SUMMARY, DISCUSSION AND FUTURE PERSPECTIVES
INTRODUCTION

Idiopathic pulmonary arterial hypertension (PH) is a pulmonary vascular disease characterized by a progressive elevation in pulmonary vascular resistance and right ventricular (RV) remodeling. Patients having PH develop fatal right heart failure for which little treatment is available. Increased understanding of the mechanisms underlying right heart failure is necessary to develop new therapeutic strategies, which can stabilize or even reverse RV failure. There is increasing evidence that the oxygen (O₂) supply as well as the O₂ utilization is altered during myocardial hypertrophy. We hypothesized that the O₂ supply and demand balance is shifted in the hypertrophied right ventricle, causing cardiomyocyte hypoxia that underlies the development of RV failure in PH. To study this, we evaluated whether alterations in O₂ metabolism were associated with the deterioration in RV function in PH patients as well in the monocrotaline (MCT)-induced PH rat model. Our translational studies provide new insights in the O₂ metabolism during RV dysfunction and failure in PH and the results have been discussed in the previous chapters and are summarized in the present chapter. Furthermore, an overview addressing the main findings is given in Figure 9.1. We close the discussion with an answer to the question whether cardiomyocyte hypoxia is present in the hypertrophied right ventricle in PH, using a mathematical approach based on the findings presented in this thesis.

Figure 9.1 The myocardial oxygen balance in the failing right heart secondary to pulmonary arterial hypertension. Included are the factors affecting the balance, indicated with numbers of the chapters in which they are discussed in the present thesis.
SUMMARY

Oxygen supply and transport to the cardiomyocyte is determined by capillary perfusion, O₂ extraction and intracellular facilitated diffusion. Myoglobin (Mb) is the intracellular protein that is responsible for diffusion. It is probable that due to cardiomyocyte hypertrophy in PH, the O₂ supply is limited by a reduction in capillary density and an increase in intracellular O₂ diffusion distance. It was therefore hypothesized that Mb concentration is upregulated to counterbalance the O₂ supply alterations during RV remodeling. In Chapter 2 we studied this hypothesis in end-stage PH by means of RV autopsy material of patients who died of PH, in comparison to RV tissue of non-PH patients who died of acute left myocardial infarction. To our surprise, Mb concentration in the RV tissue of PH patients was significantly reduced compared to control, thus suggesting decreased intracellular facilitated diffusion. Additionally, we found clear hypertrophy of RV cardiomyocytes in the PH tissue, whereas the number of capillaries per myocyte was similar in PH and control RV myocardium. This resulted in a significantly lower capillary density in the PH right ventricle. The results indicate that in end-stage PH the diffusion of O₂ is limited by the lack of all three factors. Additional analyses performed on the right myocardium of the PH rat model showed a similar reduced Mb content in progressive PH. The experimental data show, however, that these changes were already present in stable disease, although less pronounced. Since a lowered iron concentration is found in PH patients and the functionality of Mb depends on the availability of iron that is needed to be implemented in the haem molecule of Mb, iron supplementation may be an effective therapy to upregulate Mb concentration in the (cardio)myocytes and with that increase the intracellular O₂ facilitated diffusion in PH.

To study the in vivo O₂ utilization of PH patients, we studied the oxygen extraction fraction (OEF), an important determinant of myocardial oxygen consumption by means of dynamic PET scanning and bolus inhalation of the short-lived tracer [¹⁵O]O₂ in Chapter 3. The method of analysis was optimized by investigating potential re-use of myocardial blood flow (MBF), perfusable tissue fraction, and blood and lung spillover factors derived from separate [¹⁵O]H₂O and [¹⁵O]CO scans. We showed that the measurement of OEF in both left and right ventricles in PH patients is feasible using bolus inhalation of [¹⁵O]₂ and a dynamic scan protocol, with optimal accuracy and precision when other relevant parameters, such as MBF, are derived from an additional [¹⁵O]-water scan. To calculate the myocardial oxygen consumption (MVO₂ in mL/min/g) a combination of the three PET scans is necessary. However, as of yet these tracers are only available in centers that have an on-site cyclotron, like the VU Medical Center. Also, the protocol demands an invasive, often painful, placement of a canule in the brachial artery to sample O₂ metabolites and arterial O₂ content. To avoid these drawbacks in the future, we subsequently compared the hypertrophied RV MVO₂ derived from Chapter 2 with the more stable radiotracer [¹¹C]acetate in Chapter 4. The myocardial clearance rate of [¹¹C]acetate, K_monot, is known to give an estimate of MVO₂ and has been shown to correlate with left ventricular MVO₂. We found a significant correlation between the K_monot and the quantitative MVO₂ for the hypertrophied right ventricle in IPAH patients. Similar correlations were found for the [¹¹C] acetate clearance rate based on the efflux rate constant, Kₑ. These findings indicate that [¹¹C] acetate clearance rate can be used as an index for RV oxidative metabolism in PH patients in future studies.
In the following chapters, we applied the method described in Chapter 3 to investigate the RV oxidative metabolism in PH. This method yields absolute O2 extraction values that enable calculation of MVO2 using the product of MBF, O2 extraction and arterial O2 content. It was shown previously that major determinants of the left ventricular MVO2 are systolic pressure and heart rate. Using PET and 15O-tracers, we demonstrate in Chapter 5 that the major determinants of RV MVO2 are the systolic pulmonary artery pressure (PAP) and heart rate in PH. As systolic PAP is the main determinant of both the RV wall stress and rate pressure product, these parameters were also significantly related to the RV MVO2. Our findings indicate that the RV O2 consumption increases with the progression of PH, tipping over the O2 supply and demand balance to O2 demand side. Based on these findings we postulate that a reduction in the heart rate and/or PAP, by reducing the PVR, is likely to improve the balance between myocardial supply and demand of oxygen.

Arguments supporting this postulation are supplied by the Appendix of Chapter 4 and 5 that discusses the effect of PH treatment on the RV oxidative metabolism in 1 treatment-naive patient who was included in our study cohort. After one year of treatment, the PVR reduced spectacularly along with the normalization of other haemodynamical and clinical parameters. In parallel, the estimated RV MVO2 (i.e. Kmono) normalized to values similar to the baseline values of the other optimally treated PH patients. Since this is only a case report, future studies are warranted to include interventions in reducing heart rate and/or systolic PAP to study their effects on the RV MVO2 in both treatment-naive as well as optimally treated PH patients.

Myocardial O2 consumption, on the other hand, is provided for by the MBF and the OEF. MBF becomes a major determinant for the myocardial O2 metabolism in particular when O2 extraction capacity is maximized at baseline and the O2 demand is further elevated due physical exercise. When the increase in perfusion is also limited, myocardial hypoxia and ischemia may emerge during exercise. In Chapter 6, we investigated whether the MBF increases sufficiently when the baseline O2 extraction capacity is maximal in PH patients by means of the [15O]H2O-PET scans at baseline and during submaximal exercise performed on a recumbent bike (40% of maximum achieved load during cardiopulmonary exercise test). The median baseline RV OEF was 73% and the RV MBF a increased significantly during exercise. Subsequent division into a higher and a lower OEF group using a cut-off point of the median OEF value showed a similar baseline MBF in both groups. Interestingly, however, the group with a baseline OEF of more than 73% proved to have a significantly lower increase of RV MBF during exercise. Moreover, this group had poorer haemodynamics, with lower stroke volume and cardiac output, and overall a higher NYHA class compared with the lower OEF patients. The data suggest that PH patients with higher baseline OEF are prone to develop myocardial hypoxia during exercise.

In Chapter 7, we looked into RV myocardial energy metabolism at baseline in terms of the ratio of RV power (the product of cardiac output and mean PAP) to MVO2, i.e. RV mechanical efficiency. For this purpose we studied patients with mild PH (NYHA class II) and compared them with severe PH patients (NYHA class III). We found that the RV power was similar in the mild and severe PH patients, whereas NYHA III patients had a significantly higher RV MVO2. This resulted in a significantly lower RV mechanical efficiency in the severe PH patients, indicating that the failing right myocardium in severe PH requires more oxygen to generate the RV power. We additionally found that the RV mechanical efficiency was strongly related to the RV ejection fraction, indicating that RV mechanical efficiency can be seen as a measure for RV function,
reflecting the energetics of the pump. The lowered RV mechanical efficiency can be partly explained by a worsening of leftward septal bowing expressed as the post-systolic isovolumic contraction time. In contrast, tricuspid regurgitation did not appear to play a significant role in the reduction of RV mechanical efficiency. Furthermore, the lack of correlation between RV glucose uptake, measured by [18F]FDG, and mechanical efficiency, suggests that the glucose shift is a secondary process that, once heart failure has developed, cannot prevent lowering of the mechanical efficiency.

In Chapter 8 we investigated whether a reduced RV mechanical efficiency was also present in papillary muscle isolated from the hypertrophied right ventricle in the PH rat model. If so, cellular alterations due to myocardial hypertrophy and failure also underlie the reduced mechanical efficiency next to mechanical effects (leftward septal bowing, Chapter 7). RV mechanical efficiency was significantly reduced in the isolated PH rat papillary muscle compared to control. In addition, we found that the RV wall thickness and RV cardiomyocyte cross sectional area were inversely related to the mechanical efficiency, suggesting that intracellular changes have occurred during the hypertrophy process causing inefficient O2 use in relation to cardiomyocyte work. This was not due to changes in sarcomeres and myofilaments as the isometric contractions of hypertrophied muscle and controls were similar. We argue that O2-consuming-non-ATP producing reactions, due to e.g. mitochondrial dysfunction, may be possible causes for lowering the mechanical efficiency. This is currently under investigation in our laboratory.

Mathematical approach to determine cardiomyocyte hypoxia based on in vivo (PET) data and histological (post-mortem) data obtained from PH patients; a discussion

The findings of previous chapters suggest that the O2 supply and demand is imbalanced leaning over to an increased O2 demand with a reduced O2 supply in the hypertrophied and failing right ventricle in PH (Figure 9.1). However, that this results in cardiomyocyte hypoxia remains to be demonstrated. Nevertheless, in order to evaluate the risk of the occurrence of hypoxia in RV cardiomyocytes, we evaluated this hypothesis mathematically by combining data from the post-mortem data (Chapter 2) and the 15O-PET scan data of PH patients (reported in Chapter 3, 5 and 7). The combination is only justified when the hypertrophic changes (i.e. increase in myocyte cross-sectional area, decrease of Mb concentration and capillary density) observed in autopsy material are already present in severely ill patients.

The estimated MVO2_{2max} of the human heart equals 0.45 nmol/mm3/s (see supplement Chapter 2). The in vivo resting RV MVO2 was measured by means of 15O-PET (Chapters 3, 5, 7). An estimation of the MVO2 during submaximal exercise was also given. For this, we made an assumption that myocardial perfusion only increases when O2 extraction is maximal, ~80% or more. The estimated MVO2 during exercise is then calculated as the product of ‘maximal’ OEF, exercise MBF and arterial O2 content. The MVO2_{max} is not reached by the PH patients at baseline (0.08 ± 0.02 nmol/mm3/s) and during exercise (0.16 ± 0.05 nmol/mm3/s), even when the PET data are corrected for 30% interstitial space, yielding: 0.11 ± 0.03 nmol/mm3/s and 0.22 ± 0.07 nmol/mm3/s, respectively: exercise at 40% of peak VO2 doubles MVO2, but MVO2 during exercise is only half the predicted MVO2_{2max} when oxygen supply is not rate limiting. When MVO2 = 0.22 ± 0.07 nmol/mm3/s, cardiomyocyte cross-sectional area is 824 μm2 and myoglobin
concentration is 0.65 mM (Chapter 2), the required interstitial oxygen tension \( \text{PO}_{2\text{crit}} = 3.6 \text{ mmHg} \). Using Ficks law, it can be calculated that this requires a mean capillary \( \text{PO}_2 \) = 42 mmHg in the exercising patients, which is a realistic value close to the \( P_{50} \) of blood. (see supplement Chapter 2, for references). Because it is unlikely that mean capillary \( \text{PO}_2 \) during exercise can increase to values higher than 42 mmHg. We conclude that increasing the exercise load to values above 40% of peak VO\(_2\) are expected to lead to hypoxia in cardiomyocytes in severely ill patients, indicating that these patients should avoid heavy exercise. This is in agreement with the study of Handoko et al.,\(^9\) who demonstrated that exercise in progressive pulmonary hypertensive rats (the MCT 60 model) reduces survival.

**CONCLUSION AND FUTURE PERSPECTIVES**

The underlying causes that has been discussed in the present thesis for a diminished \( O_2 \) supply and increased \( O_2 \) demand during RV remodeling to RV failure can be found Figure 9.1. As remodeling and failure is a gradual process, we believe that the various causes mutually reinforce each other to contribute to the disturbance of the \( O_2 \) balance. On the one side, perfusion (\( O_2 \) supply) reserve is limited at the macroscopic level by a limited hyperemic blood flow in the severe PH patients with increased baseline \( O_2 \) extraction (Chapter 6), which is further worsened by histological changes such as increased cardiomyocyte cross sectional area (i.e. cardiac hypertrophy), reduced capillary density (or rarefaction) and reduced functional Mb concentration (Chapter 2). On the other hand, \( O_2 \) demand is increased due to an increased need of energy (ATP) to generate power to pump against the elevated RV afterload in PH (Chapter 7). Additionally, the systolic pulmonary artery pressure and heart rate are major determinants to the RV myocardial \( O_2 \) consumption in PH (Chapter 5). Furthermore, the reduced mechanical efficiency itself causes an increased myocardial \( O_2 \) demand in patients with severe PH, which is in part due to inefficient mechanical contraction by leftward septal bowing (Chapter 7). The remaining other part must be looked for at the cardiomyocyte level since isolated RV papillary muscles of PH rats also demonstrated reduced mechanical efficiency (Chapter 8). Figure 9.2 places in simplified perspective a few of the above-mentioned metabolic alterations that are likely to be involved in heart failure, mainly resulting in a reduced mechanical efficiency. The contributive role of each alteration remains unaddressed in this thesis and is subject to future studies.

The thesis provides new evidence that adds to and supports previous findings on the RV blood flow and perfusion in PH and increased myocardial \( O_2 \) consumption during heart failure.\(^1\)\(^-\)\(^4\)\(^-\)\(^6\) It brings the question forward whether restoration of the oxygen balance might prevent RV failure, warranting future clinical as well as experimental studies. The data of the Appendix indeed suggests that this balance can be reestablished by improving the RV mechanical efficiency during ordinal PH therapy in the treatment-naïve patient (similar mechanical RV power and reduced estimated RV MVO\(_2\) to almost halve).

Figure 9.1 suggests possible sites that can be intervened in order to answer this question. For instance, capillarization can upregulated by VEGF and/or erythropoietin or cardiomyocyte reactive oxygen species reduced by scavenger supplement.\(^10\)-\(^14\) As suggested earlier, iron supplement may be the proper therapy that effectively will increase the active Mb concentration in PH. Furthermore, beta-blocker treatment may be a promising drug with a wide-range of
possible positive affects that reduces the heart rate via blockage of over-stimulated adrenergic activity, increases mechanical efficiency, and in experimental PH it also reduces capillary rarefaction, RV fibrosis, cardiac inflammation and improves survival. Currently, as a result of the findings above, our group has initiated several clinical studies investigating the effect and benefit of 1. iron suppletion on the endurance of the skeletal muscles in PH patients and 2. β-blocker on the RV O₂ metabolism in PH patients.

Calculations suggest that hypoxia can occur in the hypertrophied cardiomyocyte. To study and quantify this phenomenon in IPAH in vivo, hypoxia should be demonstrated non-invasively in the failing right ventricle. PET imaging of tissue hypoxia has been described using a diversity of hypoxia tracers, for instance nitroimidazole agents (e.g. [¹⁸F]fluorodeoxyglucose, NADH nicotinamide adenine dinucleotide, [¹⁵O]O₂ O₂ oxygen-labeled oxygen molecule, [¹⁵O]H₂O O₂ oxygen-labeled water.

Figure 9.2 Adapted schematic overview of Figure 1.3 addressing the possible metabolic pathways in a dysfunctioning cardiomyocyte in heart failure. Possible causes for energy loss and reduced mechanical efficiency are indicated next to the normal metabolic pathway. For instance, dysfunctional mitochondria at the level of the respiratory chain result in misuse of oxygen molecules (radical formation) and loss of energy (uncoupling). Furthermore, misuse of energy can also result from septal bowing. Non-contractile ATP usage include futile ion pumping by the sarcoplasmic reticulum (calcium) and across the sarcolemma (calcium, sodium, potassium). On the other hand, a small amount of extra ATP may be contributed by anaerobic glycolysis. The thick broken-lined arrow represents myoglobin, O₂ buffer and transporter within the cell. Grey blocks are the PET tracers. Shaded block are haemodynamic measurements, including septal bowing. ATP adenosine triphosphate, [¹³C]acetate carbon-acetate, FADH₂ flavin adenine dinucleotide, [¹⁸F]FDG fluor-fluorodeoxyglucose, NADH nicotinamide adenine dinucleotide, [¹⁵O]O₂ O₂ oxygen-labeled oxygen molecule, [¹⁵O]H₂O O₂ oxygen-labeled water.
trapped only in viable cells that are hypoxic and it has been proposed previously as tool to identify cardiac hypoxia and/or ischemia.\textsuperscript{18-21} The application of such tracers and PET may enable future non-invasive monitoring of oxygen handling in the hypertrophied right myocardium during the treatment of IPAH patients.

In conclusion, this thesis provides a basis for future studies to improve the $O_2$ supply-demand balance in the diseased right ventricle in PH. These studies should be directed as such that a definitive answer can be given regarding the question whether restoration of the balance between oxygen supply and uptake is possible and can prevent RV failure.
REFERENCE LIST


