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GENERAL INTRODUCTION
AND OUTLINE OF THE THESIS
IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION

Idiopathic pulmonary arterial hypertension (PH) is a rare lung disease with incidence of 2-5 cases per one million per year and a high mortality rate of 20-40% 3 years after diagnosis.\textsuperscript{1,2} PH is characterized by endothelial and smooth muscle cell proliferation leading to narrowing and/or restriction of the small pulmonary vessels. This increases the pulmonary vascular resistance and the pulmonary artery pressure (PAP) that in turn increase right ventricular (RV) afterload. The diagnosis is confirmed when the mean PAP is above 25 mmHg assessed by right heart catheterization (normal is 10-12 mmHg) in the absence of an underlying disease.

Initially, the right ventricle adapts to the increased afterload by hypertrophy. However, this compensatory mechanism can progress to RV failure. Over the past two decades, the awareness of PH has increased and new therapeutic drugs have been developed that can lower, although not normalize, effectively the pulmonary vascular resistance. Despite this fact, the cause of death remains invariably right heart failure. Current treatment of RV failure only consists of symptomatic relief and the mechanisms underlying the transition from compensated RV hypertrophy to failure remain unclear. In order to develop new therapies that may prevent or even reverse RV failure in PH, knowledge on the underlying mechanism to failure needs to be increased. Until now, RV research aiming to increase the understanding of the basic mechanisms that underlie right heart failure has been mainly confined to animal studies. The present thesis aims to translate RV preclinical research in pulmonary hypertension to the clinic and vice versa.

Hypothesis

The main hypothesis of this thesis is that the transition from adaptive RV hypertrophy to fatal RV failure is caused by an imbalance of oxygen (O\textsubscript{2}) supply and demand in the hypertrophied right ventricle. This would be a result of a combined increase in myocardial O\textsubscript{2} demand and a reduction of O\textsubscript{2} supply to the hypertrophied RV cardiomyocytes in PH. Factors that influence this balance is summarized in Figure 1.1 and are discussed below.

![Figure 1.1 The myocardial oxygen balance and the factors that may affect it during RV hypertrophy and failure](image-url)
Factors determining the myocardial oxygen demand

Classic determinants of myocardial oxygen consumption (MVO$_2$) in the left myocardium are systolic (aortic) pressure, heart rate and contractility that have been repeatedly validated in both normal and ischemic or failing conditions. In PH, systolic pulmonary artery pressure and heart rate are both elevated, suggesting a similar association with RV MVO$_2$. The association was shown between the pulmonary artery pressure and RV MVO$_2$ in a canine pulmonary banding model, but it remains to be confirmed in PH patients.

Ventricular power In PH, the RV stroke work and power is increased in order to overcome the elevated pulmonary vascular resistance. This demands, at least, a proportional increase in MVO$_2$ by the right ventricle.

Wall stress Due to the increased systolic pressure, ventricular wall stress in PH is increased. It has been shown that an increase in wall stress is related to the MVO$_2$ (per gram muscle) in the left ventricle. According to Laplace’s law, hypertrophy is an adaptive response of the myocardium to reduce ventricular wall stress and with that the MVO$_2$. In PH patients, however, RV wall stress is increased compared to control subjects and it is associated with an increased serum Nt-proBNP, suggesting increased atrial and/or ventricular stretch. Administration of epoprostenol to newly diagnosed PH patients reduces RV wall stress and RV myocardial glucose uptake. Until now, the relation between wall stress and MVO$_2$ in the right ventricle has not been studied in PH.

Mechanical efficiency may also alter during the course to RV failure causing an additional elevation of the O$_2$ demand. Mechanical efficiency is defined as the ratio of ventricular power (stroke work times heart rate) to the metabolic energy calculated from MVO$_2$. In LV cardiomyopathy and failure, mechanical efficiency was found to be reduced, which may be either due to an increase in MVO$_2$ and/or a reduction in the ventricular power. The underlying cause(s) of reduced external work in relation to O$_2$ consumption remains, however, unclear. The mechanical efficiency of the hypertrophied, failing right ventricle in PH has not been studied before.

Substrate oxidation and MVO$_2$ are closely related in the production of cardiac energy. The heart normally prefers free fatty acids but also uses glucose and lactate as energy sources. During LV hypertrophy and failure, secondary to cardiomyopathy or infarction, this metabolic preference of the myocardium shifts from free fatty acids to glucose. Similar observations were reported for the right ventricle in a rodent model of PH as well as in patients with PH. Because glucose oxidation generates slightly more energy (ATP/O$_2$) compared to free fatty acid oxidation, it is generally postulated that with this glucose shift the myocardium becomes metabolically more efficient during increased O$_2$ demand. Treatment of PH indeed reduced the glucose uptake in PH patients. It is likely that an altered mechanical efficiency is related to the glucose metabolism in the failing right ventricle in PH.

Cellular alterations affecting the O$_2$ demand In addition, a study in the PH rat model using isolated papillary muscles from the hypertrophied RV reported a higher O$_2$ consumption at rest compared to control. This indicates, among other things, that cardiomyocyte mitochondria are working at a higher rate to provide for an elevated need in energy.
Determinants of myocardial oxygen supply

Myocardial blood flow (MBF) or perfusion and the amount of $O_2$ extracted by cardiomyocytes (calculated as the difference between arterial and venous $O_2$ content of myocardial blood) are the two main variables on which the myocardial $O_2$ supply depends. The third component, arterial $O_2$ content, is less variable, provided that anemia, cardiac shunting or reduced lung diffusion capacity are absent.

The normal left ventricle extracts about 60 to 80% of $O_2$ supplied by myocardial perfusion. Since this is near maximal, an increase in LV MVO$_2$ for instance during exercise, is considered proportional to the increase in MBF. The normal right heart, however, has both an $O_2$ extraction (~40%) and MBF that is lower than in the left myocardium. Therefore, the physiological response to an increase in $O_2$ demand is not similar to that of the left heart. A canine study showed that $O_2$ extraction increased first when the $O_2$ demand increased during exercise. Only when the $O_2$ extraction is maximized during strenuous exercise, the right coronary flow was drawn upon to provide for the elevated $O_2$ demand. In PH patients, right coronary arterial flow was elevated at rest, whereas RV myocardial perfusion reserve (the ratio between MBF at exercise and resting MBF, was decreased compared to controls. However, the combined effects of $O_2$ extraction and MBF in the hypertrophied right ventricle in PH patients during exercise have not been studied before.

Cellular alterations affecting the $O_2$ supply
Cardiomyocyte hypertrophy results in a reduction of the capillary density. This has consequences for the maximal capacity of $O_2$ supply. Also, the diffusion distance of $O_2$ from the capillary to the center of the cardiomyocytes is enlarged in hypertrophy. In theory, the intracellular protein myoglobin (Mb) could compensate for increased diffusion distance by facilitation of $O_2$ extraction from the capillaries into the cardiomyocytes and for intracellular $O_2$ diffusion in cardiomyocyte cytoplasm to the mitochondrial respiratory chains. It also functions as an intracellular short-term $O_2$ reservoir. In responding to the increased $O_2$ demand in PH, it may be expected that the Mb concentration is upregulated during hypertrophy. This, however, does not occur in the PH rat model, although the amount of Mb per cardiomyocyte nucleus increases.

Research approach
To gain insights in the pathophysiology of the remodeled right ventricle in PH, this thesis used both clinical and experimental material and data. Cellular alterations were studied in both necropsies of the right ventricle, obtained from patients who died of end-stage PH, as well as in RV tissue obtained from the monocrotaline-induced PH rat, a well-established experimental model for PH. RV papillary muscles of the MCT model were obtained to measure the mechanical efficiency in vitro.

RV MVO$_2$ is difficult to measure invasively due to the absence of a common drainage system like the coronary sinus for the left myocardium. Only studies on animals undergoing a heavy open-chest protocol have been performed to investigate the metabolism of the (hypertrophied) right ventricle. The ability of positron emission tomography (PET) technology, on the other hand, is to noninvasively trace biologic pathways of radiolabeled compounds. In normal condition, current PET resolution is not able to discern the thin RV...
wall. However, due to the elevated pulmonary vascular resistance in PH the hypertrophied right ventricle enables the application of PET technology to study the RV metabolism. The mechanism of PET is explained in Figure 1.2. PET was performed as a complementary study to the work-up protocol of PH. This contains amongst others right heart catheterisation, cardiac magnetic imaging resonance and 6-minute walk distance.

We studied the oxidative metabolism of the hypertrophied right ventricle in PH patients using PET and the following tracers: myocardial perfusion of the right ventricle was measured using radiolabeled water ([15O]H2O). Using the combined data obtained from three scans with [15O]O2, [15O]H2O and [15O]CO the RV oxygen extraction fraction (OEF) was determined. Acetate is a small fatty acid that is readily being taken up and oxidized by the cardiomyocytes. Its clearance rate gives an estimation of the myocardial O2 consumption and is a more widely used tracer. Acetate is a small fatty acid that is readily being taken up and oxidized by the cardiomyocytes. Its clearance rate gives an estimation of the myocardial O2 consumption and is a more widely used tracer. Finally, [18F]fluor-deoxyglucose ([18F]FDG) is radiolabeled glucose with an altered glucose structure. After being taken up by the cardiomyocyte via the glucose pathway, further metabolism is prohibited. The [18F]FDG remains trapped in the cells, and the amount of myocardial uptake of glucose can thus be calculated. An overview of the different metabolic sites discussed above and their relationship between each other is given in Figure 1.3.

Figure 1.2 The working of PET tracers and PET. Radioactive tracers are compounds that contain a radioactive atom incorporated into molecules, like water, oxygen or glucose. Small amounts of these tracers are administered into the patient. Since they are identical in chemical composition to the natural compounds of interest, the tracers are recognized and taken up as such by, in this case, the myocardium. In the cardiomyocytes, the tracers become metabolized and/or trapped. There, the radioactive atom decays with emission of positrons (A). Within its vicinity, a positron collides with an electron and they become annihilated, during which two photons are produced that move into opposite directions (B). The two photons arrive simultaneously (within nanoseconds) at the detector ring of the PET scan and are then recognized as coming from the same annihilation (C). During a scan many-thousands of such annihilations are detected, which are used to localize and reconstruct a 3-dimensional image of the myocardium based on its way of the metabolization of the tracer compound (D). Thus, a PET image provides biochemical information of the myocardium, which, however, lacks anatomically correct information. PET is usually combined with anatomical imaging, such as CT or MRI.
OUTLINE OF THE THESIS

Impaired transport of O\(_2\) due to increased diffusion distance from blood vessels to cardiomyocytes and Mb deficiency may thus play a role in the development of RV failure in PH. To investigate whether the determinants of cardiomyocyte O\(_2\) diffusion is altered in PH patients in, we studied post-mortem RV tissue of PH patients and compared it to RV tissue of control non-PH patients in Chapter 2.

In Chapter 3 we studied the feasibility to measure RV O\(_2\) extraction by means of the non-invasive PET scan and \(^{15}\)O-tracers in PH patients.

In Chapter 4, we investigated whether the \(\text{MVO}_2\) measured using the short-lived (and therefore less likely applicable) \(^{18}\)O-tracers is linear to the estimated O\(_2\) consumption obtained from \(^{13}\)C-acetate-PET for the hypertrophied right ventricle in PH patients.
In Chapter 5, we studied whether the systolic PAP and heart rate were also major determinants of myocardial O₂ consumption of the right ventricle in PH patients.

In Chapter 6, we set out to measure myocardial perfusion in relation to resting O₂ extraction during exercise.

We tested the hypothesis that a similar reduction in mechanical efficiency of the right ventricle during failure in PH patients exists as in the failing hypertrophic left heart (Chapter 7). Also, we investigated whether the RV mechanical efficiency is related to leftward septal bowing and RV myocardial glucose uptake. Then, to study whether reduced mechanical efficiency can be due to altered cardiomyocyte characteristics, we additionally investigated mechanical efficiency in the isolated rat papillary muscle that was obtained from the hypertrophied right ventricle of the rat model with progressive PH (Chapter 8).

Finally, in Chapter 9 we discuss whether the increased O₂ demand and reduced O₂ supply indeed would lead to cardiomyocyte hypoxia in the living tissue. We close the discussion with future recommendations based on the findings of the present thesis.
REFERENCE LIST


3. Vonk Noordegraaf A, Westerhof N. Right ventricular ejection fraction and NT-proBNP are both indicators of right ventricular pressure overload on myocardial glucose and free fatty acid metabolism in the conscious open-chest dogs. Jpn Circ J 1993;57:533-542.


LIST OF CALCULATIONS AND DEFINITIONS

- **CaO₂ (mL O₂/mL blood)** = O₂ content, amount of available O₂ molecules in blood
- **O₂ uptake** = Arterial CaO₂ – Venous CaO₂, also AV O₂ difference
- **O₂ extraction fraction**
- **O₂ extraction (%)** = O₂ extraction fraction · 100%
- **Myocardial O₂ supply (mL/min/g)** = Arterial CaO₂ · MBF
- **Myocardial O₂ consumption (mL/min/g)** = Arterial CaO₂ · MBF · OEF
- **Ventricular Power (J/s)** = HR · mPAP · SV · 2.22·10⁻⁶
- **Mechanical efficiency (%)** = \( \frac{\text{Power}}{\text{MVO₂} \cdot \text{RV mass} \cdot 0.34} \)
- **Systolic wall stress (mmHg)** = \( \frac{3 \cdot P \text{ systole}}{\ln (1 + \frac{\text{RV wall ES}}{\text{RV lumen ES}})} \)