## Striated muscle dysfunction in Pulmonary Arterial Hypertension

Manders, E.

2015

***document version***
Publisher's PDF, also known as Version of record

# 6

# Discussion

This chapter is based on:
E. Manders, S. Rain, H.J. Bogaard, M.L. Handoko, G.J.M. Stienen, A. Vonk-Noordegraaf, C.A.C. Ottenheijm and F.S. de Man. Back to basic - The striated muscles in pulmonary arterial hypertension: adaptations beyond the right ventricle. *European Respiratory Journal.* 2015. In revision

## INTRODUCTION

Pulmonary arterial hypertension (PAH) is a fatal lung disease with a poor prognosis. Although the origin of the disease is progressive remodeling of the small pulmonary vessels, patients eventually die of right heart failure. The right ventricle (RV), which pumps blood into the lungs for oxygenation, has to face an enormous increase in pressure in PAH. Despite initial adaptations, eventually the RV is not capable to sustain the chronic pressure overload and will dilate and fail [114, 115, 135, 147].

An increasing number of studies reveal that the impact of PAH reaches further than the pulmonary circulation. Striated muscles other than the RV are affected in PAH, such as the left ventricle (LV) (chapter 2), the diaphragm (chapters 3 and 4) and peripheral skeletal muscles (chapter 5). Alterations in these muscle-types are closely associated with clinical worsening, exercise intolerance and reduced quality of life [22, 66, 82, 83, 129]. Therefore, it is of utmost importance to obtain more insight into the pathophysiological mechanisms causing muscle dysfunction in PAH.

With this discussion on striated muscle function in PAH we aim to provide 1) an overview on cardiac muscle alterations in PAH; 2) an overview of the changes in skeletal muscle function in PAH and 3) a discussion on potential new therapeutic strategies to restore or even prevent striated muscle dysfunction in PAH.

## 6.1 CARDIAC DYSFUNCTION IN PAH, NOT LIMITED TO THE RIGHT VENTRICLE

As a consequence of pulmonary vascular proliferation and remodeling, pulmonary vascular resistance increases in PAH-patients. This results in an increased demand on the RV, leading to RV remodeling. This remodeling includes excessive fibrosis and cardiomyocyte hypertrophy; as a consequence, the RV develops both systolic and diastolic dysfunction. For further details on RV dysfunction in PAH, please see reviews [44, 113, 147].

### Structural contractile alterations in the left ventricle

RV remodeling in PAH-patients also affects LV function. LV dysfunction in PAH-patients is characterized by reduced ejection fraction and impaired diastolic function, and some studies report a reduction in LV free wall mass in PAH-patients [35, 43, 51, 68, 129]. However, the pathophysiology of LV dysfunction in PAH is incompletely understood. Recently, we obtained unique LV biopsies of PAH-patients and performed detailed analyses of LV cardiomyocyte structure, function, and protein composition (chapter 2). Our data showed that PAH LV cardiomyocyte cross sectional area (CSA) was $\sim 30\%$ smaller than control cardiomyocytes, indicating severe LV atrophy in PAH-patients. In addition, LV contractile function was reduced, even after correction for differences in cardiomyocyte CSA. This study indicated that in addition to atrophy, intrinsic dysfunction of the

sarcomeres limits the force generating capacity of LV cardiomyocytes of PAH-patients [83].

Clinically, this may explain the occurrence of LV failure in PAH-patients after lung transplantation. RV afterload restores after a lung transplant and the hypertrophic and hypercontractile RV vigorously pumps blood into the now low resistance pulmonary circulation. The LV, which was adapted to a low filling state, may not be able to cope with the sudden increase in filling and might fail [4, 67, 130, 153].

### Underlying etiology of LV dysfunction

Alterations in cardiac muscle function in PAH may be explained by systemic factors and local (loading) factors.

**Systemic factors** - To compensate for the low cardiac output, neurohormonal systems are up-regulated in PAH-patients [19, 20]. However, chronic neurohormonal stimulation leads to a compensatory down regulation of $\beta$-adrenergic receptors leading to disturbed protein kinase A (PKA) mediated phosphorylation of contractile proteins [13, 113]. Reduced contractile protein phosphorylation affects cardiomyocyte contractility and relaxation. Indeed, in both the RV and LV of PAH-patients key contractile proteins are hypophosphorylated, resulting in an increase in $Ca^{2+}$-sensitivity [83, 113, 115]. Several studies have demonstrated increased expression of systemic inflammatory markers in PAH-patients [26, 55, 112]. However, the functional consequence of inflammation on cardiomyocyte function has yet to be determined.

**Local factors** - We propose that in addition to systemic factors changes in load contribute substantially for the observed RV and LV dysfunction in PAH. The ventricles are not separate entities: the functions of the two ventricles are inextricably linked. The RV and LV are intimately attached through common muscle fibers, the interventricular septum, and share the pericardial space. Thus, adaptation of the RV induces alterations in the demand placed on the LV. An increase in RV afterload, as occurs in PAH-patients, leads to a decrease in RV output. The RV and LV are serially linked, consequently LV filling is reduced [43, 63, 80]. In addition, direct ventricular interaction might further impair LV filling. Leftward septum bulging is frequently observed in PAH-patients [35, 91, 94, 129]. This is caused by prolonged RV shortening, pushing the septum towards the LV during early diastole, hampering LV filling [91]. Thus, LV filling is impaired in PAH-patients and might affect the functioning of LV cardiomyocytes.

Exciting new data where we have compared RV and LV cardiomyocyte adaptation in the same PAH-patients further emphasizes the importance of loading on RV and LV function [83, 115]. As shown in figure 6.1A, RV cardiomyocyte CSA increased almost 2-fold, while LV cardiomyocyte CSA decreased. This was paralleled by opposing results in maximal force development, even after correction for CSA (Fig. 6.1B). Furthermore, passive tension, a reflection of diastolic stiffness, increased 3-fold in the RV whereas LV passive tension was unchanged (Fig. 6.1C). Therefore, RV cardiomyocytes are hypercontractile, whereas LV cardiomyocytes are hypocontractile in PAH-patients (Fig. 6.2).

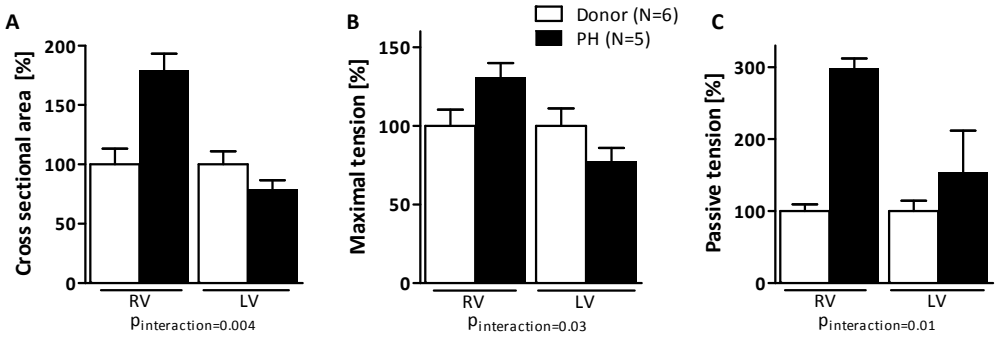


**Figure 6.1: RV and LV comparison of the same pulmonary hypertension (PH) patients.** All parameters are expressed relative to donor values of the specific ventricle. **(A)** Cardiomyocyte cross sectional area (CSA) was increased in RV cardiomyocytes of PAH-patients, while it was decreased in the LV of PAH-patients. **(B)** Maximal tension (i.e. force normalized to CSA), was increased in RV cardiomyocytes, while it was decreased in LV cardiomyocytes of PAH-patients. **(C)** Passive tension was greatly increased in the RV, while passive tension was unchanged in LV cardiomyocytes. Based on data from Rain et al. and Manders et al. (chapter 2) [83, 115]

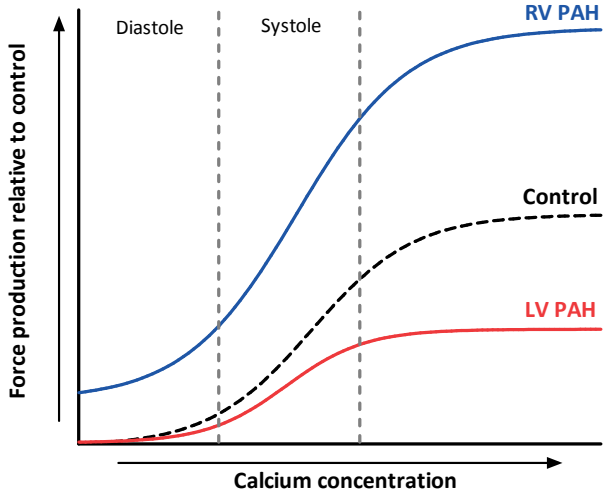


**Figure 6.2: Relative force production of RV and LV cardiomyocytes of PAH-patients compared with control.** RV cardiomyocyte are hypercontractile, while LV cardiomyocytes are hypocontractile compared with control subjects.

Since the load imposed on the ventricles is also in opposite direction this strengthens our proposition that loading is a major contributor of cardiomyocyte remodeling in PAH.

## 6.2 SKELETAL MUSCLE DYSFUNCTION IN PAH: TRIGGERED BY SYSTEMIC OR LOCAL FACTORS?

At rest, more than 20% of cardiac output (CO) goes to the skeletal muscles. This percentage can increase up to 84% upon extreme physical exertion [95]. Cardiac function is therefore a very important determinant of exercise capacity. As described above, both RV and LV function are hampered in PAH-patients, which results in a reduced capacity for stroke volume to augment CO during exercise. In addition, PAH-patients fail to maximally increase heart rate during exercise. This chronotropic impairment in PAH-patients is related to down regulation of $\beta$-adrenergic activity and is a reflection of disease severity [57, 110, 127]. Furthermore, oxygen ($O_2$) saturation decreases during exercise in PAH-patients [133]. Together with the reduction in CO, $O_2$ supply to the skeletal muscles might be limited, which may result in muscle dysfunction.

### Structural and contractile alterations in the skeletal muscles

Leg fatigue and dyspnea are the dominant symptoms causing PAH-patients to stop exercise, implying skeletal muscle dysfunction [127]. Maximal volitional and non-volitional strength of both the inspiratory and quadriceps muscles are reduced in PAH-patients, a reduction which correlates with exercise capacity [66, 82, 99]. Maximal muscle strength does not depend directly on $O_2$ supply, indicating that the intrinsic capacity of the skeletal muscle to generate force is impaired. Forearm grip strength correlates with maximal inspiratory pressure, suggesting the presence of a generalized muscle weakness in PAH-patients [6]. However, impaired $O_2$ supply to the muscles due to lowered cardiac output could also contribute to muscle weakness and fatigue during exercise. To discriminate between intrinsic muscle weakness and limitations in $O_2$ supply/extraction of the muscles, further experiments are needed. For example, by measuring exercise capacity and muscle strength under normoxia and hyperoxia (100% $O_2$) in combination with measurements on $O_2$ transport and metabolism [28].

Table 6.1 gives an overview of the structural and functional changes of the inspiratory and peripheral muscles in PAH-patients and animal models. Inspiratory muscle strength largely depends on the functioning of the diaphragm. Diaphragm muscle fibers in PAH are atrophied and hypocontractile (chapters 3 and 4) [2, 22, 84, 85]. More importantly, these changes also translated to in vivo reductions of inspiratory muscle contractility (chapter 4).

As illustrated in table 6.1, peripheral muscle structural and contractile alterations are less clear. Inconsistent data with respect to muscle fiber CSA, fiber type distribution and capillary density are found in both PAH-patients as well as PH animal models [5, 22, 82, 85, 109, 145, 152]. In addition, in PH-rats no change in force generating capacity was found, while in PAH-patients maximal tension was reduced (chapter 5) [22, 85, 86]. Thus, the underlying cause of the reduction in peripheral muscle strength

is still not completely clear, but might involve atrophy, sarcomere dysfunction, a shift towards more fast-twitch muscle fibers and capillary rarefaction.

### Underlying etiology of skeletal muscle dysfunction

It is generally believed that skeletal muscle dysfunction is the result of a systemic myopathy. We propose that systemic factors may affect the balance between protein synthesis and protein degradation, but that differences in muscle activity are an additional trigger to induce skeletal muscle dysfunction.

**Systemic factors** - Besides a diminished $O_2$ supply to the skeletal muscles as a result of cardiac dysfunction, other systemic factors can affect muscle function. Pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-$\alpha$ levels are elevated in the systemic circulation of PAH-patients [26, 55, 124]. Inflammatory cytokines affect muscle function by upregulation of proteolysis via the ubiquitin proteasome system and also induces contractile dysfunction [78, 116]. Both in the diaphragm as well as quadriceps muscle of PAH-patients and PH-rats increased levels of Atrogin-1 and MuRF-1 were found, which are key markers of proteolytic activity in muscle [5, 22]. This leads to a reduction in contractile protein content and muscle fiber atrophy. Neurohormonal over-stimulation might also affect muscle function by inducing changes in muscle metabolism and muscle regenerating capacity [40, 155].

**Local factors** - Skeletal muscles are very sensitive to changes in activity and load, and remodel accordingly [17, 59, 131]. In PAH-patients, the activity of inspiratory and peripheral muscles changes in opposite direction. PAH-patients hyperventilate during exercise, at rest and sometimes even during sleep, placing an increased demand on the inspiratory muscles [102]. On the other hand, peripheral muscle activity might decrease, as physical activity declines with disease progression.

In PH-rats, muscle fiber CSA and contractility were unaltered in a peripheral muscle

**Table 6.1: Overview skeletal muscle dysfunction in PAH**

| | **Inspiratory muscle** | | **Peripheral muscle** | |
|---|---|---|---|---|
| | **Animal** | **Human** | **Animal** | **Human** |
| **Muscle fiber size** | ⇓ [2, 22] | ⇓ [22], = [84] | ⇓ [152], = [22, 145] | ⇓ [5], = [22, 82, 109] |
| **Maximal tension** | ⇓ [2, 22, 85] | ⇓ [22, 84] | = [22, 85] | ⇓ [86] |
| **% of fast-twitch muscle fibers** | = [22] | = [84] | ⇑ [145], = [152] | ⇑ [5, 82], = [109] |
| **Capillary density** | = [22] | - | = [152] | ⇓ [109], = [82] |

while both were reduced in the diaphragm muscle (chapter 3) [22, 85]. Similarly, in 2 end-stage PAH-patients, quadriceps muscle fiber CSA was unaltered, while diaphragm muscle fiber CSA was reduced by $\sim$ 75% [22]. In addition, maximal tension was lower in muscle fibers of the diaphragm of CTEPH-patients compared to a those of a non-inspiratory muscle of the same patient, suggesting specific weakening of the diaphragm muscle (chapter 4) [84].

The diaphragm might be more vulnerable to changes in activity than the peripheral muscles. The activation of the diaphragm is very unique in relation to other skeletal muscles. Its normal daily duty cycle (ratio of active to inactive times) is $\sim$ 45%, while it is $\sim$ 15% in peripheral muscles [88]. The overstimulation of the diaphragm might lead to a greater and faster development of an imbalance between protein synthesis and protein degradation caused by systemic factors and consequently muscle dysfunction. Peripheral muscles might be less vulnerable to the systemic factors as muscle activity is lower. However, muscle disuse or unloading can by itself also induce contractile dysfunction and muscle fiber atrophy [17, 131]. A specific feature of skeletal muscle disuse and unloading is a shift towards more fast-twitch muscle fibers [17, 131]. This fiber type shift has also been observed in some PAH-patients, strengthening our proposition that muscle activity might contribute to peripheral muscle dysfunction in PAH. However, the inconsistent results on peripheral muscle function and structure in PAH might suggest that this depends more on individual variations in physical activity, disease duration and/or disease severity.

In conclusion, the differences between inspiratory and peripheral muscle adaptation suggests that the alterations are not only caused by systemic effects. Differences in muscle activity of the inspiratory and peripheral muscles are most likely an additional trigger to amplify skeletal muscle dysfunction in PAH.

## 6.3 ENCLOSING NOVEL TREATMENT TARGETS

The combination of a reduction in cardiac output, reduced $O_2$ supply to the muscles and skeletal muscle weakness leads to exercise intolerance in PAH-patients. Current disease targeted treatments are insufficient to prevent progression of right heart failure. In addition, vasodilators might improve $O_2$ supply to the muscles and thereby muscle function, but they do not sufficiently restore exercise capacity. Therefore, treatment strategies aimed at improving exercise capacity have gained importance. An overview of specific muscle treatment targets is illustrated in figure 6.3.

### Improving endurance capacity

**Exercise training** - Although it was initially thought that exercise training (ExT) could be detrimental for PAH-patients, it is now considered a valuable treatment to improve exercise capacity and quality of life. For a complete overview of ExT in PAH-patients

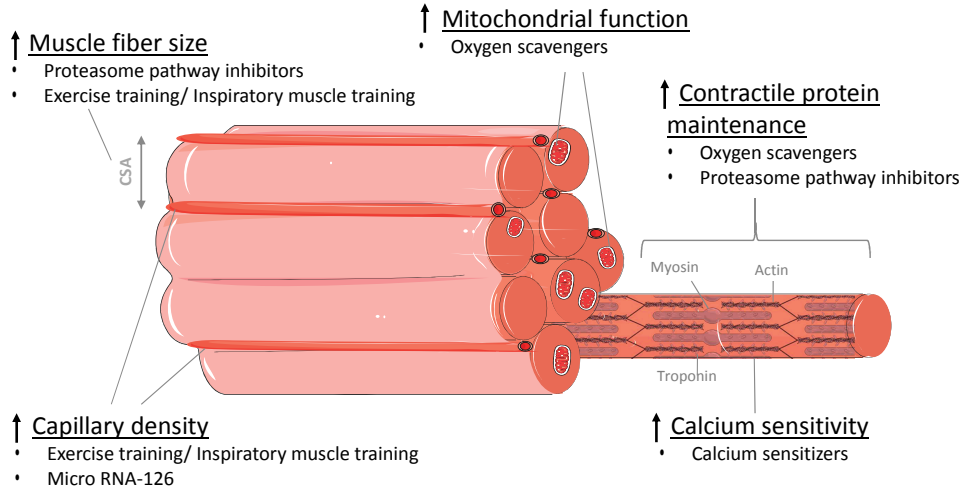


**Figure 6.3: Schematic representation of muscle fibers, including capillaries, mitochondria, contractile proteins.** Depicted are the points of application of new therapeutic options specifically targeting muscle function. Exercise and inspiratory muscle training (IMT) may increase muscle fiber cross sectional area (CSA) and capillary density. Proteasome inhibitors can improve muscle fiber CSA and contractile protein content. Oxygen scavengers can reduce oxidative stress which might improve contractile protein and mitochondrial function. Calcium sensitizers could improve submaximal force generating capacity and might improve muscle neuromechanical efficiency.

see review by Zafrir et al. [156]. Large improvements in 6MWT were reported in several studies after only 3 weeks of ExT [41, 42, 65, 97, 103]. However, most of these programs included walking training, which may directly act upon 6MWT. Nevertheless, also improvements in peak maximal heart rate, peak $VO_2$ and maximal work load have been observed after 12 weeks of training. De Man et al. demonstrated that exercise training also specifically alters quadriceps muscle function and morphology [18]. Improvements in aerobic capacity by increased capillarization and oxidative enzyme activity were found in the quadriceps muscle of PAH-patients after training. These improvements were in line with the increase in quadriceps muscle endurance [18]. Others found a reduction in fast-twitch muscle fibers and a potential increase in capillary density in the quadriceps of PAH-patients after ExT [81].

**Inspiratory muscle training** - With the inclusion of inspiratory muscle training (IMT) in the ExT program, inspiratory muscle weakness can be targeted. Improvements in inspiratory muscle strength were reported in PAH-patients, but also in a randomized control trial of IMT alone in chronic heart failure (CHF) patients [16, 65]. In addition, in CHF-patients no adverse effects have been reported indicating that IMT is a safe tool to improve inspiratory muscle strength [90]. Furthermore, IMT and ExT could also reduce sympathetic drive, which might lead to improvements in cardiac function and respiratory drive [96]. Most importantly, CHF and PAH-patients report a better quality of life and decreased sensation of dyspnea on exertion after IMT [16, 65, 97].

### Improving muscle structure and metabolism

Exercise training might not be feasible for all patients, due to for example hemodynamic instability [48]. Therefore, other strategies to improve muscle function are necessary.

**Proteasome pathway inhibitors** - In PAH, there is an imbalance between protein synthesis and degradation in peripheral and respiratory muscles, because proteolysis via the ubiquitin proteasome system is increased [5, 22]. Treating PAH-patients with proteasome inhibitors might lead to improvements in muscle fiber size and contractile function [1, 140].

**Mirco-RNA 126** - For proper muscle function, the increase in size should be accompanied with an increase in capillarization. Several studies have shown reduced capillary density in striated muscles of PAH-patients [48, 109, 117]. Potus et al. recently demonstrated that capillary rarefaction in PAH-patients was caused by down regulation of micro-RNA 126. Restoration of micro-RNA 126 in a rat model led to increased capillary density and improved endurance capacity [109]. Thus, modulation of micro-RNA 126 expression might be a new attractive therapeutic target to specifically improve capillary rarefaction in PAH.

**Oxygen scavengers** - The reduction in $O_2$ supply and capillary rarefaction could lead to oxidative stress within the muscles. Oxidative stress induces protein breakdown and mitochondrial dysfunction or damage. Oxygen scavengers, such as N-acetylcysteine, are able to reduce the levels of reactive oxygen species, thereby potentially reducing protein breakdown and ameliorate mitochondrial function [93].

### Improving sarcomere function

The aforementioned therapeutic options target muscle structure and metabolic pathways. However, sarcomere dysfunction is also an important contributor to weakness of the LV, diaphragm and quadriceps muscle in PAH-patients, making the sarcomere an attractive target for treatment.

**Calcium sensitizers** - Calcium sensitizers are small molecule drugs, which slow the dissociation of calcium from the troponin complex, thereby stabilizing the open conformation of the troponin/tropomyosin complex to enhance cross-bridge formation. Currently, Levosimendan is the only clinically available calcium sensitizer. However, Levosimendan targets slow-twitch muscle fibers and also affects cardiac muscle [27, 139]. As $Ca^{2+}$-sensitivity in the ventricles is already increased in PAH-patients, contributing to diastolic dysfunction; it could be detrimental for the heart to increase $Ca^{2+}$-sensitivity even further. Tirasemtiv is a novel calcium sensitizer that is currently in a phase 2B clinical trial. It specifically targets fast-twitch muscle fibers and has no effect on cardiac muscle [118]. As smooth muscle cells do not contain troponin, it also has no effect on the pulmonary vasculature. An analog of tirasemtiv was tested on single permeabilized muscle fibers of the diaphragm of PAH-patients. It greatly improved submaximal force generation of fast-twitch muscle fibers (chapter 4). Besides improving submaximal force

generation, calcium sensitizers might also be energetically beneficial, as less calcium is needed for a certain force response. Re-uptake of calcium from the cytoplasm into the sarcoplasmic reticulum during relaxation is a high energy consuming process. It was shown that neuromechanical efficiency improved by 21% in the diaphragm of healthy controls after acute supplementation of a calcium sensitizer [24].

In summary, exercise training including inspiratory muscle training could be beneficial for both cardiac and skeletal muscle function. Nonetheless, improving muscle oxygenation by micro-RNA 126 modulation and contractile function with calcium sensitizers might further improve exercise capacity and quality of life of PAH-patients.