shift of the pressure-area loop, whilst a decrease in preload caused a leftward shift and reduced stroke volume (**Figure 1**). These characteristic shifts were also present in the strain-area loop, with an increase in preload inducing RV longitudinal strain decline and a decrease in preload causing an

increase in peak RV longitudinal strain. The slope of the systolic phase of the strain-area loop (i.e. Sslope) during preload elevation was significantly smaller than during preload reduction (-1.8±0.7%/cm^2 *vs*. -2.9±0.9%/cm^2, P<0.05). A potential explanation of this finding is that as preload and stroke volume decreases there is a larger contribution of longitudinal fiber shortening with possible less dependency on circumferential fiber shortening to facilitate systolic volume ejection. This also may explain the paradoxical increase in peak longitudinal strain upon preload reduction as circumferential strain may be disproportionally decreased. Since we were not able to measure circumferential strain, this remains speculative. It is important to acknowledge the complexity of RV function, with changes in stroke volume potentially impacting upon various aspects of cardiac mechanics. This makes it difficult in our study to identify a single or most important factor explaining our observations.

Cardiac contractility is presented as the relation between end-systolic area (or volume) *versus* pressure. Using the non-invasive RV strain-area loop, we explored the ability to assess RV contractility by presenting the relation between end-systolic area *versus* strain. For this purpose, we used the data before and after balloon inflation. We found an excellent correlation between the slopes of the end-systolic pressure area-relation *versus* strain area-relation (r=0.98, P<0.001). This observation provides further support for the ability of strain-area loops to assess RV cardiac function.

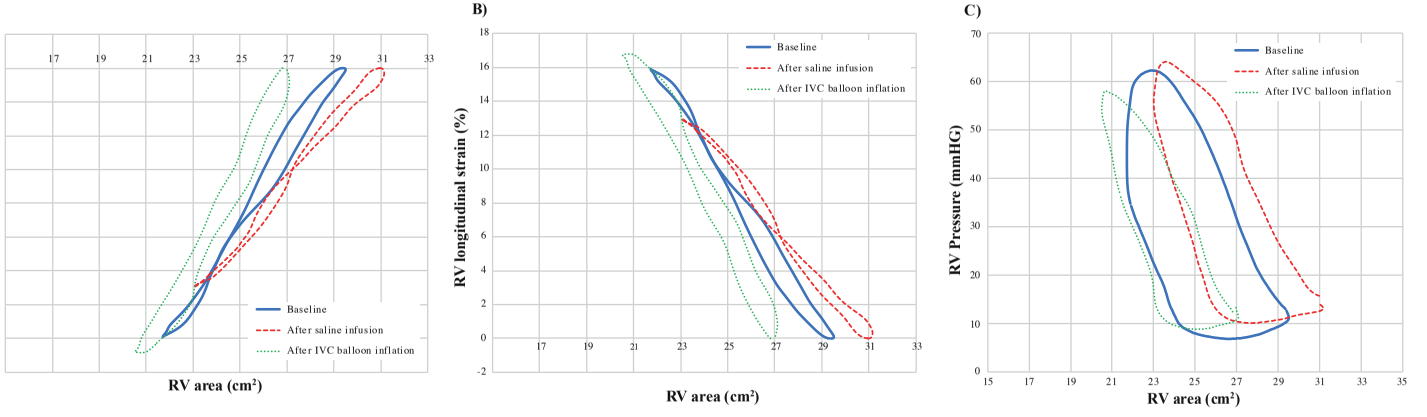

Figure 1. Mean RV strain-area (A), transformed strain-area (B) and RV pressure-area loops (C) of n=7 patients suspected of pulmonary arterial hypertension at baseline, after saline infusion and after IVC balloon inflation. For the transformed strain-area loop, positive instead of negative strain values are used-(B).

The non-invasive nature of the RV strain-area loop and its potential in assessing RV function and mechanics may contribute in evaluating and adjusting pharmacological therapy in pulmonary arterial hypertension patients, whereas right heart catherization is not ideal given its expensive, time-consuming and invasive nature. Further studies are warranted to better understand our observations, and to explore its potential (clinical) value.

Some caution must be taken when interpreting our results. The small sample size and limitations in deriving RV-area, further studies are warranted to explore and validate assessment of RV strain-area loops. Furthermore, this study is limited to patients with suspicion of PAH, therefore caution is needed in extrapolating findings to other populations. Importantly, also changes in pulmonary vascular resistance (because of preload manipulation) may contribute to our observations. For example, a decreased RV afterload (or pulmonary vascular resistance) is associated with an increase in RV longitudinal strain and *vice versa*. Measurement of pulmonary vascular resistance was not performed in this study.

In conclusion, this explorative study shows that a reduction in preload leads to a larger contribution of longitudinal myocardial strain to facilitate systolic volume ejection and *vice versa*. Most importantly, following comparison of the invasive RV strain-area and pressure-area loop, we found a strong correlation in the assessment of cardiac contractility. This suggests that both loops provide similar information, at least related to identification of loop shifts and cardiac contractility.

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Right Ventricular Strain-Area Loop following 17-weeks of Selexipag in Pulmonary Arterial Hypertension Patients: an Exploratory Study

CHAPTER 8

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a progressive pulmonary vascular disease, characterised by an increased pulmonary artery pressure, which is associated with a poor 5-year survival-rate.[1] The primary cause of death is related to deterioration of right ventricular (RV) function, caused by the inability of the RV to overcome the increased afterload.[2] For this reason, drug-induced reduction of RV afterload is the cornerstone in the treatment of patients with PAH. Selexipag (Uptravi®), an oral prostacycline-receptor agonist, resulted in a 30% reduction in pulmonary vascular resistance (i.e. afterload) upon 17 weeks treatment.[3] The GRIPHON trial showed a hazard ratio of 0.60 in time to clinical worsening after Selexipag treatment compared to placebo.[4] The clinical benefits of Selexipag may relate to improvements in RV function, given the strong relation between RV function and disease prognosis.[5] Nonetheless, echocardiography is rarely used in the follow-up of patients with PAH to evaluate the impact of (pharmacological) treatment.[6]

Recently, we introduced a non-invasive echocardiographic assessment of the dynamic relationship between RV longitudinal strain and RV area across the cardiac cycle, i.e. strain-area loop.[7] We demonstrated that RV strain-area loop characteristics; *i.* relate to increasing levels of pulmonary vascular resistance, and *ii.* predict clinical outcome in PAH.[8-10] In this study, we hypothesise that Selexipag-induced changes in the RV strain-area loop relate to clinical outcomes within individuals, which may ultimately contribute to optimal patient management. Therefore, the aim of this exploratory study was to evaluate the impact of 17-weeks Selexipag use on strain-area loop characteristics and, subsequently, relate these outcomes to changes in clinical status in patients with PAH.

METHODS

We recruited 12 individuals (59±18 years of age, 67% female) for the study. Participants were eligible to take part in this study if they were diagnosed with PAH (WHO group 1), were aged ≥18 years or older, were in NYHA class II or III and the clinical pulmonary hypertension team decided to initiate treatment with Selexipag according to current guidelines.[6] Exclusion criteria were patients with pulmonary hypertension WHO group 2 to 5, usage of another prostacyclin-analog within 1 month of inclusion, moderate or severe obstructive lung disease, severe restrictive lung disease, moderate or severe

hepatic impairment, significant left-sided heart disease, severe renal insufficiency, BMI <18.5 kg/m^2 or had a life expectancy less than 12 months. Inclusion and exclusion criteria were according to phase II and III Selexipag trials.[3, 4] The procedures were performed in accordance with institutional guidelines and conformed to the declaration of Helsinki. The study was approved by the Ethics Research Committee of the Radboud University Medical Center (2018-4036). Participants gave full written and verbal informed consent before participation.

In this prospective exploratory study, participants attended the laboratory on two separate occasions. During visit 1, after signing informed consent, baseline echocardiographic assessment, 6-minute walk test (6MWT), functional New York Heart Association (NYHA) classification and a venous blood sampling were performed. After visit 1, the participant was prescribed Selexipag. Clinical PAH nurses (NC, EdG) contacted participants for titration of Selexipag doses weekly. 17 weeks after start with Selexipag, the participant was invited for follow-up measurements (visit 2), during which all measurements were repeated.

Echocardiographic assessments, prior to and 17 weeks after start with Selexipag, were performed at rest. All examinations were performed by one highly experienced cardiologist (AvD) using a Vivid E9 ultrasound machine (GE Medical, Horton, Norway), equipped with a 1.5-4.5 MHz transducer. Images were stored in raw digital imaging and communication in medicine (DICOM) format and were exported to an offline workstation (EchoPAC, version 203, GE Medical, Horton, Norway). Data analysis was performed by a single observer with experience in echocardiography (GK). The observer was blinded for the timing (baseline vs. follow-up) under which echocardiography was performed. Cardiac structural and functional measurements were made according to the current guidelines for cardiac chamber quantification.[11] We examined the following structural and functional indices: RV basal and mid-cavity end-diastolic diameters, RV end-diastolic area (RVEDA), RV end-systolic area (RVESA), RV outflow tract (RVOT) diameter at the proximal level in the parasternal long-axis (PLAX) and parasternal short-axis (PSAX) view, right atrial (RA) area, RV fractional area change (RVFAC), tricuspid annular plane systolic excursion (TAPSE), tissue Doppler imaging (TDI) of the tricuspid annulus (RV 's, e', a'), pulmonary artery Doppler acceleration time (PAT) and maximum tricuspid regurgitation velocity (TR Vmax). The pressure gradient between the RV and RA at peak systole (TR maxPG) was estimated

using the modified Bernoulli equation: TR maxPG = 4 x (TR Vmax).[2] Echocardiographic images were acquired specifically for offline speckle tracking analysis and construction of RV strain-area loops. This involved maintaining frame rates between 40 and 90 frames/sec, depth to ensure adequate imaging of the chamber of interest, and compression and reject to ensure endocardial delineation. For methodology of derivations of strain-area loops, see Kleinnibbelink *et al*.[12]

Statistical analysis was performed using SPSS Statistics 25 (SPSS Inc., Chicago, IL, VS). All parameters were visually inspected for normality. Continuous variables were reported as mean ± standard deviation (SD) and categorical variables were presented as proportions. Paired-sampled T-tests were used to compare baseline and follow-up measurements. For all tests, we assumed statistical significance at $p<0.05$. Sub-analysis was performed by stratifying to clinically responders, defined by a decrease in NYHA classification, and clinically non-responders, defined by a similar or decreased NYHA classification, following 17 weeks of treatment with Selexipag compared to baseline.

RESULTS

Between 1st June 2018 and 31st December 2020, 12 consecutive patients with PAH started with Selexipag and participated in this study (59±18 years, 67% females, n=5 idiopathic PAH, n=5 PAH associated with connective tissue disease, n=2 PAH associated with congenital heart disease). A total of 4 participants dropped out (n=2 due to side effects, n=1 follow-up performed using non-compatible echo machine, n=1 lung transplantation). Eight participants (60±16 years, 6 females) completed the study protocol (n=3 idiopathic PAH, n=4 PAH associated with connective tissue disease, n=1 PAH associated with congenital heart disease) (**Table 1A**). At a cohort level, NYHA classification, 6MWT, NT-PRoBNP and MDRD-GFR did not significantly change following 17 weeks of treatment with Selexipag. Also, conventional structural and functional indices as well as characteristics of the RV strain-area loop did not significantly change following 17 weeks of treatment with Selexipag (**Table 1A**, **Figure 1A**).

At an individual level, stratification to NYHA classification revealed three clinical responders (65±15 years, 2 females, n=3 idiopathic PAH) and five clinical non-responders (57±18 years, 4 females, n=4 PAH associated with connective tissue disease, n=1 PAH associated

with congenital heart disease) (**Table 1B, 1C**). The clinical responders decreased in NYHA classification and levels of NT-proBNP, while 6MWT increased. In contrary, the non-responders remained in the same NYHA classification, increased in NT-proBNP levels and decreased their 6MWT. With regards to the RV strain-area loop, RV longitudinal strain and uncoupling increased in the clinical responder group (**Table 1B**, **Figure 1B**), while in the clinical non-responders, RV longitudinal strain decreased, and uncoupling decreased and a leftward shift is present (**Table 1C**, **Figure 1C**). Conventional functional and structural (with exception of RVEDA) indices did not change in both clinical responders and non-responders (**Table 1B, 1C**).

Table 1. Baseline and follow-up patient and echocardiographic characteristics of A) all participants, B) clinical responders, and C) clinical non-responders

		A) All (n=8)			B) Responders (n=3)			C) Non-Responders (n=5)		
		Pre	**Post**	**p-value**	**Pre**	**Post**	**p-value**	**Pre**	**Post**	**p-value**
	Age (yr)	60±17			65±15			57±18		
	Sex (m/f)	2/6			1/2			1/4		
	NYHA classification	2.9±0.4	2.7±0.7	0.28	2.7±0.3	2.0±0.5	0.06	3.0±0.4	3.1±0.4	0.34
	6MWT (m)	422±56	421±66	0.96	446±48	473±34	0.32	404±60	382±58	0.45
	Kreat (umol/l)	101±27	105±26	0.28	82±15	88±22	0.26	112±28	115±25	0.62
	MDRD-GFR (ml/min)	57±16	54±16	0.18	68±5	64±10	0.21	50±16	49±17	0.59
	NT-ProBNP (pg/ml)	1035±1403	1304±1735	0.19	487±393	417±260	0.53	1364±1735	1836±2071	0.14
Structural measurement	RVOT PLAX (mm)	33±4	33±5	0.48	33±4	31±2	0.44	33±4	35±7	0.13
	RVOT1 PSAX (mm)	32±5	34±4	0.07	30±5	32±1	0.59	33±5	35±4	0.40
	RVOT2 PSAX (mm)	25±5	24±3	0.50	22±2	23±1	0.23	27±5	25±4	0.26
	RV basal diameter (mm)	51±9	51±5	0.96	47±10	51±2	0.42	54±8	51±6	0.38
	RV mid-cavity diameter (mm)	40±7	42±9	0.22	36±3	39±2	0.34	42±8	43±11	0.48
	RV end-diastolic area (cm2)	29±7	27±8	0.14	24±3	24±4	0.88	31±8	29±10	0.08
	RV end-systolic area (cm2)	18±7	17±7	0.04	16±3	15±2	0.48	19±9	18±8	0.05
	RA area (cm2)	21±4	23±4	0.12	19±4	21±3	0.57	23±4	24±5	**0.01**

Table 1. (Continued)

		A) All (n=8)			B) Responders (n=3)			C) Non-Responders (n=5)		
		Pre	Post	p-value	Pre	Post	p-value	Pre	Post	p-value
Functional and Doppler measurements	RV global longitudinal strain (%)	-16.2±4.2	-16.3±3.9	0.92	-14.5±3.8	-16.7±3.6	**0.01**	-17.2±4	-16.0±4.5	**0.04**
	RV fractional area change (%)	39±11	39±5	0.98	35±5	39±3	0.14	41±13	38±6	0.53
	TAPSE (cm)	2.2±0.6	2.4±0.5	0.46	2.2±0.7	2.5±0.2	0.37	2.2±0.6	2.3±0.7	0.88
	TDI s' (cm/s)	11±3	12±4	0.79	12±1	14±1	0.20	12±4	11±4	0.11
	TDI e' (cm/s)	11±4	11±2	0.60	14±1	11±1	0.20	11±5	11±3	0.76
	TDI a' (cm/s)	15±4	17±6	0.55	13±4	19±5	0.13	16±4	15±6	0.21
	PAT (ms)	97±23	94±22	0.76	93±6	93±19	0.93	98±27	94±25	0.62
	TR Vmax (m/s)	4.3±0.6	4.0±0.8	0.27	4.5±0.3	3.8±0.6	0.33	4.2±0.7	4.1±1.0	0.74
	TR maxPG (m/s)	75±20	66±26	0.31	81±10	59±19	0.30	72±24	71±33	0.86

Data are expressed as means±SD. 6MWT, 6-minute walking distance. ED, Early diastole. ES, Early systole. GFR, glomerular filtration rate. LD, Late Diastole. PAT, pulmonary acceleration time. PG, pressure gradient. PLAX, Parasternal long axis. PSAX, parasternal short axis. RA, Right atrium. RV, Right ventricle. RVFAC, RV fractional area change. RVEDA, Right ventricular end-diastolic area. RVESA, Right ventricular end-systolic area. RVOT, Right ventricular outflow tract. TAPSE, Tricuspid annular plane systolic excursion. TDI, Tissue Doppler imaging. TR, Tricuspid. V, velocity.

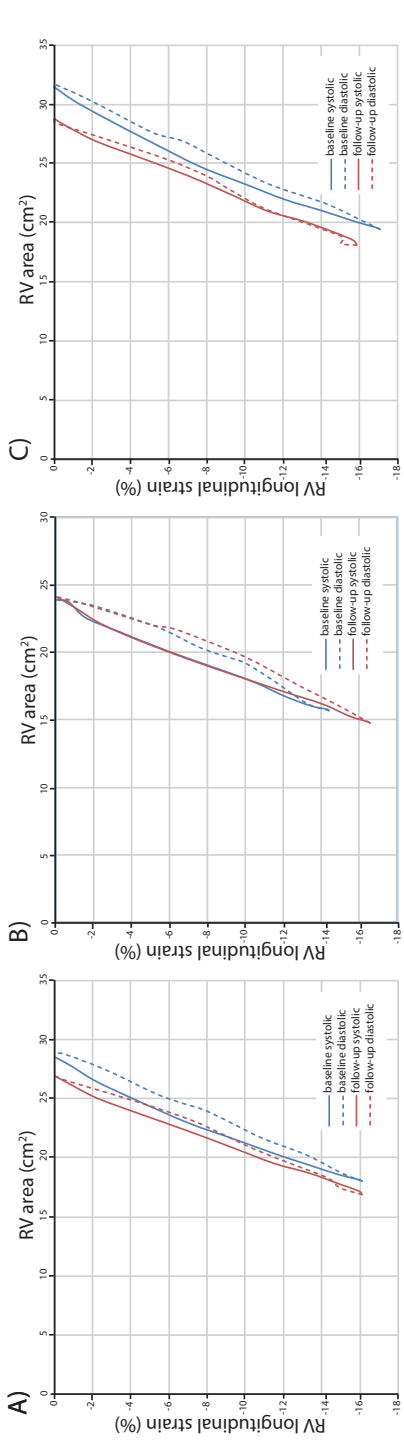

Figure 1. Mean RV strain-area loop of (A) all participants (n=8) (Uncoupling 1.3±0.7 to 1.1±1.3, p=0.81; Uncoupling ED 1.3±0.8 to 1.1±1.4, p=0.66; Uncoupling LD 1.2±0.5 to 1.3±1.2, p=0.73; Sslope 1.6±0.4 to 1.8±0.7, p=0.21), (B) clinical responders (n=3) (Uncoupling 1.3±0.7 to 1.9±1.1, p=0.56; Uncoupling ED 1.2±0.8 to 1.8±1.3, p=0.64; Uncoupling LD 1.3±0.5 to 2.0±0.6, p=0.34; Sslope 1.8±0.5 to 1.9±0.7, p=0.20), and (C) clinical non-responders (n=5) (Uncoupling 1.3±0.7 to 0.7±1.3, p=0.42; Uncoupling ED 1.4±0.8 to 0.6±1.3, p=0.32; Uncoupling LD 1.1±0.6 to 0.9±1.3, p=0.78; Sslope 1.4±0.2 to 1.7±0.7, p=0.36) at baseline and following 17-weeks of treatment with Selexipag.

DISCUSSION

The aim of the present study was to explore the impact of 17 weeks of Selexipag on the RV strain-area loop in patients with PAH and, subsequently, relate these outcomes to changes in clinical status. The key outcome, at group level, was that treatment with Selexipag did not change any RV strain-area loop characteristics nor did treatment change other measures of RV function or any clinical outcomes. When stratified to clinical responders (change in NYHA class) and non-responders, we found that the responders showed an increase in RV longitudinal strain and uncoupling, whilst the non-responders showed a decrease in RV longitudinal strain and uncoupling.

This is the first study exploring the effect of 17 weeks of Selexipag on RV function in patients with PAH. Our exploratory analysis showed that, at group level, 17 weeks of treatment with Selexipag has no impact on characteristics of the RV strain-area loop or other echocardiographic indices for RV function. This is likely related to the heterogeneous effects of Selexipag i.e. it did not induce (sufficient) afterload reduction in all individuals. To support this notion, indirect echocardiographic measures associated with RV afterload (TR Vmax, PAT, RA size) did not change following Selexipag treatment. Although echocardiography shows moderate relation with invasive assessment of RV afterload[13, 14], we cannot rule out the presence of a small effect size of Selexipag on pulmonary vascular resistance. Nonetheless, the effect size was too small and/or the duration of follow-up too short to induce relevant and significant effect on RV function across the population.

To further explore the impact of Selexipag, we categorized patients into responders and non-responders using clinical outcomes. The hypothesis being that Selexipag successfully reduced pulmonary vascular resistance, and hence improved RV function, in clinical responders. Indeed, the reduction of RV systolic pressure (reduced TR Vmax) in the responders demonstrates a decrease in RV afterload. The reduction in RV afterload, subsequently, may explain the increase in peak RV longitudinal strain and greater uncoupling of the RV strain-area loop. Indeed, previous studies have found that reduction in pulmonary vascular resistance is associated with improvement of RV longitudinal strain following treatment.[15] The greater uncoupling (in early and late diastole) of the strain-area loop suggests a decrease in longitudinal contribution to area change in diastole compared

to systole. In healthy subjects, compliance of the ventricle wall contributes to RV filling, which leads to dissociation between systolic and the diastolic phase (uncoupling). Patients with PAH present an increased RV diastolic stiffness due to (eccentric) hypertrophic remodelling to compensate for the increased afterload. To offset for the increased stiffness, increased relaxation in the longitudinal plane may be required to facilitate diastolic filling. Previously it has been shown that patients with PAH had less uncoupling, and possess therefore an increased diastolic filling drive, compared to controls.[8] Therefore, the greater uncoupling in responders following Selexipag treatment may be suggestive of increased RV compliance secondary to a reduced afterload. In contrary, the non-responders show opposite changes in these strain-area loop characteristics, suggesting further deterioration of RV function in the presence of unaltered afterload. We can speculate that a further reduction in RV compliance has led to an increased diastolic stiffness and impaired filling, hence increased coupling and a leftward shift. Previous work has demonstrated that coupling (as part of the RV-loop score) has independent predictive capacity for all-cause mortality in patients with precapillary pulmonary hypertension.[10]

Interestingly, conventional RV functional indices did not change upon treatment at group level or respective sub-groups. Nath *et al*. also addressed the relation between improvement in clinical status (NYHA classification) and change in RV function upon pharmacological treatment (with epoprostenol) in PAH patients (n=20, 16 females).[16] They failed to demonstrate an association between a change in NYHA classification and RV function measured with conventional indices (while RV strain was not assessed). This suggest a potential relation between clinical responders and improvement in RV function measured with novel echocardiographic indices such as the strain-area loop whilst absent when measured with conventional indices. However, given the limited sample size, this should be further explored in larger studies.

Clinical relevance. We demonstrated a potential relationship between improvement in clinical status and improvement in RV function measured with novel echocardiographic indices. Risk stratification and follow-up of patients with PAH is traditionally based on clinical assessment using functional NYHA classification, 6MWT and NT-proBNP assessment as conventional echocardiographic assessment of RV function fall short. Incorporation of a more comprehensive assessment of RV function using the strain-area loop may improve

risk stratification and follow-up of patients with PAH. Moreover, this may facilitate early identification of responders and non-responders upon pharmacological treatment and support the clinician to switch, add-on or increase dosage of a drug. However, this remain speculative and should be investigated in larger, well-powered studies.

Limitations. Some limitations warrant consideration. First, this exploratory study is limited by its restricted sample size of n=8 and may therefore be underpowered. Second, we did not perform right heart catheterisation preventing us to show invasive cardiopulmonary haemodynamic data and relate to echocardiographic RV function. Third, we did only include patients with a treatment of Selexipag. This prevents us to extrapolate our findings in PAH patients using other pharmacological treatment.

CONCLUSION

In conclusion, this exploratory analysis showed that 17-weeks of Selexipag does not alter RV function in all patients with PAH. However, when stratified to clinical responders vs. non-responders, we found opposite changes in peak longitudinal strain and uncoupling of the RV strain-area loop. Specifically, this means that responders to 17-week Selexipag treatment also demonstrate improvement in RV systolic and diastolic function, while such improvements are absent (or even deteriorated) for RV function in PAH patients that do not demonstrate clinical improvement. This suggests that improvement in RV function may translate to a clinical benefit upon 17-week Selexipag treatment in patients with PAH.

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Submitted

Cardiac Rehabilitation is Associated with Lower 1-year All-Cause Mortality in Primary Pulmonary Hypertension

CHAPTER 9

ABSTRACT

Importance. Although exercise-based cardiac rehabilitation (CR) improves exercise capacity, quality of life and improves symptoms in patients with primary pulmonary hypertension (PH), little is unkown whether CR improves survival in patients with PH.

Objective. To assess the association between exercise-based CR and mortality in patients with primary PH.

Design, Setting, and Participants. In this retrospective observational cohort studies, patients with primary PH (aged ≥18 years) undergoing exercise-based CR and a propensity score-matched controlled without exercise-based CR were identified using anonymized data within a large electronical medical record of TriNetX, a global federated health research network with access to EMRs from participating academic medical centres, specialty physician practices, and community hospitals.

Exposure. Exercise-based CR versus no exercise-based CR (controls).

Main Outcomes and Measures. 1-year mortality and hospitalisation.

Results. We included 632 PH patients and 632 propensity-matched controls. Groups did not differ in age, sex, race, comorbidities, cardiovascular medication or cardiovascular procedures (all $p>0.05$). CR was associated with a significantly lower 1-year mortality compared to controls (13.9% versus 21.0%; OR 0.60, 95% CI 0.45-0.81). CR was not related to different re-hospitalisation (OR 0.93, 95% CI 0.75-1.16), with re-hospitalisations rates of 55.4% in PH patients receiving CR and 57.1% in the controls.

Conclusion and Relevance. This study, consisting of 1,264 patients with primary PH, suggests that CR is associated with 40% lower odds of 1-year mortality, when compared to propensity score-matched patients without CR or exercise programmes.

INTRODUCTION

Despite introduction of pharmacological therapies to improve outcomes of pulmonary hypertension (PH), poor long-term survival remains present.[1] This highlights the need for alternative strategies to improve survival and lower morbidity, and to subsequently improve quality of life and lower socioeconomic costs. One such strategy relates to regular exercise training or prescription of cardiac rehabilitation (CR). A recent European Respiratory Society (ERS) statement in this journal highlighted the potential of CR for PH patients.[2] Specifically, CR improves exercise capacity, quality of life and improves symptoms (NYHA classification) and currently holds a class IIa recommendation.[2, 3] However, improvement of these outcomes have been observed only after relatively short time periods (3-4 months) and, more importantly, no study has examined the association between CR and mortality in PH patients. This latter aspect was identified as a key future challenge in the ERS-statement.[2] Although prospectively designed randomized clinical trials (RCTs) are preferred in this context, practical difficulties related to sample size, recruitment, and prolonged follow-up are important challenges. Therefore, adopting a retrospective observational study using a large electronic medical record (EMR) database, the objective of this study was to compare mortality between patients with primary PH with CR or exercise programme *versus* a propensity-matched control group of PH without CR or exercise programmes. We hypothesized that primary PH patients undergoing CR or exercise programmes are associated with lower mortality rates compared to primary PH patients without CR or exercise programmes.

METHODS

The retrospective analysis was conducted on December 14, 2020 using anonymized data within TriNetX, a global federated health research network with access to EMRs from participating academic medical centres, specialty physician practices, and community hospitals, predominantly in the United States. Patients with PH were identified via Centers for Disease Control and Prevention (CDC) coding using ICD-10-CM code I27.0 primary pulmonary hypertension. All patients were aged ≥18 years with primary PH recorded in EMRs at least 18-months before the search date to allow for 1-year follow-up from CR. CR was identified from ICD-10-CM code Z71.82 (Exercise counselling), HCPCS code S9472

(CR program, non-physician provider, per diem), or CPT code 1013171 (Physician or other qualified health care professional services for outpatient CR). Correspondingly, these CR-related codes were excluded in the propensity score-matched controls. At the time of the search, 27 participating healthcare organizations had data available for patients meeting the study inclusion criteria. Thus, following propensity score matching, the cohort consisted of patients with primary PH, who either were referred for CR or did not receive CR (control). Information on 1-year mortality was also retrieved from the data set. As an exploratory aim, we also compared re-hospitalisation between patients with primary PH who were referred for CR or did not receive CR.

Baseline characteristics were compared using chi-squared tests or independent-sample t-tests. Using logistic regression, patients with PH with an EMR of CR were 1:1 propensity score-matched with PH patients without CR for age, sex, race, diseases of the respiratory system, disease of the circulatory system, hypertensive disease, heart failure, diabetes mellitus, chronic kidney disease, cerebrovascular disease, cardiovascular procedures (e.g. cardiography, echocardiography, cardiac catheterization, cardiac devices, electrophysiological procedures), and cardiovascular medications (e.g. beta-blockers, antiarrhythmics, diuretics, antilipemic agents, antianginals, calcium channel blockers, ACE inhibitors). These variables were chosen because they are established risk factors for mortality or were significantly different between the two cohorts. Logistic regression produced odds ratios (OR) with 95% confidence intervals (CI) for mortality and hospitalisation at 1-year following PH diagnosis, comparing CR with propensity score-matched controls. Statistical significance was set at $p<0.05$.

RESULTS

In total, 70,875 and 637 patients with primary PH met the inclusion criteria for the control group and the CR and exercise cohort, respectively. Compared to controls, the CR and exercise cohort were older, had less females, and reported more comorbidities (**Table 1**). Following propensity score-matching, cohorts were well balanced for age, race, sex, comorbidities, cardiovascular medications and cardiovascular procedures ($p>0.05$; **Table 1**).

Using the propensity score-matched cohort, and excluding patients with outcomes outside the measurement window, mortality at 1-year from CR was proportionally lower

with mortality of 13.9% (n=87 of 628 patients) in the CR and exercise cohort compared to 21.0% (n=133 of 632 patients) in the controls (OR 0.60, 95% CI 0.45-0.81). There was no significant effect on re-hospitalisation (OR 0.93, 95% CI 0.75-1.16), with re-hospitalisations rates of 55.4% (n=350 out of 632 patients) in the CR and exercise cohort, compared to 57.1% (n=361 out of 632 patients) in the controls.

Table 1. Baseline characteristics % (n)* of the primary PH populations with and without CR before and after propensity score matching.

	Initial populations			Propensity score matched populations		
	Primary PH without CR (n=70,875)	**Primary PH with CR (n=637)**	**P-value**	**Primary PH without CR (n=632)**	**Primary PH with CR (n=632)**	**P-value**
Age (years), mean (SD)	62.0 (14.5)	60.0 (20.7)	**0.01**	62.7 (14.5)	62.2 (14.3)	0.51
Female	62.8 (43,467)	43.9 (279)	**<0.0001**	43.5 (275)	44.1 (279)	0.82
Race[a]						
White	63.6 (43,997)	71.2 (452)	**<0.0001**	73.3 (463)	71.4 (451)	0.45
Black or African American	21.6 (14,961)	19.7 (125)	0.24	20.3 (128)	19.8 (125)	0.83
Asian	1.8 (1,214)	1.7 (11)	0.97	1.6 (10)	1.7 (11)	0.83
Unknown	12.7 (8,769)	5.8 (37)	**<0.0001**	4.6 (29)	5.8 (37)	0.31
Diseases of the circulatory system	71.2 (49,286)	99.7 (633)	**<0.0001**	99.2 (627)	99.7 (630)	0.26
Diseases of the respiratory system	46.3 (32,021)	85.0 (540)	**<0.0001**	86.6 (547)	85.0 (537)	0.42
Hypertensive Diseases	43.2 (31,959)	83.9 (533)	**<0.0001**	86.2 (545)	83.9 (530)	0.24
Heart Failure	27.6 (19,076)	80.0 (508)	**<0.0001**	78.6 (497)	79.9 (505)	0.58
Diabetes Mellitus	21.5 (14,878)	47.7 (303)	**<0.0001**	48.9 (309)	47.6 (301)	0.65
Chronic Kidney Disease	14.9 (10,318)	42.4 (269)	**<0.0001**	44.8 (283)	42.6 (269)	0.43
Cerebrovascular Diseases	9.1 (6,291)	27.7 (176)	**<0.0001**	27.8 (176)	27.5 (174)	0.90
Cardiovascular Procedures[b]	47.1 (32,568)	97.3 (617)	**<0.0001**	97.6 (617)	97.3 (615)	0.72
Cardiovascular Medications[c]	56.3 (38,974)	95.1 (604)	**<0.0001**	94.5 (597)	95.1 (601)	0.61

*Values are % (n) unless otherwise stated. Baseline characteristics were compared using a chi-squared test for categorical variables and an independent-sample t-test for continuous variables. [a]Data are taken from structured fields in the electronic medical record systems of the participating healthcare organizations, therefore, there may be regional or country-specific differences in how race categories are defined. [b]Cardiovascular procedures include cardiography, echocardiography, catheterization, cardiac devices, electrophysiological procedures. [c]Cardiovascular medications include beta-blockers, antiarrhythmics, diuretics, antilipemic agents, antianginals, calcium channel blockers, ACE inhibitors. CR; cardiac rehabilitation and exercise programmes, SD; standard deviation.

DISCUSSION

Confirming our hypothesis, we provide the first evidence that CR in patients with PH is associated with significantly lower 1-year mortality compared to patients who did not receive CR. Specifically, PH patients who were prescribed CR demonstrated 40% lower odds of 1-year mortality. Previous RCTs showed improvement in exercise capacity, symptomatology, and quality of life after a relatively short-term follow-up (3-15 weeks).[4-10] However, whether these surrogate endpoints are associated with outcomes in PH has never been demonstrated.[11, 12] Our data suggests CR and exercise programmes may ultimately translate to clinically meaningful improvements in mortality. The poor long-term survival in PH, which also is demonstrated in the high 1-year mortality rate in this cohort of 21%, stresses the urgency of adding CR and exercise to existing (non)-pharmacological treatment to further improve survival. Facilitated through improved digital registration, this retrospective cohort study represents a useful and feasible alternative to the extremely challenging and demanding prospectively designed studies. Nonetheless, our unique observations highlight the need to confirm the potential of CR to reduce mortality, using RCTs.

Some limitations warrant consideration. First, characterization of disease states and therapy were based on ICD-codes, which may vary between healthcare organisations.[13] Along these lines, distinction was made between primary PH (i.e., caused by disease of the pulmonary arteries) and secondary PH (i.e., secondary to other, non-vascular causes) rather than the current WHO-classification for PH into group 1 to 5.[3] Given the complexity of PH phenotypes, some PH patients labelled as 'secondary PH' may also have a variant of primary PH. As CR is beneficial for secondary causes of PH, such as cardiovascular diseases and risk factors, the exclusion of these individuals does not necessarily alter the strength of our findings. A second limitation is that we were unable to evaluate characteristics of CR and/or adherence to CR, which limits the ability to identify successful factors of CR to reduce mortality. Third, although we matched patients for important co-morbidities and demographic factors, residual confounding and the relatively modest sample size should be considered. Finally, we were unable to evaluate the potential effect of CR on morbidity, primarily because of too small sample sizes of subgroups that were available for individual co-morbidities (e.g. stroke, myocardial infarction, heart failure). The relatively

small sample size may also explain why we could not detect a significant effect of CR on hospitalisation, despite the lower absolute prevalence of re-hospitalisation and OR in PH patients undergoing CR.

CONCLUSION

In conclusion, the present study of 1,264 patients with primary PH suggests that CR is associated with 40% lower odds of 1-year mortality, when compared to propensity score-matched patients without CR or exercise programmes. This novel data on clinical outcomes highlights the potential of CR and urgency of appropriately powered RCTs to investigate the causal effects of prescription of exercise as medicine for patients with PH.

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NIJMEGEN LIVERPOOL

General Discussion, Summary and Appendices

PART III

General Discussion

CHAPTER 10

During the past two decades, the right ventricle (RV) has received increasing scientific and medical interest. Although the importance of imaging and assessing RV structure and function is clear in multiple clinical scenarios, use of non-invasive echocardiographic techniques to follow-up patients with pulmonary hypertension (PH) is limited. Likewise, RV structure and function in the athlete's heart has been studied to aid differentiation from cardiomyopathies, such as arrhythmogenic RV cardiomyopathy, that increase the risk of sudden cardiac death and could be identified in pre-participation screening or secondary care. The general aim of this thesis was to investigate the acute and chronic effects of load challenges, either induced by pathological (e.g. PH) or physiological stimuli (e.g., exercise), on RV structure, function and mechanics. For this purpose, we have used novel echocardiographic techniques, namely speckle tracking echocardiography and the strain-area loop. In this final chapter, we will discuss and integrate the findings of this thesis with the existing literature and any future directions for research.

10.1 – Relevance of evaluation during and post-acute exercise

For many decades, cardiopulmonary exercise testing has been used to evaluate cardiovascular conditions, providing information on exercise capacity, heart rate (and rhythm) and blood pressure during exercise.[1-3] Exercise testing may have even further potential for clinical use in the diagnosis or prognosis of cardiovascular disease. Indeed, following an acute bout of exercise, a temporary increase of cardiac biomarkers is found (e.g. troponin and natriuretic peptide [BNP])[4], but also a reduction in systolic cardiac function (i.e. exercise-induced cardiac fatigue [EICF])[5] or decrease in blood pressure (post-exercise hypotension [PEH])[6] (**Figure 1**). Interestingly, exercise-related changes in these markers may have clinical potential as the magnitude of these exercise-induced changes seem to relate to clinical outcomes.[7-9] To further explore the potential of exercise-induced changes, in this thesis, we evaluated cardiac function *during* exercise to evaluate EICF (**Chapter 2**) and further investigated the relevance of PEH (**Chapter 5**). Findings of these chapters will be discussed below to support the wider use of measures of cardiovascular function *during and post*-exercise for improved (patho)physiological insight and clinical use.

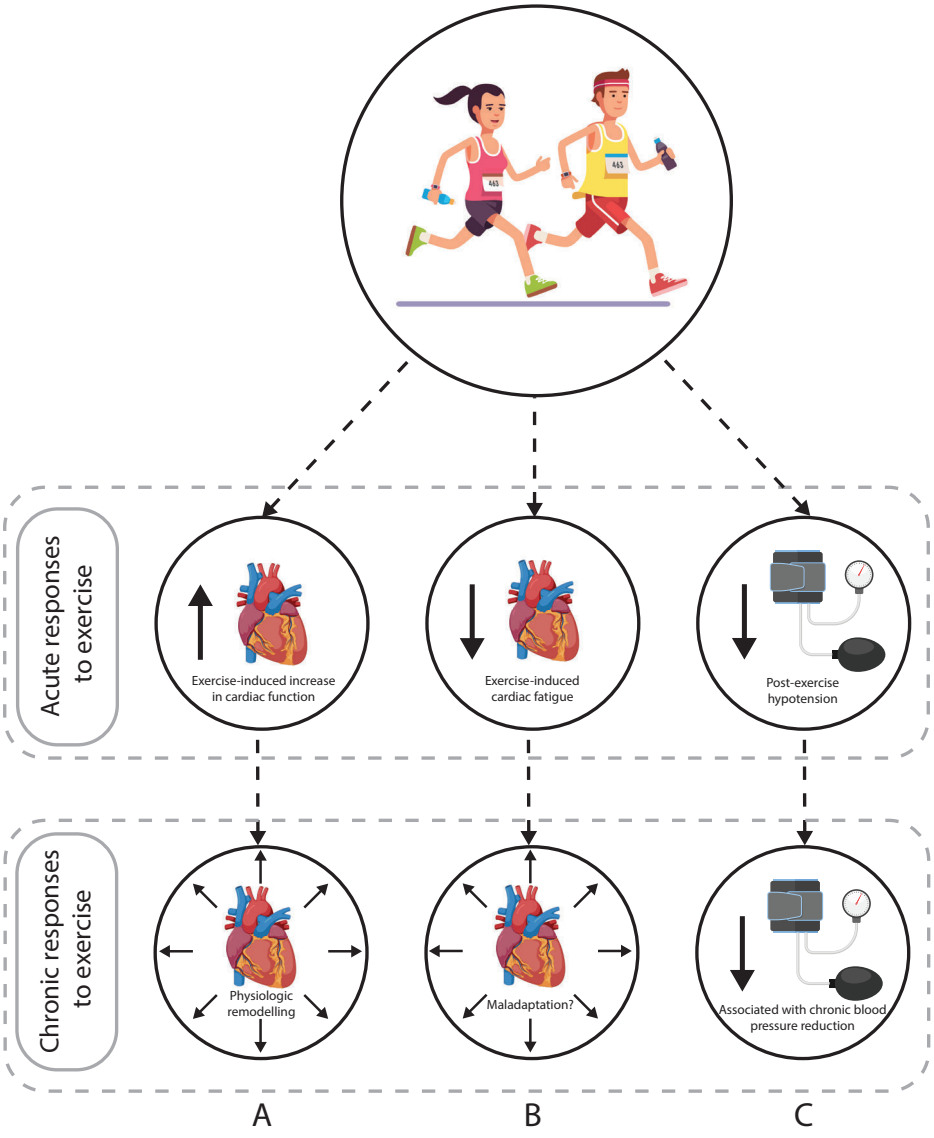

Figure 1. The relation between acute cardiac responses and chronic cardiac responses to exercise. Acute exercise leads to (A) an increase in cardiac function, (B) exercise-induced cardiac fatigue or (C) post-exercise hypotension. In this thesis, we investigated the acute and chronic effects of exercise on the cardiovascular system. Specifically, we investigated (A) the associations between exercise-induced increase in cardiac function and cardiac remodelling to exercise training (Chapter 3, discussion paragraph 10.2), (B) the effect of hypoxic exercise on and value of stress echocardiography in the assessment of exercise-induced cardiac fatigue (Chapter 2, discussion paragraph 10.1.1) and speculated on its potential meaning (discussion paragraph 10.1.1) and (C) the associations between post-exercise hypotension and chronic blood pressure reduction to exercise training (Chapter 5, discussion paragraph 10.1.2).

10.1.1 – Exercise-induced cardiac fatigue

In **Chapter 2**, we investigated the impact of a bout of 45 minutes high-intensity running exercise under hypoxia *versus* normoxia on EICF in a randomized cross-over design in 21 healthy individuals. Interestingly, one of our observations was that EICF was only present when measured during a low-to-moderate exercise challenge (stress echocardiography) and not at rest (**Figure 2**). Similarly, several studies did not observe any signs of EICF at rest following short duration and prolonged bouts of exercise in both the RV and LV.[10-16] In the context of our novel finding, it could be hypothesized that these studies may have detected EICF when using stress echocardiography. For example, researchers in our laboratory did not detect any deterioration of RV and LV function following prolonged single and three-day walking exercise in 10 cardiac patients and 10 age- and sex-matched healthy controls suggesting absence of EICF.[10] However, close inspection of the post-exercise heart rate and blood pressure data advocates a (para)sympathetic imbalance. Namely, a higher heart rate and lower blood pressure was observed post-exercise compared to baseline. These inequalities may have biased the evaluation of EICF and masked the actual presence of EICF (if assessed during exercise). By adopting a design where evaluation during exercise is integrated, a potential bias of (para)sympathetic imbalance could be prevented. Recently, others proved the value of stress echocardiography in the evaluation of the athletes' heart. Stress echocardiography has a better discriminatory capacity to differentiate physiological from pathophysiological conditions compared to rest echocardiography and has the ability to unmask any potential cardiac dysfunction.[17-19]

These novel insights contribute to the rationale that evaluation of cardiac function during the haemodynamic stress of exercise has additional value compared to evaluation at resting conditions only. Namely, such evaluation would improve its sensitivity and discriminative capacity between different types, modes or intensities of exercise. Such as an athlete is judged by his or her performance during competition, functioning of their hearts should also be judged during exercise.

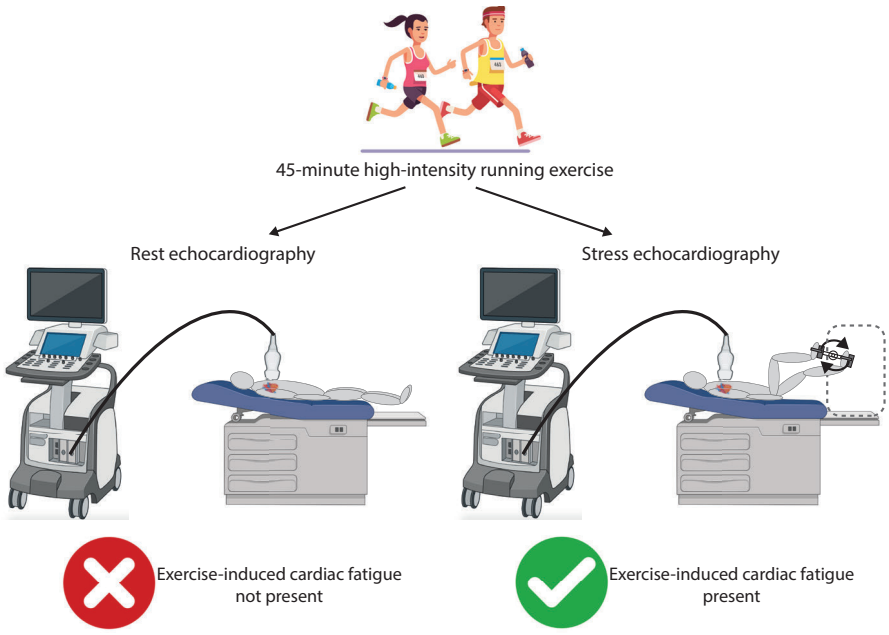

Figure 2. Exercise-induced cardiac fatigue after a bout of 45-minutes high-intensity running exercise is not present when evaluating with rest echocardiography but present when evaluated with stress echocardiography.

Meaning of exercise-induced cardiac fatigue

It is currently unclear whether EICF is a physiological or pathophysiological phenomenon. It has been hypothesized that excessive exercise training (training which is too intense and/or recovery is too short) leads to repetitive occurrence of EICF that may act as a stimulus for maladaptive remodelling (**Figure 1B**).[9, 20] However, associations between these acute exercise changes and long-term cardiovascular changes remain unknown. To understand this link, studies need to adopt a prospective design in a large cohort of individuals. However, besides studying the direct association between EICF and long-term outcome, it is important to examine the direct (patho)physiological mechanism involved. In **Chapter 2**, we demonstrated that an additional RV afterload, induced by hypoxia (FiO2 14.5%), did not exaggerate EICF after 45 minutes of high-intensity running exercise. This suggests that (after)load dependency may be less important as a contributing factor to EICF in short duration exercise as previously suggested.[21, 22] Other suggested factors that may be implicated in the development of EICF are subclinical levels of cardiomyocyte damage[23,

[24], oxidative stress[25], β-adrenergic receptor downregulation in response to sustained elevations in circulating catecholamines[26, 27] and post-exercise alterations in loading and heart rate.[5] These factors may have independent or synergistic roles in the development of EICF. These mechanisms remain poorly understood. One potential explanation is a lack of EICF studies which specifically address this question, possibly due to the field-based (and entirely human model) setting in which EICF was studied. This limits studies to control for a single factor contributing to the development of EICF. Nevertheless, EICF is highly likely to be multifactorial. This thesis shows that with use of environmental factors (i.e. hypoxic environment), a controlled study to a specific mechanism of EICF can be conducted. Further research should not only investigate the direct relation between EICF and long-term outcome but also investigate these multifactorial causes, ideally per single factor, to develop a greater understanding of the (patho)physiological mechanisms and nature of EICF.

10.1.2 – Post-exercise hypotension

Evaluation of blood pressure during or following exercise may have clinical relevance. Usually, blood pressure will drop temporarily post-exercise, for several hours to days, and is typically referred to as post-exercise hypotension (PEH).[6, 28, 29] This may be explained by a decrease in total peripheral resistance which is not compensated by adequate elevations in cardiac output. In **Chapter 5**, we demonstrated that the magnitude of PEH during the first bout of 45-minute high-intensity running exercise was positively related to the magnitude of the BP-lowering effect of 12-weeks high-intensity running exercise training under hypoxia (**Figure 1C**). This is in line with previous studies that showed that the magnitude of PEH relates to the blood pressure lowering effect of exercise training in healthy and pre-hypertension middle-aged individuals.[8, 30, 31] Previously, it was demonstrated that a hypertensive blood pressure response *during* exercise predicts future development of hypertension in young athletes and predicts cardiovascular death in middle-aged men.[32, 33] These observations underscore that studying the cardiovascular system during and after the haemodynamic stress of exercise provides a wealth of valuable information. Although further work is required, presence of PEH may be used as a proxy to identify 'responders' to the blood pressure lowering effects of exercise training. Such approaches may contribute to personalised healthcare and enlarges successes of treatment strategies.

10.2 – Relation between acute cardiac responses to exercise and subsequent cardiac remodelling

It is well established that chronic exercise will lead to physiological cardiac remodelling such as chamber enlargement and hypertrophy.[34, 35] The substrate for cardiac remodelling is the repetitive exposure to haemodynamic stress during acute bouts of exercise. Therefore, by implication, the acute cardiovascular changes in haemodynamics may be related to this chronic remodelling. Although acute and chronic exercise responses are highly relevant research topics, relatively little research has assessed the association between acute cardiac responses and chronic cardiac remodelling. Hulshof *et al*. studied the direct relationship between acute changes in cardiac haemodynamics and chronic remodelling.[36] They demonstrated that uncoupling of the LV strain-volume loop is related to reverse LV remodelling in a cohort of 30 patients undergoing aortic valve replacement due to a severe stenosis. In line with this work, we tested the hypothesis whether baseline acute cardiovascular responses to exercise are related to chronic cardiac remodelling (**Chapter 3**). We included 21 participants of whom 15 completed a 12-week training programme. In order to increase RV afterload[37, 38], the training programme was performed under hypoxic conditions (FiO_2 14.5%) to induce RV remodelling after a relatively short period of training. Indeed, we succeeded in inducing RV remodelling after 12 weeks hypoxic exercise training. At baseline, we performed stress echocardiography, at low-to-moderate intensity exercise, to explore cardiac responses to acute exercise. Interestingly, we found that augmentation in RV fractional area change to acute exercise was related to the increase in RV size following exercise training (**Figure 1A**). This association between the pre-training augmentation in RV fractional area change to acute hypoxic exercise and RV remodelling after 12 weeks of hypoxic training substantiates that acute cardiac responses of the RV to exercise are related to subsequent RV remodelling.

It has been hypothesized that repetitive exposure to high volumes of exercise may lead to maladaptive remodelling of the RV with a phenotype similar to that seen in patients with arrhythmogenic RV cardiomyopathy.[23, 39-42] There is a lack of empirical, prospective data to explore this theory. The concept explored in this thesis, i.e. exploring cardiac responses to exercise and their relation with subsequent cardiac remodelling, may be a potential strategy for future studies aiming to better understand this cardiac (patho)physiology.

10.3 – Acute and chronic exercise: right does not mirror left

La Gerche *et al*. stated: *"cardiac output is only as good as your worst ventricle"*.[43] This means that both ventricles, rather than just the LV, can serve as the potential Achilles' heel, and that both should be examined separately in relation (and in conjunction) to acute and chronic exercise responses.

10.3.1 – Exercise-induced cardiac fatigue

EICF may affect the RV and to a lesser extent the LV. It has been hypothesized that the disproportionate increase in end-systolic wall stress in the RV, when compared to the LV, during exercise explains the difference in presence and magnitude of EICF in both ventricles.[21, 23] In **Chapter 2**, we demonstrated that EICF occurred to the same extent in both RV and LV. One other study reported similar reductions in cardiac function post-exercise[22] while others showed reductions in cardiac function to a greater extend in the RV compared to the LV.[23, 44-47] These differences may be explained by difference in sport discipline, participant characteristics, exercise intensity or duration. Nevertheless, the magnitude of EICF is greater in the RV compared to the LV in the majority of the studies. This indicates that not only in-exercise responses vary between both ventricles but also affects both ventricles to a different extent (i.e. right does not *per se* mirror left).

Association of exercise-induced cardiac fatigue with level of training

A gap in the literature is the effect of the level of training on the magnitude of EICF. Whilst some have attempted to compare participants with lower or higher cardiorespiratory fitness (cross-sectional design) little data exists exploring prospective intervention studies that directly alter fitness.[22] The data of **Chapter 2** and **Chapter 3** are suited to address this question as we performed rest and stress echocardiography prior to and post a 12-week hypoxic training program. In this cohort, 15 individuals (N=6 drop-outs) completed the training program and significantly increased their cardiorespiratory fitness. Both RV free wall and LV global longitudinal strain significantly reduced post-exercise (RV: E, $p<0.001$; LV: E, $p<0.001$) but did not differ in magnitude following the 12-week training program compared to baseline (RV: E*T, p=0.36; LV: E*T, p=0.25) (**Figure 3, Table 1**). Also, when observing with stress echocardiography, there was no difference in magnitude of the EICF (RV: E*T*S, p=0.89; LV: E*T*S, p=0.28). This exploratory analysis suggests that the changing

training status, specifically via 12-weeks of high-intensity hypoxic running exercise, does not influence the magnitude of EICF. Future research, in larger populations, is warranted to confirm these findings.

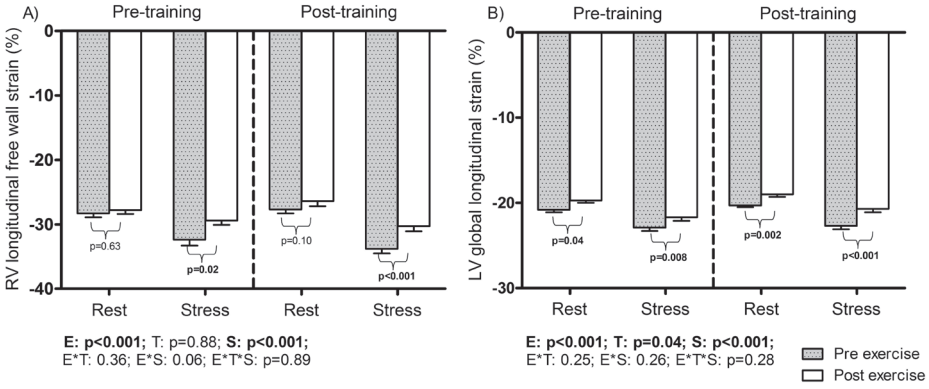

Figure 3. (A) RV longitudinal free wall strain and (B) LV global longitudinal strain pre- and post 45-minute high-intensity running exercise prior to and post a 12-week hypoxic training.

Table 1. RV and LV strain prior to and post 12-week hypoxic training program

	Pre-training				Post-training				P-Value					
	Rest echocardiogram		Stress echocardiogram		Rest echocardiogram		Stress echocardiogram							
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	E	T	S	E*T	E*S	E*T*S
RV Free Wall strain (%)	-28.3±0.6	-27.8±0.6	-32.4±0.9	-29.4±0.7	-27.7±0.6	-26.4±0.8	-33.8±0.7	-30.3±0.8	**<0.001**	0.88	**<0.001**	0.36	0.06	0.89
LV Global longitudinal strain (%)	-20.8±0.3	-19.7±0.3	-22.9±0.4	-21.7±0.4	-20.3±0.2	-19.0±0.3	-22.7±0.4	-20.7±0.4	**<0.001**	**0.04**	**<0.001**	0.25	0.26	0.28

Data are expressed as means±SEM. RV, Right ventricle. LV, Left ventricle. Linear mixed models factors: E, Exercise: Comparison between all echocardiographic measurements performed pre vs. post 45-minutes high intensity exercise. T, Training: Comparison between all echocardiographic measurements performed pre vs. post 12-week training programme. S, Stress: Comparison between all echocardiographic measurements performed under rest vs. during stress. E*T, Exercise*Training: Comparison whether the change pre- vs. post-exercise (i.e. EICF) is different pre vs. post 12-week training programme. E*S, Exercise*Stress: Comparison whether the change pre- vs. post-exercise (i.e. EICF) is different measured during rest vs. stress echocardiography. E*T*S, Exercise*Training*Stress: Comparison whether the change pre- vs. post-exercise (i.e. EICF) is different pre vs. post 12-week training programme when observed using rest vs. stress echocardiography.

10.3.2 – Cardiac remodelling to exercise training

Based on the disproportionate end-systolic wall stress experienced by the RV during exercise[21], it has been hypothesized that chronic exercise-induced remodelling may also disproportionally affect the RV. In **Chapter 3**, we studied the effect of a 12 weeks hypoxic high-intensity running exercise programme on cardiac remodelling in healthy individuals. Indeed, we observed RV structural remodelling (increase in RV dimensions) but no LV remodelling, that supports previous data and theory. Another prospective study by Arbab-Zadeh *et al*. showed that after 12 months progressive and intensive marathon training in 12 previously sedentary subjects (mean age, 29±6 years), the RV increased in size during the initial 3-month training period.[48] The LV, however, only started to remodel after 6 months of training.

In contrast to these observations, in less trained individuals, an increase in training volume in already highly-trained Olympic rowers induced LV but not RV structural remodelling (**Chapter 4**). These findings contrast with other studies performed in elite athletes.[49, 50] D'Ascenzi *et al.* reported seasonal variation in RV size in a cohort of top-level basketball and volleyball players where RV remodelling occurred from pre- to mid-season but plateaued towards the end of the season.[50] Across three consecutive Olympic Games, Aengevaeren *et al.* noted that RV remodelling occurred between the first two Olympics Games, followed by a plateau during the subsequent 4 years in a heterogeneous group of athletes (n=50, 17 different sports).[49] Data from these two studies suggests that once these athletes reach a specific level of training, the RV does not continue to remodel having potentially reached a plateau. This may explain why we did not find RV remodelling despite a gradual increase of training volume over 9 months in the cohort with Olympic rowers (**Chapter 4**).

In combination, these data suggest that when an untrained individual starts to exercise, the primary cardiac response will be RV remodelling, with LV remodelling occurring later in the training exposure. Once the individual reaches a higher level of training changes in the RV will slow or stop but the LV may continue to adapt or remodel. This hypothesis of structural cardiac remodelling pattern suggests that the RV and LV do not mirror each other and remodel differently to chronic exercise. This hypothesis is outlined in **Figure 4**.

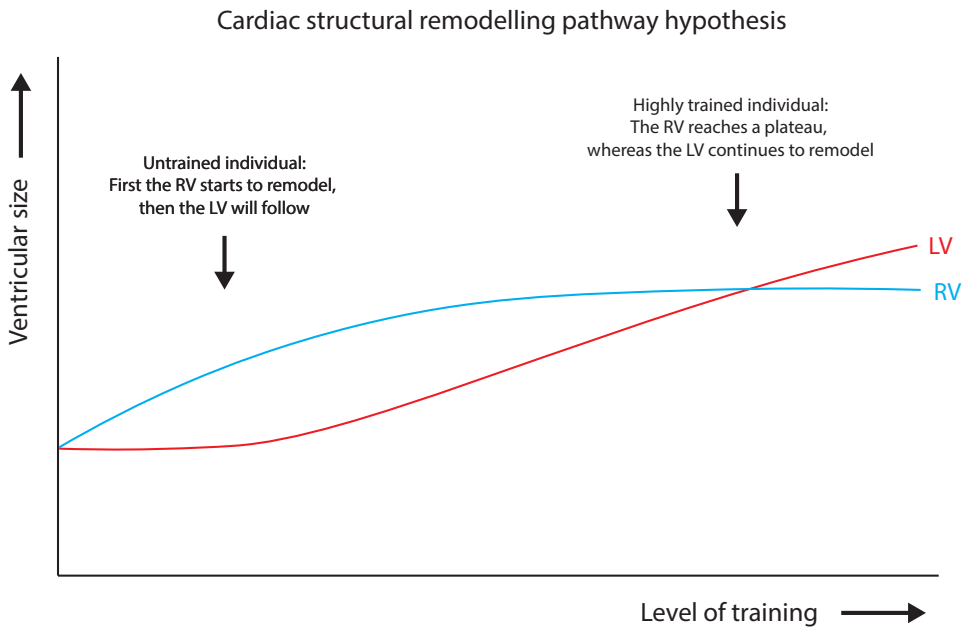

Figure 4. Structural cardiac remodelling to exercise hypothesis. When a relatively untrained individual increases their exercise training volume, the RV start to remodel earlier than the LV. When an individual reaches a more trained status the remodelling of the RV will flatten and finally plateauing whereas the LV will continue to remodel.

Sex differences in cardiac remodelling to exercise training

Although some cross-sectional studies show sex differences between the female and males athlete's heart, prospective longitudinal studies are lacking.[51, 52] To our knowledge, we explored potential sex differences in cardiac remodelling to the same exercise training for the first time in human individuals (**Chapter 4**). We demonstrated that an increase in exercise training volume induced significantly larger structural LV remodelling in female elite rowers compared to their male counter peers. Possible explanations for the distinct remodelling may be hormonal, molecular and/or genetic mechanisms. A recent study by Oláh *et al*. presented similar findings in a rat model of cardiac hypertrophy.[53] These findings should be confirmed in larger prospective cohort studies in human individuals. Female athletes are concerningly underrepresented in current sports and exercise medicine literature.[54] This sex-specific publication bias of the athlete's heart might impede the external validity of current knowledge onto female elite athletes and thus stresses the importance of highlighting any between-sex differences. Given the importance of

this topic, we are currently performing a systematic review regarding sex differences in the elite athlete's heart titled: *'The elite athlete's heart – do men and women demonstrate different cardiac adaptations?'*. With this overview, we aim to pave the way for conduction of large studies addressing sex differences in cardiac remodelling to exercise training.

Novel methods to assess cardiac remodelling

Over the years novel imaging techniques have been introduced to measure cardiac structure and function. One such technique is the novel strain-area loop which has the ability for comprehensive analysis of cardiac function, including mechanics.[55, 56] In this thesis, we applied this strain-area loop to assess cardiac remodelling to exercise training for the first time. In **Chapter 3**, we showed that exercise training induced a rightward shift (increased end-diastolic area), lessening of the systolic slope and reduced uncoupling of the RV strain-area loop. These changes suggest that with an increased RV size following exercise training, the contribution of longitudinal strain to systolic volume ejection lowers. Thereby, the longitudinal contribution to diastolic filling compared to systolic volume ejection will be more dominant. In contrast to these changes, we did not observe any changes in the RV after a 9-month increase in training volume in Olympic rowers; neither in the strain-area loop nor in conventional structural echocardiographic measurements (**Chapter 4**). This might indicate that a change in mechanics, measured with the strain-area loop, is not an isolated process but merely a consequence of cardiac structural remodelling due to exercise training and may be influenced by baseline training status. These novel insights with the strain-area loop in cardiac remodelling to exercise training substantiates its use to gain greater insight into the (patho)physiological process of cardiac remodelling to exercise training.

10.4 – Strain-area loop and exercise-based cardiac rehabilitation in cardiovascular disease

Monitoring patients with PH is predominantly based on subjective rather than objective parameters. The echocardiographic RV strain-area loop may be a suitable technique to incorporate in daily clinical practice and contribute to personalised medicine for the PH patient. It is also relevant to say that exercise-based cardiac rehabilitation (CR) may be beneficial in patients with cardiovascular disease and could also contribute to personalised medicine.

10.4.1 – Strain-area loop in pulmonary hypertension patients

In a meta-analysis in this thesis (**Chapter 6**), we demonstrated that peak RV longitudinal strain has independent prognostic value for a combined end-point and all-cause mortality in patients with PH (**Figure 5**). Recently, Hulshof *et al.* confirmed this independent prognostic value of peak RV longitudinal strain and demonstrated that the RV strain-area loop holds (independent) predictive capacity for all-cause mortality in pulmonary arterial hypertension patients (**Figure 5**).[57] Moreover, they showed the ability of the RV strain-area loop to reclassify high-risk patients to intermediate-risk.[57] In another study, Hulshof *et al*. demonstrated that characteristics of the strain-area loop are related to afterload in PAH patients (**Figure 5**).[58] In this thesis, we continued to apply this novel echocardiographic technique in PAH patients. We explored the acute and chronic cardiovascular responses to haemodynamic changes induced by invasive manipulations and pharmacological treatment. Specifically, in **Chapter 7**, we investigated the effect of acute preload manipulation on seven patients with suspected PAH on the strain-area loop (**Figure 5**). We showed that the non-invasive RV strain-area loop provides similar information compared to the invasive pressure-area loop with regards to loop shifts and the assessment of cardiac contractility. These findings show the potential of the non-invasive strain-area loop to comprehensively assess RV cardiac function and mechanics. These abilities may contribute to a potential clinical value in the risk assessment, evaluation and adjusting pharmacological therapy in PAH patients.

In **Chapter 8**, we tested the hypothesis whether the strain-area loop is able to detect changes after Selexipag induced afterload reduction (**Figure 5**). In this explorative study, we demonstrated that Selexipag induced afterload reduction does not result in any changes in characteristics in the RV strain-area loop. However, when stratified to clinical responders (decreased NYHA classification) vs. non-responders (similar or decreased NYHA classification), we found a distinct effect of Selexipag on cardiac function between the two groups. Specifically, clinical responders showed an increase in RV longitudinal strain and uncoupling while the non-responders showed a decrease in RV longitudinal strain and uncoupling. Despite the low sample size, these pilot data suggest that changes in clinical status may relate to (changes in) RV function. This highlights the potential use of non-invasive echocardiography for patients with PAH to improve and personalise treatment.

Obviously, the clinical relevance should be evaluated in a larger cohort consisting of PAH patients who start with or switch afterload reducing pharmacological treatment. At least, in combination with previous studies by Hulshof *et al.*[57, 58], this thesis shows the potential of the RV strain-area loop in evaluating patients with PH (**Figure 5**). Currently, methods to produce strain-area loops are based on manually drawing monoplane volumes. To incorporate the RV strain-area loop in daily clinical practice, development of currently used method to semi-automated strain-area loops would accelerate this process.

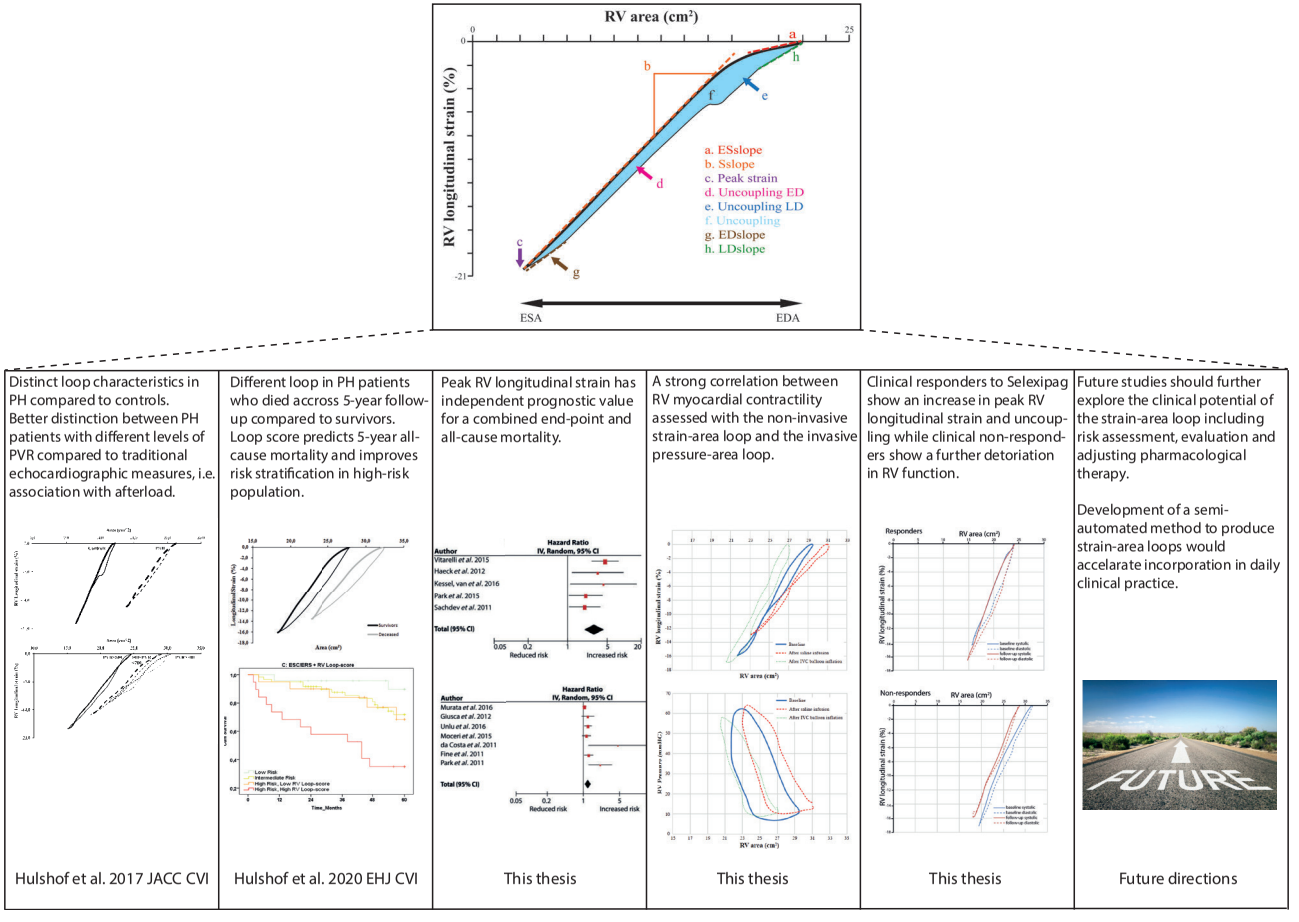

Figure 5. Outline of current evidence of the right ventricular strain-area loop in pulmonary hypertension patients.

10.4.2 – Combining exercise and cardiovascular disease

The work in my thesis has importantly contributed to a better understanding of acute and chronic responses of the cardiovascular system to exercise. To further elaborate on this, "exercise as medicine" has been one of the cornerstones in the (primary and) secondary prevention in the treatment of cardiovascular disease as it improves exercise capacity, health-related quality of life, and reductions in hospital admissions.[59-61] However, the direct association between exercise-based CR and mortality disappeared in randomized controlled trials in patients with coronary heart disease as demonstrated by the most recent Cochrane meta-analysis.[61] One possible explanation may be the heterogeneity of the coronary heart disease populations included in most recent studies. Better insight into these topics may clarify the impact of exercise-based CR on all-cause mortality and contribute to a personalised approach of CR for individual patients with coronary heart disease. Therefore, we are currently conducting a CR meta-analysis on patients with coronary heart disease using individual-participant data.[62] Adopting this approach, we are able to determine the influence of individual patient (e.g. age, sex, risk factors) or exercise characteristics (e.g. location, type, intensity, duration of exercise) on the effectiveness of CR. Improved insight into whether patient or exercise characteristics affect all-cause mortality or hospitalisation following CR, will ultimately enhance the benefits of CR. This will increase insight and provide the potential to personalise CR for coronary heart disease patients such that a more targeted approach to CR allows us to leave behind the simplicity of 'one size fits all'. With this project, I will continue my research career alongside my clinical registry to become a consultant cardiologist.

As discussed above, exercise-based CR is frequently explored in the primary, secondary and tertiary prevention of cardiovascular disease. Unfortunately, few studies have examined the impact of CR in PH. This topic is highly relevant, since long-term survival in PH remains poor.[63, 64] Several studies have shown that exercise-based CR in patients with PH improved exercise capacity, quality of life and improve symptoms[65-71] but hard clinical endpoints on mortality are lacking. It is debated whether improvement in these surrogate endpoints reflect improvement in clinical outcome.[72] In this thesis, we showed that exercise-based CR in patients with primary PH was associated with significantly better 1-year survival than those without exercise-based CR (**Chapter 9**). This is the first evidence that shows the

direct link between exercise-based CR and mortality. This may suggest that the additional "relative" load faced by the RV during exercise could have a positive effect on its function. Furthermore, this stresses the urgency of appropriately powered randomized controlled trials to investigate the causal effect of exercise as medicine for patients with PH.

10.5 – Conclusion and future perspectives

The present thesis provides novel insights into acute and chronic effects of load challenges on the RV in healthy individuals, elite athletes and patient with PH. The most important findings are;

First, cardiac function is traditionally measured under resting conditions whilst in supine position. We showed that cardiac function measured *during* exercise (i.e. stress echocardiography) yields additional information compared to resting conditions only. Namely, EICF after short duration high-intensity exercise is only present when evaluated with stress echocardiography. This observation fits with some recent observations of other athletes' heart studies, where exercise-induced responses show added (clinical) value compared to evaluation at rest alone. Therefore, we recommend the use of stress echocardiography in research, and to explore its potential in the diagnosis or prognosis of cardiovascular diseases in clinical settings.

Second, acute cardiovascular responses to exercise and altered haemodynamics are related to subsequent chronic responses (within each side of the ventricle). This highlights the need to measure and evaluate both ventricles to understand the entire heart.

Third, following the current body of evidence, including those presented in this thesis, we conclude that the RV does not simply mirror the LV in both acute and chronic responses to exercise. EICF may vary between the RV and LV. Furthermore, integration of our findings with existing literature, we hypothesize the presence of a distinct pathway of structural remodelling for the RV and LV related to the level of training of an individual.

Fourth, evolving technology in echocardiography has led to the development of novel strain-area loops, which provide a comprehensive overview of cardiac function. In this thesis, we showed that the strain-area loop detects changes in RV function following interventions to alter haemodynamics. This novel technique, therefore, may serve as a

valuable tool to follow-up patients with PAH. Future studies should explore further its clinical utility in this and other patient groups. Development of a semi-automated method is key for an eventual integration into daily clinical practice.

Lastly, we showed that exercise training causes rapid right-sided cardiac adaptation (in healthy individuals), but also that exercise-based CR reduces all-cause mortality in patients with primary PH. Moreover, we described that exercise training in coronary heart disease has strong inter-individual effects. Whilst our work highlights the role and clinical benefits of exercise, further improving these benefits would require insight into patient and exercise characteristics. Ultimately, this will enhance the benefits of CR and will contribute to exercise as personalised medicine in cardiovascular disease.

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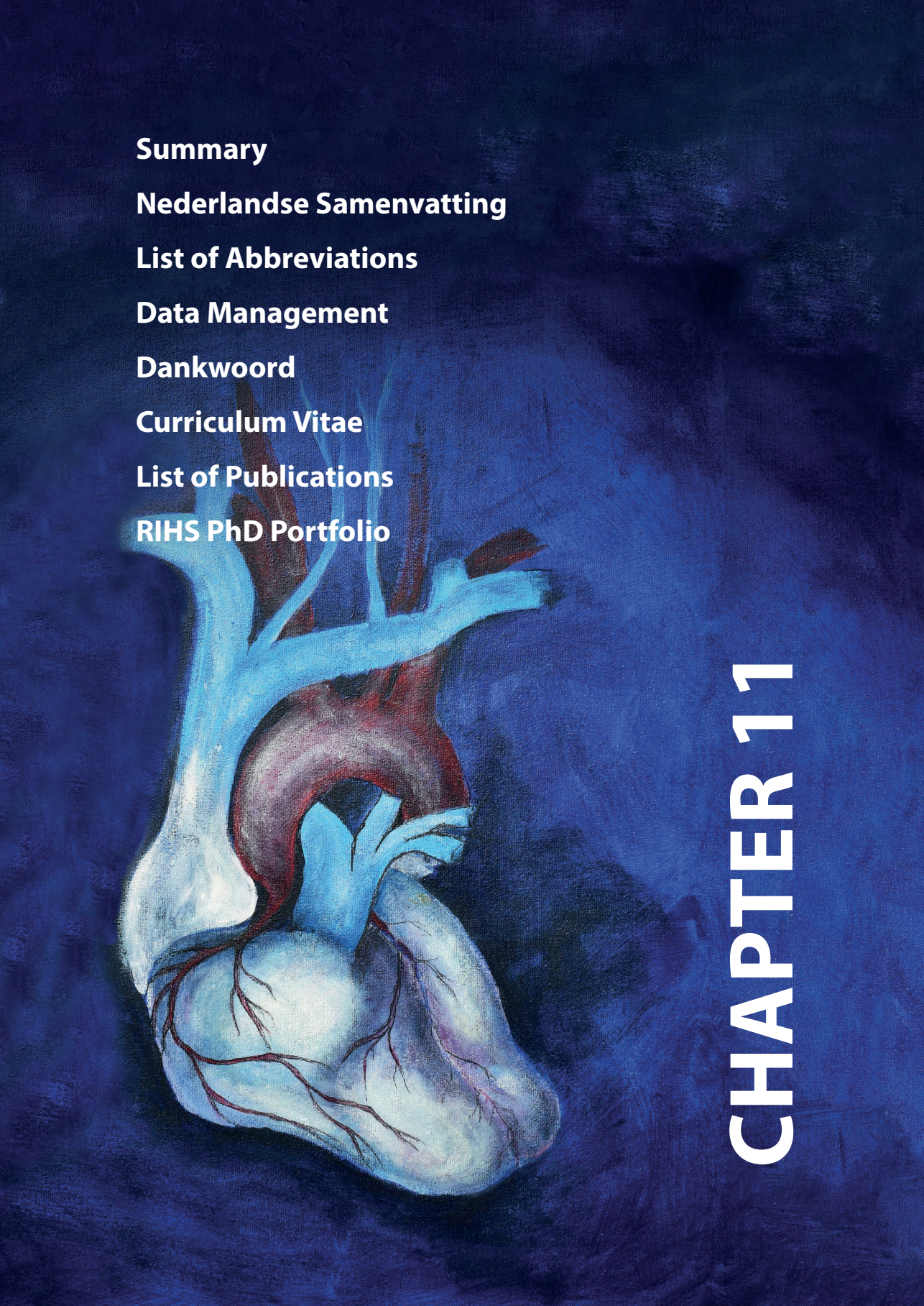

CHAPTER 11

Summary

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SUMMARY

The general aim of this thesis was to investigate the acute and chronic effects of load challenges, either induced by pathological (e.g. PH) or physiological stimuli (e.g., exercise), on RV structure, function and mechanics. For this purpose, we performed exercise-related studies in healthy individuals and elite athletes (**Part 1: Chapters 2 – 5**) and performed a series of studies in patients with PH (**Part 2: Chapters 6 – 9**) using novel echocardiographic techniques (e.g., speckle tracking echocardiography and the strain-area loop). In **Chapter 1**, we provided a general introduction on the background and rationale of this thesis. We provided an overview of the history of the cardiovascular system, followed by a discussion of the anatomical and functional differences between the RV and LV. Furthermore, we introduced novel echocardiographic techniques (i.e. speckle tracking echocardiography and strain-area loop) used in this thesis and provided an outline of this thesis.

Part I – Right ventricular responses to acute and chronic exercise

In **Chapter 2**, we compared the impact of a single bout of high-intensity exercise under hypoxia *versus* normoxia on EICF on the RV and LV in healthy individuals. We demonstrated that a bout of 45-minute high-intensity exercise induced a reduction in functional indices of right- and left-sided cardiac function and mechanics. We found no impact of hypoxia on exercise-induced reduction in right- or left-sided cardiac function and mechanics. An additional, but equally important, finding was that the reduction in right- and left-sided cardiac function was present when echocardiography was performed during a standardized low-to-moderate-intensity recumbent exercise challenge, but not when examined under resting conditions. Taken together, these data indicate that EICF after short-term high-intensity exercise is not exaggerated under hypoxia, suggesting that an additional cardiac load (induced by hypoxia) on the RV does not necessarily relate to an exaggerated EICF in this setting.

In **Chapter 3**, we aimed to relate pre-training changes in cardiac function during acute hypoxic exercise to subsequent adaptations to a 12-week hypoxic endurance exercise training program on RV cardiac structure, function and mechanics in healthy individuals. First, we showed that hypoxic exercise training increased RV size, including diameter and area. Whereas measures of RV function remained largely unchanged, exercise training

resulted in adaptations in RV mechanics, with less uncoupling and lessening of the systolic and diastolic slopes of the RV strain-area loop. Second, we found that pre-training augmentation in RV fractional area change to acute hypoxic exercise was inversely related to cardiac remodelling of the RV following 12 weeks of hypoxic endurance training in healthy individuals. Taken together, our results demonstrate that acute cardiac responses of the RV to hypoxic exercise are related to subsequent RV remodelling upon 12-weeks of hypoxic exercise training in healthy, relatively untrained individuals.

In **Chapter 4**, we evaluated the impact of an increase in Training volume (across 9-months) in elite rowers on left- and right-sided cardiac structure, function and mechanics. We showed that an increase training volume in elite rowers across 9-months resulted in significant structural adaptation of the left ventricle and atrium, with no adaptations observed on the right side. Left-sided structural cardiac adaptation was accompanied by an increase in LV twist, but no other left- or right-sided functional adaptations. This highlights the plasticity of the heart for remodelling in response to exercise training, even in elite athletes. The finding that left-sided cardiac remodelling was found in elite athletes across 9-months training, and the observation from **Chapter 3** that right-sided remodelling is present in untrained individuals, suggests a time-dependent adaptation of the heart to exercise training (see **Chapter 10**).

In **Chapter 5**, we investigated the impact of hypoxia on PEH, and whether the magnitude of PEH relates to the BP-lowering effect of 12-week hypoxic endurance exercise-training. We demonstrated that the magnitude of PEH does not differ when exercise, matched at relative intensity, is performed under hypoxia or normoxia. The magnitude of PEH during the first exercise bout was positively related to the magnitude of the BP-lowering effect of 12-weeks high-intensity running exercise training under hypoxia. Taken together, our results demonstrate that hypoxia does not alter the PEH response, whilst we reveal the close relationship between acute and chronic changes in BP in response to high-intensity running exercise in healthy individuals.

Part II – Altered haemodynamics and exercise in patients with pulmonary hypertension

In **Chapter 6**, we performed a systematic review and meta-analysis to examine whether RV longitudinal strain, i.e. a relatively novel measure of RV function, has prognostic value for future events in patients with PH. The key finding was that RV longitudinal strain has independent prognostic value for all-cause mortality. To a lesser extent, RV longitudinal strain also demonstrated independent predictive capacity for the combined endpoint of mortality and PH-related events. Taken together, these findings emphasize that RV longitudinal strain is a valuable tool with independent prognostic value for all-cause mortality in PH patients.

In **Chapter 7**, we compared the impact of preload manipulation on RV strain-area loop (another novel measure of RV function) *versus* pressure-area loop, and subsequently compared invasive and non-invasive assessment of cardiac contractility. We showed that a reduction in preload leads to a larger contribution of longitudinal myocardial strain to facilitate systolic volume ejection and *vice versa*. Following comparison of the RV strain-area and pressure-area loop, we found a strong correlation in the assessment of cardiac contractility. This suggests that the invasive and non-invasive loops provide comparable information, at least information related to identification of loop shifts and cardiac contractility.

In **Chapter 8**, we explored the impact of 17-weeks Selexipag on strain-area loop characteristics and, subsequently, relate these outcomes to changes in clinical status in patients with PAH. At group level, we found that treatment with Selexipag did not change any RV strain-area loop characteristics nor did treatment change other measures of RV function or any clinical outcomes. When, stratified to clinical outcome, we found that PH patients who improved their clinical status following Selexipag treatment showed an increase in RV longitudinal strain. In contrast, PH patients that showed no benefit of treatment revealed a decrease in RV longitudinal strain. These opposite changes in RV longitudinal strain between PH patients who clinically improved *versus* deteriorated may be coupled to successful decline or non-decline in pulmonary vascular resistance, respectively. These changes may, subsequently, explain the clinical (non-)response to Selexipag.

In **Chapter 9**, we compared 1-year mortality rates between patients with primary PH who were prescribed CR or exercise programmes *versus* a propensity-matched control group of primary PH without prescription for CR or exercise programmes. We demonstrated that CR in patients with PH is associated with a 40% lower odds of 1-year mortality compared to patients who did not receive CR.

Part III – General discussion

In **Chapter 10**, we integrated findings of this thesis with insights from other studies, and discussed directions for future research. We have identified five key messages.

First, the additional value of stress echocardiography in the evaluation of cardiac responses to exercise was discussed. Based on this thesis, and recent other studies, stress echocardiography shows additional value in the detection of EICF after short duration high-intensity exercise but also in the discrimination of physiological from pathophysiological cardiac conditions. Further studies should explore its potential in the diagnosis or prognosis of cardiovascular disease.

Second, acute cardiovascular responses to exercise seem to closely relate to adaptations to chronic exercise training. Adopting this approach may be a potential strategy to better understand the hypothesis that repetitive exposure to high training volumes of exercise may lead to maladaptive remodelling of the RV in certain individuals. Furthermore, PEH may be used as a proxy to identify responders to the blood pressure lowering effect of exercise training.

Third, we concluded that the RV does not simply mirror the LV in both acute and chronic responses to exercise. Not only the in-exercise response vary but also the magnitude of EICF may differ between the RV and LV. Integrating our findings with existing literature, we discussed the cardiac structural remodelling hypothesis suggesting the presence of a distinct pathway of structural RV and LV remodelling to exercise related to the level of training of an individual. Moreover, we discussed the added value of the strain-value loop in providing insight into cardiac mechanics upon exercise training.

Fourth, we summarized and integrated our findings of the echocardiographic RV strain-area loop in patients with PH. The strain-area loop has potential but future studies should

assess its clinical value in monitoring PH patients. Also, development of a semi-automated method is key for an eventual incorporation into daily clinical practice.

Fifth, we discussed the added value of exercise-based CR in patients with PH but also in patients with cardiovascular disease in the reduction of all-cause mortality. Future studies should encounter the influence of individual patient and exercise characteristics on the effectiveness on CR. Ultimately, this information will contribute to exercise as personalised medicine in cardiovascular disease.

NEDERLANDSE SAMENVATTING

Het doel van dit proefschrift was om de acute en chronische effecten van druk- en volumebelasting op de rechterventrikel te onderzoeken. Deze belasting kan worden veroorzaakt door zowel fysiologisch als pathologische stimuli waardoor de structuur, functie en mechanica van de rechterventrikel beïnvloedt wordt. Voor dit doel hebben we verschillende inspanning gerelateerde onderzoeken uitgevoerd bij gezonde individuen en topsporters (**Deel I: Hoofdstukken 2 – 5**) en daarnaast een reeks onderzoeken bij patiënten met pulmonale hypertensie (hoge bloeddruk in de longslagaderen) (**Deel II: Hoofdstukken 6 – 9**). Hierbij hebben we gebruik gemaakt van nieuwe echocardiografische technieken. In **Hoofdstuk 1** hebben we een algemene inleiding gegeven over de achtergrond en grondgedachte van dit proefschrift. We gaven een overzicht van de geschiedenis van het cardiovasculaire systeem, gevolgd door een bespreking van de structurele en functionele verschillen tussen de rechter- en linkerventrikel. Verder hebben we nieuwe echocardiografische technieken beschreven, waaronder speckle-tracking-echocardiografie en de strain-volume curve, welke in dit proefschrift zijn gebruikt.

Deel I – Reactie van de rechterventrikel op acute en chronische inspanning

Hoe hoger je komt in de bergen, des te lager is de zuurstofspanning. Deze lagere zuurstofspanning zorgt voor een hogere bloeddruk in de longslagaders, en daardoor een hogere drukbelasting van de rechterventrikel. Onze hypothese in **Hoofdstuk 2** was dat door de lagere zuurstofspanning op hoogte, de rechterventrikelfunctie na inspanning meer afneemt in vergelijking met de linkerventrikelfunctie. Om dit te onderzoeken hebben we bij gezonde individuen de verandering in de rechter- en linkerventrikelfunctie na een hardloopsessie van 45 minuten op 3,000m gesimuleerde hoogte in de hoogtekamer vergeleken met 45 minuten hardlopen op zeeniveau. We zagen dat er sprake was van een afname in het functioneren van zowel de rechter- als linkerventrikel, maar dat de grootte van deze afname niet verschillend was tussen de rechter- en linkerventrikel. We verwierpen dan ook onze hypothese dat de afname in rechterventrikelfunctie groter zou zijn na 45 minuten hardlopen op 3,000 meter gesimuleerde hoogte in vergelijking met hardlopen op zeeniveau. Een aanvullende, maar minstens zo belangrijke bevinding was dat de rechter- en linkerventrikelfunctie gemeten tijdens inspanning afnam,

terwijl de functie gelijk bleef wanneer gemeten in rust. Concluderend, de afname van de rechterventrikelfunctie na (kortdurende) intensive inspanning wordt niet groter onder zuurstofarme omstandigheden. Dit suggereert dat extra drukbelasting van de rechterventrikel niet per se leidt tot een grotere afname in functie.

In **Hoofdstuk 3** bestudeerden we de relatie tussen de rechterventrikelfunctie tijdens inspanning en de adaptatie van de rechterventrikelstructuur na een 12 weken durend trainingsprogramma op 3,000m gesimuleerde hoogte bij gezonde individuen. Ten eerste toonden we aan dat 12 weken training op hoogte resulteert in toename van de rechterventrikelgrootte. De conventionele maten voor rechterventrikelfunctie bleven onveranderd, terwijl er aanpassingen optraden in de eigenschappen van onze strain-volume curve (d.w.z. meer systolisch-diastolische koppeling). Ten tweede vonden we dat de toename in rechterventrikelfunctie tijdens inspanning omgekeerd evenredig was met de toename van de rechterventrikelgrootte na 12 weken training. Met andere woorden, een relatief kleine toename in rechterventrikelfunctie tijdens inspanning leidt tot grotere toename in rechterventrikel grootte, en omgekeerd. Deze bevindingen suggereren een relatie tussen de acute en chronische reacties van de rechterventrikel ten gevolge van inspanning bij gezonde individuen.

In **Hoofdstuk 4** onderzochten we de impact van een toename in trainingsvolume (over 9 maanden) bij Olympische roeiers op de linker- en rechterhartstructuur, -functie en -mechanica. We toonden aan dat een toename van het trainingsvolume resulteerde in een significante toename van de linkerventrikelgrootte en -atriumgrootte, zonder dat daarbij veranderingen werden waargenomen aan de rechterzijde van het hart. De toename in linkerventrikelgrootte ging gepaard met een toename van de linkerventrikeldraaiing. Verder werden er geen links- of rechtszijdige functionele adaptaties gevonden. Tezamen benadrukt dit de vormbaarheid van het hart als reactie op een toename van het traingingsvolume, zelfs bij topsporters die reeds erg goed getraind zijn. Eerder werd in **Hoofdstuk 3** beschreven dat training leidt tot enkel rechtzijdig structurele adaptaties van het hart bij ongetrainde individuen. Samen met de bevinding uit **Hoofdstuk 4**, suggereert dit een tijdsafhankelijke adaptatie van het hart ten gevolge van training.

In **Hoofdstuk 5** onderzochten we de impact van een hardloopsessie op 3,000m gesimuleerde hoogte in de hoogtekamer op bloeddrukverlaging, en of de mate van deze bloeddrukverlaging gerelateerd is aan de daling van de bloeddruk na 12 weken training. Ons onderzoek toonde aan dat de mate van bloeddrukdaling niet verschilt wanneer er wordt hardgelopen op 3,000 gesimuleerde hoogte in vergelijking met hardlopen op zeeniveau. De mate van bloeddrukverlaging na de eerste hardloopsessie was evenredig met de grootte van het bloeddrukverlagende effect van een 12 weken durend trainingsprogramma. Concluderend, onze resultaten laten zien dat sporten op hoogte het bloeddrukverlagende effect na een enkele hardloopsessie niet verandert maar dat er wel een nauwe relatie is tussen acute en chronische veranderingen in bloeddruk als reactie op een trainingsprogramma bij gezonde individuen.

Deel II – Veranderde haemodynamiek en lichamelijke inspanning bij patiënten met pulmonale hypertensie

In **Hoofdstuk 6** hebben we een systematische literatuurreview en meta-analyse uitgevoerd om te onderzoeken of rechterventrikel strain, een relatief nieuwe maat voor rechterventrikelfunctie, prognostische waarde heeft voor het optreden van gezondheidsproblemen en overlijden bij patiënten met pulmonale hypertensie. De belangrijkste bevinding was dat rechterventrikel longitudinale strain prognostische waarde heeft voor overlijden. In mindere mate vertoonde rechterventrikel strain ook voorspellend vermogen voor het gecombineerde eindpunt van overlijden en het optreden van gezondheidsproblemen.

In **Hoofdstuk 7** hebben we de impact van de *preload* (ook wel vullingsdruk genoemd) op de eigenschappen van de niet-invasieve rechterventrikel strain-volume curve vergeleken met de eigenschappen van de conventionele invasieve druk-volume curve. We toonden aan dat een vermindering van de *preload* leidt tot een grotere bijdrage van longitudinale myocardiale strain om de systolische volume-ejectie te faciliteren en omgekeerd. Daarnaast vonden we een sterke correlatie tussen de strain-volume en de druk-volume curve in de beoordeling van cardiale contractiliteit. Dit suggereert dat de invasieve en niet-invasieve curves vergelijkbare informatie verschaffen met beiden waardevolle informatie met betrekking tot de identificatie van verschuivingen van de curves en cardiale contractiliteit.

In **Hoofdstuk 8** hebben we de impact van de behandeling met Selexipag gedurende 17 weken bij patiënten met pulmonale hypertensie op de eigenschappen van de strain-volume curve onderzocht. Vervolgens hebben we de eigenschappen gerelateerd aan de verandering in klinische symptomen gemeten middels de NYHA-classificatie. Op groepsniveau ontdekten we dat behandeling met Selexipag de eigenschappen van de strain-volume curve niet veranderde, evenmin andere conventionele maten voor rechterventrikelfunctie. Stratificatie naar klinische uitkomsten liet zien dat bij patiënten met een verbetering van klinische symptomen (afname NYHA-classificatie) na behandeling met Selexipag een toename in rechterventrikel longitudinale strain optrad. Daarentegen vertoonden patiënten met verergering van klinische symptomen (toename NYHA-classificatie) een afname in rechterventrikel longitudinale strain. Deze tegengestelde veranderingen in rechterventrikel longitudinale strain zouden mogelijk gekoppeld kunnen worden aan, respectievelijk, een succesvolle af- of toename van de pulmonale vasculaire vaatweerstand. Deze veranderingen zouden vervolgens de klinische *responders* (patiënten met positief effect van Selexipag) en *niet-responders* (patiënten zonder gunstig effect van Selexipag) kunnen verklaren. In de toekomst zou het gebruik van de strain-volume curve een potentiële strategie kunnen zijn bij het personaliseren van medicamenteuze therapie bij patiënten met pulmonale hypertensie.

In **Hoofdstuk 9** vergeleken we 1-jaars sterftecijfers tussen patiënten met pulmonale hypertensie die deelnamen aan hartrevalidatie met een controlegroep van patiënten met pulmonale hypertensie zonder deelname aan hartrevalidatie. We toonden aan dat hartrevalidatie bij patiënten met pulmonale hypertensie geassocieerd is met een 40% lagere kans op overlijden 1 jaar na start van hartrevalidatie in vergelijking met patiënten die geen hartrevalidatie kregen.

Deel III – Algemene discussie

In **Hoofdstuk 10** hebben we de bevindingen van dit proefschrift geïntegreerd met inzichten uit andere studies, en hebben we richtingen voor toekomstig onderzoek besproken. We identificeerden vijf kernboodschappen.

Ten eerste werd de toegevoegde waarde van echocardiografie tijdens inspanning besproken. Gebaseerd op dit proefschrift en recente andere studies, laat echocardiografie

tijdens inspanning een toegevoegde waarde zien bij de detectie van een afname in functie van het hart na kortdurende, intensieve inspanning, maar ook bij het onderscheiden van fysiologische en pathofysiologische hartaandoeningen (zoals aritmogene en gedilateerde cardiomyopathie). Verdere studies moeten het potentieel diagnostische en prognostische nut binnen de verscheidende hart- en vaatziekten onderzoeken.

Ten tweede lijken acute cardiovasculaire reacties op inspanning nauw verband te houden met cardiovasculaire adaptatie ten gevolge van chronische training. Het gebruik van echocardiografie tijdens inspanning is een mogelijke strategie om de hypothese dat herhaalde blootstelling aan hoge trainingsvolumes kan leiden tot een pathologische adaptatie van de rechterventrikel beter te begrijpen. Daarnaast kan de mate bloeddrukverlaging na inspanning worden gebruikt als middel om personen te identificeren die goed reageren op het bloeddrukverlagende effect van training.

Ten derde concludeerden we dat de rechterventrikel simpelweg niet de linkerventrikel spiegelt in zowel acute als chronische reacties op training. Niet alleen de acute reactie van beide ventrikels op inspanning varieert, maar ook de afname in ventrikelfunctie na inspanning verschilt tussen de rechter- en linkerventrikel. Door onze bevindingen te integreren in de bestaande literatuur, bespraken we de structurele adaptatie hypothese van het hart. Deze hypothese suggereert de aanwezigheid van een tijdsafhankelijke structurele rechter- en linkerventrikel adaptatie ten gevolge van training. Daarnaast bespraken we de toegevoegde waarde van de strain-volume curve bij het verschaffen van inzicht in de adaptatie van cardiale mechanica ten gevolge van training.

Ten vierde hebben we onze bevindingen van de echocardiografische strain-volume curve bij patiënten met pulmonale hypertensie samengevat en geïntegreerd in de reeds bestaande literatuur. De strain-volume loop heeft potentie, maar toekomstige studies moeten de klinische waarde ervan beoordelen bij het monitoren van patiënten met pulmonale hypertensie. De ontwikkeling van een semi-automatische methode is essentieel voor uiteindelijke integratie in de dagelijkse klinische praktijk.

Ten vijfde bespraken we de toegevoegde waarde van hartrevalidatie bij patiënten met pulmonale hypertensie maar ook bij patiënten met andere hart- en vaatziekten.

Toekomstige studies zouden de invloed van individuele patiënt- en inspanningskenmerken op de effectiviteit op hartrevalidatie moeten onderzoeken. Uiteindelijk zal deze informatie bijdragen aan het belang van lichamelijke inspanning als gepersonaliseerde geneeskunde bij hart- en vaatziekten.

LIST OF ABBREVIATIONS

6MWT	6-minute walk test
A2C	Apical 4-chamber
A2C	Apical 2-chamber
AMS	Acute mountain sickness
BMI	Body mass index
BP	Blood pressure
Bpm	Beats per minute
BSA	Body size area
CHD	Coronary heart disease
CR	Cardiac rehabilitation
CO	Cardiac output
CO_2	Carbon dioxide
CPET	Cardiopulmonary exercise test
DBP	Diastolic blood pressure
DICOM	Digital imaging and communication in medicine
ECG	Electrocardiogram
EICF	Exercise-induced cardiac fatigue
EDslope	The early linear strain-area slope during first 5% of volume increase in diastole
ESslope	Early strain-area slope during first 5% of volume ejection in systole
LDslope	Late linear strain-area slope during first 5% of volume increase in diastole

FiO_2	Fraction of inspired oxygen
HR	Heart rate
ICC	Intra-class correlation coefficient
IVSd	Interventricular septum thickness end diastole
LLS	Lake Louis Score
MAP	Mean arterial pressure
LA	Left atrium/atrial
LOA	Limits of agreement
LV	Left ventricle/ventricular
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVESV	Left ventricular end-systolic volume
LVGLS	Left ventricular global longitudinal strain
LVIDd	Left ventricular internal diameter end diastole
O_2	Oxygen
PAH	Pulmonary arterial hypertension
PAP	Pulmonary artery pressure
PAT	Pulmonary artery Doppler acceleration time
PEH	Post-exercise hypotension
PH	Pulmonary hypertension
PLAX	Parasternal long-axis

PSAX	Parasternal short-axis
PVR	Pulmonary vascular resistance
PWd	Posterior wall thickness end diastole
RA	Right atrium/atrial
ROI	Region of interest
RPE	Rate of perceived exertion
RV	Right ventricle/ventricular
RVEDA	Right ventricular end-diastolic area
RVESA	Right ventricular end-systolic area
RVFAC	Right ventricular fractional area change
RVFWS	Right ventricular free wall strain
RVOT	Right ventricular outflow tract
RWT	Relative wall thickness
SBP	Systolic blood pressure
SD	Standard deviation
SEM	Standard error of the mean
SpO_2	Oxygen saturation
Sslope	The linear strain-area slope
SV	Stroke volume
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler imaging

TPR	Total peripheral resistance
VO_2max	Maximal oxygen consumption

DATA MANAGEMENT

The data used within this thesis are collected and stored according to the Findable, Accessible, Interoperable and Reusable (FAIR) principles.[1] Appropriate data management is important for 1) knowledge discovery and innovation, 2) protecting scientific integrity and 3) preservation and reuse of data sets. This thesis is based on results of human studies, which were conducted in accordance with the principles of the Declaration of Helsinki. Additionally, a local Medical Ethics Committee approved the study protocols. All subjects were well informed about the study using an information package and all subjects gave written informed consent prior to participation in the study. The paper data for this PhD project are stored in a closed cabinet in a room at the department of Physiology, Radboud University Medical Center, or at the Research Institute for Sport and Exercise Sciences, Liverpool John Moores University. The raw and processed digital data are stored on the server of the department of Physiology, Radboud University Medical Center which are backed-up on daily basis to prevent data loss. The processed data that were generated have been stored in encoded Microsoft Excel or SPSS data files. The privacy of the participants in this study is warranted by use of encrypted and unique individual subject codes. The code was stored separately from the study data. In data files and case report forms the individual subject code is used, which allows us to share the data if necessary. The encryption key is only available to the research team. In order to ensure that the data is generally accessible and interoperable, file names and data which were used to produce the final results, were documented using applicable language for knowledge representation. The data will be available for further analyses for at least 15 years. Furthermore, the datasets generated and analyzed during these studies are available from the corresponding author upon reasonable request.

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DANKWOORD

Met dit proefschrift komt er een einde aan een intensief, leerzaam maar bovenal erg leuke periode. Ik ben ontzettend dankbaar dat mij de mogelijkheid werd gegeven om een duaal promotietraject aan te gaan in Nijmegen en Liverpool. Met Nijmegen als uitvalsbasis, bracht ik korte bezoekjes aan Liverpool in het eerste jaar, gevolgd door twee langere periodes in het tweede en derde jaar. De laatste periode in Liverpool werd helaas abrupt onderbroken door de COVID-19 pandemie. Sindsdien is het helaas onmogelijk geweest om terug te keren. Desondanks heeft de COVID-19 pandemie mij ook ontzettend veel gebracht waarover later meer. Alles tezamen kan ik niet anders dan concluderen dat mijn onderzoeksperiode een fantastische tijd was. Ik wil hieronder graag de mensen bedanken die dit mede mogelijk hebben gemaakt.

Allereerst wil ik **alle deelnemers** van de onderzoeken bedanken. Jullie motivatie, inzet en tijd hebben mede gezorgd voor de totstandkoming van dit proefschrift.

Tijdens mijn promotie had ik geen beter begeleidingsteam kunnen wensen. Een goede mix tussen fulltime onderzoekers en clinici, met eenieder zijn specifieke kwaliteiten, zorgden voor een uitgebalanceerd team dat altijd voor mij klaar stond.

Dick, jouw begeleiding als professor bij de totstandkoming van dit proefschrift is ongekend. Ik moet nog een professor gaan tegenkomen die meer is toegewijd, en meer tijd investeert in zijn promovendi dan jij. Om iets concreets te noemen, ondanks jouw drukke agenda heb ik letterlijk nooit langer dan drie dagen gewacht op feedback. Niet alleen de inhoudelijke begeleiding, maar ook jouw aandacht voor persoonlijke ontwikkeling en oog voor privézaken heb ik enorm gewaardeerd de afgelopen jaren. Achteraf kijk ik terug op een mooie periode. Echter was het bij tijden ook erg druk en stressvol. Ook hier had jij oog voor en bood mij ondersteuning daar waar nodig. Dick, enorm bedankt voor je toewijding en inzet. Ik durf te stellen dat zonder jou, en zeker niet in 3 jaar tijd, het niet was gelukt om dit proefschrift af te ronden. Hopelijk lukt het mij om de kliniek met wetenschap te kunnen combineren komende jaren en zullen onze wegen elkaar blijven kruisen.

Dave, with regards to this dissertation I cannot express my gratitude enough. Your continuous support, guidance and encouragement were absolutely essential in this PhD. Along the process, your knowledge and enthusiasm about echocardiography and sports

cardiology were extremely inspirational and had a contagious effect on me. Thank you. But it is not only this, it stretches further. You made me feel very welcome in Liverpool from the very first start. We met many times outside the Tom Reilly Building for 'progress meetings'. But to admit, these took place in the pub most of the times. Of course, we philosophized about our studies and ideas came up for more studies, however, quite often we also had a lot of fun about many other things in life. Not uncommon, these pub sessions ended up dancing in a club till the early morning. Dave, matey, also many thanks for your friendship these three years. Unfortunately, due to the COVID-19 pandemic, I have had to leave Liverpool suddenly and earlier than expected. It feels as unfinished business. I will be back!

Arie, tijdens ons eerste gesprek, op 18 september 2015, uitte ik voor het eerst mijn interesse in de cardiologie. We hadden direct een klik en je gaf aan overeenkomsten tussen ons te zien. Dat je uiteindelijk naast mijn opleider ook nog mijn co-promotor zou worden had ik destijds niet kunnen vermoedden. Jouw fysiologische kennis was onmisbaar om de onderwerpen aangesneden in dit proefschrift naar een hoger niveau te tillen. Hartelijk dank voor al je inzet en toewijding. Ook bedankt voor al je vertrouwen die je altijd in mij hebt gehad. Ik hoop nog veel van je te leren zodra ik terug ben in het Radboudumc voor het academische deel van de opleiding tot cardioloog.

Keith, you always managed to find the weak spots and inconsistencies in my work. Your valuable feedback significantly improved the quality of this PhD dissertation. Many thanks for the opportunity to work with you.

John (In memoriam), your enthusiasm and expertise in sports cardiology has been really inspirational. Many thanks for all your advice and the opportunity you gave me to get involved in the cardiac screening programs. The last time I spoke to you, we talked about your retirement plans in a couple of years. I was literally dreaming to become your successor. It wasn't meant to be. May you rest in peace.

Naast mijn promotieteam heb ik ook te maken gehad met hele leuke en fijne collega's op de afdeling fysiologie van het Radboudumc. **Maria**, ondanks dat je niet actief betrokken was bij mijn promotieonderzoek heb ik veel gehad aan je adviezen. Jij hielp mij gemotiveerd te blijven tijdens het thuiswerken in het laatste jaar. Dank daarvoor. **Thijs E**,

gaandeweg mijn promotie kwam de interesse in de inspanningsfysiologie en met name de sportcardiologie. Eigenlijk helemaal jouw straatje. Hopelijk gaat het me lukken om kliniek en wetenschap te combineren en gaat ons review er komend jaar ergens van komen. **Bregina**, heel erg bedankt voor het maken van de voorkant van mijn boekje. Hopelijk was mijn perfectionisme een beetje dragend ;-) Het eindresultaat is werkelijk prachtig geworden! Uiteraard mijn grote dank aan mijn kamergenoten en medebestuursleden van de Mancave Research Group B.V.: **Bram** en **Thijs L**. Het was werkelijk genieten om met jullie de Mancave op de afdeling te mogen delen. Ondanks dat het soms meer op Jiskefet leek, hebben we (op zijn tijd) hard gewerkt maar toch vooral veel en hard gelachen. Op naar een reünie met kapsalon en pils. Ondanks mijn aftocht, hebben jullie een meer dan waardig opvolger weten te strikken: **Thijs K**. Thijs, veel succes met die mannen en je prachtige project. Uiteraard mag **Hugo** niet ontbreken in dit dankwoord. Jij was er nooit vies van om te komen buurten in de Mancave om een lolletje te trappen. Door jouw hulp heeft mijn promotie een soepele start gehad. Heel erg bedankt daarvoor. **Paul**, jouw komst op de afdeling heeft elke PhD'er op de afdeling een vreugdedansje laten doen. Er kwam topdocent en de continuïteit werd gewaarborgd waardoor wij, de PhD's, minder tijd kwijt waren aan het geven van onderwijs. Hopelijk lukt het je om de komende jaren ook te gaan promoveren naast je onderwijstaken. Het is je gegund. **Vincent**, medesportcardiologie liefhebber, grote klasse wat jij afgelopen jaren hebt weten neer te zetten. Dank dat ik in de laatste fase van mijn promotie bij de MARC-EXERSCIENCE-studie heb kunnen helpen. Nu we samen zijn begonnen aan de opleiding tot cardioloog zullen onze wegen elkaar vast nog vaak gaan kruisen. Daarnaast kan **Esmee** niet ontbreken. Ingewikkelde mixed-models uitleggen zodat het simpel lijkt. Echter, eenmaal de deur uit was ik vaak het spoor weer bijster. Dank voor je geïnvesteerde tijd. Het fietsclubje 'Fysiolofiets': **Cindy**, **Femke**, **Thijs V** en **Yannick**. Wat was het heerlijk om met jullie tijdens of na werktijd te wielrennen of mountainbiken. **Ellis** en **Nicole**, enorm bedankt voor al jullie hulp bij het rekruteren van patiënten voor de Selexipag studie. **Josephine**, bedankt dat je elke keer weer klaarstond om mijn ICT-sores op te lossen die ik met de diverse beeldvormingsapplicaties had. **Laurien**, **Coen**, **Ralf**, **Eline**, **Carlijn**, **Lando**, **Jenske**, **Malou**, **Merle**, **Sylvan**, **Yvonne**, **Biba**, **Lisa**, **Isa** en **Laween**, ook jullie hartelijk dank voor de gezelligheid op de afdeling. Aan de nieuwe garde van de afdeling fysiologie: veel succes met jullie promoties! Hierbij wil ik één iemand uitlichten. **Niels**, als bachelor student durfde jij het aan om 5 maanden stage te

komen lopen in Liverpool. Zondag arriveren, direct door naar de pub om de stageplanning door te nemen, en maandag volle bak te beginnen met de *Exercise at Altitude* studie. Pure klasse. Mooi om te zien dat je uiteindelijk bent gaan promoveren. Veel succes.

I want to say a huge thank you to **all the people from the Research Institute for Sport and Exercise Sciences at the Liverpool John Moores University** who made me feel very welcome during my time in Liverpool. It was extremely inspirational and motivating to be surrounded by people who are completely engaged into sport physiology in an environment with just top-class equipment. **George**, **Gemma** and **Dean**, many thanks for all your supportive help with the *Exercise at Altitude study*. My request to use the altitude chamber for 35 hours a week stirred things up but finally we managed it, many thanks. I have worked in Liverpool with great people from all around the world. **Guilherme**, **Fabio**, **Thiago** and **Marcelle** from Brazil. **Matteo** and **Alessandro** from Italy. **Jarret and Barb** from Canada. My dear friends **Andrea** from Cyprus and **Niels** from Denmark. And of course, the British, **Dan**, **Joe**, **Lucy**, **Maddie**, **Katie**, **Ben** and **Chris**. We not only worked together, but we also had a lot of fun together outside the Tom Reilly Building. Thank you all for making my time in Liverpool unforgettable.

Ook mijn Achterhoekse vrienden van **'t Zomerhuus** wil ik bedanken. Toen wij vanaf onze 14e levensjaar elk weekend op de kop naar huis gingen na een avondje Ivarca of Radstake had ik nooit kunnen vermoeden dat ik ooit op het punt zou staan om te gaan promoveren. Het blijft altijd genieten om weer terug te gaan naar Varsseveld om een biertje met jullie te drinken. Dank daarvoor.

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CURRICULUM VITAE

Geert Kleinnibbelink was born on 9 November 1989 in Doetinchem and he grew up in Varsseveld, the Netherlands. In 2008, he graduated from pre-university secondary school (Almende College, Silvolde). Thereafter, he combined studies in Technical Medicine at the University of Twente, Enschede and Medicine at the Radboud University, Nijmegen. In 2011, he obtained a Bachelor of Science (BSc) degree in Technical Medicine with a scientific internship titled: *"Time lag determination between glucose concentration peaks in plasma and brain, measured in humans using ^{13}C MR spectroscopy"*. This internship was supervised by Dr. Ir. B. ten Haken, Dr. Ir. K.C.C. van de Ven and Dr. B.E. de Galan. After obtaining his Bachelor of Science (BSc) degree in Medicine (2013), he continued his study in Medicine. In 2016, he obtained his medical degree (MD). During his time at the university, Geert worked at PROVA, Erasmus MC and the Dutch College of General Practitioners where he was involved in the development of several Dutch evidence-based guidelines. After obtaining his medical degree, he worked at the department of cardiology at Rijnstate hospital and Radboudumc for 1 year. In 2018, he started his dual PhD at the Radboud University Medical Center, the Netherlands and the Liverpool John Moores University, United Kingdom under the supervision of Prof. Dr. D.H.J. Thijssen, Dr. D.L. Oxborough, Prof. Dr. K.P. George and Dr. A.P.J. van Dijk. The focus of his PhD dissertation was on the effect of exercise and altered haemodynamics in healthy individuals and patients with pulmonary hypertension. To carry out his research, Geert stayed alternately in Nijmegen and Liverpool and collaborated with other researchers and clinicians from the Netherlands, the United Kingdom, Italy and Brazil. Besides performing his research, Geert also supervised students during their scientific internships. The work described in this dissertation has been presented at various national and international conferences and has received several awards. In 2021, Geert started his cardiology

residency at the Radboud University Medical Center in Nijmegen under the supervision of Dr. A.P.J. van Dijk and Prof. R. Nijveld. Geert is currently working in the Rijnstate hospital as internal medicine resident, as part of the cardiology residency, under the supervision of Dr. L.J.M. Reichert and Dr. A.C. van Bon.

Geert Kleinnibbelink werd geboren op 9 november 1989 in Doetinchem en groeide op in Varsseveld. In 2008 behaalde hij zijn Atheneum diploma aan het Almende college, locatie Isala te Silvolde. Vervolgens startte hij met de opleidingen Technische Geneeskunde aan de Universiteit van Twente te Enschede en Geneeskunde aan de Radboud Universiteit te Nijmegen. In 2011 ronde hij de Bachelor of Science Technische Geneeskunde af met een onderzoekstage getiteld: *"Time lag determination between glucose concentration peaks in plasma and brain, measured in humans using ^{13}C MR spectroscopy"* onder supervisie van Dr. Ir. B. ten Haken, Dr. Ir. K.C.C. van de Ven en Dr. B.E. de Galan. Na het behalen van zijn Bachelor of Science Geneeskunde in 2013, startte Geert zijn Master of Science opleiding Geneeskunde welke hij in 2016 afrondde. Gedurende zijn tijd op de universiteit was Geert werkzaam bij PROVA, Erasmus MC en het Nederlands Huisartsen Genootschap waar hij actief betrokken was bij de ontwikkeling en totstandkoming van meerdere landelijke evidence-based richtlijnen. Na het afronden van de opleiding Geneeskunde, heeft Geert ruim een jaar als basisarts gewerkt op de afdeling cardiologie in het Rijnstate ziekenhuis en het Radboudumc. In 2018, startte Geert met zijn duaal promotieonderzoek aan het Radboudumc en aan de Liverpool John Moores University in het Verenigd Koninkrijk. De focus van het proefschrift lag op de effecten van lichamelijke inspanning en veranderingen in hemodynamiek op het rechter ventrikel in gezonde individuen en patiënten met pulmonale hypertensie. Voor de uitvoering van zijn promotieonderzoek verbleef Geert afwisselend in Nijmegen en Liverpool en werkte hij samen met andere onderzoekers en clinici uit Nederland, het Verenigd Koninkrijk, Italië en Brazilië. Naast het uitvoeren van zijn onderzoek, heeft Geert studenten begeleid bij hun wetenschappelijke stages. Het werk beschreven in dit proefschrift is gepresenteerd op verschillende nationale en internationale congressen en is beloond met diverse prijzen. In 2021 is Geert gestart met de opleiding tot cardioloog aan het Radboudumc te Nijmegen onder supervisie van dr. A.P.J. van Dijk en prof. dr. R. Nijveld. Momenteel werkt Geert in het Rijnstate ziekenhuis in het kader van de vooropleiding interne geneeskunde onder supervisie van dr. L.J.M. Reichert en dr. A.C. van Bon.

LIST OF PUBLICATIONS

Kleinnibbelink G*, Panhuyzen-Goedkoop NM*, Hulshof HG, van Dijk APJ, George KP, Somauroo JD, Oxborough DL, Thijssen DHJ. Exercise Training Induces Left- but not Right-Sided Cardiac Remodelling in Olympic Rowers. *Int J Sports Med. 2021. Online ahead of print.*

*Shared first authorship

Kleinnibbelink G, van Dijk APJ, Fornasiero A, Speretta GF, Johnson C, Sculthorpe N, George KP, Somauroo JD, Thijssen DHJ, Oxborough DL. Acute Exercise-Induced Changes in Cardiac Function Relates to Right Ventricular Remodeling Following 12-weeks Hypoxic Exercise Training. *J Appl Physiol. 3032;131:511-519.*

Kleinnibbelink G, van Dijk APJ, Fornasiero A, Speretta GF, Johnson C, Hopman MTE, Sculthorpe N, George KP, Somauroo JD, Thijssen DHJ and Oxborough DL. Exercise-Induced Cardiac Fatigue after a 45-min Bout of High-Intensity Running Exercise Is Not Altered under Hypoxia. *J Am Soc Echocardiogr*. 2020;34:511-521.

Kleinnibbelink G*, Hulshof HG*, van Dijk APJ, Ten Cate T, George KP, Oxborough DL and Thijssen DHJ. Effects of Preload Manipulation on Right Ventricular Contractility: Invasive Pressure-Area Loop versus Non-invasive Strain-Area Loop. *J Am Soc Echocardiogr*. 2020;34:447-449.

*Shared first authorship

Kleinnibbelink G, Stens NA, Fornasiero A, Speretta GF, Van Dijk APJ, Low DA, Oxborough DL and Thijssen DHJ. The acute and chronic effects of high-intensity exercise in hypoxia on blood pressure and post-exercise hypotension: A randomized cross-over trial. *Medicine (Baltimore)*. 2020;99:e22411.

Buckley BJR, **Kleinnibbelink G**, Lip GYH, Taylor RS and Thijssen DHJ. Cardiac rehabilitation meta-analysis of trials in patients with coronary heart disease using individual participant data (CaReMATCH): Project protocol. *Int J Cardiol Heart Vasc*. 2020;31:100616.

Nas J, **Kleinnibbelink G**, Hannink G, Navarese EP, van Royen N, de Boer MJ, Wik L, Bonnes JL and Brouwer MA. Diagnostic performance of the basic and advanced life support

termination of resuscitation rules: A systematic review and diagnostic meta-analysis. *Resuscitation*. 2020;148:3-13.

Hulshof HG, Eijsvogels TMH, **Kleinnibbelink G**, van Dijk AP, George KP, Oxborough DL and Thijssen DHJ. Prognostic value of right ventricular longitudinal strain in patients with pulmonary hypertension: a systematic review and meta-analysis. *Eur Heart J Cardiovasc Imaging*. 2019;20:475-484.

SUBMITTED PUBLICATIONS

Buckley JR*, **Kleinnibbelink G***, Harrison SL, Williams N, Fazio-Eynullayeya E, Underhill P, van Dijk APJ, Lip GYH**, Thijssen DHJ**. Cardiac Rehabilitation is Associated with Lower 1-year All-Cause Mortality in Primary Pulmonary Hypertension. *Submitted*

*Shared first authorship **shared last authorship

Kleinnibbelink G, van Dijk APJ, Coenen NPW, de Groot-Vos EH, George KP, Oxborough DL, Thijssen DHJ. Right ventricular strain-area loop following 17-weeks of Selexipag in pulmonary arterial hypertension: an exploratory study. *Submitted*

OTHER SCIENTIFIC OUTPUT

Book chapter: **Kleinnibbelink G**, Baten A. Systematische beoordeling van het electrocardiogram. 2020. *Pocket Acute Geneeskunde (1st edition)*.

Guideline: Tuut MK, **Kleinnibbelink G**, Schellevis FG, Burgers JS. NHG generieke module 'comorbidititeit'. 2017. https://www.ggzstandaarden.nl/generieke-modules/comorbiditeit/inleiding

Guideline: Driessen GJA, **Kleinnibbelink G**, Bogaert D, van Ewijk B, Mathot F, Noordzij J, Oostenbrink R, Rippen H, van der Schroeff MP, Vaessen-Verberne A, Venekamp RP, Versteegh FGA, de Vries E, van Well G, van Wermeskerken A, Tuut MK. Richtlijn 'diagnostiek naar recidiverende luchtweginfecties bij kinderen'. 2016. https://www.kinderinfectieziekten.nl/

RIHS PHD PORTFOLIO

Name PhD candidate: Geert Kleinnibbelink	**PhD period:** 01-01-2018 to 31-03-2021
Graduate Schools:	**Promotors:**
Radboud Institute for Health Sciences, Department of Physiology	Prof. dr. D.H.J. Thijssen, Prof. dr. K.P. George
Liverpool John Moores University, Research Institute for Sport and Exercise Sciences	**Co-promotors:** Dr. D.L. Oxborough, Dr. A.P.J. van Dijk

		Year(s)	ECTS
	TRAINING ACTIVITIES		
a.	**Courses & Workshops**		
-	Introduction course, Radboud University Medical Center	2018	0.5
-	Radboud Institute for Health Sciences Introduction course for PhD students	2018	0.5
-	Induction course, Liverpool John Moores University	2018	0.5
-	BROK (basic course regulations and organization for clinical researchers)	2018	1.5
-	Echocardiography summer school, Liverpool John Moores University	2018	1.5
-	Dutch Heart Foundation PhD course	2018	2.0
-	Presentation Skills Training, Amsterdam, Partners in Training	2018	0.5
-	Biometrics statistical course	2018	4.0
-	Scientific writing days, Liverpool John Moores University	2018-2019	1.5
-	Scientific Integrity, Radboud University Medical Center	2019	1.0
-	Football Association (FA) ECG and Echo for Athletes course	2020	1.0
-	Thesis Bootcamp, Liverpool John Moores University	2020	1.5
-	Gett Media Savvy! Effective Communicating via Lens and Microphone, Liverpool John Moores University, by Julian Dismore	2020	0.5
b.	**Seminars & lectures**		
-	Vascular Damage meetings, Radboud University Medical Center	2018-2021	0.5
-	Radboud Research Rounds, Radboud University Medical Center	2018-2021	0.5
-	RISES seminars, Liverpool John Moores University	2019-2021	0.5
c.	**Symposia & congresses**		
-	Dutch Society of Cardiology Congress (no presentation)	2018	0.5
-	Symposium at Vitesse Football club (invited oral presentation)	2018	1.0
-	Dutch Society of Cardiology Congress (oral presentation)	2019	1.0
-	EuroEcho congress, Vienna, Austria (poster presentation)	2019	1.5
-	PhD retreat 2019, Radboud Institute for Health Sciences (oral presentation)	2019	0.5
-	Faculty research day, Liverpool John Moores University (poster presentation, 1st prize)	2019	0.5
-	Research and innovation day, Liverpool John Moores University (poster presentation, 1st prize)	2019	0.5
-	Cardiovascular symposium, Florianópolis, Brazil (invited oral presentation	2019	1.0
-	Three Minute Thesis Competition Faculty of Science, Liverpool John Moores University (oral presentation, 1st prize)	2020	1.0
-	Three Minute Thesis Competition University Finals, Liverpool John Moores University (oral presentation, 3rd prize people's vote)	2020	1.0
-	ESC Preventive Cardiology digital congress (oral presentation)	2021	1.0
d.	**Other**		
-	Review scientific publication (3x)	2018-2020	0.3
-	Second assessor of internship reports of MSc student (1x)	2019	0.2

Name PhD candidate: Geert Kleinnibbelink	**PhD period:** 01-01-2018 to 31-03-2021
Graduate Schools:	**Promotors:**
Radboud Institute for Health Sciences, Department of Physiology	Prof. dr. D.H.J. Thijssen, Prof. dr. K.P. George
	Co-promotors:
Liverpool John Moores University, Research Institute for Sport and Exercise Sciences	Dr. D.L. Oxborough, Dr. A.P.J. van Dijk

TEACHING ACTIVITIES

e.	**Lecturing**		
-	Teaching Biomedical Sciences and Medicine, Radboud University Medical Center. Lectures, group assignments and practicals in following Courses: Circulation and Respiration, Clinical Exercise Physiology, Applied Exercise Physiology.	2018-2020	6.0
-	Meet the PhD, Radboud University Medical Center	2018-2020	0.5
f.	**Supervision internships**		
-	Stella Timmerman, Master of Science Biomedical Science – Literature thesis: The Acute Effects of Hypoxic Exercise on Right Ventricular Function and Structure	2018	0.5
-	Niels Stens, Bachelor of Science Biomedical Sciences - The Impact of High-Intensity Exercise in Hypoxia on Post-Exercise Hypotension	2019	2.0
-	Manon van Steen, Bachelor of Science Medicine (Honours) - The Impact of Normoxic versus Hypoxic Endurance Exercise on left Ventricular Strain-Volume Loops	2019	2.0
-	Jack Homans, Bachelor of Science Sport and Exercise Sciences – The Acute Effects of Hypoxic Exercise on Right Ventricular Structure and Function	2019	1.5
-	Lewis Clark, Bachelor of Science Sport and Exercise Sciences – The Acute Effects of Hypoxic Exercise of Left Ventricular Structure and Function	2019	1.5
-	Nadya Waner, Master of Science Clinical Exercise Physiology – The Acute and Chronic Effects of Hypoxic Exercise on Right Ventricular Structure and Function	2019	2.0
-	Ellie Colquitt, Bachelor of Science Sport and Exercise Sciences – The Impact of 12 weeks of High-Intensity Running Exercise on Electrocardiographic Changes	2019	0.5
-	Michiel Ewalts, Master of Science Biomedical Sciences - Left Ventricular Exercise-Induced Cardiac Fatigue after 45-Minute High-Intensity Running Exercise under Hypoxia versus Normoxia in Recreational Athletes	2020	2.0
-	Joost Biere, Master of Science Biomedical Sciences – Literature thesis: The Elite Athlete's Heart: do Men and Women Demonstrate Different Cardiac Adaptations?	2020	0.5
TOTAL			**47.0**

NIJMEGEN LIVERPOOL