Striated muscle dysfunction in Pulmonary Arterial Hypertension

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Summary

Pulmonary arterial hypertension (PAH) is a rare and detrimental disease, in which vascular proliferation and remodeling leads to a progressive increase in pulmonary vascular resistance. The right ventricle (RV) adapts to this increase in pulmonary artery pressure by hypertrophy, but is eventually not capable to sustain the chronic pressure overload and will dilate and fail. Quality of life of PAH-patients is reduced as a result of a reduction in exercise capacity, fatigue and the sensation of dyspnea. With current therapy, partial reductions in RV afterload can be achieved, however the reduction is not sufficient to prevent RV failure. Therefore, treatment strategies for PAH-patients aimed to improve everyday quality of life, have gained importance.

Previous studies have indicated that the symptoms described above are not only caused by a reduction in RV function. Evidence is accumulating that also other striated muscles such as the left ventricle (LV), respiratory and peripheral muscles play a role in the symptoms observed in PAH-patients. The aim of the present thesis was to unravel the underlying pathophysiology of striated muscle dysfunction in PAH. We specifically focused on the contractile function of the sarcomeres, the smallest contractile unit of a muscle.

Due to the increase in pulmonary artery pressure, the RV of PAH-patients hypertrophies and eventually dilates and fails. In addition, there are indications that also LV function is altered in PAH-patients. The ventricles are not separate entities; the function of the two ventricles is inextricably linked in both the healthy and diseased heart. Thus, adaptations of the RV can induce alterations in the demand placed on the LV. This may be caused by reduced filling of the LV, as a consequence of decreased RV output and subsequently reduced LV input. Furthermore, leftward septum bulging is a well-known characteristic in PAH which can further hamper LV filling. This may lead to the

observed reduction in LV ejection fraction, LV free wall mass and LV dysfunction.

In **chapter 2** we investigated whether LV cardiomyocyte atrophy and reduced contractility contributes to LV dysfunction in PAH-patients. To this end, biopsies were obtained from the LV of PAH-patients and donor subjects. A 30% reduction in cardiomyocyte cross sectional area (CSA) was observed in PAH-patients, reducing the force generating capacity to the same extend. In addition, maximal tension (force normalized to CSA) was decreased by 30% in PAH-patients, which was most likely caused by a reduction in the major contractile protein myosin. This leads to a total reduction of ∼50% in total force generating capacity of LV cardiomyocytes of PAH-patients compared with donor subjects. However, calcium (Ca^{2+})-sensitivity of force generation was increased in PAH-patients, thereby partly compensating for the reduction in force generating capacity at physiological submaximal calcium concentrations. These results suggest that cardiomyocyte contractile dysfunction could contribute to the reduction in LV ejection fraction and LV strain in these patients. Furthermore, we propose that this also contributes to the post-operative complications observed in PAH-patients after a lung-transplant when the hypertrophic and hypercontractile RV vigorously pumps blood into the low resistance pulmonary circulation. The LV, which was adapted to a low filling state, cannot cope with the sudden increase in LV filling and might fail.

At rest, more than 20% of cardiac output goes to the skeletal muscles, this percentage can increase up to 84% upon extreme physical exertion. Cardiac function is therefore a very important determinant of exercise capacity. RV and LV function is reduced in PAH-patients, leading to a reduction in cardiac output and reduced O_2 supply to the muscles which may affect skeletal muscle function. In addition, PAH-patients hyperventilate at rest, during exercise and sometimes even during sleep. Consequently, inspiratory muscle activity increases, which may ultimately lead to over-loading of the inspiratory muscles and inspiratory muscle weakness. Indeed, maximal inspiratory pressure is lower in PAH-patients compared with control subjects. This indicates that the force generating capacity of the inspiratory muscles is impaired.

In PAH-rats it was previously shown that the diaphragm muscle, the main inspiratory muscle, was weakened. In **chapter 3** we have shown that breathing frequency increases in these PAH-rats with disease progression. In addition, we demonstrated that the reduction in diaphragm muscle contractility was partly caused by sarcomeric dysfunction of diaphragm muscle fibers. A significant reduction of 15-25% in maximal tension was found in especially fast-twitch diaphragm muscle fibers of PAH-rats. This was most likely caused by a reduction in force generated per cross-bridge. In addition, Ca^{2+}-sensitivity of force generation was significantly lower in fast-twitch PAH-diaphragm fibers, which could contribute to diaphragm muscle weakness at physiological calcium concentrations. These findings strongly suggest that sarcomere function is impaired in the diaphragm of PAH-rats.

Encouraged by the findings of **chapter 3**, we investigated in **chapter 4** whether this sarcomeric dysfunction was also present in the diaphragm muscle of PAH-patients. We combined *in vivo* inspiratory muscle function with *ex vivo* diaphragm muscle fiber contractility in chronic thromboembolic pulmonary hypertension (CTEPH) patients. In addition, to augment diaphragm fiber contractile strength of CTEPH-patients, we tested the ability of a novel, small molecule drug CK-2066260 to improve Ca^{2+}-sensitivity of force generation. Histology revealed that muscle fiber CSA was not significantly different between CTEPH-patients and controls subjects, in both slow-twitch and fast-twitch muscle fibers. However, diaphragm contractile function in CTEPH-patients was significantly reduced. A 15% reduction in maximal tension was observed in slow-twitch muscle fibers of CTEPH-patients compared with control subjects. This was most likely caused by a reduction in the number of available cross-bridges, caused by a reduction in the major contractile protein myosin. In addition, Ca^{2+}-sensitivity of force generation was significantly reduced in fast-twitch muscle fibers, leading to a reduction of ∼25% in submaximal force development in CTEPH-patients. The fast troponin activator CK-2066260 could markedly increase the contractile strength at physiological calcium concentrations in fast-twitch diaphragm muscle fibers of CTEPH-patients, to levels that exceeded those observed in control subjects. Interestingly, sarcomeric weakness correlated with *in vivo* inspiratory muscle function, suggesting that diaphragm sarcomeric dysfunction contributes to the reduced contractile strength of the inspiratory muscles in CTEPH-patients. This may contribute to the sensation of dyspnea, which is most likely caused by an imbalance between the demand placed on the inspiratory muscles and the capacity of the inspiratory muscle to generate force.

Leg fatigue is one of the dominant symptoms of PAH-patients to stop cycling exercise, suggesting that PAH-patients suffer from peripheral muscle dysfunction. Indeed, maximal quadriceps muscle strength is reduced in PAH-patients. The underlying cause of the reduction in muscle strength is unclear. Some studies have reported muscle fiber atrophy and a shift towards more fast-twitch fatigable fibers in skeletal muscle of PAH-patients; however these are not consistent findings. We have shown in **chapter 4** that weakness of the respiratory muscles in PAH-patients was at least partly caused by impaired contractility of the sarcomeres. Physical activity declines with disease progression in PAH-patients, and muscle disuse is known to affect sarcomere function. Therefore, we investigated in **chapter 5** whether sarcomeric contractility is also affected in peripheral muscles of PAH-patients.We obtained biopsies of the quadriceps muscle of idiopathic PAH-patients and healthy controls and measured the contractile properties of permeabilized muscle fibers. A significant reduction in maximal tension was found in fast-twitch muscle fibers of PAH-patients. This weakness was caused by a reduction in the number of available cross-bridges, which could be the result of a loss in the major contractile protein myosin. Of note, the reduction in maximal tension (∼15%) was less than the reduction in muscle strength measured *in vivo* (20-30%), suggesting that extrasarcomeric

changes, for example in the process of excitation-contraction coupling, and/or muscle atrophy also contribute to peripheral muscle weakness.

Thus, sarcomeric dysfunction contributes to impaired functioning of the LV, diaphragm and quadriceps muscle in PAH-patients. Together with a reduction in cardiac output, this may contribute to exercise intolerance of PAH-patients. These findings provide rational to test therapeutic strategies targeting striated muscle function to improve muscle contractility and quality of life of PAH-patients.