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RĪGA STRADIŅŠ UNIVERSITY

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Effects of Meldonium on Right Ventricular Failure in a Preclinical Model and Patients

Doctoral Thesis for obtaining the scientific degree
"Doctor of Science (*PhD*)"

Sector Group – Medical and Health Sciences
Sector – Clinical Medicine
Sub-Sector – Internal Medicine

Riga, 2023

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Abstract

Right ventricular failure (RVF) is associated with poor prognosis and currently has no known treatment. RVF is characterised by pathologically altered myocardial energy metabolism. In turn, modulation of myocardial energy metabolism pathways in heart muscle has been suggested as a promising therapeutic option. Meldonium is a cardiometabolic drug that improves cardiac function in preclinical models of the left-sided heart failure as well as improves the clinical status of heart failure patients.

This study was conducted to analyse the incidence of two RVF-inducing diseases, pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH), in the Latvian population and to evaluate the effects of meldonium in the preclinical RVF model and in RVF patients.

The incidence of PAH and CTEPH was characterised by analysing data from the Latvian PH Registry. The effects of meldonium administration on the development of pulmonary hypertension-induced RVF was assessed in rats after monocrotaline administration. The safety and effects of meldonium treatment on functional capacity and dyspnoea were assessed in patients with PAH-induced RVF.

The estimated incidence of PAH in 2021 was 9.5 per million inhabitants and 11.7 per million adult population. The incidence of CTEPH was 4.2 per million inhabitants and 5.0 per million adult population. In preclinical setup, in Sprague-Dawley rats, treatment with meldonium reduced lung-to-body weight index, right ventricle-to-body mass index along with Fulton index ($p < 0.05$), reduced RV end-systolic area ($p < 0.05$) and increased RV fractional area change ($p < 0.05$). In addition, meldonium treatment improved altered mitochondrial bioenergetics in the right ventricular cardiomyocytes. In RVF patients, meldonium treatment significantly improved WHO functional class and SF-36 scores ($p < 0.05$). Significant increase in walking distance in 6-minute walking test ($p = 0.021$) and decrease in Borg dyspnoea score after the 6MWT ($p = 0.003$) were also observed in patients with RVF.

The overall incidence of PAH and CPTEH in Latvia has remained stable over the last five years and is in line with other European countries, but the majority of patients are still diagnosed at an advanced stage of the disease. In an experimental model of RVF, treatment with meldonium prevents the development of RVF by improving mitochondrial bioenergetics. In patients with chronic RVF, meldonium treatment is safe and well tolerated and increases functional capacity and decreases dyspnoea.

Keywords: right ventricular failure; meldonium; mitochondria; energy metabolism; PAH incidence; CTEPH incidence

Anotācija

Meldonija efekti preklīniskā modelī un pacientiem ar labā kambara mazspēju

Labā kambara (LK) mazspēja (LKM) ir saistīta ar sliktu klīnisko prognozi, un tās ārstēšanas iespējas ir ierobežotas. LKM gadījumā ir patoloģiski izmainīts miokarda enerģijas metabolisms. Savukārt enerģijas metabolisma signālceļu regulācija sirds muskulī tiek uzskatīta par daudzsološu terapijas iespēju. Meldonijam piemīt kardiometaboliska aktivitāte preklīniskajos sirds kreisā kambara mazspējas modeļos, kur tas uzlabo sirds funkciju, savukārt pacientiem ar sirds mazspēju ir novērota klīnisko rādītāju uzlabošanās.

Šī pētījuma ietvaros tika analizēta divu LKM izraisošu slimību, pulmonālās arteriālās hipertensijas (PAH) un hroniskas trombemboliskas plaušu hipertensijas (HTEPH), incidence Latvijas populācijā un izvērtēta meldonija farmakoloģiskā ietekme preklīniskā modelī žurkām ar monokrotalīna inducētu PAH un LKM.

PAH un HTEPH incidence tika noteikta, analizējot Latvijas Pulmonālās hipertensijas (PH) reģistra datus. Meldonija ietekme uz PH inducētu LKM modeli tika noteikta žurkās, kurām tika injicēts monokrotalīns. Meldonija ietekme uz pacientu funkcionālo kapacitāti un elpas trūkumu, kā arī medikamenta panesamība, tika analizēta pacientiem ar PAH inducētu LKM.

PAH incidence 2021. gadā bija 9,5 uz 1 miljonu iedzīvotāju un 11,7 uz 1 miljonu pieaugušo iedzīvotāju. Savukārt, HTEPH incidence bija 4,2 uz 1 miljonu iedzīvotāju un 5,0 uz 1 miljonu pieaugušo iedzīvotāju. Terapija ar meldoniju preklīniskā pētījumā ar *Sprague-Dawley* žurkām samazināja plaušu pret ķermeņa masas indeksu, LK pret ķermeņa masas indeksu, Fultona indeksu un beigu sistolisko laukumu ($p < 0,05$), un palielināja LK frakcionētās laukuma izmaiņas ($p < 0,05$). Terapija ar meldoniju uzlaboja mitohondriju bioenerģētiku LK kardiomiocītos. Terapija ar meldoniju pacientiem ar LKM būtiski uzlaboja PVO funkcionālo klasi un SF-36 anketas vērtības ($p < 0,05$). Pēc meldonija ordinēšanas 6 minūšu iešanas testa noietā distance būtiski pieauga ($p = 0,021$), savukārt Borga dispnojas skalas vērtība samazinājās pēc 6 minūšu iešanas testa ($p = 0,003$).

Gan PAH, gan HTEPH incidence pēdējo 5 gadu laikā Latvijā ir stabila un atbilst citu Eiropas valstu incidencei, tomēr lielākā daļa pacientu vēl arvien tiek diagnosticēti vēlīnā slimības stadijā. Eksperimentālā labā kambara mazspējas modelī meldonijs novērš LKM attīstību, uzlabojot mitohondriju bioenerģētiku. Terapija ar meldoniju pacientiem ir droša un labi panesama. Tā palielina pacientu funkcionālu kapacitāti un samazina dispnoju pacientiem ar hronisku LKM.

Atslēgvārdi: labā kambara mazspēja; meldonijs; mitohondriji; enerģijas metabolisms; PAH incidence; HTEPH incidence

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Abbreviations used in the Thesis

6MWT	the six-minute walk test
ACE2	angiotensin-converting enzyme 2
ARVC	arrhythmogenic right ventricular cardiomyopathy
ATP	adenosine triphosphate
BAB	β-blockers
BBOX	γ-butyrobetaine hydroxylase
BDS	Borg dyspnoea score
BMI	body mass index
BNP	brain natriuretic peptide
BP	blood pressure
CACT	carnitine/acylcarnitine translocase
CAMPHOR	Cambridge Pulmonary Hypertension Outcome Review
CHD	coronary heart disease
CHF	chronic heart failure
CI	cardiac index
CYP3A4	cytochrome P-450 3A4
CMP	cardiomyopathy
CO	cardiac output
CPT1	carnitine palmitoyltransferase-1
CrAT	carnitine acetyltransferase
CTEPH	chronic thromboembolic pulmonary hypertension
DCA	dichloroacetate
e.g.	exempli gratia
ECG	electrocardiogram
EF	ejection fraction
ET-1	endothelin-1
ERA	endothelin receptor antagonists
ESC	European Society of Cardiology
etc.	Et Cetera
FA	fatty acid
FAC	fractional area change
FAO	fatty acid oxidation
FC	functional class
Fio2	fraction of inspired oxygen
FWLS	free wall longitudinal strain

GLUT	glucose transporter
GO	glucose oxidation
HF	heart failure
HR	heart rate
HIF1-α	Hypoxia-inducible factor 1-alpha
IPAH	Idiopathic pulmonary arterial hypertension
IVC	inferior vena cava
KCCQ	Kansas City Cardiomyopathy Questionnaire
kg	kilograms
LV	left ventricle
LVEDD	left-ventricular end-diastolic diameter
MCS	mental component score
MCT	monocrotaline
mg	miligrams
mPAP	mean pulmonary arterial pressure
MRA	Mineralocorticoid receptor antagonists
MRI	magnetic resonance imaging
n	number
NYHA	New York Heart Association
NO	nitric oxide
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OCTN2	organic cation/carnitine transporter type 2 protein
PAB	pulmonary artery banding
PAH	pulmonary arterial hypertension
PCS	physical component score
PCWP	pulmonary capillary wedge pressure
PDK	pyruvate dehydrogenase kinase
PH	pulmonary hypertension
PKG	protein kinase G
PGC-1α	peroxisome proliferator-activated receptor-gamma coactivator 1 alpha
PPARα	peroxisome proliferator activated receptor-alpha
PVR	pulmonary vascular resistance
QoL	quality of life
RAP	right atrial pressure
RAAS	renin-angiotensin-aldosterone system
RHF	right heart failure

RIMP	right ventricular index of myocardial performance
RV	right ventricle
RVD	right ventricular dysfunction
RVEDD	right ventricular end diastolic diameter
RVEF	right ventricular ejection fraction
RVF	right ventricular failure
RVH	right ventricular hypertrophy
RVSP	right ventricular systolic pressure
S'	tissue Doppler imaging of the basal free lateral wall of the RV
SF-36	Short Form-36
SuHx	Sugen hypoxia
TAPSE	tricuspid annular plane systolic excursion
TMAO	N-oxide-trimethylamine
TZD	thiazolidinediones
VEGF	vascular endothelial growth factor

Introduction

Right ventricular (RV) failure (RVF) is a clinical syndrome characterised by reduced right ventricular function leading to suboptimal blood delivery to the pulmonary circulation and/or elevated venous pressure at rest or during exercise (Mehra et al., 2014; Galiè et al., 2019). The reduced life expectancy (Voelkel et al., 2006) and poor quality of life (Winter et al., 2008) of RVF patients reflect the severity and clinical importance of the syndrome. Recently, there has been an increase in the number of publications emphasising the significance of right ventricular failure and the urgent need for the targeted treatment of RVF (Voelkel et al., 2006; Houston, Brittain, and Tedford, 2023). There are multivariate causes of RVF, such as left ventricular failure, chronic lung diseases, cardiomyopathies, right ventricular infarction, valvular diseases, pulmonary hypertension etc. (Voelkel et al., 2006). Pulmonary arterial hypertension (PAH) and chronic thromboembolic pulmonary hypertension (CTEPH) are two rare pulmonary hypertension (PH) diseases that induce RVF, and early diagnosis plays a crucial role in initiating treatment for PH and preventing the progression to RVF.

Therapy for RVF is based on 3 options: reducing afterload, optimising preload, and increasing contractility (Lahm et al., 2010; Houston, Brittain, and Tedford, 2023). Currently, there are no specific drugs for the treatment of RVF (Prisco, Thenappan, and Prins, 2020). In addition, drugs used to treat conditions that induce RVF may attenuate the development of RVF but do not improve the right ventricular function (Prisco, Thenappan, and Prins, 2020). Specifically, etiological therapy for pulmonary arterial hypertension (PAH) targets the pulmonary circulation and reduces blood pressure in the pulmonary arteries, but has little or no effect on RVF (Ren, Johns, and Gao, 2019).

The development of RVF is characterised by altered myocardial energy metabolism, and improving mitochondrial function in experimental models of RV dysfunction has been associated with improved ventricular function (Sun et al., 2016; Fowler et al., 2019). To date, some metabolic modulators have shown some promising results in experimental RVF models (Guarnieri and Muscari, 1990; Guarnieri and Muscari, 1988; Archer et al., 2013; Fang et al., 2012; Prins et al., 2019; Paffett, Lucas, and Campen, 2012; Ren, Johns, and Gao, 2019). However, few of them improved quality of life and cardiac function in RVF patients (Khan et al., 2015; Han et al., 2021; NCT03273387, 2017).

Meldonium is a clinically used cardioprotective drug that reduces the levels of L-carnitine and its fatty acid ester, acylcarinite levels, thereby modulating energy metabolism by preventing mitochondrial overload of fatty acid (FA) metabolism intermediates and redirecting FA flux to peroxisomal metabolism (Dambrova et al., 2016; Liepinsh et al., 2013).

The addition of meldonium to existing treatment in patients with LV heart failure improved their quality of life and functional class (Dzerve et al., 2010), improved chronic heart failure (CHF) outcomes (Dzerve et al., 2005), and induced favourable changes in cardiac structural and functional parameters (Statsenko, Shilina, and Turkina, 2014).

So far, there is no information on the effects of meldonium on ventricular function and mitochondrial energetics in right ventricular failure, nor on the quality of life of RVF patients. The Thesis is divided into three sections: 1) analysis of the incidence of PAH and CTEPH patients, 2) evaluation of the effects of meldonium on the right ventricle in the preclinical model of RVF, 3) the assessment of the clinical effects of meldonium in patients with RVF.

Aim of the Thesis

The study was conducted to analyse the incidence of two RVF-inducing diseases, PAH and CTEPH, in the Latvian population and to assess the effects of meldonium in preclinical model of RVF and in RVF patients.

Objectives of the Thesis

1. To analyse the incidence and characteristics of PAH and CTEPH patients in the Latvian population
2. To assess the effects of meldonium on experimentally induced RVF in a preclinical model
3. To assess the safety and clinical efficacy of meldonium in patients with PAH-induced RVF.

Hypotheses of the Thesis

Meldonium enhances the mitochondrial bioenergetics in the cardiomyocytes of the right ventricle and improves RV function and overall physical well-being in both experimental model rats and RVF patients.

Novelty of the Thesis

A major burden in the management of rare diseases, including PAH and CTEPH, is the lack of data. A significant majority of patients with both PAH and CTEPH are typically diagnosed at an advanced stage of the disease, adding to the complexity of treatment. More consistent data on the baseline characteristics of PAH and CTEPH patients could contribute to a better understanding of these diseases. Initiating specific treatment at an earlier stage could help preserve RV function and reduce the pressure overload.

Currently, the RV therapy is based on symptomatic relief and treatment of the primary disease, but there is a lack of cardiospecific treatment focused on RV dysfunction and progressive RVF. Novel therapeutic approaches are essential in the treatment of the RV. The dysregulation of energy metabolism, including mitochondrial dysfunction, is a key component of RV progression to failure. We are the first to show that the treatment with meldonium attenuates the development of pulmonary arterial hypertension-induced RVF. Moreover, our results are the first to show that the stimulation and restoration of decreased mitochondrial FA metabolism in the right ventricle is capable of improving the function of the ventricle.

The conducted clinical trial demonstrates that treatment with meldonium significantly increases daily exercise capacity and reduces objective and subjective shortness of breath in patients with chronic RVF due to PAH. Our study demonstrates that meldonium is safe in patients with chronic RVF. No serious adverse events were observed during the 60-day treatment period. Overall, the results of the research bring to the forefront a promising cardiometabolic treatment option and drug for the management of RVF, offering hope to patients with progressive RVF.

Practical significance of the Thesis

Our preclinical results show that meldonium improves RV function by modifying energy metabolism in the myocardium. The results of the clinical study indicate that meldonium may be a viable novel drug treatment to improve the quality of life (QoL) and functional capacity, and reduce dyspnoea in patients with chronic RVF, making it suitable for people with RVF.

1 Literature

1.1 Right ventricular failure

The right ventricle (RV) is a thin-walled, crescent-shaped structure that, under physiological conditions, is coupled to the low-resistance pulmonary circulation. Increased pressure overload in the RV is commonly observed in conditions such as pulmonary hypertension (PH) and congenital heart disease. The RV is more susceptible to pressure-induced damage than the LV because of the relatively greater adverse changes in coronary perfusion with increased afterload (Ryan and Tedford, 2015). Various terms have been used to describe the failing RV: adaptive and maladaptive RV, right ventricular dysfunction (RVD) and failure (RVF). However, the lack of a strict definition, as in the case of LV failure, leads to lower prevalence rates and difficulties in diagnosing RVF (Haddad et al., 2008; Ryan and Archer, 2014). RVF has been described as a clinical syndrome characterised by the presence of heart failure (HF) symptoms and signs resulting from RVD (Mehra et al., 2014; Konstam et al., 2018). According to Mehra et al, 2015, the right heart failure is a dysfunction in any of the components that make up the right heart circulatory system, which includes the systemic veins extending to the pulmonary capillaries, but the right ventricular failure is one component of a pathophysiological entity that can result in right heart circulatory failure. The systemic circuit of the right heart includes the systemic veins, right atrium, coronary arteries, tricuspid valve, right ventricular free wall, right ventricular outflow tract and pulmonic valve. The pulmonary circuit of the right heart includes the main pulmonary artery post-pulmonic valve and the secondary and tertiary branches of the pulmonary arteries (Mehra et al., 2014).

1.1.1 Definition of right ventricular failure

Over the past 20 years, several definitions of RVF have been presented by various cardiology societies and associations. The European Society of Cardiology (ESC) Heart Failure Guidelines in 2008 proposed the definition of RVF as a common clinical manifestation of heart failure characterised by breathlessness, fatigue, evidence of RV dysfunction, elevated jugular venous pressure, peripheral oedema, hepatomegaly and gut congestion (Dickstein et al., 2008). In 2014, the International Right Heart Failure Foundation Scientific Working Group proposed the definition of RVF as a clinical syndrome caused by an alteration in the structure and/or function of RV circulatory system that leads to suboptimal delivery of blood flow (high or low) to the pulmonary circulation and/or elevated venous pressures at rest or during exercise (Mehra et al., 2014; Ashraf and Rosenthal, 2020). According to the Heart Failure Association's 2018 Position Paper on Right Heart Dysfunction and Failure, RV dysfunction is defined as

a measure of RV function that is outside the recommended range of normal. The definition includes the following parameters: 1) presence of RV systolic dysfunction; 2) signs of right-sided pressure and volume overload; 3) clinical evidence of right heart failure. The presence of RV systolic dysfunction is measured by the echocardiography and is characterised by the following parameters: tricuspid annular plane systolic excursion (TAPSE) < 17 mm, RV fractional area change (FAC) < 35 %, tissue Doppler imaging of the basal free lateral wall of the RV (S') < 9.5 cm/s, RV free wall longitudinal strain (FWLS) > −20 % (< 20 in magnitude with the negative sign), right ventricular index of myocardial performance (RIMP) (pulsed Doppler) > 0.43 (pulsed Doppler) or > 0.54 (tissue Doppler), 3D RV ejection fraction (EF) < 45 %. Signs of right-sided pressure / volume overload are: tricuspid regurgitation, RV basal end-diastolic diameter > 41 mm, right-ventricular end-diastolic diameter (RVEDD) / left-ventricular end-diastolic diameter (LVEDD) > 1.0, septal shift or D-shaped LV in systole and/or diastole, RV wall thickness > 5 mm, inferior vena cava (IVC) diameter > 21 mm, IVC collapsibility < 50 %, tricuspid regurgitation peak systolic velocity > 2.8 m/s, right atrial end-systolic area > 18 cm^2 (Lang et al., 2015; Gorter et al., 2018). It is also clearly stated that there are no clear reference values, meaning that the range can vary significantly depending on the agreement of normal and abnormal (Gorter et al., 2018). The assessment and evaluation of RVF by echocardiography is possible but there is still a lack of standardization of the parameters (Konstam et al., 2018). It is mentioned that the RV maladaptive phenotype in patients with pulmonary arterial hypertension (PAH) can be defined by assessing RV free-wall longitudinal strain and end-systolic dimensions (such as the RV end-systolic remodelling index) (Fine et al., 2013; Amsallem et al., 2017). The 2018 American Heart Association scientific statement on RVF is as follows: “RVF is a clinical syndrome with signs and symptoms of heart failure resulting from abnormal RV structure or function, caused by the inability of the RV to support optimal circulation in the presence of adequate preload.” (Konstam et al., 2018). RVF is a clinical syndrome characterised by reduced RV function that leads to insufficient blood flow and/or increased filling pressures at rest or during physiologically demanding conditions such as exercise, developmental growth or pregnancy in accordance with the 6th World Symposium on Pulmonary Hypertension (Galiè et al., 2019; Ashraf and Rosenthal, 2020). In conclusion, the definition of RVF is not widely accepted and varies from statement to statement, taking into account the aetiology of RVF. There are several variations on how to differentiate RVD, RVF, RVH and right heart failure (RHF). Authors form the Mayo Clinic have proposed that the term RVF refers to the clinical syndrome of HF with the concomitant right ventricular structural and physiological changes, whereas RVD refers only to pathological structural changes in the RV (Ashraf and Rosenthal, 2020).

1.1.2 Aetiology of right ventricular failure

The aetiology influences the progression of RVF. Despite similar RV pressure and afterload, PAH can lead to adaptive or maladaptive right ventricular hypertrophy (RVH) (Ryan and Archer, 2014). RVH is the response to haemodynamic stress characterised by reduced RV wall pressure and oxygen consumption, with a concomitant increase in the ability to maintain the flow in the pulmonary vascular bed (de Man, Louis Handoko, and Vonk-Noordegraaf, 2019). The hypertrophy is described as the concentric hypertrophy with increased relative wall thickness and cardiac mass with little or no change in chamber volume (Llucià-Valldeperas, de Man, and Bogaard, 2021; Hill and Olson, 2008). The normal RV muscular wall is 3–5 mm thick (Ho and Nihoyannopoulos, 2006) with a RV mass of 19–23 g depending on sex and a normal RV EDV value of 108–140 ml depending on sex (Kawut et al., 2011). Adaptive RVH is defined by preservation of normal cardiac output (CO) 4–8 L/minute, right ventricular ejection fraction (RVEF) ~ 62 %, RV filling pressures and exercise capacity (Ryan and Archer, 2014). The definition of adaptive and maladaptive RVH according to the Ren at al., 2019, depends on the level of the organisation: 1) anatomy and function, 2) biochemistry and metabolism, 3) molecules and pathways. According to the level of anatomy and function, adaptive RVH is increased RV chamber size, cardiac sympathetic activity, RV mass, increased or unchanged ejection fraction, ventriculo-arterial coupling, myocardial perfusion, diastolic function (Ren, Johns, and Gao, 2019). Maladaptive RVH is described as increased RV chamber size, cardiac sympathetic activity and fibrosis, but decreased RV mass, ejection fraction, ventriculo-arterial coupling, ventricular reserve, myocardial perfusion, diastolic function. Moreover, the definition of adaptive and maladaptive RVH cannot be based only on anatomical changes of the RV, there is also dysregulation at the metabolic and molecular level (Ren, Johns, and Gao, 2019).

According to the "Evaluation and management of right-sided heart failure: a scientific statement from the American Heart Association", the aetiology of RVF is acute and chronic and can be divided into 3 groups: 1) reduced RV contractility, 2) RV volume overload, 3) RV pressure overload (see Table 1.1

). Decreased RV contractility can be caused by RV cardiomyopathy or arrhythmogenic right ventricular cardiomyopathy (ARVC). Ebstein's anomaly can cause chronic RVF due to reduced RV contractility and RV volume overload. Chronic RVF in case of RV volume overload is caused by transposition of the great arteries or tricuspid regurgitation. RV pressure overload is caused by PAH, chronic thromboembolic pulmonary hypertension (CTEPH), pulmonary stenosis, left-sided valvular heart disease, restrictive cardiomyopathy (CMP) or pericardial disease. A combination of RV volume overload and RV pressure overload may

cause chronic RVF due to left-sided heart disease or single ventricle (Konstam et al., 2018). Another approach to classify the causes of chronic right heart failure is: 1) primary RV myocardial disease (e.g. RV CMP, restrictive CMP, myocarditis, ARVC), 2) PH (e.g. PAH, congenital heart disease, LV systolic or diastolic dysfunction, left-sided valvular disease, CTEPH, chronic lung disease), and 3) right-sided valvular heart disease (e.g. tricuspid regurgitation, Ebstein's anomaly, pulmonary regurgitation, pulmonary stenosis, tricuspid stenosis) (Ashraf and Rosenthal, 2020).

Table 1.1

Major causes of chronic right ventricular failure

Pressure overload	Volume overload	Depressed contractility
PH	Transposition of the great arteries	Right ventricular myocardial ischemia
pulmonary stenosis	Tricuspid valve regurgitation	ARVC
Left-sided HF	–	RV CMP
Restrictive CMP	–	Myocarditis
Pericardial diseases	–	–

ARVC – arrhythmogenic right ventricular cardiomyopathy, CMP – cardiomyopathy, HF – heart failure, PH – pulmonary hypertension, RV – right ventricle

1.1.3 Pathophysiology of right ventricular failure

RVF can present acutely, with haemodynamic instability and cardiogenic shock, or chronically, as a result of a gradual increase in RV afterload (Konstam et al., 2018). An initial adaptive response is RVH caused by pressure overload, but this often leads to RVF. Myocardial hypertrophy allows the right ventricle to remain isovolumic, but it is followed by contractile dysfunction. As a result RV systolic pressure and RV end-diastolic volume increase, leading to chamber dilatation (Cassady and Ramani, 2020; Voelkel et al., 2006; Konstam et al., 2018). Decompensated and dilated RV is characterised by tricuspid annular dilatation, poor leaflet coaptation and functional tricuspid regurgitation, as well as rising filling pressures, diastolic dysfunction and reduced cardiac output (Rana et al., 2019; Cassady and Ramani, 2020; Voelkel et al., 2006; Konstam et al., 2018). RV pressure and volume overload also compress the LV, causing diastolic dysfunction (Cassady and Ramani, 2020; Voelkel et al., 2006; Konstam et al., 2018).

1.1.4 Symptoms and signs of right ventricular failure

The symptoms of *cor pulmonale* are non-specific and often result from both RVF and progression of the primary disease. Clinical evidence of RVF is described as the combination of evidence structural and/or functional abnormalities of the RV combined with symptoms such

as dyspnoea, fatigue, dizziness, chest pain, syncope on exertion, ankle swelling, epigastric fullness, right upper quadrant abdominal pain and anorexia, cyanosis, hoarseness, also known as Ortner's syndrome. Rarely, cough and haemoptysis may occur. Signs of right ventricular failure include jugular venous distention, increased jugular venous pressure on inspiration (Kussmaul sign), peripheral oedema, sacral oedema, hepatic congestion, ascites and a right ventricular third heart sound. Signs of associated tricuspid regurgitation are a holosystolic murmur best heard at the left parasternal border, a prominent V-wave on the jugular venous pulse, and pulsatile hepatomegaly. The symptoms observed vary according to the aetiology of RVF (Voelkel et al., 2006; Gorter et al., 2018; Galie et al., 2016; Konstam et al., 2018).

RVH appears on on electrocardiogram (ECG) as a rightward shift of the QRS vector. The abnormalities are described as tall R waves in the anterior and right leads (leads aVR, V1, and V2) without normal R wave progression in the precordial leads, and deep S waves and small R waves in the left leads (I, aVL, and lateral precordial leads). S waves in leads I, II, and III are common (the SI SII SIII pattern). Common diagnostic criteria for RVH are tall R in V1 > 0.6 mV, increased R/S in V1 > 1, deep S in V5 > 1.0 mV, deep S in V6 > 0.3 mV, tall R in aVR > 0.4 mV, small S in V1 < 0.2 mV, small R in V5–6 < 0.3 mV, reduced R/S ratio in V5 < 0.75, reduced R/S ratio in V6 < 0.4, reduced R/S in V5 to R/S in V1 < 0.04, (RI + SIII) − (SI + RIII) < 1.5 mV, max R V1–2 + max SI, aVL − SV1 > 0.6 mV, R V1 + S V5–6 > 1.05 mV, R peak V1 > 0.035 msec, QR in V1 present (Mirvis and Goldberger, 2012; Hancock et al., 2009). QT interval prolongation in case of electrical remodelling of the RV has been reported in the literature (Archer et al., 2013).

Biomarkers such as N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and brain natriuretic peptide (BNP) to assess RV function are similar to LV failure. According to the ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure published in 2021, the reference values for normal plasma natriuretic peptide concentrations in the non-acute setting are 35 pg/mL for BNP, and 125 pg/mL for NT-proBNP (McDonagh et al., 2021). In patients with PAH and RVF BNP is mentioned as a useful prognostic marker (Benza et al., 2012; Konstam et al., 2018).

The screening of several blood markers such as transaminases, increased bilirubin usually, hypoalbuminemia already suggest the advanced stage of the chronic RVF and the progression of haemodynamic imbalance related to congestion and increased systemic venous pressures (Konstam et al., 2018; Poelzl et al., 2012; Poelzl and Auer, 2015). The aforementioned haemodynamic congestion in combination with systemic hypoperfusion can lead to a chronic cardiorenal syndrome, observed as changes in serum creatinine, cystatin C and other specific renal markers. (Galie et al., 2016; Mullens et al., 2009; Konstam et al., 2018).

1.1.5 Epidemiology right ventricular failure

Epidemiology of RVF, RVD is mainly mentioned in the literature in the setting of LV heart failure. Data are available on RVF in acute LV failure, and according to the European CHARITEM registry, RVF was present in 2.2 % of all acute decompensated HF admissions (Ashraf and Rosenthal, 2020). The prevalence of RVD in patients with HF with reduced EF is reported to be 48 % (Iglesias-Garriz et al., 2012; Konstam et al., 2018). In patients with HF with preserved EF, the RVD has been reported in 33–50 % of patients, depending on the definition (Konstam et al., 2018). RVD and RVF are caused by a diverse group of underlying pathologies, so the prevalence depends on the prevalence of the primary disease and the definition of the RVD and RVF used. In the case of RVF due to PAH, the prevalence is difficult to determine due to multiple and not widely used definitions of RVF, but RV dysfunction is a strong predictor of PAH progression with a high mortality rate (Cassady and Ramani, 2020).

1.2 Quality of life in patients with RVF

Many tools are available to assess quality of life (QoL), but the challenge is to compare results in the context of the disease. One of the criteria for selecting an instrument is comparability. The Short Form-36 (SF-36) QoL questionnaire is used in the PAH patient population and could therefore be used in RVF (Rival et al., 2014). Measuring QoL as an outcome is complex and refers to an individual's satisfaction with physical, social, and psychologic domains, and unfortunately, the improvement in objective physical functioning does not always lead to improvement in QoL (Chen, Taichman, and Doyle, 2008; Halank et al., 2013; Rival et al., 2014). A meta-analysis showed that in the patients with PAH, the mean physical component score (PCS) of the SF-36 was 37.2 points (95 % CI: 33.24–41.16) and the mean mental component score (MCS) was 46.38 points (95 % CI: 44.21–48.56). The PAH therapy prolongs survival, but there are some controversial data on the improvement of quality of life (Sarzynska et al., 2021). An improvement over pre-treatment SF-36 scores, particularly in physical function, may be associated with increased exercise capacity (Taichman et al., 2005). In 2017, Morris et al. published data on the effects of exercise-based rehabilitation programmes on patients with PH. It was demonstrated that the exercise group had a 60.12 metre improvement in 6MWT compared to the control group, as well as the SF-36 physical component score and MCS were 4.63 and 4.17 points higher than the control group, respectively (Morris, Kermeen, and Holland, 2017). Whether QoL scores can predict mortality or disease worsening is still a matter of debate and different conclusions, but Blok et al. showed

that a decrease in SF-36 PCS was a determinant of mortality in PAH associated with congenital heart disease patients (Blok et al., 2015). Moreover, Mathai et al. showed that SF-36 scores are associated with survival in patients with PAH (Mathai et al., 2016); and Johansson et al. demonstrated in the Global Congestive Heart Failure Study that lower health-related QoL is associated with a higher risk of all adverse outcomes (Johansson et al., 2021). Jorge et al. compared QoL data between patients with and without heart failure, independent of syndrome phenotype; they found significantly higher mean SF-36 scores in patients without HF than in those with HF, and the functional capacity of patients with HF was significantly worse than that of patients without HF (Jorge et al., 2017).

1.3 Metabolic remodelling of right ventricular failure

Apart from anatomical changes in the RV, there are cellular and metabolic changes, extracellular matrix remodelling, abnormalities in natriuretic peptide levels, cardiomyocyte apoptosis and inflammatory markers (Singh et al., 2019; Ryan and Archer, 2014). PAH-induced right ventricular (RV) metabolic remodelling results in an imbalance of fatty acid and glucose metabolism for energy generation and altered lipid deposition in RV cardiomyocytes. Despite the reciprocal relationship between fatty acid oxidation (FAO) and glucose oxidation (GO), the effect is more composite as other mechanisms are activated in case of RVD (e.g. activation of the fetal gene programme, neurohormonal activation, impaired vascularisation, mitochondrial dysfunction, oxidative stress, inflammation) (Talati and Hemnes, 2015; Llucià-Valldeperas, de Man, and Bogaard, 2021). In addition, metabolic dysregulation depends on the preclinical PH model (e.g. monocrotaline (MCT) model, pulmonary artery banding (PAB) model, Sugen hypoxia (SuHx) rat model) or the primary disease causing RVF (Koop et al., 2019). PH models aim to replicate the characteristics of one of the five groups of PH in patients; however, it is important to note that different models share both common and distinct metabolic dysregulations.

In PH there are alterations in the mitochondrial bioenergetics of pulmonary arterial smooth muscle cells, pulmonary arterial endothelial cells and in RV myocytes. PH-induced RVH is characterised by a shift from fatty acid to glucose metabolism, and a shift from glucose oxidation to glycolysis. There are also alterations in the tricarboxylic acid (TCA) cycle. Long chain acetyl-coenzyme A (acetyl-CoA) is derived from fatty acids undergoing β-oxidation. This, in turn, leads to the activation of pyruvate dehydrogenase kinase (PDK) that inhibits pyruvate decarboxylation. Pyruvate undergoes a transformation into lactate instead of entering the Krebs cycle after decarboxylation by pyruvate dehydrogenase (PDH) (Archer et al., 2013;

Fessel et al., 2012; Piao, Marsboom, and Archer, 2010) (see Figure 1.1). Changes in RV energy metabolism pathways include upregulation of glucose transporter (GLUT) GLUT-1 activity, glucose uptake, glutaminolysis, hypoxia-inducible factor 1-alpha (HIF1-α), c-Myc (the multifunctional protein controlling cell growth and vitality (Kuzyk and Mai, 2014)), Max (present in the network with c-Myc (Madden et al., 2021)), pyruvate dehydrogenase kinase (PDK) activity and reduction of enzyme activity involved in FAO (e.g., acetyl-CoA dehydrogenase), and PDH activity. HIF-1α stimulates glycolysis by inducing transcription of glucose transporters, hexokinase and lactate dehydrogenase kinase, and inhibits glucose oxidation by activating PDK which decreases PDH activity in mitochondria (Freund-Michel et al., 2014). Upregulation of c-Myc and Max proteins leads to increased expression of glutamine transporters and glutaminolysis (Freund-Michel et al., 2014; Piao et al., 2013). Glycolysis produces pyruvate, which cannot be used by mitochondrial PDH to fuel oxidative phosphorylation, so it is reduced and lactate accumulation is observed. Adenosine triphosphate (ATP) is produced sixteen times less during the aerobic glycolysis than during oxidative metabolism, leading to an upregulation of glucose uptake. One cause of impaired GO in RVH is increased PDK activity or expression (Sugden et al., 2000; Archer et al., 2013). There are 4 PDK isoforms in cardiomyocytes and they all inhibit PDH by phosphorylating its E1-α subunit (Piao et al., 2013 b). As a result, the formation of acetyl-CoA is inhibited and the Krebs cycle is impaired, explaining the reduced oxygen consumption and reduced energy production (Sugden et al., 2000; Archer et al., 2013). As mentioned above, the shift towards cardiac glycolysis and glutaminolysis could be explained by capillary rarefaction and RV ischaemia, and impaired angiogenesis due to VEGF/HIF-1a dysregulation and Akt1 activation (Bogaard et al., 2009 a; Talati and Hemnes, 2015).

Fatty acid synthase (FAS) synthesises long chain FFAs like palmitate from malonyl-CoA. Free fatty acids such as palmitate cause mitochondrial membrane depolarisation, increase the intracellular second messenger ceramide synthesis, and concomitantly decrease complex III activity, leading to cytochrome c release and apoptosis in cardiomyocytes (De Vries et al., 1997; Sparagna et al., 2001; Hickson-Bick, Buja, and McMillin, 2000; Marín-García and Goldenthal, 2002; Sparagna et al., 2000). Inhibition of FAS by the pharmacological inhibitor C75 reverses the pulmonary vascular remodelling, reduces the right ventricular pressure and hypertrophy, and improves cardiac function in MCT-treated rats (Singh et al., 2016; Singh et al., 2019).

Lipid deposition is a result of decreased FAO and increased FA uptake in the hereditary PAH model (Brittain et al., 2016; Talati and Hemnes, 2015; Koop et al., 2019), whereas ceramide deposition is present in the hypoxic PH model (Bitar et al., 2002; Koop et al., 2019).

Thus, different forms of lipids may accumulate depending on the PH model. In addition to this accumulation of lipotoxic compounds, insulin resistance is induced as a result of an altered insulin signalling pathway, although the sequence of the previously described metabolic interactions still needs to be fully confirmed. (Talati and Hemnes, 2015; Koop et al., 2019). Overload of FA and its metabolism intermediates which results due to changes in activity of enzymes involved in energy metabolism leads to ventricular dysfunction. However, a complete explanation for the progression of RV dysfunction is still under the investigation (Talati and Hemnes, 2015).

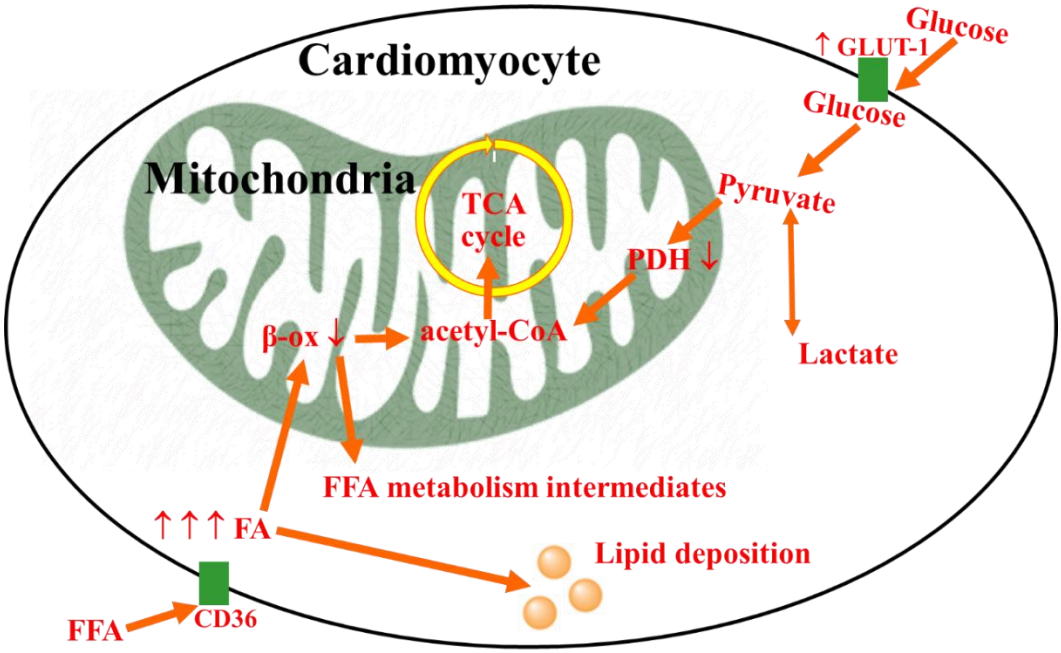

Figure 1.1 **Summary of the right ventricular cardiomyocyte changes in RVH**

acetyl-CoA – acetyl-coenzyme A, CD36 – fatty acid transporte molecule, FA – fatty acids, FFA – free fatty acid, GLUT-1 – glucose transporter 1, PDH – pyruvate dehydrogenase, TCA – tricarboxylic acid cycle, β-ox – β-oxidation

1.4 Experimental models of the right ventricular failure

Several animal models of acute or chronic RVF have been described. (Andersen et al., 2020). In experimental models, chronic RVF is characterised by volume overload-induced RVF, pressure overload-induced RVF or a combination of both (Andersen et al., 2020). Different experimental models have been described to study PH induced RVH and RVF. Genetic models are also widely used in PH molecular studies of PH, however, a single gene mutation is rarely sufficient to induce severe PH (Voelkel et al., 2012; Dignam et al., 2022).

Current animal models of PH range from transgenic mice to ungulates. Each model generally provides insight into one of the key histologic or molecular features of PH. Laboratory rats have become the preferred animals for studying the pathophysiology of PAH and new drugs

for the treatment of PH-induced RVD and RVF. Three main methods have been described to induce pulmonary hypertension in animals: 1) prenatal constriction or occlusion of the ductus arteriosus; 2) exposure to low oxygen (equivalent to 10 % of the fraction of inspired oxygen (Fio2)), hyperoxia (80 % to 100 % Fio2), 3) administration of the toxin monocrotaline or Sugen 5416 (Rabinovitch, 2017; Wadia et al., 2018). The pressure overload models of RVF are the pulmonary artery occlusion model and PH model. In rodents PH can be induced in various ways, moreover, the PH-inducing factors can be combined, for example, monocrotaline administration can be combined with hypoxia and Sugen 5416 or pneumonectomy. In addition, PH can be induced in certain strains of rats, such as Fawn-hooded or athymic rats (Andersen et al., 2020).

As far as PH is categorised into five groups that differ significantly from each other, it is difficult to create a universal PH model. In addition, even pulmonary arterial hypertension or group 1 PH is heterogeneous. There is no ideal experimental PH model that fully represents all the symptomatic, histopathological, molecular etc. features of human PH. Ryan et al. suggested that histologically, chronic hypoxia plus SU-5416 best matches PAH in that it develops complex vascular lesions. On the other hand, the monocrotaline model can be induced to manifest complex vascular lesions and does manifest the tendency of PAH patients to die of RVF. However, the use of murine models is recommended to elucidate the molecular pathogenesis (Ryan, Marsboom, and Archer, 2013). The most commonly used animal/rodent models in PH are listed in Table 1.2. Animal models show different degrees of progression from RVH to RVF (Bogaard et al., 2009 a).

Table 1.2

Rodent models of pulmonary hypertension

Method of PH induction	Model of PH	Species	Condition	RV remodeling	RV function
Monocrotaline	Inflammatory PAH	Rat	High dose (60–80 mg·kg^{-1})	Maladaptive	Reduced
		Rat	Low dose (20–40 mg·kg^{-1})	Adaptive	Maintained
Chronic hypoxia	High-altitude, chronic lung disease	Rat	–	Adaptive	Maintained
		Mouse	–	Adaptive	Maintained
Sugen + hypoxia	PAH	Rat	–	Maladaptive	Reduced
		Mouse	–	Adaptive	Moderately reduced
Pulmonary artery binding	Isolated RV pressure overload	Rat/mouse	Mild constriction	Adaptive	Maintained
		Rat/mouse	Severe constriction	Maladaptive	Reduced

PAH – pulmonary arterial hypertension; PH – pulmonary hypertension. Adapted from: (Dignam et al., 2022)

1.4.1 Monocrotaline-induced right ventricular failure model

Monocrotaline (MCT) is a pyrrolizidine alkaloid derived from the plant *Crotalaria spectabilis*. The MCT alkaloid is activated to the reactive pyrrole metabolite dehydromonocrotaline (MCTP) in the liver by cytochrome P-450 3A4 (CYP3A4) (Wilson et al., 1992; Reid et al., 1998). Once metabolised in the liver, MCTP is harmful to pulmonary endothelial cells and induces pulmonary arterial hypertension in rats (Bueno-Beti et al., 2018). A single subcutaneous injection of 60–80 mg/kg of monocrotaline in rats results in the development of pulmonary hypertension within 2 to 3 weeks. MCT causes disruption of intracellular membrane trafficking, disruption of endothelial nitric synthase, dysregulation of nitric oxide (NO) signalling, pulmonary mononuclear vasculitis, induction of endothelial cell apoptosis, pulmonary arterial medial hypertrophy and smooth muscle cell proliferation, obstructive pulmonary vascular remodelling characterised by vascular lumen narrowing and obliteration, and right ventricular hypertrophy (Bueno-Beti et al., 2018; Mathew and Lakshminrusimha, 2018; Dignam et al., 2022). RVH is present by week three (Meyrick, Gamble, and Reid, 1980; Dignam et al., 2022), and rapid progression of RVF is further noted, with a 100 % mortality rate at the week 6–8 (Urboniene et al., 2010; Dignam et al., 2022) (Figure 1.2). The MCT injury is not limited to the pulmonary arterial vasculature, but also causes alveolar oedema, interstitial fibrosis, pulmonary venous occlusion, and myocarditis. The advantages of this model are its technical simplicity, reproducibility, and low cost (Bueno-Beti et al., 2018; Mathew and Lakshminrusimha, 2018; Dignam et al., 2022). Nevertheless, it is crucial to mention the MCT PH model has some disadvantages or additional features, for example, the lung parenchyma – fibrosis, hypoxaemia, alveolar oedema and septal cell hyperplasia – is more involved when compared to human PAH. A marked perivascular inflammatory component and no complex lesions are characteristic of the MCT model (Carman et al., 2019; Wilson et al., 1992). In conclusion, MCT injection causes a broad spectrum of toxicity, the so called monocrotaline syndrome, which includes acute lung injury, interstitial pulmonary fibrosis, necrotizing pulmonary arteritis, pulmonary hypertension, RVH, myocarditis, hepatic venooclusive disease, renal insufficiency (Gomez-Arroyo et al., 2012; Dignam et al., 2022). Monocrotaline administration reduces cardiac mitochondrial function at the level of complexes I, II and IV of the respiratory chain. The submitochondrial particles produced more O2- and there was increase in the malondialdehyde content of the cardiac tissue (Guarnieri and Muscari, 1988).

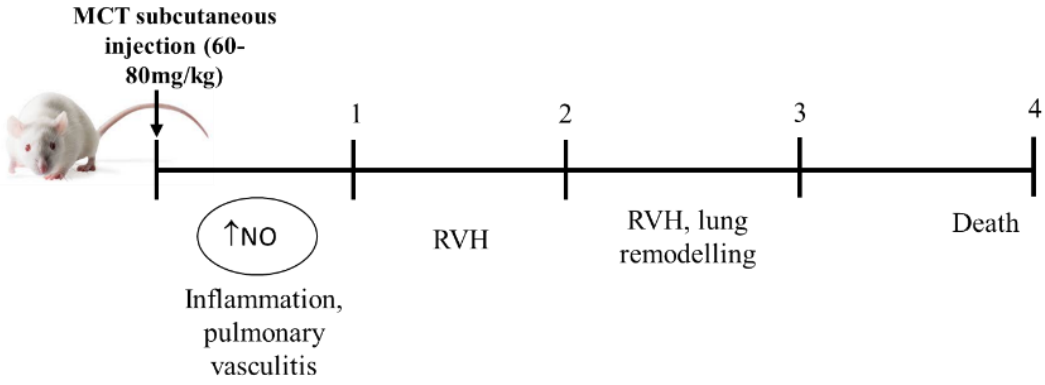

Figure 1.2 **The progression of pulmonary arterial hypertensions and right ventricular failure in monocrotaline rat model**

MCT – monocrotaline, NO – nitric oxide, RVH – right ventricular hypertrophy

1.5 Right ventricular hypertrophy and right ventricular failure assessment in small animals

The adaptive RVH in small animals is characterised by RV hypertrophy and largely preserved RV volumes. (Bogaard et al., 2009 b; Dignam et al., 2022). Assessment of failing RV in animal studies is done by echocardiography or cardiac magnetic resonance imaging. RV function and hypertrophy should be assessed by the Fulton index (the weight ratio of right ventricle/left ventricle + septum), RV volumes, RVEF, RVFAC TAPSE and cardiac output. In animal models of RVF, forced exercise capacity can be assessed by a treadmill test and voluntary exercise capacity by spontaneous activity in a running wheel (Dignam et al., 2022).

1.6 Therapy for chronic right ventricular failure

Therapy for RVF is based on 3 options: reducing afterload, optimising preload, and increasing contractility (Lahm et al., 2010). The best described treatment option in the literature is reduction of afterload, particularly in the patients with pulmonary hypertension. Etiological therapy for PAH and CTEPH targets the pulmonary circulation but has limited effect on RVF (Ren, Johns, and Gao, 2019). Treatment modalities for RVF depend on whether the RVF is acute or chronic, and can be categorised into pharmacotherapy and mechanical circulatory support (Ren, Johns, and Gao, 2019).

To prevent progression of RVF, the primary goal is to treat the underlying disease. Pharmacological treatment of the PAH is divided into 4 groups targeting different mechanisms: 1) calcium channel blockers, 2) nitric oxide bioavailability/synthesis enhancers, 3) endothelin receptor antagonists (ERAs), 4) activation of prostacyclin receptors. Treatment of CTEPH is based on pulmonary endarterectomy; inoperable disease is treated with riociguat and pulmonary

balloon angioplasty. PH due to left heart disease and lung diseases is based on optimisation of the underlying condition (Humbert et al., 2022).

RVF is associated with volume overload leading to RV dilatation, increased myocardial wall tension, tricuspid regurgitation and venous congestion. Optimisation of intravascular fluid status to minimise RV dilatation is essential and is achieved with diuretics. There are no randomised controlled trials evaluating the benefit, type, or dose of diuretic to be administered in patients with RVF (Chizinga and Fares, 2018). Oral torasemide is the treatment of choice to reduce volume overload. The activation of the renin–angiotensin-aldosterone system in case of RVF necessitates the addition of aldosterone antagonists to loop diuretics in order to increase diuresis (Chizinga and Fares, 2018; Sauler, Fares, and Trow, 2013).

1.6.1 Beta-blockers and chronic RVF

β-blockers (BABs) are the cornerstone in patients with left-sided systolic heart failure, but the failing RV may not tolerate BABs due to their negative inotropic and antihypertensive effects (Ren, Johns, and Gao, 2019). The variations in treatment options differ between different PH-induced RVF groups. For example, in heart failure patients with reduced ejection fraction, β-blocker therapy has significant efficacy in limiting RV remodelling and improving RV function (Galves et al., 2020). According to the 2015 European Society of Cardiology and European Respiratory Society guidelines for the diagnosis and treatment of pulmonary hypertension, patients with severe PH and RV dysfunction rely heavily on changes in heart rate to maintain CO. Therefore, it is generally considered that BABs are contraindicated in these patients unless required to treat a comorbidity (Galie et al., 2016). No clear data are available to determine if these short-term haemodynamic risks might be offset by the potential positive long-term beneficial neurohormonal, vascular and cardiac remodelling effects during long-term BAB therapy in RVF (Zelt et al., 2019). Different BABs have been tested in pre-clinical models to elucidate the benefits of BABs in RVF, however a role for β-blockade in the treatment of RVF remains undetermined.

Beta-blockers in preclinical models for right ventricular failure

In rats with induced angioproliferative pulmonary hypertension, carvedilol treatment resulted in increased exercise endurance, improved RV function (increased tricuspid annular plane systolic excursion and decreased RV dilatation) and an increased cardiac output. The effects of carvedilol were associated with an enhancement of RV fetal gene reactivation, increased protein kinase G (PKG) activity and a reduction in capillary rarefaction and fibrosis.

The effects of metoprolol were similar (Bogaard et al., 2010). In an experimental model of PH induced in rats by a single injection of monocrotaline (60 mg/kg), bisoprolol delayed the time to right ventricular failure ($p < 0.05$), increased RV contractility and filling (both $p < 0.01$), and partially restored right ventriculo-arterial coupling and cardiac output (both $p < 0.05$). Bisoprolol restored RV β-adrenergic receptor signalling. Histology revealed significantly less RV fibrosis and myocardial inflammation in bisoprolol-treated PH rats (De Man et al., 2012). Andersen et al. in 2015 summarized some animal studies on the effects of β-blocker treatment on RVF by preventing or reversing it. The different models of RVF were used in the animal studies, the study duration also varied from 2-6 weeks with mostly beneficial effects on RVF (Andersen et al., 2015). However, existing animal models mimicking PAH all have limitations and therefore these preclinical results do not justify treating patients with PAH with β-blockers before appropriate clinical trials.

Beta-blockers in PAH patients with right ventricular failure

The potential choice of β-blockers in PAH patients may be determined by their specificity (Perros et al., 2017). Different β-blockers may not only have different effects on beta-1 and beta-2 receptors (Perros et al., 2017), but some of them (nebivolol and carvedilol) also have NO-stimulating effects leading to vasodilatation in pulmonary arteries (Perros et al., 2015; Perros et al., 2017). Andersen et al. in 2015 also focused on PAH-induced RVF and provided a summary of published data on the use of BABs in case of RVF. Peacock et al. in 2010 and Provencher et al. in 2006 studied patients with portopulmonary hypertension, however data from Grinnan et al. 2014 and So et al. 2012 in patients with PAH showed no clear result about the positive effect of BABs on RVF. The summary of BABs in PAH and RVF populations is summarised in Table 1.3.

Table 1.3

The summary of the treatment with BAB in PAH and RVF patient population

Study population	N, Number of patients	Treatment	Study design	Findings
PAH patients (Grinnan et al., 2014)	6	Carvedilol, 6 months	Single-arm, prospective pilot study	• improvement in RVEF and RV stroke volume • increase in BNP levels • trend (p = 0.22) toward increase in 6MWT
PAH patients (So et al., 2012)	94	Various AR-blockers, 20 months	Two-arm, prospective cohort study	No differences in: • adverse events including PAH-related hospitalization or all-cause mortality (p = 0.19) • presence of right HF by last visit (p = 0.75) • change in 6MWT (p = 0.92)
PAH patients (Bandyopadhyay et al., 2015)	568 (508 analysed)	Different BAB	Observational analysis	• No significant difference was noted in probability of survival and time to clinical worsening events. • Patients on BAB walked a shorter distance on follow-up 6MWT. • Follow-up NYHA class was similar between groups.
PAH (Thenappan et al., 2014)	564	Different BAB	Observational analysis	No difference in absolute mortality between those with and without BAB
IPAH patients (J.S.J.A. et al., 2016)	18	6 months of bisoprolol	Randomised, placebo-controlled, crossover, single centre study	Bisoprolol was associated with: • lower HR (17 beats per minute, p = 0.0001) • unchanged RVEF • a drop in CI (p = 0.015) • a trend towards a decreased 6MWT
IPAH patients (Martynyuk, Konosova, and Chazova, 2012)	12	24-week therapy with nebivolol	–	• increased 6MWT distance • a drop in BDs and FC • reduced the anteroposterior dimensions of the RV • reduction in the level of ET-1
PH patients, mainly PAH (Farha et al., 2017)	30	Carvedilol, 24 weeks	A single-center, double-blind, randomized, controlled trial	No change in: • 6MWT • CAMPHOR questionnaire

AR-blockers – adrenergic receptor blockers; BAB – β-blockers; BDS – Borg dyspnoea score; BNP – brain natriuretic peptide; CAMPHOR – Cambridge Pulmonary Hypertension Outcome Review; CI – cardiac index; ET-1 – endothelin-1; FC – functional class; HR – heart rate; IPAH – Idiopathic pulmonary arterial hypertension; NYHA – New York Heart Association; PAH – pulmonary arterial hypertension; RVEF – right ventricular ejection fraction; RV – right ventricle; 6MWT – the six-minute walk test. Partly adapted from: (Perros et al., 2017; Andersen et al., 2015)

1.6.2 Mineralocorticoid receptor antagonists and RVF

Mineralocorticoid receptor antagonists (MRA) are the cornerstone of HF treatment in patients with LV HF. So far, the evidence from preclinical trials with MRA and PH-induced RVF are debatable. MRA attenuates RV fibrosis, hypertrophy, Fulton index and reduces right ventricular systolic pressure, (Boehm et al., 2018; Kowalski et al., 2021; Preston et al., 2013), however, these data were only observed in one PH model (Mamazhakypov, Hein, and Lother, 2022).

The retrospective data on the addition of spironolactone to ambrisentan for the treatment of PAH from the Ambrisentan in Pulmonary Arterial Hypertension, Randomized, Double-Blind, Placebo-Controlled, Multicenter, Efficacy Study (ARIES) 1 and 2 trials showed that ambrisentan plus spironolactone had a trend towards improvement in the change in 6MWT and plasma B-type natriuretic peptide concentration. Improvement in WHO FC was also observed (Maron et al., 2013). To summarise other clinical studies, it seems that MRA are prescribed for patients with severe and progressive PH (Corkish et al., 2019; Lahm et al., 2021; Andersson et al., 2019). There are 2 ongoing clinical trials: the STAR-HF trial and the Spironolactone for Pulmonary Arterial Hypertension trial to evaluate the efficacy of spironolactone in patients with PAH as well as impact on RV function and 6MWT (NCT03344159, 2017; NCT01712620, 2012; Mamazhakypov, Hein, and Lother, 2022). The summary of MRA in the PAH and RVF populations is shown in Table 1.4.

Table 1.4

The summary of the treatment with MRA in PAH and RVF patient population

Study population	N, number of patients	Treatment	Study design	Findings
PAH patients (Corkish et al., 2019)	69	MRA	a single-center, retrospective review	• higher rate of all-cause hospitalization during the treatment period with MRA • no difference in the rates of hospitalization for worsening HF or PH
PH patients (Lahm et al., 2021)	24221	MRA	Retrospective study	• worse survival in MRA group • not associated with worse survival after adjustment for disease severity • MRA indicates severity of PH

Table 1.4 continued

Study population	N, number of patients	Treatment	Study design	Findings
PAH patients (Maron et al., 2013)	88	spironolactone	Retrospective analysis from the ARIES-1 and ARIES-2 trials	Improved in MRA group: • 6MWT • BNP concentration • WHO FC
PAH patients (Safdar et al., 2020)	(42 enrolled) 35 completed	spironolactone	randomized double-blinded crossover clinical trial	• No change in 6MWT • Safe and well-tolerated

BNP – brain natriuretic peptide; FC – functional class; PAH – pulmonary arterial hypertension; PH – pulmonary hypertension; WHO – World Health Organisation; 6MWT – the six-minute walk test. Partly adapted from: (Mamazhakypov, Hein, and Lother, 2022)

1.6.3 Treatment with digoxin in patients with chronic right ventricular failure

In case of pulmonary arterial hypertension, digoxin is used as adjunctive therapy along with diuretics, oral anticoagulants and calcium channel blockers if the vasoreactivity test is positive. Digoxin has been shown to improve CO acutely in IPAH, although its efficacy in chronic use is unknown (Rich et al., 1998). It may be given to slow the ventricular rate in patients with PAH who develop atrial tachyarrhythmias (Humbert et al., 2022). There are a few studies on the use of digoxin in patients with PH and RVF (Table 1.5).

Table 1.5

The summary of the digoxin effects on PAH and RVF patient population

Author	Study population	Study design and period	Findings	Source
Saucedo et al.	275 PAH patients with right ventricular dysfunction	Retrospective cohort study – 1984 - 2016	No benefit on survival, except for rehospitalization and FC	ERS International Congress abstract, 2019 (Saucedo et al., 2019)
Eshtehardi et al.	2,208 patients with isolated RVD secondary to severe PH	From 2002 to 2012 consecutive patientsfrom 3 hospitals	Lower 1-year mortality	(Eshtehardi et al., 2014)
Rich et al.	17 patients with primary PH and RVF	Prospective study	↑ CO and ↓ circulating norepinephrine, ↑ atrial natriuretic peptide	(Rich et al., 1998)

CO – cardiac output; FC – functional class; PAH – pulmonary arterial hypertension; PH – pulmonary hypertension; RVD – right ventricular dysfunction; RVF – right ventricular failure

1.7 Therapy for altered metabolism in patients with right ventricular failure

Ranolazine, a persistent or late inward sodium current (IN_a) inhibitor and chronic angina treatment (Coppini et al., 2013), is a partial inhibitor of FAO that has been shown to have a beneficial effect on RV function, decrease RVH and increase exercise capacity in PH models due to a shift from FAO to GO (Fang et al., 2012). Khan et al. conducted a 3-month, prospective, open-label pilot study of ranolazine therapy at a dose of 1,000 mg twice daily in patients with symptomatic PAH (n = 11). Three months of ranolazine therapy improved patients' FC, reduced RV size, and improved RV strain during exercise. In the same study, ranolazine therapy tended to increase patients' exercise capacity in the bicycle echocardiography test, 6-minute walk test (6MWT) from 383 ± 60 m to 419 ± 80 m, and KCCQ (Kansas City Cardiomyopathy Questionnaire) summary score, but there were no improvement in haemodynamic parameters assessed by RHC (Khan et al., 2015). In patients with precapillary PH and RV dysfunction, ranolazine improved the RV ejection fraction (RVEF) but had no effect on New York Heart Association (NYHA) class, 6MWT scores or NT-proBNP levels (Han et al., 2021).

Trimetazidine, an anti-ischemic (anti-anginal) metabolic agent (Marzilli et al., 2019), is also a partial FAO inhibitor (Fang et al., 2012). The beneficial effects of repeated administration of trimetazidine in MCT-induced RVH have been shown to increase O_2-consumption and improve cardiac mitochondrial function, while reducing the formation of oxygen free radicals (Guarnieri and Muscari, 1990; Guarnieri and Muscari, 1988; Archer et al., 2013). Phase 2 randomised controlled trial "The Role of Trimetazidine on Right Ventricle Function in Pulmonary Arterial Hypertension Patients in National Cardiovascular Center Harapan Kita Hospital Indonesia" evaluates whether trimetazidine 35 mg bid for 3 months versus placebo 1 pill bid for 3 months on top of their regular PAH specific therapy will change right ventricular ejection fraction assessed by cardiac magnetic resonance imaging (MRI) at 3 months intervention minus with RVEF at baseline as the primary outcome. A total of 26 patients were enrolled in a female-dominated study, 2 patients in both groups died due to right ventricular failure and one patient in each group withdrew from the study. RVEF as assessed by cardiac MRI at 3 months after the intervention minus with RVEF at baseline was higher in the trimetazidine group than in the placebo group (3.9 versus –2.8, respectively, $p = 0.008$). Functional capacity as assessed by SF-36 score after 3 month intervention minus functional capacity at baseline was significantly higher in trimetazidine group compared to the placebo group (11.5 versus –17.73, respectively, $p = 0.0002$). The mean baseline SF-36 functional capacity score for the trimetazidine group was 46.54, compared to 61.15 for the placebo group (NCT03273387, 2017; Sakti Muliawan et al., 2020). Another study assessing the efficacy

of trimetazidine showed an improvement in myocardial velocity on tissue Doppler imaging in both ventricles of patients with heart failure (Gunes et al., 2009). Metformin therapy for PH in preclinical models has shown benefits for the RV through various molecular mechanisms, however, there is still lack of clinical data (Prins et al., 2019). Targeting oxidative stress, which plays a central role in the development of RVH, is also a novel direction for the treatment of RVF. Resveratrol is one of the medications that showed efficacy in the preclinical studies, however, no data from the clinical setting (Paffett, Lucas, and Campen, 2012). Similar results are mentioned for dichloracetate, angiotensin-converting enzyme 2 (Ren, Johns, and Gao, 2019).

To sum up, repurposing medications to treat RVF seems to be a safe approach, but it usually targets a single pathway that causes RVF, as demonstrated in one of the preclinical models of PAH. However, many of these drugs fail to demonstrate efficacy in clinical models due to the complexity of the underlying disease and concomitant RV failure. New potential therapeutic targets for RVFinclude metabolic imbalance, inflammation and oxidative stress (Klinke et al., 2020). A summary of the treatment of RVF is presented in Figure 1.3.

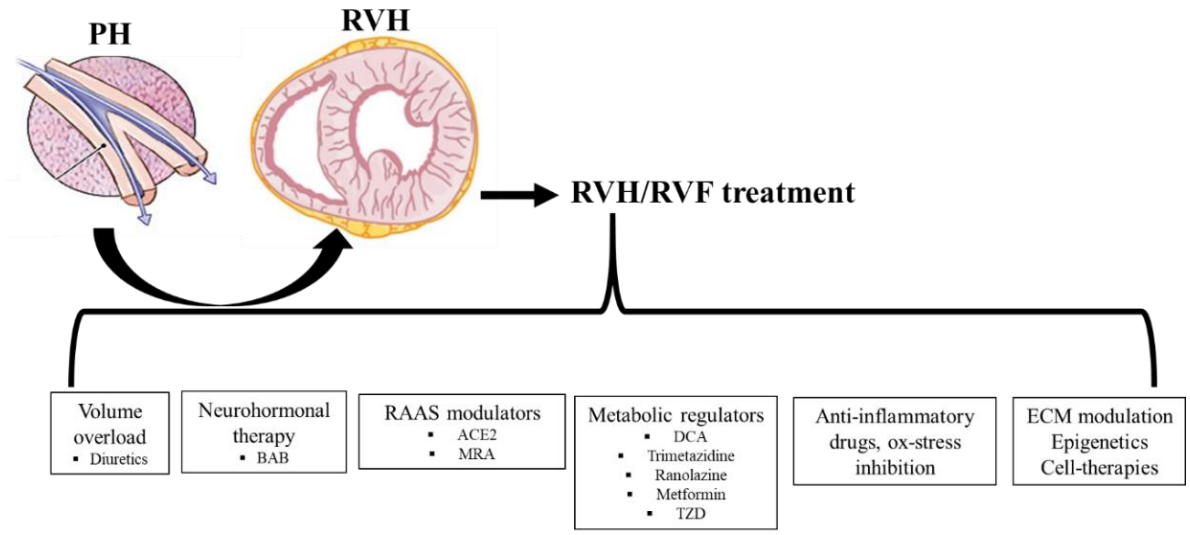

Figure 1.3 **Therapy options for RVF**

ACE2 – angiotensin-converting enzyme 2, BAB – β-blocker, DCA – dichloroacetate, MRA – Mineralocorticoid receptor antagonist, RAAS – renin-angiotensin-aldosterone system, TZD – thiazolidinediones, ox-stress – oxidative stress

1.8 Meldonium

Meldonium, also known as mildronate, MET-88 or 3-(2,2,2-trimethylhydrazinium) propionate dihydrate, is the cardioprotective drug. The mechanism of action of meldonium is based on the regulation of energy metabolism pathways through an L-carnitine and acylcarnitine level-lowering effects. The mechanism of action of meldonium is the competitive

inhibition of the enzyme γ-butyrobetaine hydroxylase (BBOX), which suppresses L-carnitine biosynthesis, inhibition of the organic cation/carnitine transporter type 2 protein (OCTN2) in kidneys, which reduces L-carnitine levels, as well as at higher concentrations meldonium inhibits carnitine acetyltransferase (CrAT) and carnitine/acylcarnitine translocase (CACT) (Dambrova et al., 2016).

The cardioprotective mechanism of action is based on the ability of the meldonium to redirect long-chain FA metabolism from mitochondria to peroxisomes. Together with the meldonium-induced decrease in L-carnitine, there is a decrease in CPT1 and CACT activity, which inhibits long-chain fatty acids from overloading mitochondria and facilitates β-oxidation. At the same time, meldonium stimulates glucose utilisation, which helps to restore the balance of lipid and glucose metabolism balance in the cardiomyocytes (Dambrova et al., 2016).

Meldonium has shown cardioprotective effects in various preclinical models (Dambrova et al., 2016). Occlusion of the left coronary artery followed by reperfusion in rats has shown that 10 days of treatment with meldonium reduces the size of myocardial infarction in an experimental model (Sesti et al., 2006). In another model, meldonium was also shown to protect the outer mitochondrial membrane and reduce fatty acid transport in cardiac mitochondria, which is the anti-infarction activity (Kuka et al., 2012). In both models, meldonium administration had no effect on haemodynamic parameters (Sesti et al., 2006; Kuka et al., 2012). In rats with congestive heart failure after myocardial infarction, treatment with meldonium prevented left ventricular remodelling, reduced the increase in right atrial pressure (RAP) in rats with heart failure, enhanced the functional adaptability of the heart to increased stress and improved myocardial energetics (Hayashi et al., 2000). In an experimental model of type 2 diabetes in Goto-Kakizaki rats, meldonium was shown to have cardioprotective effects, reduce blood glucose concentrations and prevent loss of pain sensitivity (Liepinsh et al., 2009).

1.9 Meldonium in the clinical trials

Meldonium is registered in Latvia and approved for clinical use in patients with stable angina in Latvia, Lithuania, Russia, Ukraine and Georgia. No serious adverse effects have been reported by the manufacturer so far (Schobersberger et al., 2017). Allergic reactions (redness and itching of the skin, urticaria, rash, and/or angioedema), dyspepsia, tachycardia, and changes (increase or decrease) in blood pressure are reported as rare adverse reactions (Berlato and Bairros, 2020). Evidence from clinical trials reports effects of meldonium in coronary heart disease and left-sided heart failure.

Additional therapy with meldonium at 500 mg BID for 3 months in patients with coronary heart disease (CHD) showed an increase in the mean change in the total exercise time confirmed by bicycle ergometry (Dzerve, 2011). Long-term outcomes in patients with stable angina pectoris showed that the treatment with meldonium 1 g daily for 12 months in combination with a standard therapy was superior to placebo for the exercise tolerance (Dzerve et al., 2010).

The evaluation of oxidative stress, haemodynamics and quality of life in patients with cardiovascular disease under extreme climatic conditions reported that meldonium 500 mg dayly reduced systolic blood pressure (BP) and heart rate during heat, increased sodium levels and improved quality of life (M.d et al., 2014). The cardioprotective effect of meldonium was also demonstrated in terms of secondary prevention after percutaneous coronary intervention, increasing exercise duration, decreasing maximum ST-segment depression, decreasing ST segment recovery time to baseline on ECG (Lyamina et al., 2014). Statsenko et al. studied the use of meldonium 1000 mg/day intravenously in patients with CHF as part of combination therapy in the early post-infarction period in addition to the basic therapy. Clinical improvement and the favourable changes in cardiac structural and functional parameters and heart rate variability were observed in the meldonium group (Statsenko, Shilina, and Turkina, 2014).

In 2005, it was reported that the efficacy of combined treatment of chronic HF with mildronate and the angiotensin-converting enzyme inhibitor lisinopril is superior to the treatment of chronic HF with lisinopril alone. The study group included the patients with chronic HF NYHA class I-III due to coronary heart disease. Symptom severity was determined by assessing the functional class of chronic HF, and symptoms were rated on a five-point scale at each visit. Exercise tolerance was assessed by bicycle ergometry, and the quality of life was assessed using the Minessota Living With Heart Failure questionnaire and the Health Satisfaction Score. The study group concluded that the addition of meldonium to lisinopril in left heart failure due to CHD improves the quality of life, exercise capacity and peripheral circulatory mechanisms (Dzerve et al., 2005). The improvement in the six-minute walk test (6MWT) with meldonium 1000 mg daily plus lisinopril 20 mg daily was 25 metres in comparison to 14 metres in the control group. One publication showed that in the patients (n = 16) with CHF, the addition of meldonium increased the pulmonary arterial flow acceleration time and shortened RV isovolumic relaxation time and TEI index (Tsverava, 2013). Meldonium 1000 mg a day for 3 months improved the 30-item Multidimensional Fatigue Symptom Inventory-Short Form (MFSI-SF) score, particularly reducing symptoms such as fatigue in HF patients with reduced ejection fraction (Key et al., 2021).

Besides the use of meldonium in clinics it is widely used among athletes for hypothetical benefits on physical performance and has been associated with doping in the world of sports. The ergogenic effects are considered to be due to the redirection of long-chain fatty acids from mitochondria to peroxisomes, decreased lactic acid production, increased glycogen storage and utilisation, increased glucose availability in muscles, decreased oxidative stress, and therefore improved cardiac activity, improved circulation and oxygenation at the CNS level, improved post-exercise recovery and aerobic resistance (Berlato and Bairros, 2020; Lippi and Mattiuzzi, 2017). Thus, these favourable effects of meldonium could potentially be applied to patients suffering from RVF.

Taken together previously published results from preclinical experiments and clinical studies, meldonium could improve the altered energy metabolism in cardiomyocytes and thus improve RV function in case of RVF and, moreover, enhance the quality of life of RVF patients. Thus, these favorable effects of meldonium could potentially be applied in patients suffering from RVF.

2 Methods

The Thesis is structured into three sections, as presented in Figure 2.1.

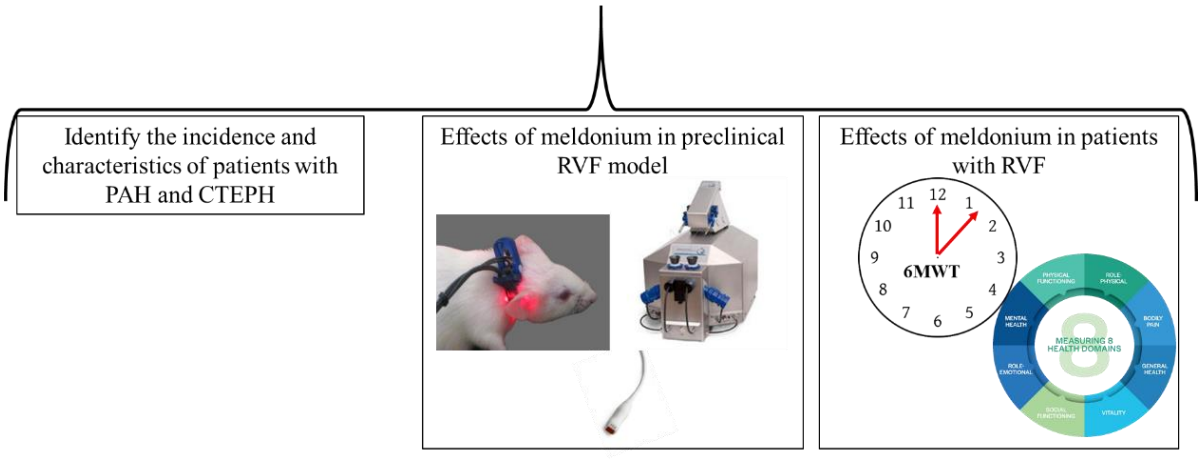

Figure 2.1 **Schematic representation of the thesis structure**

The visual representation partly adapted from: https://www.qualitymetric.com/health-surveys/the-sf-36v2-health-survey/

2.1 PAH and CTEPH incidence in Latvia

Between January 1 and December 31 of 2021, a prospective observational study conducted at Pauls Stradiņš Clinical University Hospital in Riga examined the Latvian Pulmonary Hypertension (PH) registry. Ethical approval was obtained from the Pauls Stradins Clinical University Hospital (Atzinums Nr. 250123-12L) before the initiation of the study. The study included a total of 18 patients with pulmonary arterial hypertension (PAH) and 8 patients with chronic thromboembolic pulmonary hypertension (CTEPH). Patients were included in the study based on diagnostic criteria consistent with the 6th World Symposium on Pulmonary Hypertension Task Force (Condon et al., 2019). To confirm the diagnosis of PH the right heart catheterization was performed in all patients, which measured the following haemodynamic parameters: RAP, mPAP, pulmonary capillary wedge pressure (PCWP), PVR, CO and cardiac index (CI).

2.2 Effects of meldonium in preclinical RVF model

Eight-week-old 100 male Sprague-Dawley rats were obtained from Charles River Laboratories (Sulzfeld, Germany). Animals were housed in individually ventilated cages (three rats per cage) with unlimited access to food (R70 diet, Lantmännen Lantbruk, Sweden) and water. Standard housing conditions (temperature of 21–23 °C, 12-hour light/dark cycle and relative humidity of 50 ± 5 %). Rats were adapted to these housing conditions for at least one

week before the beginning of the experiments. The experimental procedures were performed in accordance with the guidelines of the European Community as well as local laws and policies, and the procedures were approved by the Latvian Animal Protection Ethical Committee of the Food and Veterinary Service, Riga, Latvia. All studies involving animals were reported in accordance with the ARRIVE guidelines (Kilkenny et al., 2010; McGrath et al., 2010). The experiments evaluating right ventricular functionality found that due to interindividual variability, a sample size of 8–10 animals per group is needed to obtain statistically significant results. As a result, n = 10–12 per group was chosen. The statistical power analysis of data from previous experiments investigating mitochondrial energy metabolism showed that a sample size of at least n = 5 or 6 per group is necessary to achieve significant results with a power > 0.95 in the mitochondrial respiration assay (Videja et al., 2021).

The meldonium at a dose of 200 mg/kg was chosen as meldonium at this dose exert cardioprotective effects and modifies energy metabolism in accordance with the previous invivo models. This dose is equivalent to the dose 1000 mg a day in humans (Dambrova et al., 2016).

To study the effects of meldonium on RVF development, 34 rats were used. To study the effects of meldonium on the development of endothelial dysfunction in pulmonary arteries, 36 rats were used and to study the effects of meldonium on blood oxygen saturation with experimental pulmonary hypertension models, 30 rats were used. All experimental procedures and analyses were performed by a scientific staff blinded to the treatment groups and the experimental groups were uncovered only after summarizing results.

2.2.1 Study design of pulmonary hypertension and right ventricular failure model

Pulmonary hypertension and RVF in rats were induced by a single subcutaneous injection of monocrotaline (MCT) at a dose of 60 mg/kg in 24 animals. The induction of PAH in rats by a single MCT injection results in notable elevation of RV pressure and remodeling of pulmonary vasculature, along with increased RVH after 4 weeks (Bueno-Beti et al., 2018). The MCT model at a dose of 60 mg/kg causes the RVSP to fluctuate between 34.9–79.2 mmHg and results in in a range of 0.51–0.95 for the right ventricle/left ventricle + septum (Benoist et al., 2011; Handoko et al., 2009; Kasahara et al., 1997; Mitani, Maruyama, and Sakurai, 1997; Gomez-Arroyo et al., 2012). Control group rats (n = 10) received an injection of an equal volume of saline. Rats that received MCT were randomly allocated to two equal groups (n = 12). The animals in the MCT group continued to receive purified drinking water, while the rats in the MCT + Meldonium group started to receive meldonium at a dose of 200 mg/kg

together with purified drinking water for four weeks (Figure 2.2). The weight of the animals was monitored twice per week after the administration of MCT or vehicle.

After 4 weeks of treatment, the echocardiography and direct measurement of systolic RV pressure were performed. The rats were euthanized, and the pulmonary and cardiac tissues were harvested for histological analysis and assessment of mitochondrial functionality. The heart was cut out and divided into the right ventricle and the left ventricle with the septum. Both parts were weighted separately to calculate the Fulton index. Additional heart tissues from both ventricles were used to prepare permeabilized cardiac fibers to assess the functionality of mitochondria. The lungs were collected and weighed to calculate the lung-to-body weight index and prepared for histological analysis to visually assess the extent of fibrosis.

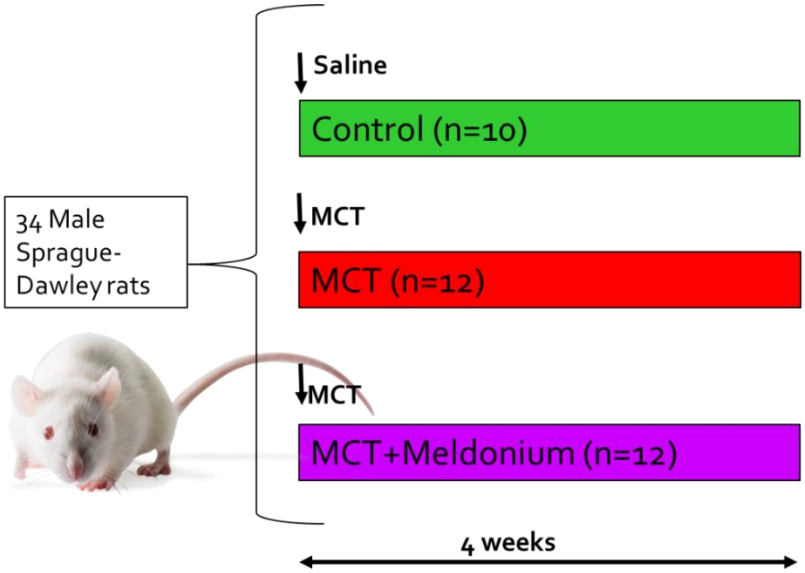

Figure 2.2 **Schematic representation of the study design**

2.2.2 Echocardiographic assessment of cardiac function in rats

Echocardiography was performed using Philips iE33 ultrasonograph (Philips Healthcare, Andover, MA) 28 days after the administration of MCT. The rats were anaesthetized with isoflurane (2 %) dissolved in 100 % oxygen. After the onset of anesthesia, the chest and upper part of the abdomen were shaved, and the animals were connected to the Philips ultrasound system to record the ECG from the second standard lead. ECG was used to determine the exact time of RV systole and diastole. Animals were placed on the left side, and an apical 4-chamber view was recorded to analyse the dimensions and functioning of the right ventricle (end-diastolic area (EDA) of the right ventricle, end-systolic area (ESA) of the right ventricle and fractional area change of the right ventricle (RVFAC) using an S12-4 sector array transducer. Next, the animals were placed in the decubitus position. M-mode tracings of the left

ventricle were recorded at the papillary muscle level with a linear L15-7io transducer. The following parameters of the LV were obtained – ejection fraction (EF), fractional shortening (FS), cardiac output (CO), LV posterior wall thickness at end-systole (LVPWs), stimulated posterior wall thickness at end-diastole (LVPWd), interventricular septal thickness at end-systole (IVSs), interventricular septal thickness at end-diastole (IVSd), LV internal dimension at end-systole (LVIDs), LV internal dimension at end-diastole (LVIDd), end systolic volume (ESV) and end diastolic volume (EDV).

2.2.3 Systolic right ventricular pressure

After the echocardiographic parameters were recorded, the rats were intubated using a 16G intravenous catheter and artificially ventilated with a tidal volume of 1.5 ml/100 g animal with 2 % isoflurane dissolved in 100 % oxygen. Median sternotomy was performed, and a 21G needle connected to a pressure transducer (ADInstruments) was inserted into the right ventricle and fixated while the RV systolic pressure reached a plateau.

2.2.4 Vascular reactivity of pulmonary arteries

The vascular reactivity was evaluated in isolated pulmonary arteries using methods previously described with minor adjustments (Nakazawa et al., 1999; Mathew et al., 1995). Twenty-four rats received subcutaneous injection of MCT at a dose of 60 mg/kg. Control group animals (n = 12) received an injection of an equal volume of saline. Rats that received MCT were randomly divided into two equal groups (n = 12). The animals from the first group (MCT group) continued to receive purified drinking water, while the rats from the second group (MCT + Meldonium) started to receive meldonium at a dose of 200 mg/kg together with purified drinking water for two weeks. Rats were sacrificed by decapitation and exsanguination. The heart and lungs were removed en bloc, and the right and left extrapulmonary arteries were cleaned from surrounding tissues, dissected and cut into ~3 mm width rings. The obtained artery rings were mounted between two platinum hooks and incubated in organ baths in Krebs–Henseleit (K-H) solution (composition (in mM): NaCl 118, $CaCl_2$ 2.5, $MgCl_2$ 1.64, $NaHCO_3$ 24.88, KH_2PO_4 1.18, glucose 10.0, and EDTA 0.05), pH 7.4 at 37 °C. The pulmonary artery rings were stretched to a resting tension of 0.5 g and equilibrated to the new conditions for 60 min. During the adaptation period, the incubation buffer solution was changed every 15 min. At the beginning of each experiment, the maximal contraction force of each ring was determined by adding 80 mM potassium chloride. Afterwards, the artery rings were washed until the resting tension was restored. After that, the pulmonary artery rings were precontracted

with phenylephrine to 60 %-80 % of maximal contraction until a stable plateau contraction was reached. Endothelium-dependent relaxation was assessed by adding cumulative concentrations of acetylcholine (10^{-9} to 10^{-5} mol/L). Furthermore, the pulmonary artery rings were washed until the resting tension was restored and once again precontracted with phenylephrine to 60–80 % of maximal contraction until a stable plateau contraction was reached. Endothelium-independent relaxation was assessed by adding cumulative concentrations of sodium nitroprusside (10^{-10} to 10^{-5} mol/L).

2.2.5 Oxygen saturation in arterial blood

To study the effects of meldonium treatment on blood oxygen saturation in an experimental model of RVF, 30 male Sprague-Dawley rats were used. Twenty animals received subcutaneous injection of MCT at a dose of 60 mg/kg. Control group animals (n = 10) received an injection of an equal volume of saline. Rats that received MCT were randomly divided into two equal groups (n = 10). The animals from the first group (MCT group) continued to receive purified drinking water, while the rats from the second group (MCT + Meldonium) started to receive meldonium at a dose of 200 mg/kg together with purified drinking water for two weeks. Blood oxygen saturation (SpO_2), respiratory rate and heart rate were measured before the administration of saline or MCT and once every week for 4 weeks using a pulse oximeter (MouseOx® Plus Pulse Oximeter for Rodents). Measurements were performed in awake and freely moving rats using a specialized collar (MouseOx - Collar clip Sensor), which was placed on the neck of the rat.

2.2.6 Mitochondrial functionality assessment

Mitochondrial function was assessed in permeabilized cardiac fibers that had been prepared as previously described (Kuka et al., 2012). Mitochondrial respiration was measured at 37 °C using an Oxygraph-2k respirometer (O2k; Oroboros Instruments, Innsbruck, Austria) in MiR05 medium (110 mM sucrose; 60 mM K-lactobionate; 0.5 mM EGTA; 3 mM $MgCl_2$; 20 mM taurine; 10 mM KH_2PO_4; 20 mM HEPES, pH 7.1; and 0.1 % BSA essentially free of FA).

The following protocol was used to evaluate mitochondrial functionality in the MCT experiments. Palmitoylcarnitine and malate (10 μM and 0.5 mM, respectively) were added to measure fatty acid oxidation (FAO)-dependent mitochondrial respiration (F(N)-pathway) in the LEAK (L) substrate-dependent, state. Next, ADP was added at a concentration of 5 mM to initiate oxidative phosphorylation-dependent respiration (OXPHOS state). Then, pyruvate

(5 mM, complex I substrate, N-pathway) was added to reconstitute FN pathway-linked respiration. Succinate (10 mM, complex II substrate, S-pathway) was added to reconstitute convergent FNS-linked respiration. Then, rotenone (0.5 μM, an inhibitor of complex I) and antimycin A (2.5 μM, an inhibitor of complex III) were added to determine the S-linked respiration and residual oxygen consumption (ROX), respectively.

To determine the contribution of each substrate to the respiration rate, the flux control factor was calculated as follows:

$$1 - \frac{\text{Resp.rate before the addition of substrate}}{\text{Resp.rate after the addition of substrate}}.$$

In addition, mitochondrial function during in vitro anoxia-reoxygenation was determined in permeabilized cardiac fibers prepared from the left ventricle of the MCT study animals as described previous. Respiration measurements with simultaneous H_2O_2 flux detection were performed in MiR05 using an Oxygraph-2k respirometer. To induce anoxia, the maximal respiration rate of the sample was induced by the addition of substrates succinate (10 mM) with rotenone (0.5 μM) and ADP (5 mM), and the preparation was allowed to consume all the O_2 in the respiratory chamber (within 10–20 min), thereby entering an anoxic state. After 30 min of anoxia, O_2 was reintroduced to the chamber by opening the chamber. After 8 min of reoxygenation, the chamber was closed, and O_2 flux was monitored for an additional 2 min. At the end of the experiment, antimycin A (2.5 μM) was added to determine the ROX level. H_2O_2 flux (ROS flux) was measured simultaneously by respirometry as described previously (Makrecka-Kuka, Krumschnabel, and Gnaiger, 2015). The effect of anoxia-reoxygenation-induced damage was calculated as the ratio from baseline values (i.e., from normoxia, the state before anoxia induction).

2.2.7 Histological preparations

Lungs and part of the myocardium of the right ventricle from the MCT-treated rats were embedded in OCT freezing media, frozen and cut into serial 10-μm thick sections, and processed for staining. Masson's trichrome staining was performed to confirm fibrosis in the lungs and right ventricle. The stained slides were analysed under a Nikon Eclipse TE300 inverted microscope.

2.3 Effects of meldonium in patients with RVF

This was an investigator-initiated observational study conducted in P. Stradins Clinical University Hospital, Riga, Latvia. Patients ≥ 18 years of age who fulfilled the criteria for right ventricular failure due to Group 1 PAH and were classified as World Health Organization (WHO) FC I to III were enrolled in the study. Each patient with symptomatic PAH belonged to one of the following 2018 Clinical Group 1 subtypes: idiopathic PAH (IPAH), heritable PAH, or PAH associated with connective tissue disease (CTD PAH). Written informed consent was obtained from all patients before the study. Ethical approval was obtained from the Pauls Stradins Clinical University Hospital (Atzinums Nr. 030221-8L) and the State Agency of Medicines of the Republic of Latvia (16.03.2021) before the initiation of the study. All experiments were performed in accordance with the State Agency of Medicines of the Republic of Latvia. Meldonium was provided by JSC Grindeks without any financial support during the study.

The study consisted of an initial visit, a safety control visit after 14 days, an examination of the patient after 30 days of treatment ± 7 days, and a health checkup after a washout period of 30 days ± 7 days. The study procedures included a clinical visit with a physical examination; completion of the SF-36; assessment of the Borg dyspnoea score (BDS); administration of the 6MWT; laboratory testing, such as full blood count, liver and kidney function tests and B-type natriuretic peptide (BNP); assessment of WHO FC; and determination of adverse events. The 6MWT was assessed in a flat, straight, enclosed corridor that was 30 metres long and clearly marked with the start point, a distance marker every 3 metres, and the end point. The 6MWT was performed as described in the guidelines (Crapo et al., 2002). Study participants completed a quality-of-life questionnaire (SF-36) at three time-points: before treatment, just after treatment, and one month after the end of treatment. The Medical Outcomes Study Short-Form 36 general health survey (SF-36) is a generic, coherent questionnaire widely used in PH patients to measure health-related quality of life (QoL). The SF-36 questionnaire consists of eight health concepts or domains: physical functioning (10 items), social functioning (2 items), role limitations due to physical health (4 items), role limitations due to emotional problems (3 items), emotional well-being (5 items), energy/fatigue (4 items), pain (2 items), general health (5 items) and a single health transition item. The domain scores are converted into a range of 0-100, where higher scores indicate better health status (Ware and Sherbourne, 1992; Hays et al., 2016). I state that in producing the translation in Latvian and Russian languages, I followed the specifications provided by RAND Health Care. In addition, at the same time

points, all participants underwent a standardized 6MWT, BDS evaluation and assessment of WHO FC; laboratory testing, however, was performed only at visit 3 (Figure 2.3).

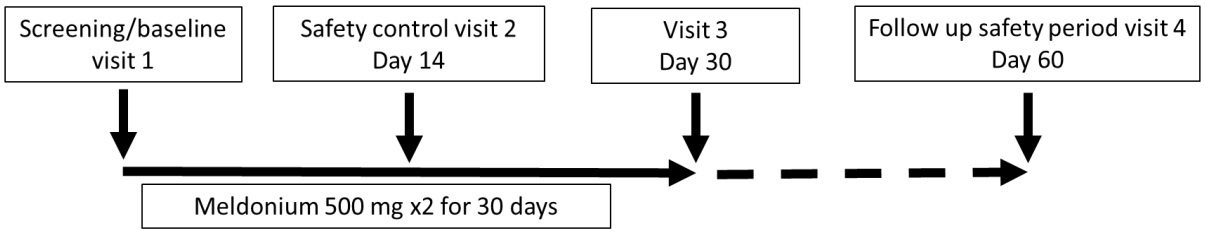

Figure 2.3 **Study schematic**

Right heart catheterization (RHC) was completed prior to the study in all patients to confirm the diagnosis of PAH according to the 6th World Symposium on Pulmonary Hypertension Task Force criteria: mean pulmonary arterial pressure (mPAP) of ≥ 20 mmHg and PCWP or LV end-diastolic pressure ≤ 15 mmHg and pulmonary vascular resistance ≥ 240 dynes/cm^{-5} (3 Wood units). Patients were on a stable treatment regimen with one or more treatments approved for primary disease and CHF. Stable therapy was defined as constant therapy for ≥ 12 weeks before the screening visit and a stable dosage of each medication for ≥ 8 weeks before the screening visit. Patients remained on their previously prescribed background medications for PH, heart failure and comorbidities without changing the dosage during the study period. None of the echocardiograms of the enrolled patients showed evidence of LV heart failure.

During the initial visit, patients received 60 meldonium (500 mg) capsules, which they were to take orally twice a day for the next 30 days. The treatment period with meldonium was selected based on the guidelines set by the State Agency of Medicines of the Republic of Latvia. In the event of a missed dose, patients were instructed to take the next dose as scheduled and not to compensate for the missed dose. After 30 days, patients returned the dispensed meldonium pack, and the return was recorded in the medical documentation. The next follow-up visits were scheduled one month and two months after the first visit. During the study, the occurrence of serious adverse effects was assessed. Serious adverse events were defined as a fatal or serious deterioration of health resulting in death, risk of death, hospitalization for > 24 hours, disability or incapacitation, or intervention to prevent a life-threatening condition.

3 Statistical analysis

Continuous variables in the PAH and CTEPH incidence study were presented as the mean ± standard deviation, while categorical variables were reported as counts and percentages. Statistical analysis was performed using SPSS version 22.0 (IBM Corp., USA).

Preclinical data were expressed as the means ± standard error of mean. Shapiro-Wilk normality test was used to assess the data distribution. One-way ANOVA with Dunnett's multiple comparisons test was used for data that were distributed normally. Kruskal-Wallis with Dunn's multiple comparison test was used for cases where the data were not distributed normally. Two-way repeated measures ANOVA with Tukey's multiple comparison test was used to compare the differences in weight gain, vascular reactivity and blood oxygen saturation between the experimental groups. P values < 0.05 were considered to indicate statistical significance. The statistical calculations and the creation of the figures were performed using GraphPad Prism software.

Continuous variables from the clinical study were expressed as the mean ± standard deviation. Categorical variables are displayed as counts and percentages. Differences in SF-36 scores, 6-minute walk distance, and laboratory parameter assessments were tested using one of two significance tests for continuous variables: the dependent-samples t test or the nonparametric Wilcoxon test. A p value < 0.05 was considered significant. Statistical analysis was performed using SPSS version 22.0 (IBM Corp., USA).

4 Results

4.1 Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension incidence in Latvia

The mean age at diagnosis of the PAH and CTEPH patients was 71.7 ± 12.2 and 72.8 ± 8.0 years, respectively. The mean body mass index of the PAH and CTEPH patients was 29.8 ± 6.1 kg/m^2 and 27.3 ± 5.1 kg/m^2, respectively. The female sex was predominant in the PAH patient group (72 % in PAH and 50 % in CTEPH group). The patient haemodynamic parameter analysis in the two study groups showed elevated pressures in the right heart catheterization (RAP of 5.8 ± 5.2 mmHg in the PAH patient group, 7.3 ± 5.8 mmHg in the CTEPH patient group and a mean pulmonary arterial pressure (mPAP) of 38.8 ± 15.2 mmHg in the PAH patient group, 45.1 ± 8.9 mmHg in the CTEPH patient group), as well as an increased vascular resistance in the pulmonary arterial tree (and a pulmonary vascular resistance (PVR) of 8.1 ± 4.6 Wood Units in the PAH patient group and 8.6 ± 3.3 Wood Units in the CTEPH patient group). Additionally, a notable portion of enrolled patients showed a decreased functional capacity (low 6-min walking test and high proportion of NYHA class III). The increased haemodynamic parameters and the decreased functional capacity for the PAH and the CTEPH patients indicate of a severe progression of the disease at the time of the diagnosis. The detailed baseline characteristics are shown in Table 4.1.

Risk stratification of PAH patients was performed analysing non-invasive parameters (6-min walking test, BNP and an echocardiography) and two variables derived from the right heart catheterization (CI, RAP). Patients were categorized as low, intermediate, or high risk, according to the method used by the Swedish Pulmonary Arterial Hypertension Registry (SPAHR) registry (Leuchte et al., 2018). At baseline 22.2 % of the patients were in the low-risk group, 72.2 % in the intermediate risk and 5.6 % in the high-risk group.

Table 4.1

Baseline characteristics of Latvian PAH and CTEPH patients

Parameter	PAH	CTEPH
Number of patients, n	18	8
Age, years	71.7 ± 12.2	72.8 ± 8.0
Females, n (%)	13 (72)	4 (50)
BMI, kg/m2	29.8 ± 6.1	27.3 ± 5.1
6 MWT, m	255.2 ± 100.1	296.6 ± 155.3
NYHA class I-II/III/IV, %	44.4/44.4/11.2	37.5/37.5/25
RAP, mmHg	5.8 ± 5.2	7.3 ± 5.8
RVSP, mmHg	65.7 ± 22.6	72.6 ± 13.6
mPAP, mmHg	38.8 ± 15.2	45.1 ± 8.9

Table 4.1 continued

Parameter	PAH	CTEPH
PCWP, mmHg	10.6 ± 5.7	6.7 ± 3.6
PVR, Wood units	8.1 ± 4.6	8.6 ± 3.3
CO, l/min	4.1 ± 1.3	4.7 ± 0.9
CI, l/min/m^2	2.3 ± 0.7	2.5 ± 0.4
BNP, pg/ml	359.9 ± 248.3	607.7 ± 421.7

Values are shown as frequencies and proportions (%) or mean ± standard deviation. CI – cardiac index; CO – cardiac output; BMI – body mass index; BNP – B-type Natriuretic Peptide; mPAP – mean pulmonary artery pressure; NYHA – New York Heart Association; PCWP – pulmonary capillary wedge pressure; PVR – pulmonary vascular resistance; RAP – right atrial pressure; RVSP – right ventricular systolic pressure; 6MWT – 6-min walking test.

After the confirmation of PAH diagnosis 15 patients (83.3 %) received a monotherapy of phosphodiesterase type 5 inhibitors (PDEi) and 2 patients (16.7 %) received a combination of PDEi and endothelin receptor antagonists. All CTEPH patients received a monotherapy of PDEi.

According to the official data acquired from http://data.csb.gov.lv, website accessed 16 March 2022, the number of the residents of Latvia in 2021 was 1.89 million (1.53 million of whom ≥ 18 years old). The estimated incidence of PAH in 2021 was 9.5 per million inhabitants, 11.7 per million adult population, respectively; whereas the incidence of CTEPH was 4.2 per million inhabitants, 5.0 per million adult population, respectively. During the study period one patient in each group died. The incidence of the PAH and the CTEPH patients included in the Latvian PH registry (data for the past five years) is summarized in Table 4.2.

Table 4.2

The last 5-year incidence of PAH and CTEPH patients from Latvian Pulmonary hypertension registry

	2021	2020	2019	2018	2017
PAH incidence (per million residents)	9.5	12.6	11.4	7.2	9.2
PAH incidence (per million adult residents)	11.7	15.5	14.1	9.0	11.3
CTEPH incidence (per million residents)	4.2	3.7	3.6	3.6	11.8
CTEPH incidence (per million adult residents)	5.0	4.5	4.5	4.5	14.5

Values are shown as frequencies. CTEPH – chronic thromboembolic pulmonary hypertension; PAH – pulmonary arterial hypertension. Table is published in: (Kigitovica et al., 2019).

4.2 Effects of meldonium in preclinical RVF model

4.2.1 Overall animal well-being

The health of the animals was monitored every day, and none of the animals died during the four-week treatment. The weight gain of the animals that received MCT injection was slower than that of the control group animals (Figure 4.1); however, the weight gain in the MCT + Meldonium group in the final week of the experiment was significantly faster than that

in the MCT group. During the 28-day observation period, the weight gain in the animals in the control, MCT and MCT + Meldonium groups was 86 ± 5, 26 ± 7 and 47 ± 4 g, respectively.

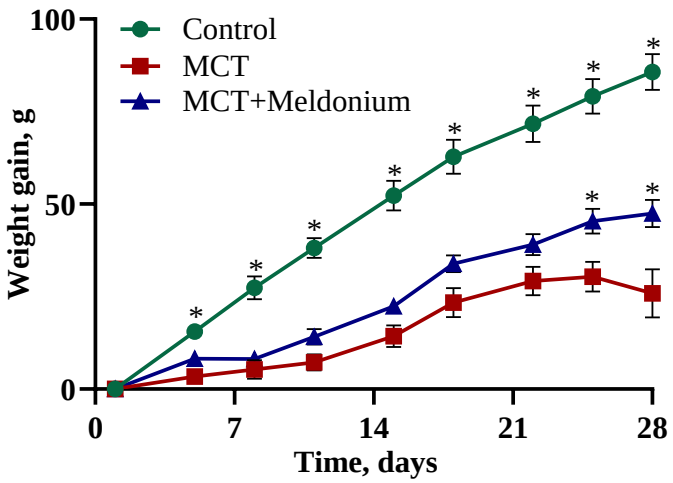

Figure 4.1 **Effects of treatment with meldonium on the weight gain of the animals**

Rats receiving meldonium gained weight significantly faster than animals from the MCT group. The results are shown as the mean ± SEM of 10-12 animals. *p < 0.05 vs. MCT group, two-way repeated measures ANOVA with Tukey's multiple comparisons test. MCT – monocrotaline

4.2.2 Effects of meldonium on ventricular size and function in the right ventricular failure model

RV systolic pressure was significantly increased in the MCT group compared to the control group, and treatment with meldonium did not attenuate the elevation in RV systolic pressure (Table 4.3). Administration of MCT induced the development of RVH, which was evident by an elevated right ventricle-to-body mass index and Fulton index (right ventricle/ (left ventricle + septum)). Treatment with meldonium attenuated the development of RVH. As shown in Table 4.3, the administration of MCT did not increase the left ventricle-to-body mass index, and the size of the left ventricle was not influenced by treatment with meldonium.

Table 4.3

Effects of meldonium administration on RV pressure, right ventricle-to-body, right ventricle-to-left ventricle and left ventricle-to-body weight indexes

	Control	MCT	MCT + Meldonium
RV systolic pressure, mmHg	19 ± 1*	52 ± 5	41 ± 4
RV-to-body mass index, mg/g	0.50 ± 0.01*	1.13 ± 0.06	0.88 ± 0.08*
Fulton index, g/g	0.27 ± 0.01*	0.53 ± 0.03	0.43 ± 0.04*
LV-to-body mass index, mg/g	1.8 ± 0.1	2.0 ± 0.1	2.0 ± 0.1

Treatment with meldonium significantly attenuated the development of RV hypertrophy and had no effect on RV pressure. The results are shown as the mean ± SEM of 10 to 12 animals. *$p < 0.05$ vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. LV – left ventricle, MCT – monocrotaline, RV – right ventricle

Echocardiograms of the right ventricle from five animals in the MCT group and four animals in the MCT + Meldonium group were excluded from the analysis due to poor echocardiogram quality, probably due to enlarged and fibrotic lungs that shadowed the heart during echocardiography. The analysis of echocardiographic parameters revealed that administration of MCT significantly increased the end-diastolic area (EDA) and end-systolic area (ESA) of the right ventricle (Figure 4.2 A). Treatment with meldonium attenuated the development of dilatation of the right ventricle and significantly decreased ESA; moreover, the results showed a tendency of meldonium treatment to decrease the EDA compared with that of the MCT group. The EDAs in the control, MCT and MCT + Meldonium groups were 0.5 ± 0.02, 0.9 ± 0.02 and 0.7 ± 0.07 cm^2, respectively. The ESAs in the control, MCT and MCT + Meldonium groups were 0.3 ± 0.02, 0.7 ± 0.06 and 0.5 ± 0.06 cm^2, respectively (Figure 4.2 B). In addition, MCT administration significantly decreased the RV fractional area change (RVFAC) by 42 %. Four weeks of treatment with meldonium significantly improved the functioning of the right ventricle and increased RVFAC by 40 % compared with the MCT group (Figure 4.2C).

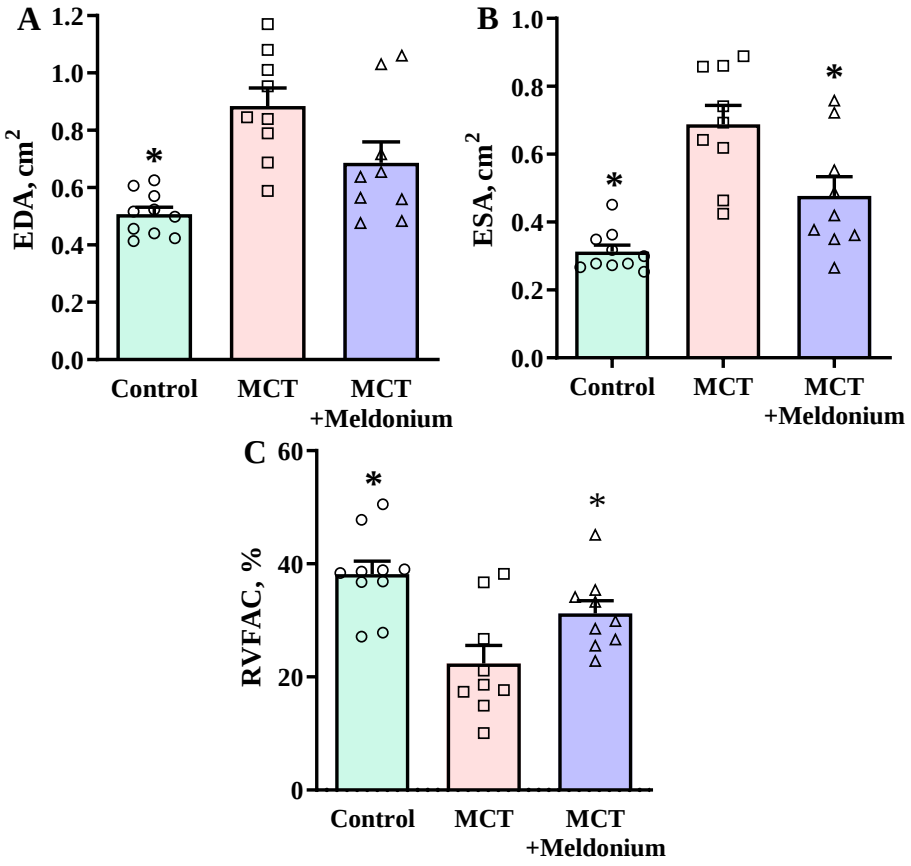

Figure 4.2 **Effects of meldonium administration on the (A) end-diastolic area (EDA) of the right ventricle, (B) end-systolic area (ESA) of the right ventricle and (C) fractional area change of the right ventricle (RVFAC)**

Treatment with meldonium significantly decreased ESA and increased RVFAC compared with the MCT group. The data are shown as the mean ± SEM of 7 to 10 animals. *p < 0.05 vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. MCT – monocrotaline

The analysis of the echocardiograms of the left ventricle demonstrated that 4 weeks after the administration of MCT, the rats had decreased end-diastolic volume, LV diameter at the end of diastole and cardiac output, although the ejection fraction and fractional shortening were unchanged (Table 4.4). Treatment with meldonium did not affect the dimensions or functioning of the left ventricle. In summary, these results indicate that treatment with meldonium attenuated the development of pulmonary hypertension-induced RV hypertrophy and failure.

Table 4.4

Effects of meldonium administration on the dimensions and functioning of the left ventricle

	Control	MCT	MCT + Meldonium
IVSs, mm	3.1 ± 0.1	3.3 ± 0.1	3.0 ± 0.2
IVSd, mm	1.6 ± 0.1	1.8 ± 0.2	1.6 ± 0.1
LVPWs, mm	3.2 ± 0.1	2.9 ± 0.1	3.0 ± 0.1
LVPWd, mm	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1
LVIDs, mm	4.9 ± 0.1	3.9 ± 0.3	4.3 ± 0.5
LVIDd, mm	9.0 ± 0.2*	7.2 ± 0.5	7.7 ± 0.5

	Control	MCT	MCT + Meldonium
ESV, ml	0.30 ± 0.02	0.19 ± 0.04	0.27 ± 0.06
EDV, ml	1.55 ± 0.09*	0.96 ± 0.15	1.10 ± 0.15
EF, %	81 ± 3	82 ± 4	80 ± 10
FS, %	45 ± 3	46 ± 4	46 ± 11
HR, bpm	310 ± 8	298 ± 8	305 ± 6
CO, l/min	0.39 ± 0.02*	0.26 ± 0.03	0.25 ± 0.03

Treatment with meldonium had no effect on MCT-induced alterations in functioning of the left ventricle. Heart rate (HR), ejection fraction (EF), fractional shortening (FS), cardiac output (CO), LV posterior wall thickness at end-systole (LVPWs), as well as stimulated posterior wall thickness at end-diastole (LVPWd), interventricular septal thickness at end-systole (IVSs), interventricular septal thickness at end-diastole (IVSd), LV internal dimension at end-systole (LVIDs), LV internal dimension at end-diastole (LVIDd), end systolic volume (ESV) and end diastolic volume (EDV) of the animals from all three groups. The data are shown as the mean ± SEM of 10 to 12 animals. *$p < 0.05$ vs. the MCR group, one-way ANOVA with Dunnett's multiple comparison test. MCT-monocrotaline

4.2.3 Effects of meldonium on mitochondrial function in the right ventricular failure model

In the MCT group, FAO (F(N)) pathway-dependent respiration in the OXPHOS state was significantly decreased, by 46 % (Figure 4.3 A), which resulted in a 23 % decrease in FAO-dependent OXPHOS coupling efficiency (Figure 4.3 B) compared with that in the control group. Moreover, despite stimulation of pyruvate metabolism, as indicated by flux control factor analysis (Figure 4.3 B), MCT administration decreased the FN and FNS pathway-linked respiration rates in the OXPHOS state (Figure 4.3 A). In addition, in the MCT group, partial dysfunction of complex I was observed, as indicated by a decrease in flux control factor upon rotenone treatment (Figure 4.3 B). Treatment with meldonium restored FAO-dependent OXPHOS coupling efficiency and subsequently decreased pyruvate metabolism and prevented complex I dysfunction (Figure 4.4 B). As a result, F(N), FN and FNS pathway-linked respiration in the OXPHOS state was improved in the MCT + Meldonium group (Figure 4.3 A). These results show that meldonium treatment normalizes mitochondrial function in the heart in conditions of pulmonary hypertension and RVF.

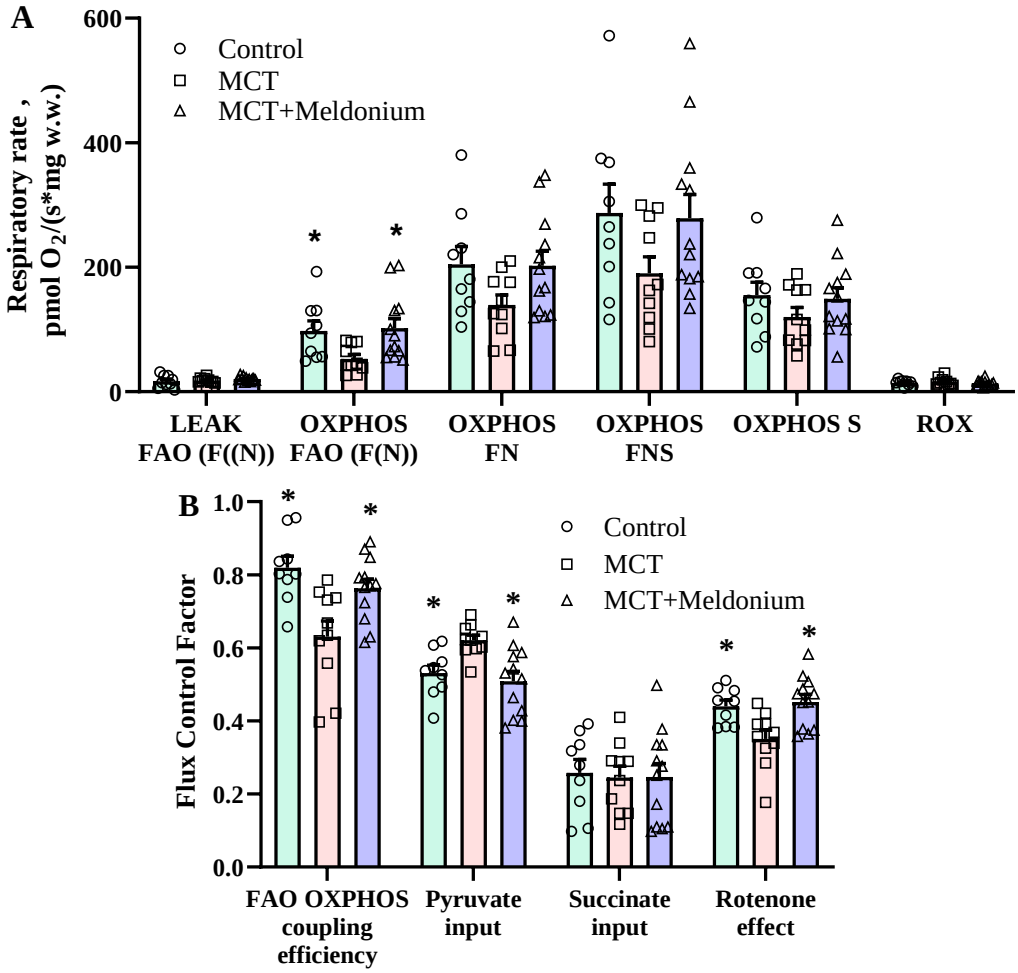

Figure 4.3 **The effects of meldonium treatment (200 mg/kg for 4 weeks) on the mitochondrial respiration rate (A) and flux control factors (B) in RV cardiac fibers 4 weeks after MCT injection**

MCT administration induced inhibition of FA-dependent oxidative phosphorylation, stimulation of pyruvate metabolism and partial complex I dysfunction. Treatment with meldonium restored mitochondrial functionality in the heart. The results are presented as the mean ± SEM of 9 to 12 animals. *p < 0.05 vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. Flux control factor – the contribution of each substrate/pathway to the respiration rate, S – succinate, F – FA oxidation-dependent pathway, N – NADH pathway, LEAK – substrate-dependent state, MCT – monocrotaline, OXPHOS – oxidative phosphorylation-dependent state, ROX – residual oxygen consumption

4.2.4 Effects of meldonium on mitochondrial H_2O_2 production after anoxia-reoxygenation in the right ventricular failure model

The next step was to evaluate the effects of meldonium treatment on ischemia-reperfusion-related conditions. Mitochondrial functionality was evaluated after *in vitro* anoxia-reoxygenation in left ventricular cardiac fibers. As shown in Figure 4.4, anoxia-reoxygenation induced an increase in the H_2O_2 production rate and H_2O_2/O flux ratio compared to normoxia in the control group. In the MCT group, the anoxia-reoxygenation-induced increases in the H_2O_2 production rate and H_2O_2/O ratio were 1.7- and 1.6-fold higher than those in the control group (Figure 4.4). Treatment with meldonium prevented the burst of H2O2 after anoxia-reoxygenation, as indicated by decreases in the H2O2 production rate and H2O2/O ratio

(Figure 4.4), suggesting that meldonium protected cardiac mitochondrial function against ischemia-reperfusion-induced injury in conditions of pulmonary hypertension and RVF.

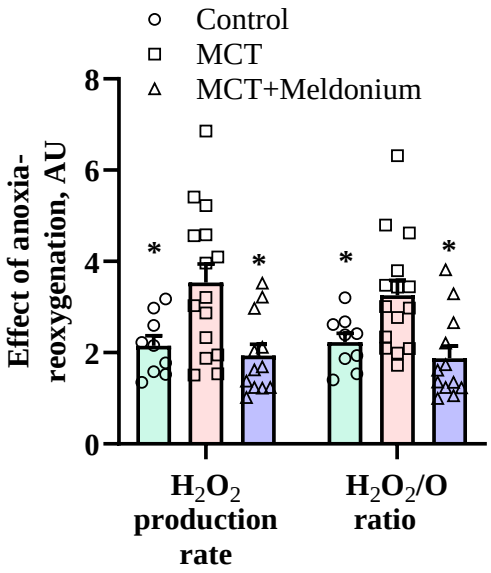

Figure 4.4 **The effects of meldonium treatment (200 mg/kg for 4 weeks) on mitochondrial function/ROS production after in vitro anoxia-reoxygenation in LV cardiac fibers in a model of pulmonary hypertension-induced right ventricle heart failure**

Anoxia-reoxygenation induced a significantly higher H_2O_2 production rate and H_2O_2/O ratio in the MCT group than in the control group. Treatment with meldonium decreased anoxia-reoxygenation-induced H_2O_2 production to the level of the control group. The data are shown as the mean ± SEM of 9 to12 experiments.
*$p < 0.05$ vs. MCT group, one-way ANOVA with Dunnett's multiple comparisons test. MCT – monocrotaline

4.2.5 The effects of meldonium on lung morphology, endothelial function and blood oxygen saturation in a right ventricular failure model

The analysis of lung-to-body weight indexes revealed that MCT administration increased the lung weight of the animals twofold (Figure 4.5). Treatment with meldonium for 4 weeks reduced the lung-to-body weight index by 16 %. ($p < 0.05$). The lung-to-body weight index in the control, MCT and MCT + Meldonium groups was 3.0 ± 0.1, 6.4 ± 0.4 and 5.4 ± 0.3, respectively. Moreover, Masson's trichrome-stained pulmonary slides revealed the development of massive fibrosis in the lungs of the animals in both the MCT and MCT + Meldonium groups (Figure 4.6 A, B, C).

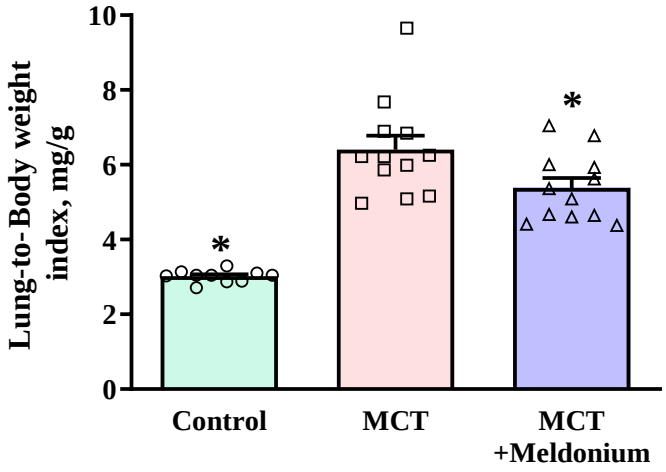

Figure 4.5 **Effects of treatment with meldonium on the lung-to-body weight index**

Four weeks after the admistration of MCT, animals from the MCT group had increased Lung-to-Body weight index. Treatment with meldonium decreased the elevated index. The data are shown as the mean ± SEM of 10 to 12 animals. *p < 0.05 vs. the MCT group, one-way ANOVA with Dunnett's multiple comparison test. MCT – monocrotaline

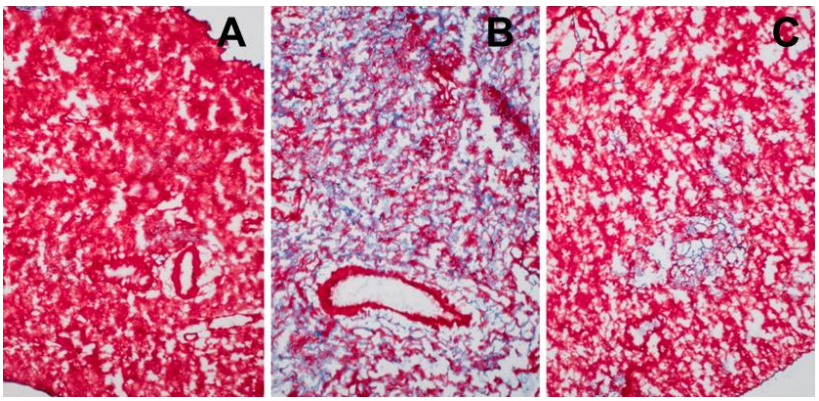

Figure 4.6 **Representative images of Masson's trichrome-stained sections of the lungs obtained from the (A) control, (B) MCT and (C) MCT + Meldonium groups**

Tissues stained in red represent normal lung tissue, but the blue tissues are connective tissues that form lung fibrosis. Scale bar denotes 200 μm. MCT – monocrotaline

Two weeks after administration of MCT, dysfunction of the vascular endothelium and smooth muscle cells was observed in the rings of pulmonary arteries (Figure 4.7 A, B). Treatment with meldonium did not improve MCT-altered vascular reactivity in pulmonary artery rings.

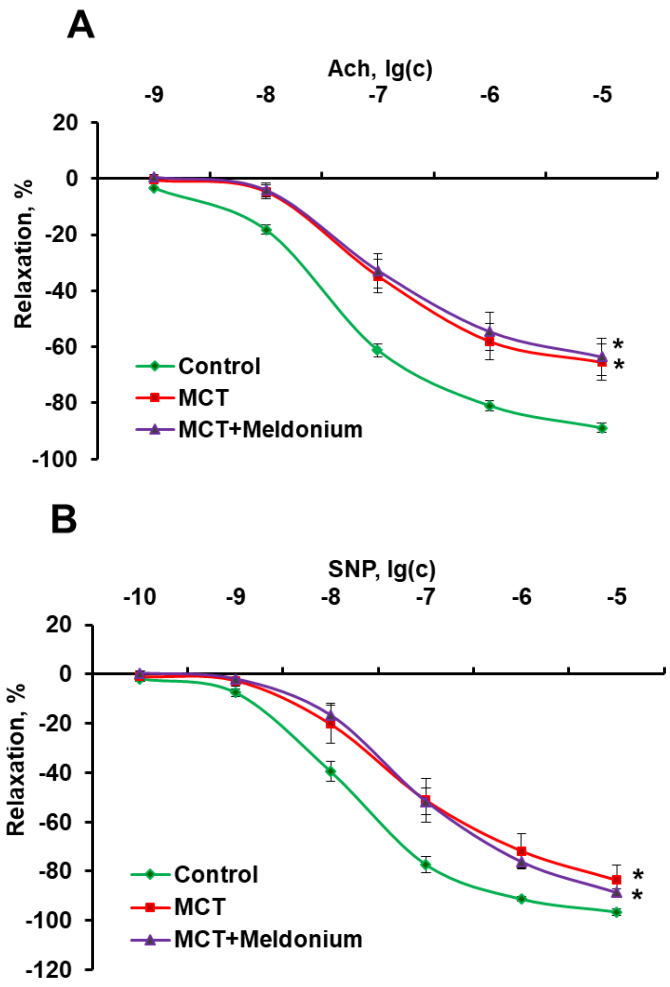

Figure 4.7 **Effects of meldonium treatment on endothelium-dependent (A) and endothelium-independent (B) relaxation of pulmonary artery rings**

Administration of MCT reduced endothelium-dependent and endothelium-independent relaxation of pulmonary artery rings. Treatment with meldonium had no effect on MCT-altered vascular reactivity. The results are shown as the mean ± SEM of 12 rats. *$p < 0.05$ vs. the MCT group, two-way repeated measures ANOVA with Tukey's multiple comparisons test. Ach – acetylcholine, MCT – monocrotaline, SNP – sodium nitroprusside

Before the administration of MCT, the average blood oxygen saturation was 96.3 ± 0.2 % (Figure 4.8). A significant decrease in blood oxygen saturation was achieved only four weeks after the injection of MCT. Treatment with meldonium did not prevent a decrease in arterial blood oxygen saturation levels. The SpO_2 level in the control, MCT and MCT + Meldonium groups after 4 weeks of treatment was 95.6 %, 94.2 % and 94.1 %, respectively. Administration of MCT or meldonium treatment did not affect the heart or respiratory rate.

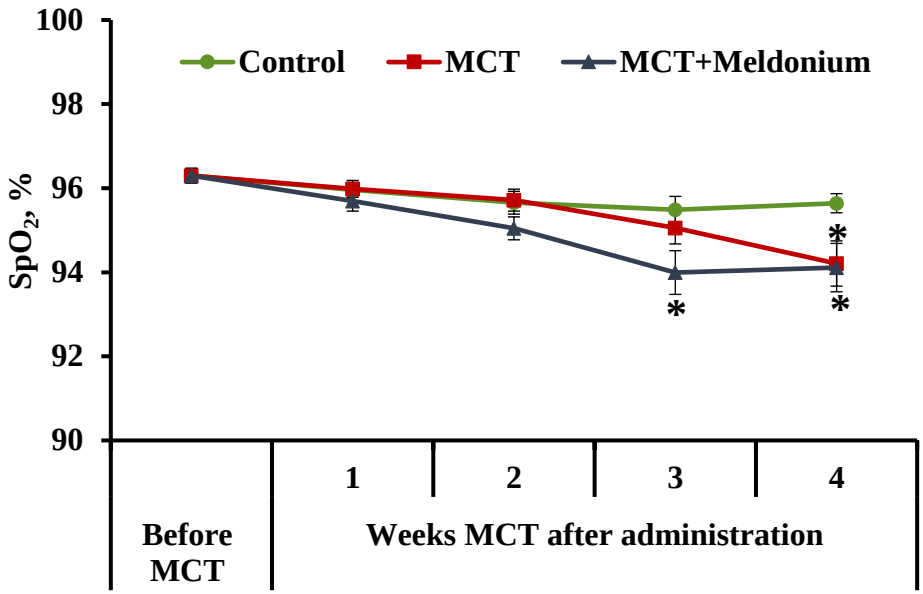

Figure 4.8 **Effects of meldonium treatment on oxygen saturation (SpO_2) in the blood of awake and freely moving rats**

Four weeks after administration of MCT, a significant reduction in SpO_2 level was noted. Treatment with meldonium did not increase the SpO_2 level four weeks after administration of MCT. The results are shown as the mean ± SEM of 10 rats. *$p < 0.05$ vs. the control group, two-way repeated measures ANOVA with Tukey's multiple comparison test. MCT – monocrotaline

4.3 Effects of meldonium in patients with RVF

A total of 22 patients who met the inclusion criteria were enrolled in the study from 2021 to 2022. Two patients refused to continue the study because they were unable to make onsite visits due to the COVID-19 pandemic. During the 2 months of the study, enrolled patients did not report any SAEs or other AEs. After 30 days, patients returned the dispensed meldonium pack. There were no instances of missed doses recorded. The mean age of the patients at the beginning of the study was 70.4 ± 13.2 years, the majority of patients (75 %) were female, and PAH was the primary disease – IPAH (n = 14) and CTD PAH (n = 6). The most common WHO FC at baseline was class III (65 %). The demographic and clinical characteristics of the patients, as well as their echocardiography and RHC, are presented in Table 4.5 and Table 4.6.

Table 4.5

Clinical characteristics of the patients

Characteristics	Overall study group; N = 20
Women, No. (%)	15 (75)
Age, mean ± SD, years	70.4 ± 13.2
BMI, kg/m^2	28.1 ± 6.0
Time from PAH diagnosis, median (IQR) range, days	834.5 (331.8–1488.5)
WHO FC II/III, (%)	35/65

Table 4.5 continued

Characteristics	Overall study group; N = 20
Comorbidity	
Arterial hypertension, No. (%)	13 (65)
CHD, No. (%)	4 (20)
VTE in anamnesis, No. (%)	0 (0)
Diabetes mellitus, No. (%)	3 (15)
Thyroid dysfunction, No. (%)	2 (10)
OSA, No. (%)	1 (5)
Current smoking, No. (%)	1 (5)
Medication	
Anticoagulants, No. (%)	12 (60)
Digoxin, No. (%)	11 (55)
Spironolactone, No. (%)	18 (90)
Loop diuretics, No. (%)	18 (90)
Statins, No. (%)	14 (70)
ACEis, ARBs, or ARNIs, No. (%)	6 (30)
ACEis or ARBs plus thiazide, No. (%)	3 (15)
PDE5is, No. (%)	10 (50)
PDE5is plus ERAs, No. (%)	4 (20)
PDE5is, ERAs, and CCBs, No. (%)	3 (15)
PDE5is plus CCBs, No. (%)	1 (5)
ERAs plus CCBs, No. (%)	2 (10)
Use of psychopharmacological drugs	
Benzodiazepines, No. (%)	6 (30)
Antipsychotics, No. (%)	2 (10)
Hypnotics, No. (%)	3 (15)

ACEis – angiotensin-converting enzyme inhibitor, ARBs – angiotensin receptor blockers, ARNIs – angiotensin receptor-neprilysin inhibitor, BMI – body mass index, CCBs – calcium channel blockers, CHD – coronary heart disease, ERAs – endothelin receptor antagonists, OSA – obstructive sleep apnea, PAH – pulmonary arterial hypertension, PDE5is – phosphodiesterase type 5 inhibitors, VTE – venous thromboembolism, WHO FC – World Health Organization functional class

Table 4.6

The findings from RHC and transthoracic echocardiography of the patients

Right heart catheterization	Measure, ± SD
RAP, mmHg	8.2 ± 4.7
Mean pulmonary arterial pressure, mmHg	42.0 ± 13.4
Cardiac output, L/min	4.6 ± 1.1
Cardiac index, L/min/m^2	2.2 ± 0.9
Pulmonary capillary wedge pressure, mmHg	12.4 ± 5.6
Pulmonary vascular resistance, Wood units	7.3 ± 5.1
Transthoracic echocardiography	
Aorta, mm	33.6 ± 4.5
LAVI, mL/m^2	44.4 ± 18.2
IVSd, mm	11.0 ± 2.0
PWd, mm	10.3 ± 1.5
LVEF, %	60.0 ± 4.2
LVEDD, mm	49.0 ± 6.8
LVESD, mm	30.6 ± 7.0
LVMI, g/m^2	107.2 ± 29.5

Table 4.6 continued

Transthoracic echocardiography	Measure, ± SD
RVOT, mm	30.7 ± 2.1
RVD, mm	41.3 ± 8.3
RVSP, mmHg	66.8 ± 16.2
TAPSE, mm	20.3 ± 3.4
IVC, mm	18.7 ± 4.2
IVC collapse, > 50 %	N = 13
Pericardial effusion	N = 0

IVC – inferior vena cava, IVSd – interventricular septum at end-diastole, LAVI – left atrial volume index, LVEDD – left ventricular end-diastolic diameter, LVEF – left ventricular ejection fraction, LVESD – left ventricular end-systolic diameter, LVMI – left ventricular mass index, PWd – posterior wall thickness, RAP – right atrial pressure, RVD – right ventricular diameter, RVOT – right ventricular outflow tract, RVSP – right ventricular systolic pressure, SD – standard deviation, TAPSE – tricuspid annular plane systolic excursion

The analysis of the 6MWT results revealed that patients were able to walk significantly longer distances after meldonium treatment than before (Figure 4.9 A). Before treatment, patients were able to walk 352.2 ± 114.8 m, but after 30 days of meldonium treatment, the walking distance increased to 398.9 ± 128.5 m ($p = 0.021$). On day 60 of the study, the results from the 6MWT demonstrated that the walking distance returned to the pretreatment value (376.7 ± 113.8 m, $p > 0.05$). In addition, treatment with meldonium markedly decreased the BDS (Figure 4.9 B) from the baseline score of 5.4 ± 2.2 to 3.4 ± 2.5 at day 30 ($p = 0.003$), and the effect persisted at day 60 with a score of 3.7 ± 2.5 ($p = 0.004$) (Figure 4.9 B).

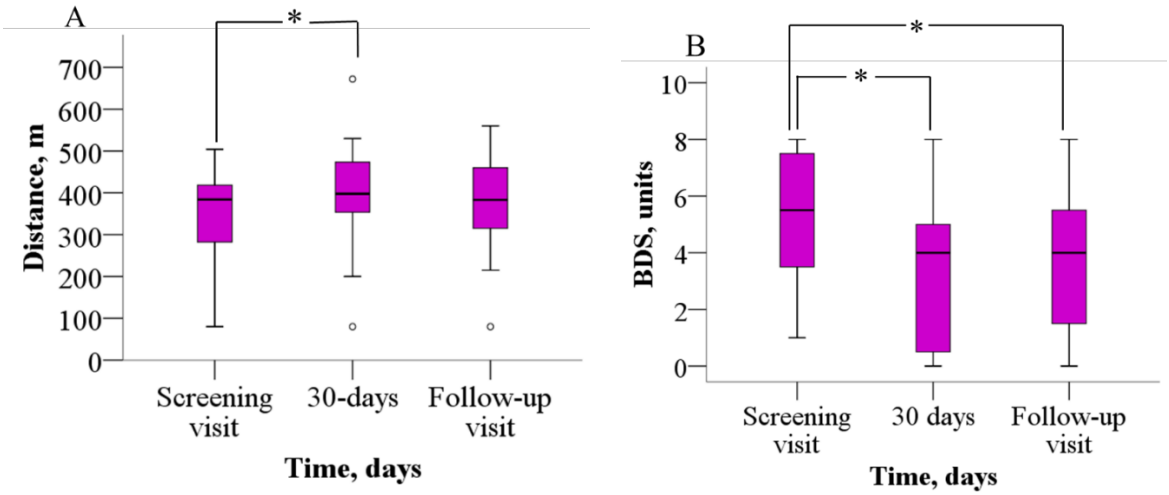

Figure 4.9 **The effect of meldonium on (A) 6MWT performance and (B) BDS**

The graphs represent the increase in 6MWT and decrease in BDS after treatment with meldonium. The results are shown as the mean ± SD of 20 patients; *$p < 0.05$ vs. the value as of the screening visit, paired-sample t test for 6MWT, Wilcoxon signed-rank test for BDS. BDS – Borg dyspnoea score, 6MWT – six-minute walk test

Thirteen study participants (65 %) reported advanced FC III symptoms at baseline, of whom 65 % improved to FC II symptoms (p = 0.031) by day 30, while the rest remained WHO FC III. After a 30-day washout period, 55 % of the patients were in WHO FC III (p > 0.05) (Figure 4.10).

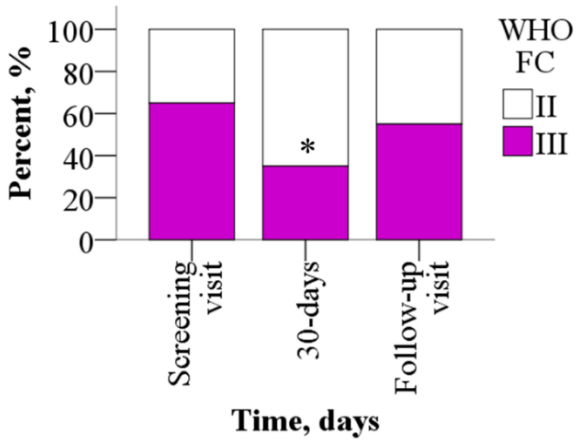

Figure 4.10 **WHO FC before and after treatment with meldonium**

Treatment with meldonium improved the functional class of the patients. The results are shown as percentages of 20 patients, *p < 0.05 vs. screening visit, chi-square test.
WHO FC – World Health Organization functional class

Heart rate and systemic blood pressure were within the normal range in the majority of patients (Table 4.7). No significant changes were noticed in the vital parameters after treatment with meldonium, except that there was a marked decrease in diastolic pressure after the 6MWT (p = 0.03) at day 30 and a decrease in heart rate after the 6MWT (p = 0.04) at day 60.

Table 4.7

Vital parameters of the patients during the study period

	SBP, mmHg	**DBP, mmHg**	**Heart rate, bpm**	**SpO2, %**	**RR, rpm**
Baseline measurement before 6MWT	134.6 ± 15.3	79.4 ± 9.7	71.4 ± 8.7	95.8 ± 2.2	17.9 ± 1.8
Baseline measurement after 6MWT	141.7 ± 18.9	80.5 ± 10.8	85.7 ± 13.9	92.2 ± 5.7	19.8 ± 3.5
Measurement before 6MWT after 30 days	132.1 ± 19.8	79.9 ± 10.9	76.2 ± 13.4	95.3 ± 3.9	18.1 ± 0.5
Measurement after 6MWT after 30 days	139.1 ± 19.6	74.4 ± 12.7*	82.9 ± 14.8	93.6 ± 4.3	20.5 ± 3.3

Table 4.7 continued

	SBP, mmHg	DBP, mmHg	Heart rate, bpm	SpO2, %	RR, rpm
Measurement before 6MWT after 60 days	130.6 ± 16.9	77.1 ± 11.8	72.9 ± 10.2	95.6 ± 2.3	17.9 ± 0.8
Measurement after 6MWT after 60 days	137.9 ± 19.2	80.1 ± 10.0	80.0 ± 14.3*	94.2 ± 4.0	20.3 ± 2.1

The results are shown as the mean ± SD of 20 patients. * $p < 0.05$ vs. baseline measurement; the parametric t test was used for heart rate, SBP and DBP before and after the 6MWT at baseline, 30 days and 60 days, and the nonparametric Wilcoxon signed-rank test was used for SpO_2 and RR at baseline, 30 days and 60 days. bpm – beats per minute, DBP – diastolic blood pressure, SBP – systolic blood pressure, rpm – respirations per minute, RR – respiration rate, SpO_2 – oxygen saturation, 6MWT – six-minute walk test

The SF-36 health-related quality of life scores for each patient are shown in Figure 4.11. SF-36 scores in 15 patients increased after 1 month of therapy with meldonium. However, 5 patients reported a decrease in their SF-36 score (Figure 4.11). The total SF-36 score increased after from 72.6 ± 17.7 points during the initial visit to 82.1 ± 14.8 points ($p = 0.009$) after 30 days of meldonium treatment. After the washout period, the SF-36 score decreased to 77.1 ± 17.7 points.

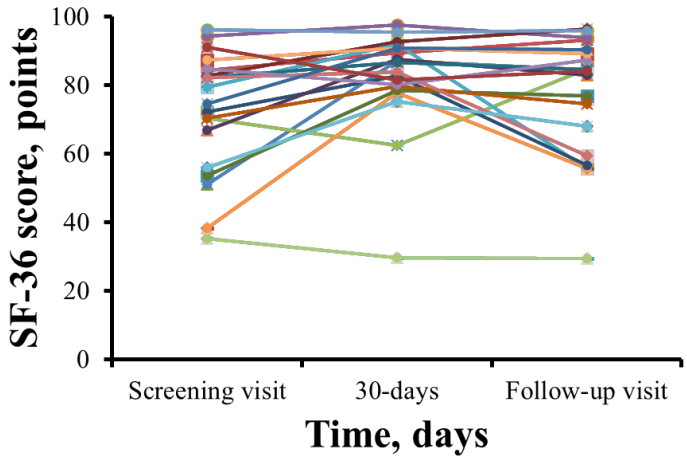

Figure 4.11 **Summary of 36-Item Short Form Health Survey results before and after treatment with meldonium**

In most patients, treatment with meldonium improved the SF-36 score at day 30, and a steady decline was noted at the follow-up visit. The results are shown for 20 patients

The mean mental component summary (MCS) of the SF-36 was 84.9 ± 15.6 points before the treatment, 87.7 ± 13.2 points ($p > 0.05$) after the 30-day treatment, and 83.6 ± 19.8 points at day 60 ($p > 0.05$). As shown in Figure 4.12, the physical component summary (PCS) score was 60.3 ± 23.1 points before treatment, but after 30 days and 60 days, scores decreased to 75.1 ± 17.6 and 70.7 ± 21.2 points, respectively ($p < 0.05$). Treatment with meldonium induced improvement in the Physical Functioning, Role–Physical and Bodily Pain domains of the SF-36.

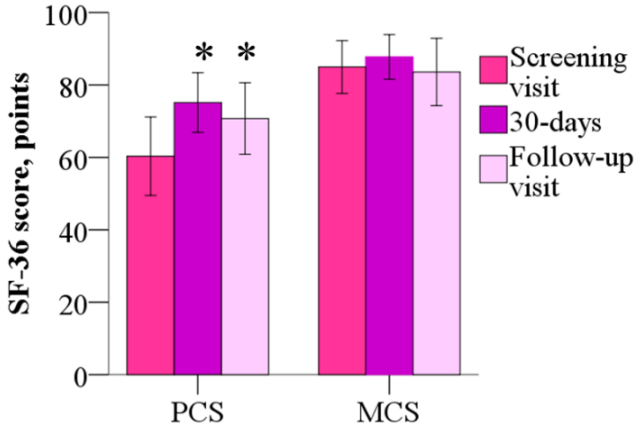

Figure 4.12 **The 36-Item Short Form Health Survey mental component summary and physical component summary before and after 30 days of treatment with meldonium**

Treatment with meldonium improved the functional capacity of the patients but had no effect on MCS. The results are shown as the mean of 20 patients; *p < 0.05 vs. PCS before the treatment, Wilcoxon signed-rank test. MCS – mental component summary; PCS – physical component summary

The MCS is composed of role limitations due to emotional problems, social functioning, emotional well-being, and energy/fatigue; none of these subscales showed improved scores after 30 days of treatment with meldonium or after 60 days from the beginning of the study. No changes were noted in the subscore for general health. PCS parameters such as physical functioning and bodily pain improved after 30 days and remained above the screening score at day 60 ($p < 0.05$), but role limitations due to physical health were improved only at day 30 (Table 4.8).

Table 4.8

SF-36 subscale scores at the baseline visit, after 30 days and at the follow-up visit

	Baseline measurement, points	**Measurement after 30 days, points**	**Measurement at follow-up, points**
Physical functioning	58.0 ± 25.9	71.0 ± 23.0*	67.8 ± 25.8*
Role limitations due to physical health	60.0 ± 42.5	85.0 ± 28.6*	73.8 ± 40.1
Role limitations due to emotional problems	90.0 ± 26.7	95.0 ± 16.3	88.3 ± 27.1
Energy/fatigue	76.5 ± 21.0	82.3 ± 15.2	76.0 ± 21.9
Emotional well-being	86.4 ± 15.9	86.2 ± 16.7	81.8 ± 17.1*
Social functioning	86.9 ± 21.6	92.5 ± 19.6	88.1 ± 23.5
Pain	67.5 ± 30.6	83.3 ± 24.1*	89.1 ± 16.7*
General health	55.8 ± 23.2	61.3 ± 20.5	52.3 ± 23.6

The results are shown as the mean ± SD of 20 patients. * p < 0.05 vs. baseline measurement, Wilcoxon signed-rank test.

There were no differences in blood cell counts, liver and kidney functional parameters or BNP levels across the three time points; thus, it can be inferred that these parameters were not influenced by meldonium treatment (Table 4.9).

Table 4.9

Results of the blood analysis of the patients before and after treatment with meldonium

	Baseline measurement	**Measurement after 30 days**
Erythrocytes, 10^12/L	4.5 ± 0.6	4.5 ± 0.6
Hemoglobin, g/L	135.0 ± 16.3	133.6 ± 14.3
Platelets, 10^9/L	219 ± 50.4	224.6 ± 54.3
Leucocytes, 10^9/L	7.1 ± 2.1	7.0 ± 1.7
Neutrophils, 10^9/L	4.7 ± 1.7	4.7 1.5
Urea, mmol/L	8.8 ± 2.9	8.8 2.7
Creatinine, μmol/L	103.1 ± 29.7	103.5 ± 32.5
Bilirubin, μmol/L	13.4 ± 6.9	14.6 ± 5.7
ALAT, U/L	22.9 ± 13.0	22.9 ± 12.3
ASAT, U/L	28.7 ± 8.1	28.0 ± 8.1
BNP, pg/ml	159.0 ± 133.9	165.3 ± 135.9

The results are shown as the mean ± SD of 20 patients. ALAT – alanine transaminase, ASAT – aspartate transaminase, BNP – B-type natriuretic peptide.

5 Discussion

The present work provides evidence about the incidence of PAH and CTEPH which are both diseases that can lead to RVF, as well as the beneficial effect of meldonium on RVF in both preclinical model and patients with RVF.

Our results reveal that the overall incidence of both RVF-inducing diseases in Latvia has remained stable over the past five years, and their incidence is comparable to that in other European countries. However, a significant portion of patients in Latvia are still being diagnosed at advanced stages of the disease, with more than 50 % of them classified as NYHA functional class III and IV.

The results of the present preclinical model study demonstrate that treatment with meldonium attenuated the development of pulmonary hypertension-induced RVF. Administration meldonium attenuated the development of RVH and increased RVFAC by 50 %. The improvement of ventricular function was attributed to the improved mitochondrial bioenergetics in the cardiomyocytes of the right ventricle. The positive effects of meldonium in preclinical study prompted the initiation of the clinical observational study involving patients with chronic RVF.

Our clinical results are the first to demonstrate that treatment with meldonium significantly increases daily physical performance and diminishes shortness of breath in patients with chronic RVF due to PAH. Meldonium treatment improved BDS and parameters characterizing objective and subjective physical functioning. At baseline, 65 % of patients in this study were in WHO FC class III, but after the 30-day meldonium treatment, WHO FC class III included only 35 % of the patients. In addition, our study demonstrates that meldonium is safe in patients with chronic right heart failure. No major AEs were observed during the 60-day period.

Global trends of the incidence of PAH and CTEPH

Nowadays, PAH is no longer considered a disease primarily affecting young females. Recent data reveal that the majority of the PAH patients are actually aged over 60 years at the time of diagnosis (Rådegran et al., 2016; Ling et al., 2012). Similar data are presented also from COMPERA registry data (Hoeper et al., 2013). In 2021, our data indicate that the average age at which PAH and CTEPH are diagnosed is 71 and 72 years, respectively. However, when examining the data from 2018, the average age at diagnosis for PAH and CTEPH was 65 and 64 years, respectively (Kigitovica et al., 2019). According to the PH registry data collected from September 1, 2007, to December 31, 2016, the mean age at the time of diagnosis for patients

with PAH and CTEPH was 65 and 67 years, respectively (Skride et al., 2018). In all cases, a significant portion of patients were identified as being in an advanced stage of the disease, with over 50 % classified as NYHA functional class III and IV and low 6MWD (Kigitovica et al., 2019; Skride et al., 2018). The incidence of PAH and CPTEH has remained relatively stable since 2016 (Skride et al., 2018), however, there was unexpectedly high incidence of CTEPH noted in 2017. The estimated prevalence of PAH and CTEPH in Latvia in 2016 was 45.7 and 15.7 cases per million residents, respectively (Skride et al., 2018).

The global prevalence of PH is approximately 1 % with an increase up to 10 % in patients aged 65 and above. The leading causes contributing to PH are left-sided heart and lung diseases (Hoeper et al., 2016). Between 2010 and 2020, the national PH unit in Ireland recorded a total of 163 cases of PAH with the annual incidence of PAH to be 3.11 cases per million population. The annual incidence of the idiopathic PAH was estimated 0.63 cases per million (Cullivan et al., 2022). The annual incidence of PAH in other European countries vary from 1.5 to 10.7, with the minimum value in Portugal and maximal incidence in Czech republic (Peacock et al., 2007; Billings et al., 2019; Humbert et al., 2006; Escribano-Subias et al., 2012; Jansa et al., 2014; Gall et al., 2017; Hoeper, Huscher, and Pittrow, 2016; Korsholm et al., 2015; Baptista et al., 2013). In general, the incidence in Latvia is comparable to that of other European countries currently. The peak was between 2007 and 2016 with an estimated incidence of idiopathic PAH to be 7.6 cases a year (Skride et al., 2018). To sum up, PAH is a rare disease with a prevalence estimated around 10–50 cases per million inhabitants and an incidence of 5–10 cases per million per year (Lau et al., 2017). It is also noted that PAH affects predominantly females, and women have better right ventricular function that leads to favourable overall prognosis (Cheron et al., 2021).

Effects of meldonium on the development of right ventricular failure in MCT model

Right ventricular dysfunction can be prevented by decreasing etiological factors or by directly stimulating ventricular contractility. It has been demonstrated that attenuation of RV remodeling and improvement of RV function can be achieved by reducing the increase in pulmonary vascular resistance (Bhat et al., 2018) or by direct RV pharmacological stimulation by inotropic drugs (Tavares-Silva et al., 2017). In general, the available treatment choices are influenced by the underlying cause of the RV dysfunction and ineffective compensatory mechanisms (Houston, Brittain, and Tedford, 2023). Our results show that MCT administration induced RVH and increased its mass, but the treatment with meldonium decreased RV-to-body mass index and Fulton index which might indicating the reversibility or attenuation of the development of RVH. Treatment with meldonium improved the functioning of pulmonary

hypertension-induced RVF; however, the enhanced contractility of the right ventricle was not related to endothelial function in pulmonary vessels, RV pressure or blood oxygen saturation. Thus, it can be concluded that meldonium acts directly on the myocardium by modulating its energy metabolism and thus improving ventricular function. This result indicates that meldonium is suitable for combination treatments with drugs that decrease pulmonary vascular resistance to reduce RV remodeling and improve RV function, as well as in conjunction with drugs that reduce preload and afterload, and provide inotropic support.

The development of RVF is characterised by altered myocardial energy metabolism, such as downregulation of FA oxidation, altered oxidative metabolism and subsequent upregulation of glucose uptake and glycolysis (Koop et al., 2019). In our study, we observed cardiac FA oxidation disturbances and complex I partial dysfunction after the development of RVF (Vilskersts et al., 2022). FA metabolism disturbances in the failing myocardium of the right ventricle have been attributed to the downregulation of peroxisome proliferator activated receptor-alpha (PPARα)/PPAR-gamma coactivator-1alpha (PGC-1α) expression and decreased expression of several PGC-1α target genes encoding key enzymes that regulate FA oxidation (Gomez-Arroyo et al., 2013). Previously it was showed that treatment with meldonium activated the PPARα/PGC1α pathway, increased the expression of genes involved in FA metabolism and stimulated mitochondrial β-oxidation (Liepinsh et al., 2013). In the present study, treatment with meldonium restored FA oxidation-dependent OXPHOS coupling efficiency in fibers obtained from the right ventricle of hearts with RVF, which can be explained by activation of the PPARα/PGC1α pathway. In addition, treatment with meldonium decreased the accumulation of FA intermediates, thus facilitating the electron transfer system (Nouws et al., 2014; Liepinsh et al., 2016) and protecting mitochondrial function. It has been suggested that, at the mitochondrial level, loss of complex I assembly may be involved in the switching of energy metabolism to glycolysis (Rafikov et al., 2015). Another study proposed that the alterations in mitochondrial function observed in RVF can be mainly attributed to complex I dysfunction (Wüst et al., 2016). Treatment with meldonium reversed complex I dysfunction in the fibers of the myocardium of the right ventricle and thus restored the functionality of the electron transfer system. Overall, meldonium treatment maintained the function of the right ventricle due to the preservation of FA metabolism and complex I function. The previous data showed that RVH is characterized by increased FAO due to the elevated expression of carnitine palmitoyltransferase-1 (CPT-1) (Bruce et al., 2009; Singh et al., 2019). Meldonium due to its L-carnitine lowering effect reduce CPT-1 dependent mitochondrial FA oxidation that is compensated by an increase in peroxisomal FA metabolism (Liepinsh et al., 2013). Therefore,

the summary FAO activity is not decreased and hypothetically this can lead to lower levels of toxic long-chain FFAs metabolism intermediates.

The protective effects of metabolic modulators on altered energy metabolism and the function of the right ventricle have been studied previously (Koop et al., 2019). Most of the previous studies have primarily focused on the activation of glucose metabolism resulting in the reduction of glycolysis. This process can be triggered by recoupling glycolysis with glucose oxidation due to inhibition of phosphorylation of the pyruvate dehydrogenase complex or indirectly by inhibition of FA metabolism (Koop et al., 2019). However, there is experimental evidence that in case of RVF fatty acids and various their metabolism intermediates accumulate in cardiomyocyes causing lipotoxicity (Talati and Hemnes, 2015). Thus, stimulating fatty acid metabolism should also be a promising mechanism to enhance ATP synthesis. Meldonium has previously demonstrated its effectiveness promoting the utilization of FA in mitochondria simultaneously redirecting them from mitochondria to peroxisomes (Dambrova et al., 2016). Our results show that treatment with meldonium restored FA oxidation-dependent OXPHOS coupling efficiency to the level of healthy controls and improved the function of the right ventricle. We are the first to show that stimulation and restoration of decreased mitochondrial FA metabolism in the right ventricle is capable of improving the function of the ventricle. In contrast to stimulation of glucose oxidation, intensifying of FA oxidation would resemble more physiological energy metabolism in the right ventricle, as more than 70 % of the ATP in the myocardium of the healthy right ventricle is produced by FA metabolism (Fillmore, Mori, and Lopaschuk, 2014). Moreover, stimulation of fatty acid metabolism would decrease synthesis of lipotoxic substances. Taken together, the results demonstrate that the restoration of mitochondrial bioenergetics is sufficient to improve ventricular function.

To date, few drugs have been available to directly stimulate the function/contractility of a failing right ventricle (Konstam et al., 2018). Some clinical studies have shown that treatment with meldonium enhances the function of the left ventricle in heart failure patients (Statsenko, Shilina, and Turkina, 2014; Statsenko et al., 2007). This does not guarantee that meldonium treatment will improve the function of the right ventricle in patients as left and right ventricle in many aspects are not the same; however, our results show that administration of meldonium modifies energy metabolism in the myocardium of the right ventricle and, thus, can improve its function and decrease mortality in patients with RVF. The data obtained in preclinical setup lay grounds for the further clinical trials or observations to test whether similar effects are observed in RVF patients after meldonium treatment.

Impact of meldonium on patients with right ventricular failure

The 6MWT is a commonly used test for the objective assessment of functional exercise capacity for the management of patients with moderate to severe pulmonary disease and is especially widely used in patients with PH and RVF (Holland et al., 2014). 6MWT performance in PAH patients with RVF can be increased by drugs that alter the function of the pulmonary vasculature. All currently used PAH treatment produce an observable increase in 6MWT performance from baseline to the endpoint of the study (Sommer et al., 2021). In addition, 24 weeks of treatment with the β-blocker nebivolol was found to increase 6MWT distance, induce a drop in BDS, and lower the FC of the patients (Martynyuk, Konosova, and Chazova, 2012). The effects of nebivolol can be attributed to the vasoprotective effects induced by its β_2 and β_3 agonist properties (Perros et al., 2015), as other classical β-blockers had no effect on 6MWT distance in RVF patients (Perros et al., 2017; Andersen et al., 2015). The results from a preclinical PAH-induced RVF model revealed that meldonium treatment had no effect on pulmonary vascular reactivity (Vilskersts et al., 2022). Thus, it can be concluded that the improvements induced by meldonium treatment are due to the modification of energy metabolism pathways. The functional status of RVF patients can also be improved by other drugs that modulate energy metabolism. In a study that administered ranolazine to PAH patients for a three-month period, there was an increase in 6MWT performance from 383 ± 60 m to 419 ± 80 m, along with a slight, statistically nonsignificant increase in the Kansas City Cardiomyopathy Questionnaire summary score (Khan et al., 2015). Similar findings were reported in a randomized double-blind placebo-controlled trial in PAH patients with trimetazidine: the trimetazidine-treated patients showed an improvement in functional capacity (NCT03273387, 2017). In our study, meldonium produced remarkable improvements in 6MWT performance (from 352 ± 115 to 399 ± 129 m) and in the physical component summary of the SF-36 after only 1 month of therapy. Moreover, some subscale SF-36 scores and BDS were significantly improved even after a 30-day washout period. This can be explained by the months-long elimination period of meldonium (Liepinsh and Dambrova, 2016; Rabin et al., 2019) and the prolonged presence of meldonium in tissues. In addition, two-thirds of the included patients were in WHO FC III, with marked limitation of physical activity and correspondingly high BNP; nevertheless, meldonium therapy significantly lowered their WHO FC, which demonstrates that meldonium treatment improves conditions after a relatively short treatment period even in patients with severe disease.

The majority of studied patients continued to use more than one drug during the study and had various comorbidities. Meldonium treatment was started as an additional therapy, but nevertheless, it induced significant improvements in the context of other drugs and

comorbidities without producing significant AEs during the treatment or follow-up period; thus, meldonium can be safely combined with other drugs used by RVF patients, and its efficacy may not be influenced by comorbidities. Hypothetically, the effects of meldonium may not be observed in patients who are already using metabolic modulators, as their energy metabolism has already changed; however, none of the participants in this study reported the use of any other metabolic modulator.

Our observational study shows that meldonium increases exercise capacity in patients with RVF. However, from the existing results, it is not possible to indicate whether the effects of meldonium were due to modified energy metabolism in the myocardium, the skeletal muscles, or both. The increase in 6MWT performance was not accompanied by tachycardia or blood pressure elevation, which indirectly demonstrates an increase in physical tolerance, as well as stable cardiac output. A previous clinical study showed that 12 months of meldonium therapy in patients with stable angina pectoris increased total exercise time and time to the onset of angina, which may indicate modified energy metabolism in the heart muscle (Dzerve et al., 2010). On the other hand, another study showed improvement of exercise tolerance with 24 weeks of meldonium treatment in patients with peripheral arterial disease and intermittent claudication with meldonium (Dzerve et al., 2011). In the second clinical trial, the improvement in exercise tolerance can be attributed to improved energy metabolism in skeletal muscles and their ability to perform better under partially ischemic conditions. In addition, as corroborated by some of the findings from the present study, exercise tolerance in patients with peripheral arterial disease was still improved one month after the discontinuation of meldonium therapy (Dzerve et al., 2011). More detailed studies are needed to understand the exact site of action of meldonium in patients with RVF.

QoL is a complex outcome that consists of an individual's satisfaction in the physical, social, and psychological domains; unfortunately, an improvement in objective physical functioning does not always lead to an improvement in QoL (Chen, Taichman, and Doyle, 2008; Halank et al., 2013; Rival et al., 2014). An improvement of SF-36 scores after the treatment, especially in physical functioning, might be associated with increased exercise capacity (Taichman et al., 2005). There is still a debate among various conclusions regarding whether the QoL score can predict mortality or deterioration of disease; however, Blok et al. showed that in PAH CHD patients, a decrease in SF-36 PCS is a determinant of mortality (Blok et al., 2015); Mathai et al. showed that SF-36 scores are associated with survival in patients with PAH (Mathai et al., 2016); and Johansson et al., in the Global Congestive Heart Failure Study, demonstrated that lower health-related QoL is associated with a higher risk of all unfavorable outcomes (Johansson et al., 2021). Jorge et al. compared QoL data among patients

with and without heart failure, independent of the syndrome phenotype; they found significantly greater mean SF-36 scores in patients without HF than in those with HF, and the functional capacity of patients with HF was notably worse than that of patients without HF (Jorge et al., 2017). Therefore, we can hypothesize that treatment with meldonium positively influences SF-36 physical subscale scores toward those of the general population, which might mitigate HF patients' increased risk for mortality due to primary disease.

In 2011, the SF-36 was used to determine health-related QoL in the Latvian population (Ivanovs, Eksteina, and Viksna, 2011). The physical functioning, role–physical, bodily pain, and general health parameters were significantly decreased in the RVF group in comparison to the population data. Treatment with meldonium increased SF-36 subscale scores as it increased exercise capacity, and RVF patients were able to perform their everyday duties. The emotional parameters did not differ greatly from those of the general population; however, 55 % of the RVF patients were actively using psychopharmacological drugs that might improve their emotional well-being. After meldonium treatment, patients' SF-36 scores even more closely approached those of the overall population.

Limitations of this observational study include its relatively short timeframe (limited to 60 days), particularly bearing in mind the chronicity of the disease, as well as the lack of a placebo control. The observed findings in this study might be due to the composite effect of PAH-specific therapy and heart failure therapy received in addition to meldonium; on the other hand, the concomitant treatment was stable during the last 3 months with no augmentation of the functional parameters. Another limitation is the small sample size; however, the overall prevalence of the disease is low, and the patients, who were enrolled from the national PH registry, were representative of the average patient with RVF caused by PAH. Further clinical trials including a placebo group are needed to study the efficacy and safety of longer treatment periods, as well as to understand in more detail the mechanism and site of action of meldonium and its effects on RV function.

Conclusions

In summary, the obtained results confirm the proposed hypothesis that meldonium enhances the mitochondrial bioenergetics in the cardiomyocytes of the right ventricle and improves RV function as well as enhance overall physical well-being of RVF patients and animals with RVF.

1. The overall incidence of PAH and CTEPH in Latvia has remained stable over the past five years and it is comparable to that of other European countries. However, more than 50 % of patients are still diagnosed at advanced stages of the diseases.
2. In experimental RVF model, meldonium treatment prevents the development of RVF by improving mitochondrial bioenergetics. Modulators of mitochondrial energy metabolism could be a potential treatment option for RVF.
3. Meldonium treatment increases functional capacity and decreases dyspnoea in patients with chronic RVF, and it is safe and well tolerated.

Practical recommendations

Early diagnosis and treatment initiation of PAH and CTEPH treatment preserves from RVF. Initiating therapy at an early stage is a hope of enhancing survival rates, attaining the most effective therapeutic equilibrium, and preventing the progression of RVF.

Metabolic modulation with meldonium in the cardiomyocyte shows positive effects on mitochondrial bioenergetics and overall RV function. The favorable effects of meldonium targeting RV are considered safe for use in clinical practice without any significant adverse events mentioned so far. Meldonium in the combination with the treatment for the primary disease improves the QoL and functional capacity in patient with chronic RVF.

The QoL questionnaire serves as a valuable tool to evaluate the effectiveness of RVF therapy, particularly showing improvements in physical domains. Nevertheless, it is important to validate these subjective findings with objective clinical changes.

Publications

Articles

1. **Kigitovica D**, Rusa E, Rudzitis A, Skride A. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension incidence in Latvia in 2021 according to the new definition. *Eur J Intern Med*. 2022;106:152-153. Doi:10.1016/j.ejim.2022.08.030
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3. **Kigitovica D**, Dzirnieks K, Lejnieks A, Dambrova M, Skride A, Vilskersts R. Meldonium improves functional capacity in patients with right heart failure. *Submitted.*
4. Kauliņš R, Rudzītis A, Lejnieks A, **Kigitoviča D**, Skride A. Baseline Clinical Characteristics and Incidence of Chronic Thromboembolic Pulmonary Hypertension Patients in Latvia, 2019-2020. *Medicina (Kaunas).* 2023;59(8):1426. Published 2023 Aug 6. doi:10.3390/ medicina59081426
5. **Kigitovica D**, Sablinskis M, Sablinskis K, Rudzitis A, Skride A. Pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension incidence in Latvia in 2018. *Eur J Intern Med.* 2019;65:e9-e10. doi:10.1016/j.ejim.2019.04.022

Presentations at international conferences (Oral presentations)

1. **Kigitovica, D.**, Korzh, S., Videja, M., Vilks, K., Makrecka-Kuka, M., Liepins, E., Skride, A., Dambrova, M., Vilskersts, R. Meldonium Therapy Improves the Right Ventricle Function. 4th Baltic Pulmonary Hypertension Conference, Rīga, Latvia: 1st Place Award Certificate.
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Scientific awards

1. L'Oréal Baltic, in cooperation with the Baltic Academies of Sciences and the UNESCO National Commissions, Award for Women in Science, 2021

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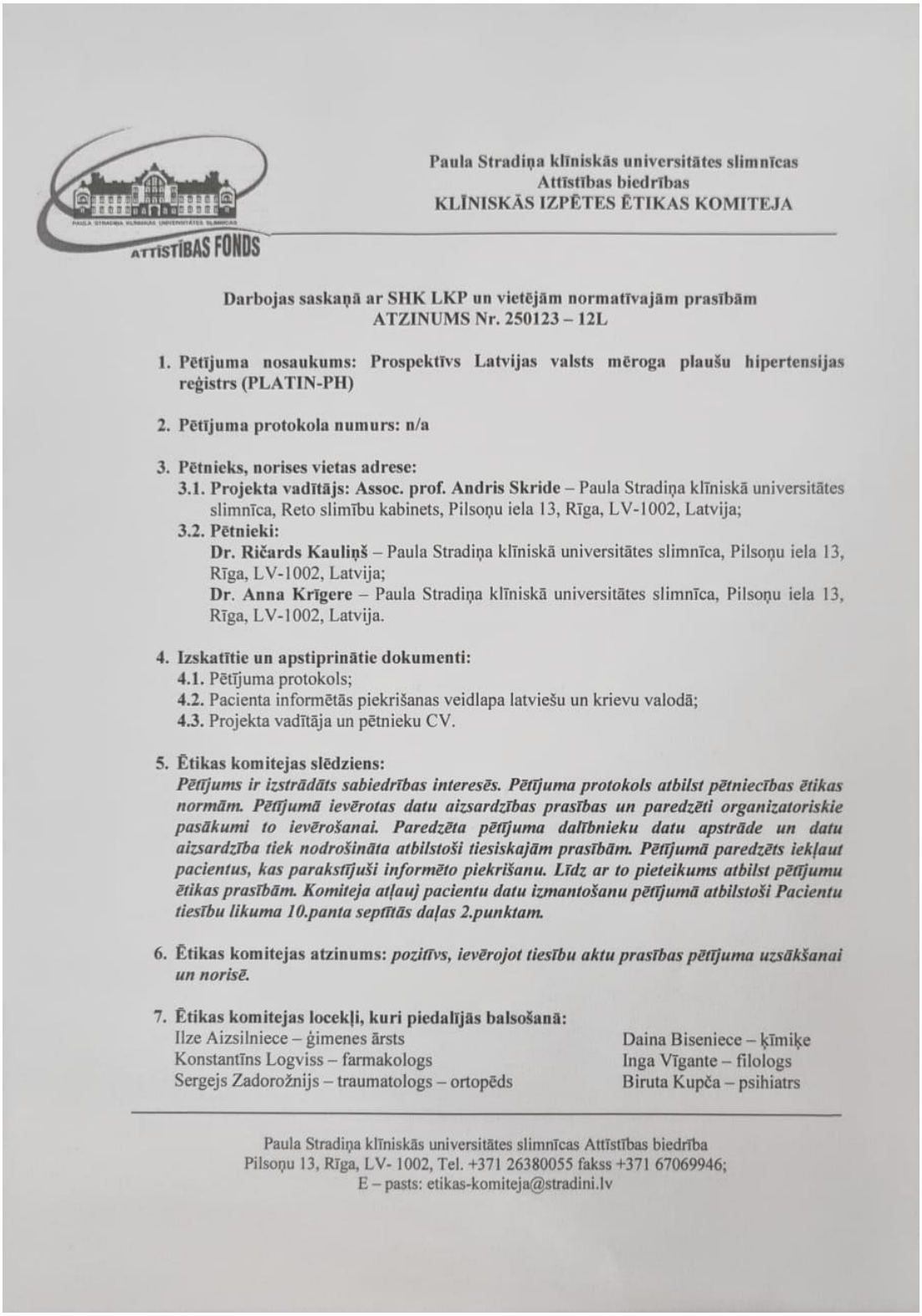
Annexes

Ethics Committee Permission



Paula Stradiņa klīniskās universitātes slimnīcas
Attīstības biedrības
KLĪNISKĀS IZPĒTES ĒTIKAS KOMITEJA

ATTĪSTĪBAS FONDS

Darbojas saskaņā ar SHK LKP un vietējām normatīvajām prasībām
ATZINUMS Nr. 250123 – 12L

1. **Pētījuma nosaukums: Prospektīvs Latvijas valsts mēroga plaušu hipertensijas reģistrs (PLATIN-PH)**

2. **Pētījuma protokola numurs: n/a**

3. **Pētnieks, norises vietas adrese:**
   3.1. Projekta vadītājs: Assoc. prof. Andris Skride – Paula Stradiņa klīniskā universitātes slimnīca, Reto slimību kabinets, Pilsoņu iela 13, Rīga, LV-1002, Latvija;
   3.2. Pētnieki:
   Dr. Ričards Kauliņš – Paula Stradiņa klīniskā universitātes slimnīca, Pilsoņu iela 13, Rīga, LV-1002, Latvija;
   Dr. Anna Krīgere – Paula Stradiņa klīniskā universitātes slimnīca, Pilsoņu iela 13, Rīga, LV-1002, Latvija.

4. **Izskatītie un apstiprinātie dokumenti:**
   4.1. Pētījuma protokols;
   4.2. Pacienta informētās piekrišanas veidlapa latviešu un krievu valodā;
   4.3. Projekta vadītāja un pētnieku CV.

5. **Ētikas komitejas slēdziens:**
   Pētījums ir izstrādāts sabiedrības interesēs. Pētījuma protokols atbilst pētniecības ētikas normām. Pētījumā ievērotas datu aizsardzības prasības un paredzēti organizatoriskie pasākumi to ievērošanai. Paredzēta pētījuma dalībnieku datu apstrāde un datu aizsardzība tiek nodrošināta atbilstoši tiesiskajām prasībām. Pētījumā paredzēts iekļaut pacientus, kas parakstījuši informēto piekrišanu. Līdz ar to pieteikums atbilst pētījumu ētikas prasībām. Komiteja atļauj pacientu datu izmantošanu pētījumā atbilstoši Pacientu tiesību likuma 10.panta septītās daļas 2.punktam.

6. **Ētikas komitejas atzinums:** ***pozitīvs, ievērojot tiesību aktu prasības pētījuma uzsākšanai un norisē.***

7. **Ētikas komitejas locekļi, kuri piedalījās balsošanā:**
   Ilze Aizsilniece – ģimenes ārsts
   Konstantīns Logviss – farmakologs
   Sergejs Zadorožnijs – traumatologs – ortopēds
   Daina Biseniece – ķīmiķe
   Inga Vīgante – filologs
   Biruta Kupča – psihiatrs

Paula Stradiņa klīniskās universitātes slimnīcas Attīstības biedrība
Pilsoņu 13, Rīga, LV- 1002, Tel. +371 26380055 fakss +371 67069946;
E – pasts: etikas-komiteja@stradini.lv

Ethics Committee Permission approved by the Latvian Animal Protection Ethical Committee of the Food and Veterinary Service, Riga, Latvia

Pārtikas un veterinārais dienests

IZMĒĢINĀJUMA PROJEKTA ATĻAUJA Nr. 105 DZĪVNIEKA IZMANTOŠANAI PROCEDŪRĀ

Atļaujas saņēmējs
Latvijas Organiskās sintēzes institūts, Nr. 022842
Izmēģinājuma dzīvnieku lietotājs (nosaukums, PVD reģistra Nr.)

Izmēģinājuma projekta/procedūras nosaukums
Jaunu zāļu vielu izpēte preklīniskajos *in vivo* pētījumos

Reinis Vilšķērsts
Par visa izmēģinājuma projekta īstenošanu atbildīgā persona

Reinis Vilšķērsts, atļauja Nr. 5
Par izmēģinājuma projekta atbildīgā persona, PVD atļaujas Nr.

Izmēģinājuma projekta norises vieta
Aizkraukles iela 21, Rīga, LV-1006
faktiskā adrese

Īpašie nosacījumi:
līdz 2024. gada 30. aprīlim iesniegt PVD 2019. gada 14. maija lēmuma Nr. 1.1-13E/19/888 rezolutīvajā daļā pieprasīto informāciju. Par jebkurām procedūrās ieviestām izmaiņām nekavējoties ziņot PVD!
Retrospektīvā izvērtēšana u.c.

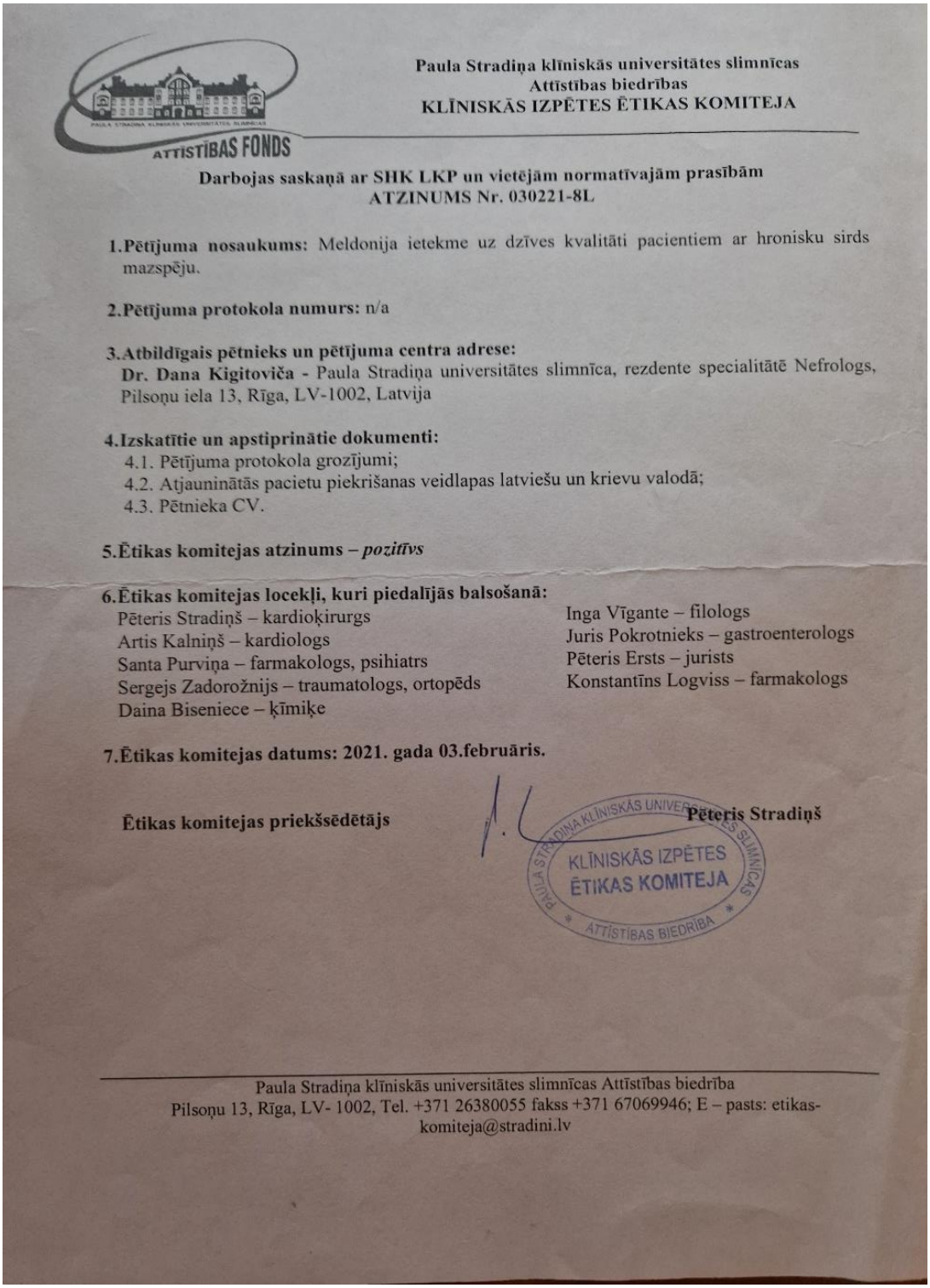
Atļaujas darbības laiks no 14.05.2019. līdz 31.03.2024.

Pārtikas un veterinārā
dienesta ģenerāldirektors M.Balodis

LATVIJAS REPUBLIKAS PĀRTIKAS UN VETERINĀRAIS DIENESTS

AA 090747

Research Permission approved by Pauls Stradins Clinical University Hospital



Paula Stradiņa klīniskās universitātes slimnīcas
Attīstības biedrības
KLĪNISKĀS IZPĒTES ĒTIKAS KOMITEJA

ATTĪSTĪBAS FONDS

Darbojas saskaņā ar SHK LKP un vietējām normatīvajām prasībām
ATZINUMS Nr. 030221-8L

1.Pētījuma nosaukums: Meldonija ietekme uz dzīves kvalitāti pacientiem ar hronisku sirds mazspēju.

2.Pētījuma protokola numurs: n/a

3.Atbildīgais pētnieks un pētījuma centra adrese:
Dr. Dana Kigitoviča - Paula Stradiņa universitātes slimnīca, rezdente specialitātē Nefrologs, Pilsoņu iela 13, Rīga, LV-1002, Latvija

4.Izskatītie un apstiprinātie dokumenti:
4.1. Pētījuma protokola grozījumi;
4.2. Atjaunināts pacietu piekrišanas veidlapas latviešu un krievu valodā;
4.3. Pētnieka CV.

5.Ētikas komitejas atzinums – ***pozitīvs***

6.Ētikas komitejas locekļi, kuri piedalījās balsošanā:
Pēteris Stradiņš – kardioķirurgs
Artis Kalniņš – kardiologs
Santa Purviņa – farmakologs, psihiatrs
Sergejs Zadorožnijs – traumatologs, ortopēds
Daina Biseniece – ķīmiķe
Inga Vīgante – filologs
Juris Pokrotnieks – gastroenterologs
Pēteris Ersts – jurists
Konstantīns Logviss – farmakologs

7.Ētikas komitejas datums: 2021. gada 03.februāris.

Ētikas komitejas priekšsēdētājs **Pēteris Stradiņš**

PAULA STRADIŅA KLĪNISKĀS UNIVERSITĀTES SLIMNĪCAS
KLĪNISKĀS IZPĒTES ĒTIKAS KOMITEJA
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Annex 4

Research Permission approved by the State Agency of Medicines of the Republic of Latvia (16.03.2021)

Zāļu nosaukums	Farmācijas firma-ražotājs/Novērojuma veicējs	Pieteikums saņemts ZVA	Statuss
1	3	4	5
Adaptol 500 mg tabletes	AS Olainfarm	12.05.2023	atļauts 25.05.2023.
Noofen 250 mg cietās kapsulas	AS Olainfarm	12.05.2023	atļauts 25.05.2023.
Dapagliflozinum, 5 mg, 10 mg apvalkotās tabletes	AstraZeneca	18.03.2022	atļauts: 11.05.2022.
Mildronāts® (Meldonium dihydricum), 250 mg un 500 mg cietās kapsulas	AS GRINDEKS	11.02.2022	atļauts: 15.03.2022.
		01.12.2021	atļauts: 07.01.2022.
Cilvēka albumīns BTS 50 g/l šķīdums	Rīgas Stradiņa universitāte	06.09.2021	atļauts: 20.09.2021
Bericox (Etoricoxibum) 60 mg, 90 mg, 120 mg apvalkotās tabletes	KRKA, d.d., Novo mesto Slovenia	15.07.2021	atļauts: 23.08.2021.
Levofloxacin 250 mg un 500 mg tabletes Bedaquiline 100 mg tabletes Linezolide 600 mg apvalkotās tabletes Clofazimine 50 mg kapsulas Cycloserine 250 mg kapsulas Terizidon 250 mg cietās kapsulas Delamanid 50 mg apvalkotās tabletes	SIA RAKUS Stacionārs „Tuberkulozes un plaušu slimību centrs"	13.04.2021	atļauts: 11.05.2021.
Mildronāts® (Meldonium dihydricum) 500 mg cietās kapsulas	AS GRINDEKS	04.03.2021	atļauts: 16.03.2021.
Mildronāts® (Meldonium dihydricum) 0,5g/5ml šķīdums injekcijām un 500mg cietās kapsulas	AS GRINDEKS	24.11.2020.	atļauts 18.12.2020.
Foster 100/6 mikrogrami/izsmidzinājumā aerosols inhalācijām, zem spiediena, šķīdums, Foster Nexthaler 100 mikrogrami/6 mikrogrami izsmidzinājumā inhalācijas pulveris	Chiesi Pharmaceuticals GmbH/ SAS Noramed	31.08.2020.	atļauts 08.10.2020.
Boncel 25 000 SV šķīdums iekšķīgai lietošanai	SIA Orivas	20.12.2018.	atļauts 01.02.2019.

Informed Consent Form

Informētas piekrišanas veidlapa Meldonija ietekme uz dzīves kvalitāti pacientiem ar hronisku sirds mazspēju

Nozīmīga informācija pacientiem

Pētījumos pacientiem ar sirds mazspēju meldonija pievienošana terapijā uzlabo sirds mazspējas funkcionālo klasi, 6 minūšu iešanas testu. To lieto kombinētas terapijas veidā hroniskas sirds mazspējas (NYHA I-III funkcionālā klase) gadījumā un pazeminātas darbaspējas, fiziskas un psihoemocionālas pārslodzes gadījumā.

Pieejamā pamatslimības terapija uzlabo dzīvildzi, bet slimība bieži provocē trauksmi, depresiju un nedrošības sajūtu ikdienā. Tādēļ aicinām Jūs piedalīties šajā pētījumā, kura **mērķis** ir noskaidrot, vai meldonijam ir ietekme uz dzīves kvalitāti pacientiem ar hronisku sirds mazspēju I-III funkcionālā klasi pēc NYHA.

Šī novērojuma gaitā paredzēts analizēt pacientu fizikālos un laboratoros izmeklējumus pirms un pēc meldonija saņemšanas 4 nedēļu laikā vai novērošanos pie ārsta 3 mēnešus. Novērojuma gaitā tiks apkopoti arī Jums veikto laboratorisko un instrumentālo izmeklējumu rezultāti, kuri tiks izmantoti apkopotā veidā tikai pētījuma vajadzībām. Novērojums tiek veikts Paula Stradiņa Klīniskās universitātes slimnīcas kardiologa Andra Skrides vadībā Danas Kigitoviča promocijas darba ietvaros. Paredzamais novērojuma ilgums no Jūsu iesaistes brīža līdz atkārtotajai vizītei ir 3 mēneši.

Novērojuma norise.

Jums ir diagnosticēta sirds mazspēja un Jūs saņemat terapiju. Jums tiek sniegta iespēja piedalīties novērojumā, kur Jums nozīmēs vienu no divām iespējām meldoniju kursa veidā 4 nedēļas 500 mg 2 reizes dienā vai novērošanos pie ārsta 3 mēnešus. Pēc 2 nedēļām no novērojuma sākuma paredzēta telefoniska konsultācija ar ārstu, un pēc 4 nedēļām un 3 mēnešiem Jūs tiksiet aicināts uz atkārtotu konsultāciju, lai izvērtētu fizikālos, laboratoros un funkcionālos rādītājus.

Jūsu veselības novērtējums tiks noteikts, izmantojot subjektīvu elpas trūkuma novērtējuma skalu, 6 minūšu iešanas testu un un dzīves kvalitātes novērtējuma anketu.

Iespējamie riski.

Asins paraugu noņemšana. Procedūras risks no asins parauga noņemšanas pielīdzināms standarta asins analīžu iegūšanas riskam. Mirstība pēc procedūras ir samērā maza jeb 0,05 %.
Meldonija lietošana. Biežākās blaknes meldonija lietošanas laikā var būt alerģiskas reakcijas, galvassāpes, dispepsija. Zāles Mildronāts (meldonijs) lieto kombinētas terapijas veidā hroniska sirds mazspēja (NYHA I-III funkcionālā klase) gadījumā un pazeminātas darbaspējas, fiziska un psihoemocionāla pārslodzes gadījumā.

Konfidencialitāte

Novērojuma gaitā tiks ievēroti konfidencialitātes principi un personas datu aizsardzība atbilstoši LR likumdošanai. Iegūtie dati tiks kodēti un izmantoti apkopotā veidā.

Novērojuma dokumentos, izņemot primāro medicīnisko dokumentāciju, pacientu personas dati tiek atspoguļoti šifrētā veidā – ar iniciāļiem un pacientu kodu. Reālie personas dati (vārds, uzvārds, personas kods, dzīves vietas adrese, kontakttālrunis) tiek reģistrēti Pacientu identifikācijas sarakstā, kas atrodas pie atbildīgā pētnieka (Dr.med., doc.Andris Skride) un tiek uzglabāts klīnikā

(Reto slimību kabinets) vēl 10 gadus pēc novērojuma pabeigšanas. Primārā medicīniskā dokumentācija tiek uzglabāta klīnikā atbilstoši Latvijas valsts likumdošanas prasībām.

Ar pētījuma norisi saistītu jautājumu gadījumā lūdzu sazināties ar Danu Kigitoviču. Epasts danakigitovica@gmail.com, tel.29421903

Tiesības atsaukt piekrišanu

Ar savu parakstu apliecinu, ka esmu iepazīstināts (-ta) ar pētījuma norisi, to saprotu un piekrītu pētījumā piedalīties. Es saprotu, ka mana dalība šajā pētījumā ir brīvprātīga un man ir tiesības pārtraukt dalību tajā jebkurā laikā.

Vieta, datums	Vārds, uzvārds (ar drukātiem burtiem)	Personas paraksts

Ārsta paziņojums

Es apstiprinu, ka esmu izskaidrojis novērojuma norisi. Esmu sniedzis informāciju par ierobežojumiem un pēc labākās sirdsapziņas atbildējis uz šī cilvēka uzdotajiem jautājumiem. Pacients ir informēts par tiesībām jebkurā laikā mutiski vai rakstiski atsaukt savu piekrišanu dalībai novērojumā. Par diagnostisko pārbaužu pieprasīšanu lemj un atbildību uzņemas ārsts. Par visiem terapeitiskajiem lēmumiem, kas pieņemti pamatojoties uz šīm pārbaudēm, atbildīgs ir vienīgi ārsts.

Vieta, datums	Vārds, uzvārds (ar drukātiem burtiem)	Ārsta paraksts

Форма информированного согласия на участие в в исследовании «Влияние мельдония на качество жизни пациентам с сердечной недостаточностью»

Важная информация для пациентов

В исследованиях больных с сердечной недостаточностью мельдоний улучшает функциональный класс сердечной недостаточности, тест 6-минутной ходьбы. Мельдоний используют при хронической сердечной недостаточности (функциональный класс I-III) и при пониженной работоспособности, физической и психоэмоциональной перегрузки.

Доступная терапия улучшает качество жизни, но болезнь сама по себе часто провоцирует тревогу, депрессию и чувство неуверенности ежедневно. Цель исследования выявить, может ли мельдоний улучшить качество жизни пациентов с сердечной недостаточностью (функциональный класс I-III).

В течении наблюдения будут анализированы Ваши физикальные и лабораторные показатели до и после 4-ёх недельного курса с мельдонием или наблюдение у врача. В течении наблюдения будут регистрированы сделанные лабораторные и инструментальные результаты, которые будут использованы только в целях этого наблюдения в обобщенном виде. Наблюдения проходит в Больнице Имени Паула Страдиня под руководством кардиолога Андрис Скриде в целях докторской работы Даны Кигитович. Длительность участия в наблюдении 3 месяца.

Процесс наблюдения.

У Вас доказан диагноз сердечной недостаточности, и Вы принимаете терапию. У Вас есть возможность участвовать в наблюдении, где Вам назначут лечение мельдонием 4 недели 500 мг 2 раза в день или наблюдение у врача. Через 2 недели после начала курса лечения у Вас будет телефонная консультация с врачом, а через 4 недели и 3 месяца приём у врача, чтобы повторно оценить физикальные, лабораторные и функциональные показатели.

Возможные риски.

Анализ крови. Риск взятия крови в целях исследования стандартный. Смертность после такого вида процедуры очень маленький - 0,05 %.

Употребление мельдония. Побочные эфекты мельдония могут быть аллергическая реакция, головная боль, диспепсия. Медикамент мельдоний используют при хронической сердечной недостаточности (функциональный класс I-III) и при пониженной работоспособности, физической и психоэмоциональной перегрузки

Конфиденциальность

Для нас очень важно, чтобы Ваша личная и медицинская информация осталась конфиденциальной. Она будет защищена в соответствии с нормативными актами, в т.ч. Законом о правах пациентов и Законом о защите данных физических лиц Латвийской Республики. Полученные медицинские данные далее будут обработаны анонимно и использованы только обобщенном виде.

Если возникнут вопросы по поводу исследования, просим связаться с Dana Kigitoviča. Eпочта - danakigitovica@gmail.com, тел.29421903

Право отозвать согласие

Я осознаю, что участие в регистре не обязательно, что я в любой момент могу отозвать своё согласие, не указывая конкретной причины и без каких-либо далеко идущих и влияющих на меня последствий.

_____	_____	_____
Имя пациента (печатными буквами)	Подпись	Дата

Document of the clinical observational study for the data registration

Novērojuma datu reģistrācijas dokumenta paraugs.

Novērojuma protokola raksturlielums	Skrīningvizīte
Laika logs	1 nedēļa
Informētās piekrišanas forma	X
Iekļaušanas kritēriju izpilde	X
Primārā diagnoze	X
Pacienta demogrāfiskie dati (rase, svars, augums, smēķēšanas anamnēze, alkohola lietošana, diētas paradumi)	Svars:_____ Augums: _____ Smēķē joprojām/nekad nav bijis smēķētājs/izbijis smēķētājs Paciņgadi_____ Alkohola lietošana_____ Diētas paradumi/gaļas patēriņš dienā_____
Anamnēzes dati, ieskaitot alerģijas	**Komorbiditātes:** KSS _____ AH_____ Venoza trombembolija _____ CD _____ Vairogdziedzera patoloģija _____ Obstruktīva miega apnoja _____ Citas____ Alerģijas_____
Pacienta fizikālā izmeklēšana	Tiek piefiksētas novirzes no normas.
Funkcionālā klasifikācija pēc PVO	X
Vitālie parametri	Sirdsdarbības frekvence Asinsspiedines SpO2 Elpošanas frekvence Temperatūra
12 novadījumu EKG (pēdējo 3 gadu laikā pirms skrīningvizītes)	X

Pārskata plaušu rentgenogramma (pēdējo 3 gadu laikā pirms skrīningvizītes)	X
Laboratoriskās analīzes – pilna asins aina, ALAT, ASAT, bilirubīns, kreatinīns, urīnviela, BNP	X
SF-36 anketa	X
6 minūšu iešanas tests	X
BORG dispnojas skala	X
Pamatslimības specifiskā terapija, tās ilgums	**PH specifiskā terapija** (medikaments; cik mg/dnn.? ; terapijas uzsākšanas datums)
Paralēli lietoto medikamentu saraksts	**Antikoagulanti (terapijas uzsākšanas datums):** _____ **Antiagreganti (terapijas uzsākšanas datums):**_____ Digitalis _____;Diurētiķi_____; BAB_____; AKEI/ARB _____; Amiodarons_____; **Citi medikamenti:** _____
Alerģiskās reakcijas novērojuma laikā	
Hospitalizācija un tās cēlonis	
Nāve, tās cēlonis	

Novērojuma protokola raksturlielums	14 diena - telefoniska vizīte
Laika logs	± 3 dienas
BORG dispnojas skala	X
Alerģiskās reakcijas novērojuma laikā	X
Hospitalizācija un tās cēlonis	X
Nāve, tās cēlonis	X

Novērojuma protokola raksturlielums	30 diena
Laika logs	± 7 dienas
Pacienta fizikālā izmeklēšana	X
Funkcionālā klasifikācija pēc PVO	X
Vitālie parametri	Sirdsdarbības frekvence Asinsspiedines SpO2 Elpošanas frekvence Temperatūra
Laboratoriskās analīzes – pilna asins aina, ALAT, ASAT, bilirubīns, kreatinīns, urīnviela, BNP	X
SF-36 anketa	X
6 minūšu iešanas tests	X
BORG dispnojas skala	X
Pamatslimības specifiskā terapija, tās ilgums	**PH specifiskā terapija** (medikaments; cik mg/dnn.? ; terapijas uzsākšanas datums)
Paralēli lietoto medikamentu saraksts	**Antikoagulanti (terapijas uzsākšanas datums):** _____ **Antiagreganti (terapijas uzsākšanas datums):**_____ Digitalis _____;Diurētiķi_____; BAB_____; AKEI/ARB _____; Amiodarons_____; **Citi medikamenti:** _____
Alerģiskās reakcijas novērojuma laikā	X
Hospitalizācija un tās cēlonis	X
Nāve, tās cēlonis	X

Novērojuma protokola raksturlielums	60 diena
Laika logs	± 7 dienas
Pacienta fizikālā izmeklēšana	X
Funkcionālā klasifikācija pēc PVO	X
Vitālie parametri	Sirdsdarbības frekvence Asinsspiedines SpO2 Elpošanas frekvence Temperatūra
SF-36 anketa	X
6 minūšu iešanas tests	X
BORG dispnojas skala	X
Pamatslimības specifiskā terapija, tās ilgums	**PH specifiskā terapija** (medikaments; cik mg/dnn.? ; terapijas uzsākšanas datums)
Paralēli lietoto medikamentu saraksts	**Antikoagulanti (terapijas uzsākšanas datums):** _____ **Antiagreganti (terapijas uzsākšanas datums):**_____ Digitalis _____;Diurētiķi_____; BAB_____; AKEI/ARB _____; Amiodarons_____; **Citi medikamenti:** _____
Alerģiskās reakcijas novērojuma laikā	X
Hospitalizācija un tās cēlonis	X
Nāve, tās cēlonis	X

36-Item Short Form Survey Instrument (SF-36)

RAND HEALTH

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36-Item Short Form Survey Instrument (SF-36)

RAND 36-Item Health Survey 1.0 Questionnaire Items

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- ○ 1 - Excellent
- ○ 2 - Very good
- ○ 3 - Good
- ○ 4 - Fair
- ○ 5 - Poor

2. **Compared to one year ago**, how would you rate your health in general **now**?

- ○ 1 - Much better now than one year ago
- ○ 2 - Somewhat better now than one year ago
- ○ 3 - About the same
- ○ 4 - Somewhat worse now than one year ago
- ○ 5 - Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports	○ 1	○ 2	○ 3
4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○ 1	○ 2	○ 3
5. Lifting or carrying groceries	○ 1	○ 2	○ 3
6. Climbing **several** flights of stairs	○ 1	○ 2	○ 3
7. Climbing **one** flight of stairs	○ 1	○ 2	○ 3
8. Bending, kneeling, or stooping	○ 1	○ 2	○ 3
9. Walking **more than a mile**	○ 1	○ 2	○ 3
10. Walking **several blocks**	○ 1	○ 2	○ 3
11. Walking **one block**	○ 1	○ 2	○ 3
12. Bathing or dressing yourself	○ 1	○ 2	○ 3

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

	Yes	No
13. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
14. **Accomplished less** than you would like	○ 1	○ 2
15. Were limited in the **kind** of work or other activities	○ 1	○ 2
16. Had **difficulty** performing the work or other activities (for example, it took extra effort)	○ 1	○ 2

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

	Yes	No
17. Cut down the **amount of time** you spent on work or other activities	○ 1	○ 2
18. **Accomplished less** than you would like	○ 1	○ 2
19. Didn't do work or other activities as **carefully** as usual	○ 1	○ 2

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- ○ 1 - Not at all
- ○ 2 - Slightly
- ○ 3 - Moderately
- ○ 4 - Quite a bit
- ○ 5 - Extremely

21. How much **bodily** pain have you had during the **past 4 weeks**?

- ○ 1 - None
- ○ 2 - Very mild
- ○ 3 - Mild
- ○ 4 - Moderate
- ○ 5 - Severe
- ○ 6 - Very severe

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- ○ 1 - Not at all
- ○ 2 - A little bit
- ○ 3 - Moderately
- ○ 4 - Quite a bit
- ○ 5 - Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
23. Did you feel full of pep?	1	2	3	4	5	6
24. Have you been a very nervous person?	1	2	3	4	5	6
25. Have you felt so down in the dumps that nothing could cheer you up?	1	2	3	4	5	6
26. Have you felt calm and peaceful?	1	2	3	4	5	6
27. Did you have a lot of energy?	1	2	3	4	5	6
28. Have you felt downhearted and blue?	1	2	3	4	5	6
29. Did you feel worn out?	1	2	3	4	5	6
30. Have you been a happy person?	1	2	3	4	5	6
31. Did you feel tired?	1	2	3	4	5	6

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- 1 - All of the time
- 2 - Most of the time
- 3 - Some of the time
- 4 - A little of the time
- 5 - None of the time

How TRUE or FALSE is **each** of the following statements for you.

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
33. I seem to get sick a little easier than other people	1	2	3	4	5
34. I am as healthy as anybody I know	1	2	3	4	5
35. I expect my health to get worse	1	2	3	4	5
36. My health is excellent	1	2	3	4	5