Clinical relevance of exercise testing in pulmonary hypertension

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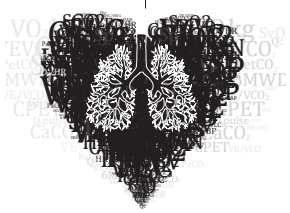
CONTENTS



1 General introduction and outline of the thesis

DEFENITION

Pulmonary hypertension (PH) is an increased blood pressure in the pulmonary circulation. PH leads to breathlessness, dizziness, fainting and other symptoms, all of which are provoked by exertion. PH occurs in many different disease states and its presence usually results in a markedly decreased exercise tolerance. Pulmonary hypertension is diagnosed by right hart catheterization and defined by a mean pulmonary arterial pressure of more than 25 mmHg (11).

Classification and disease severity

PH is classified into five different clinical groups, recognizing similar pathophysiological mechanisms, clinical presentations and therapeutic options (15):

1. Pulmonary arterial hypertension (PAH), characterized by a normal pulmonary capillary wedge pressure
2. PH associated with left heart disease
3. PH associated with lung diseases and/or hypoxemia
4. PH due to chronic thrombotic and/or embolic disease (CTEPH)
5. Miscellaneous causes of PH

PAH includes various forms of PH of different aetiologies but similar clinical presentation, and in many cases similar response to medical treatment. PAH is characterized by pathological changes in the different components of the pulmonary vascular bed (arterioles, capillaries or veins). PAH is associated with the development of right ventricular failure, which leads to breathlessness, exercise limitation and, ultimately, death (5). PAH patients are studied in all of the chapters of this thesis with the exception of chapters 7 and 8, in which clinical group 3 PH patients (associated with lung disease and/or hypoxemia) are included. PAH is a rare but life threatening complex disorder. The prognosis of treated PAH patients is still very poor with a median five year survival of 50% (11).
The New York Heart Association (NYHA) Functional Classification provides a simple way of classifying the extent of heart failure. It places patients in one of four categories based on the degree of limitation during physical activities (*see Table 1.1)*. In PAH, NYHA Functional class is strongly related to prognosis. Consequently, exercise tolerance has an important prognostic value (11) and is put forward as a meaningful clinical end-point in PAH drug trials (13)

NYHA class I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity.
NYHA class II	Mild symptoms and slight limitation during ordinary activity.
NYHA class III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity. Comfortable only at rest.
NYHA class IV	Severe limitations. Experiences symptoms even while at rest.

Table 1.1 *The New York Heart Association (NYHA) Functional Classification.*

PATHOPHYSIOLOGY OF EXERCISE LIMITATIONS WITH PAH

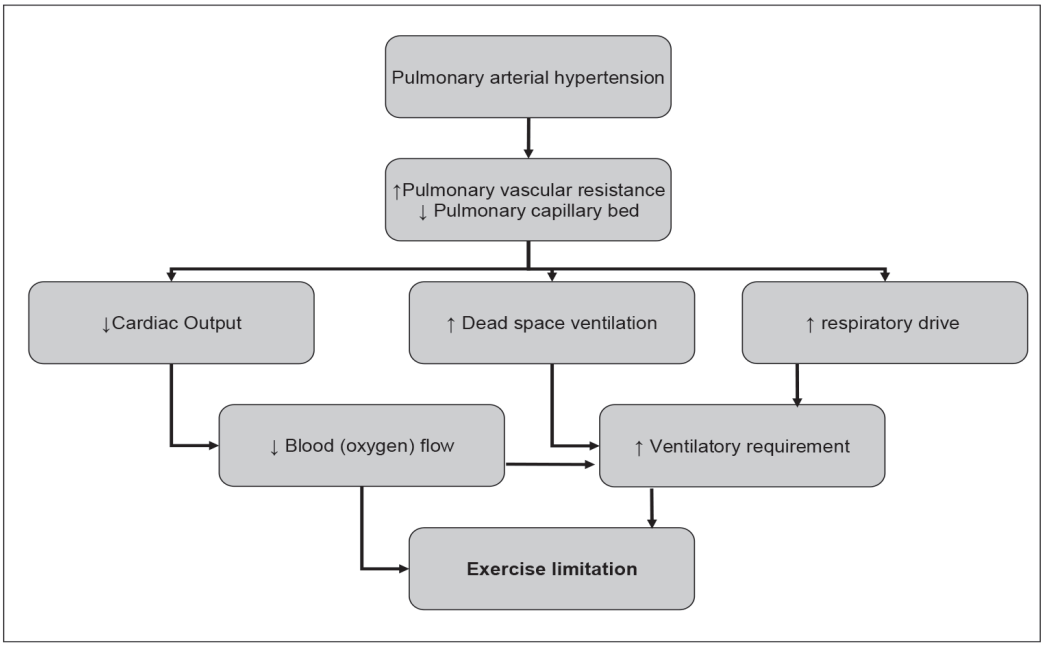


Figure 1.1 *Pathophysiology of exercise limitation of PAH patients. (adapted from Sun et al. (19))*

The two most important pathophyiological causes for exercise limitation in patients with PAH are an impaired blood flow to the peripheral muscles and an increased ventilatory requirement (Figure 1.1) (19).
In PAH, the increased afterload and reduced ventricular function impair cardiac output (8). In the normal healthy situation, maximal exercise is accompanied by an eight-fold increase in ventilation and a four-fold increase in cardiac output, together ensuring an increase in oxygen delivery. Because of insufficient oxygen delivery in PAH patients, aerobic ATP generation is impaired and lactic acidosis occurs at a lower exercise intensity.
The increased ventilatory requirement in patients with PAH is caused by increased dead space ventilation due to hypoperfusion of normally ventilated alveoli (20) and an increased respiratory drive. The causes of an increased ventilatory drive are poorly understood, but likely involve stimulation of the respiratory center in the brain stem by acidosis, hypoxemia and increased sympathetic activation (7, 21). An increased respiratory drive in PAH leads to hypocapnia, the degree of which is related to the extend of cardiac dysfunction and predict clinical deterioration (7).

Monge's disease

This thesis also includes a study (chapter 8) on patients with chronic mountain disease (CMS). CMS was first recognized by Carlos Monge M. in Peru and is also known as Monge's disease. CMS is a clinical syndrome that occurs in residents living above an altitude of 2500m. As more than 140 million people worldwide live above 2500m, CMS is a significant public health problem. The disease is characterized by excessive erythrocytosis ([HB]≥ 11.8 mmol*L^{-1} for females and ≥ 13 mmol*L^{-1} for males), severe hypoxemia and in some cases PH classified in clinical group 3 (9).

The normal adaptive response to the decreased inspired oxygen pressure at altitude is hyperventilation (25). As their hypoxic ventilatory drive seems hampered, CMS patients respond to a decrease in arterial oxygen pressure by excessive erythrocytosis, leading to an increased blood viscosity, PH and impaired blood flow to the brain and working muscles. Hence, a lowered exercise tolerance without dyspnea on exertion is a common symptom of CMS (24). It is expected that the CPET profile of CMS patients differs from the profiles of healthy subjects or PAH patients.

WHY IS EXERCISE TOLERANCE A MEANINGFUL CLINICAL END-POINT IN PAH?

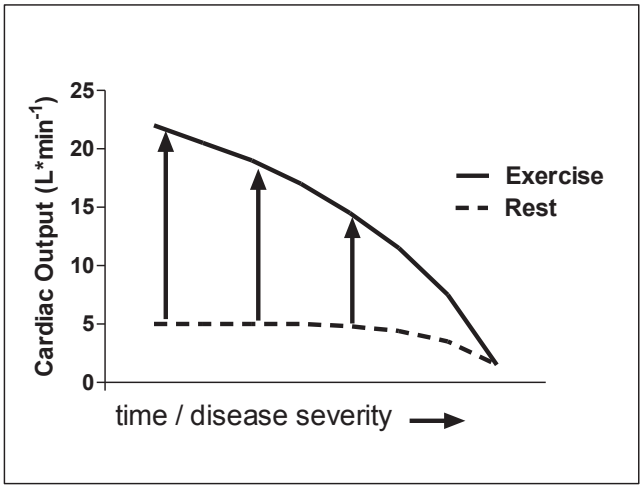


Figure 1.2 *Disease progression in pulmonary arterial hypertension. As the disease progresses, resting cardiac output (– – – –) remains stable for a long time but the ability to increase cardiac output on exercise (↑) is progressively impaired. Maximal exercise cardiac output (———) (Adapted from Peacock et al.(13))*

Resting cardiac output remains stable for a long time during the progression of PAH. In contrast, the increase in cardiac output during exercise is impaired early in the course of the disease (Figure 1.2), which explains symptoms of progressive breathlessness and fatigue. If therapeutic interventions in PAH aim to improve functional status, they should improve cardiac output during exercise. A meaningful clinical end-point in a drug trial should therefore reflect the cardiac output response during exercise (13).

EXERCISE TESTING IN PH

The six minute walk test and the maximal cardio-pulmonary exercise test (CPET) are the most frequently used exercise modalities in PAH clinical trials (13) and both are associated with functional status (12, 19). Although the six minute walk test and CPET have their own merits and disadvantages, both tests showed their clinical value in PAH trials. The six minute walking distance (6MWD) and several CPET parameters are significantly associated with mortality in PAH (12, 14, 16, 23).

Six minute walk test

The 6MWD is a relatively simple and inexpensive, well tolerated measure of functional exercise limitation (17) and therefore most often used as a clinical end-point in drug studies (13). The six minute walk test is adapted from the Cooper test in which the maximal distance run by athletes in 12 minutes is used as a surrogate for maximal oxygen uptake (VO_{2max})(2). In contrast with the Cooper test, running is not allowed during the six minute walk test. Accordingly, the 6MWD is a simple measure of the distance walked by patients within six minutes on a flat and hard surface (1). In contrast to the common believe that in healthy subjects the six minute walk test is not a maximal test but just an estimate of daily functional capacity, in heart failure patients the 6MWD is associated with peak aerobic capacity (3, 6, 12). The 6MWD reflects the sum of the integrated responses of all physiological systems involved to perform the work during this maximal walk test. Due to its simplicity, the six minute walk test provides no pathophysiologic insights into the mechanisms of exercise limitation in a tested subject. As a surrogate for VO_{2max} and by nature of the Fick equation (VO_2 = cardiac output x peripheral oxygen extraction) (4), the 6MWD is associated to cardiac output.
The six minute walk test has the disadvantage of a "ceiling effect", because patients are not allowed to run. Therefore, the six minute walk test is only indicated for patients with a moderate to severe heart- or lung disease. Patients with milder conditions are not able to increase their 6MWD according to their VO_{2max}, resulting in a curvilinear relationship between the 6MWD and VO_{2max} as shown by Lipkin et al.(10). In conclusion, because of its simplicity the 6MWD seems an ideal surrogate clinical end-point for PAH trials involving NYHA class III and IV patients. However, the 6MWD is less useful in patients with less severe disease and provides no physiological information about the nature of the exercise limitation.

Cardio-pulmonary exercise test

During CPET maximal VO_{2max} is measured and therefore CPET is the "gold" standard measure of maximal functional capacity. During CPET all ventilatory parameters can be measured together with blood pressure, arterial blood gasses and heart rate (HR), thereby providing insight into mechanisms of functional limitations. Hallmark abnormalities of PAH during CPET are decreases in VO_{2max}, oxygen pulse, and ventilatory efficiency (19), all reflecting an impaired exercise cardiac output and hampered O_2 delivery (Figure 1.1). VO_{2max} is related to cardiac output by the Fick equation: VO_2 = cardiac output x peripheral oxygen extraction (4). A decreased VO_{2max} in PAH quantifies the inability of the circulatory system to increase cardiac output due to the pulmonary vasculopathy. It follows from a rearrangement of the Fick equation (VO_2/HR = stroke volume x peripheral oxygen extraction) that a low oxygen pulse (=VO_2/HR) provides an indication of a low stroke volume (18).
An important function of the ventilation is regulation of the arterial carbon dioxide pressure to maintain body homeostasis. As a consequence, the decreased ventilatory efficiency in PAH patients is reflected during CPET by an increased ventilation relative to carbon dioxide production (VE/VCO_2). An increased VE/VCO_2 is caused by a combination of lactic acidosis, arterial hypoxemia, increased dead space ventilation and increased sympathetic drive (19, 26) (see above).
In conclusion, maximal CPET is a measurement of maximal functional capacity and has the advantage of showing a specific cardio-pulmonary exercise profile which may have clinical value: for establishing a diagnosis, for estimating disease severity, for predicting survival and for monitoring responses to treatment (13, 19, 23).

AIM AND OUTLINE OF THE THESIS

Because an impaired cardiac response to exercise reflects the severity of the disease (19) and has prognostic relevance (23), it is clear that in PH exercise testing has a clinical potential. Several important research questions pertaining the pathophysiology of exercise intolerance in PH remain. Some of these questions are topics of the research outlined in this thesis.

Although CPET has the potential to evaluate cardiac function in heart failure (19, 22), none of the measured CPET variables are direct measurements of cardiac-output or stroke volume. **Chapter 2** focuses on the validation of a relatively simple, non-invasive method to estimate the stroke volume response during exercise. In **chapter 3**, oxygen pulse is used to compare the exercise stroke volume responses between patients with right- and left-sided heart failure. Research in patients with congestive heart failure demonstrated that the 6MWD as well as several CPET variables have prognostic value (12, 23). It is unclear whether CPET variables have an additional prognostic value when 6MWD is already known. This question is subject of study in **chapter 4**. In newly diagnosed PAH patients, the outcome of CPET holds prognostic power (23). In **chapter 5**, it is studied whether CPET parameters have additional prognostic value when measured as changes over time. Because of the demonstrated beneficial effects of exercise training in patients with congestive heart failure, we investigated

the effects of training in idiopathic PAH, and the results are described in **chapter 6**. Chronic obstructive pulmonary disease (COPD) and chronic mountain disease (CMS) are two significant global clinical problems and are classified in PH clinical group 3: PH associated with lung disease and/or hypoxemia. The extent to which PH influences well known abnormal exercise responses (ventilatory limitation) in COPD is studied in **chapter 7**. CMS patients are expected to have a lowered exercise tolerance due to PH in combination with a blunted hypoxic ventilatory response. In **chapter 8**, pathophysiological exercise responses are studied in patients with CMS. In **chapter 9** we summarize our findings and discuss future perspectives.

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2 Stroke volume response during exercise measured by acetylene uptake and MRI

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ABSTRACT

The intra-breath technique to measure acetylene absorption offers the possibility to determine augmentation of the pulmonary blood flow per heart beat (Q_C) as an estimate of the stroke volume response during exercise. However, this method has not been compared with a validated test until now. Therefore, the aim of this study was to compare Q_C with stroke volume (SV_{MRI}) determined by magnetic resonance imaging (MRI) at rest and during exercise in healthy subjects and patients. For this purpose, 10 healthy subjects and 10 patients with idiopathic pulmonary arterial hypertension (iPAH) with expected impaired stoke volume response during exercise were measured by both methods. Exercise induced changes in Q_C and SV_{MRI} were correlated in healthy controls ($r = 0.75$, $p < 0.05$). Compared to healthy controls, Q_C increased less during exercise in iPAH patients (11 ± 17 ml vs. 33 ± 12 ml, $p < 0.05$). A similar difference in stroke volume response to exercise between the two groups was measured by MRI (– 0.6 ± 8 ml vs. 23 ± 12 ml, $p < 0.05$ respectively). Hence, intra-breath and MRI measurements showed similar differences in exercise-induced changes in stroke volume between controls and patients. From these results it can be concluded that intra-breath measurement of acetylene absorption might be of value as a non-invasive tool to estimate stroke volume augmentation during exercise and can detect differences in stroke volume responses between iPAH patients and healthy subjects.

INTRODUCTION

Physical exercise requires the ability of the cardiovascular system to increase stroke volume (SV) to support the increased energy demands of the contracting muscles. Determination of the SV response during exercise can contribute to the detection of circulatory failure. In heart failure, the SV response during exercise is inversely related to disease severity (Metra et al., 1999). Hence, non-invasive assessment of the blood flow per heart beat as an estimate of SV during exercise by a simple inhalation of a gas, would be useful to the evaluation of patients with a possible cardiovascular exercise limitation. It has been suggested that the determination of acetylene absorption might accurately indicate pulmonary blood flow. Acetylene is a very soluble gas whose absorption in the lung is limited by perfusion and is diffusion independent. In the absence of a significant pulmonary or cardiac shunt, pulmonary blood flow will equal cardiac output. Hoeper and colleagues (Hoeper et al., 1999) showed that acetylene absorption measured by means of the rebreathing technique (Sackner et al., 1975;Triebwasser et al., 1977) can be used as an accurate measure for cardiac output in healthy subjects and idiopathic pulmonary arterial hypertension (iPAH) patients at rest. However, due to the need for expensive equipment such as the mass spectrometer this rebreathing technique has not been widely used. Another disadvantage of this technique is that patients need to empty the rebreathing bag with a volume of 60% of patient's vital capacity for 30 seconds during the rebreathing maneuver. This hyperventilation maneuver is difficult to perform during exercise. An alternative method to measure acetylene absorption is the intra-breath technique developed by Newth and Ramage (Newth et al., 1977;Ramage, Jr. et al., 1987). The principle is based on the measurement of acetylene during a single slow exhalation which is simple during exercise. Despite the fact that principles of this technique have been known for a long time, the validation of this technique during exercise is lacking. A likely explanation is the fact that measurements of gas exchange during exercise are usually performed in pulmonary patients. In many of this patients, uneven and delayed emptying of lung compartments would limited the accuracy of the intra-breath technique. This problem is not encountered in iPAH patients and therefore determination of the validity of this technique in iPAH is warranted. Clinically accepted methods to measure cardiac function like direct Fick (Hoeper et al., 1999) method, thermodilution (Sadeh et al., 1997) or radionuclide angiography (Grimby et al., 1966) are technical demanding, invasive, time consuming and expensive. Though magnetic resonance imaging (MRI) is an expensive and technically demanding alternative to measure SV,it's non-invasive nature makes it much more patient friendly. This technique, not only allows the accurate assessment of SV, but can also be used during exercise.(Firmin et al., 1987;Kondo et al., 1991;Rebergen et al., 1993;Holverda et al., 2006;Roest et al., 2001). Therefore, this study was conducted to compare the pulmonary blood flow by heart beat measured by intra-breath acetylene absorption to SV measured by MRI at rest and during exercise in healthy subjects and patients with a severe exercise limitation caused by an impaired SV response during exercise.

MATERIALS AND METHODS

Subjects

We included two groups with expected different SV responses: 10 healthy subjects and 10 patients with iPAH. iPAH patients have severe exercise limitation due to increased pulmonary artery pressure and absence of the normal increase in SV during exercise (Holverda et al., 2006). Since iPAH patients have, by definition, no parenchymal lung disease the disease is considered a true model of cardiocirculatory exercise limitation (Sun et al., 2001). IPAH was defined as a mean pulmonary arterial pressure of more than 25 mm Hg at right heart catheterisation and a pulmonary capillary wedge pressure < 15 mmHg. (Barst et al., 2004)
The control group consisted of 10 healthy nonsmoking subjects with pulmonary functions results within the normal range, including forced expiratory volume in 1 second (FEV1) and transfer factor for carbon monoxide (TLCO) above 80% of predicted (Quanjer et al., 1994). In the iPAH group resting spirometry and total lung capacity (TLC) measurements, were within the normal range (Quanjer et al., 1994) and showed no significant differences compared to the healthy control group. The TLCO values were moderately reduced in the iPAH patients group. These values were similar to the pulmonary function results measured in iPAH patients as described in previous studies (Sun et al., 2003;Steenhuis et al., 2000). None of the iPAH patients showed evidence of parenchymal lung disease on the high resolution computed tomography (HRCT) scan. Hemodynamic characteristics of the patients obtained from right heart catheterization performed within one week after the pulmonary function tests showed a mean pulmonary artery pressure of 51 ± 18 mmHg, a cardiac output of 5.6 ± 1.3 L/min, a cardiac index of 3.1 ± 0.9 L/min/m^2 , a pulmonary vascular resistance of 736 ± 417 dyne · sec/cm^5 and a wedge pressure less than 15 mm Hg. Based on the resting hemodynamics and functional characteristics, the iPAH patients can be classified as having moderate pulmonary hypertension. The iPAH patients were stable on daily treatment at the time of examination, which consisted of i.v. epoprostenol in four patients and oral bosentan in six patients. To continue epoprostenol administration during MRI measurements the connection hose between the epoprostenol delivery pump and the patient was extended since this pump is not MRI compatible. The demographic characteristics of both study groups are summarized in Table 2.1. The VU University Medical Center Institutional Review Board approved of the study and informed consent was obtained in all patients.

DEMOGRAPHIC CHARACTERISTICS OF STUDY SUBJECTS

	HC	iPAH	*p*-value
Gender (f/m)	6/4	5/5	
Age (y)	41 ± 13	38 ± 16	ns
Height (cm)	175 ± 8	177 ± 9	ns
Weight (kg)	74 ± 10	78 ± 11	ns

Table 2.1 *Definition of abbreviations: HC = healthy controls; iPAH = pulmonary arterial hypertension; ns = not significant.*

Pulmonary function test and cardiopulmonary exercise test

All iPAH patients and healthy controls underwent a pulmonary function test consisting of spirometry, bodyplethysmography and TLCO and a cardiopulmonary exercise test (CPET). Standard pulmonary function equipment (Vmax 229 and 6200, SensorMedics, Yorba Linda, USA) was used. Heart rate (HR) was measured by pulse oximetry (9600, Nonin, Plymouth, USA). Vital capacity (VC), FEV_1, TLCO, alveolar volume (VA) and TLC were measured in accordance with European Respiratory Society (ERS) criteria (Quanjer et al., 1994).
Physician supervised CPET was performed with gas exchange measurements during 3 minutes of rest followed by progressively increasing work load of 5 to 20 W/min to maximum tolerance and 3 minutes of recovery on an electromagnetically braked cycle ergometer (Rehcor, Lode, Groningen, The Netherlands).

Intra-breath method for acetylene

Intra-breath acetylene absorption was measured with a standard pulmonary function equipment (Vmax 229 legacy, SensorMedics, Yorba Linda, USA).Acetylene as well as methane was measured by a non-dispersive infrared, thermopile flash multi-gas analyzer. (range 0 – 0.33%, resolution 0.0005% and accuracy ± 0.0003%). Flow was measured by a mass flow sensor (range 0-960 L/min, resolution 0.18 L/min from 12 -960 L/min and accuracy ± 3%). Pulmonary blood flow per heart beat (Q_c) by intra-breath acetylene absorption in the healthy controls was measured in upright and supine positions at rest and during submaximal exercise on the cycle ergometer. Since the MRI measurements could only be performed in the supine position, whereas the intra-breath technique is most conveniently performed in a sitting position, we validated the influence of body position on the intra-breath outcomes in the healthy persons. For patient convenience the iPAH patients were measured in the upright position only.

The principles of the intra-breath method have been described previously (Ramage, Jr. et al., 1987;Huang et al., 1994;Huang et al., 2002;Martonen & Wilson, 1982). Briefly, the subject performed a rapid inhalation from residual volume to TLC of dilute (i.e. 0.3% each) methane and acetylene. After a brief breath holding (i.e. ± 1 s), the subject slowly exhaled, for a period of 5 to 10 seconds, at a relatively constant flow rate (0.5 L/s) with the aid of a flow restrictor (5 mm critical orifice) and an on-screen flow indicator. During the slow exhalation methane and acetylene concentrations and flow rate were measured continuously at the mouth, enabling calculation of the pulmonary blood flow values. Under these circumstances it is been shown that Q_C values can be calculated using the following equations (Martonen & Wilson, 1982):

$$Q_c = \frac{1}{HR} \cdot \frac{60 \cdot 1000}{P_B - 47} \cdot \frac{V_E}{\alpha_B} \cdot \frac{\ln F_{A,C_2H_2,0} / F_{A,C_2H_2}}{\ln\left(\frac{V_A + \alpha_t V_t}{V_{A_0} + \alpha_t V_t}\right)}$$

where:

$$F_{A,C_2H_2,0} = F_{I,C_2H_2} \frac{F_{E,CH_4}}{F_{I,CH_4}}$$

$$V_{A_0} = (V_I - V_D) \frac{F_{I,CH_4}}{F_{E,CH_4}}$$

Where V_E is expiratory flow rate; ln is the natural logarithm; VA is alveolar volume; VAO is alveolar volume at full inspiration; V_I and V_D are inspired volume and dead space volume respectively; F_{AC2H2} is the alveolar concentration acetylene prior to the beginning of gas absorption; F_{IC2H2} and F_{ICH4} are inspired concentrations of acetylene and methane, respectively; F_{ECH4} is expired concentration of methane; P_B is barometric pressure; α_B and α_t are Bunsen coefficients for acetylene solubility in blood and tissue (0.739 and 0.768 ml/ml at 1 atm, respectively)(Meyer & Scheid, 1980). V_t is pulmonary parenchymal tissue volume. Since V_t has only a small effect on the calculation of Q_C, it is assumed to be 700 ml. Linear regression with least squares analysis was used to calculate the slope of log fractional acetylene uptake versus log exhaled alveolar plus tissue volume between 20 and 80% of exhaled vital capacity. Q_C was corrected for phase 3 (alveolar phase) slope of methane by expressing acetylene concentrations relative to methane. Mean Q_C values were used from at least two measurements,

both of which had to be within 10% of the other, according to ERS guidelines for TLCO measurements (Quanjer et al., 1994).

Because SV reaches a plateau when workload exceeds 40% of maximum exercise intensity (Spina et al., 1992), the submaximal exercise was performed in both groups at 40% of maximal workload as determined during CPET. The exercise protocol consisted of 3 minutes period of cycling, reaching the predetermined exercise work load within the first minute. In the upright position the same bicycle as during CPET was used. A recumbent bicycle (Circular, Lode, Groningen, The Netherlands) was used in the supine position.

MRI studies

MRI measurements were performed in supine position 24 ± 12 hours after the intra-breath measurements. SV_{MRI} was acquired at rest and during submaximal exercise on a 1.5-T Siemens Sonata whole body system (Siemens Medical Solutions, Erlangen, Germany) as previously described (Holverda et al., 2006,(Marcus et al., 1998). The blood flow in the main pulmonary artery (MPA) was measured in an image plane perpendicular to the MPA, at the position halfway between the pulmonary valve and the pulmonary bifurcation. Two dimensional gradient echo imaging with through-velocity sensitivity of 150 cm/s was applied to obtain a one dimensional velocity measurement perpendicular to the image plane and thus parallel to the flow in the MPA. Three phase-encoding lines per heartbeat were acquired, and with echo-sharing the temporal resolution was 35 ms. Volumetric flow in ml/s was calculated by multiplying the spatial mean velocity in the MPA with the cross-sectional area of the MPA. SV_{MRI} was calculated by integrating the flow curve over systole (Roeleveld et al., 2004). For the exercise measurements, the number of time phases in the cardiac cycle was adapted to the patient's heart rate, and the through-plane velocity sensitivity was increased to 180 cm/s. The MRI measurements were performed during an identical exercise protocol as during intra-breath measurements on the same bicycle. Resting and exercise MRI data of the patients and controls have been reported previously (Holverda et al., 2006).

Statistical Analysis

The exercise –induced responses of the Q_C and SV_{MRI} were related by calculation of Spearman rank correlations. The agreement between Q_C and SV_{MRI} responses were analyzed as described by Bland and Altman (Bland & Altman, 1986). The Mann-Whitney U test was used to check for significant differences in PFT, CPET, O_2-pulse, Q_C and SV_{MRI} between the iPAH patients and healthy controls. Values below 0.05 (2-tailed) were considered to indicate statistical significance.

PFT AND CPET CHARACTERISTICS OF STUDY SUBJECTS

	HC	iPAH	*p*-value
VC (%)	111 ± 17	105 ± 19	
FEV_1 (%)	106 ± 14	94 ± 15	
FEV_1/VC (%)	80 ± 7	74 ± 6	
TLC (%)	106 ± 14	102 ± 15	
TLCO (%)	92 ± 16	72 ± 19	< 0.05
KCO(%)	87 ± 33	78 ± 17	
VA (%)	96 ± 10	91 ± 15	
VO_{2MAX} (L/min, stpd)	3.0 ± 1.2	1.3 ± 0.6	< 0.05
VE_{MAX} (L/min, btps)	100 ± 32	72 ± 28	< 0.05
HR_{REST} beats/min)	73 ± 11	84 ± 13	
HR_{MAX} (beats/min)	174 ± 16	155 ± 16	< 0.05
$Watt_{MAX}$	248 ± 93	105 ± 53	< 0.05

Table 2. 2 *Definition of abbreviations: PFT = pulmonary function test; CPET = cardio pulmonary exercise test; HC = healthy controls; iPAH = idiopathic pulmonary arterial hypertension; % = percentage of predicted; VC = vital capacity; FEV_1 = Forced expiratory volume in one second; TLC = total lung capacity; TLCO = transfer factor for carbon monoxide; KCO = TLCO/VA; VA = alveolar volume; VO_2 = oxygen uptake; VE = ventilation; stpd = standard temperature pressure dry; btps = body temperature pressure saturated; HR = heart rate.*

RESULTS

Pulmonary function test and cardiopulmonary exercise test

The PFT and CPET characteristics of the iPAH patients and the healthy controls are summarized in Table 2.2. A significant difference between both groups was found in the TLCO values. The CPET results showed that the healthy controls reached a higher maximum exercise tolerance with a higher maximum oxygen uptake (VO_{2max}), HR and maximum ventilation (VE_{MAX}) compared to the iPAH patients. Figure 2.1 shows that although the O_2-pulse (a measure of stroke volume) at rest was not different between both groups, O_2-pulse was significant lower during submaximal exercise and maximal exercise tolerance in the iPAH patients.

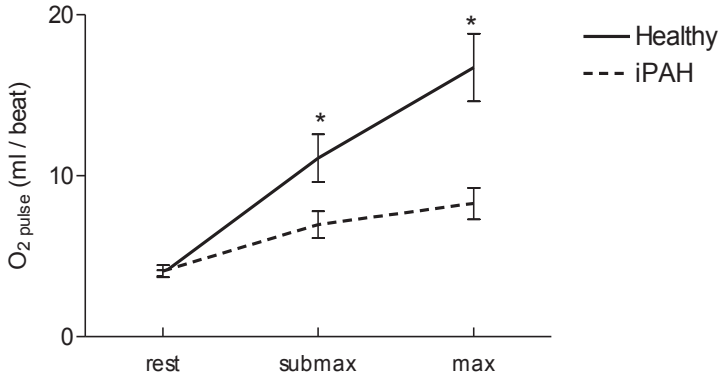

Figure 2.1 *Oxygen pulse measured during CPET in healthy controls and iPAH patients at rest, at 40% of maximal exercise tolerance (submax) and maximal (max) exercise tolerance. * p < 0.05, healthy versus iPAH.*

Upright versus supine position

In the healthy controls, resting and exercise HR's were independent from body position (upright and supine position 71 ± 9 and 70 ± 12 beats/min, respectively, and exercise HR 109 ± 10 and 111 ± 15 beats/min, respectively). The Q_C responses to exercise measured in both positions were related to the SV_{MRI} response (r = 0.64, p = 0.05 and r = 0.75, p < 0.05 respectively for upright and supine positions, respectively).

The absolute Q_C at rest in the upright position was 9.9 ml lower (not significant) compared to supine position (65.7 ± 16 and 75.6 ± 18 ml, respectively) while there was no difference during submaximal exercise (98.8 ± 24 and 98.3 ± 25 ml, respectively). The Bland Altman plot in Figure 2.2 shows a mean difference of 10 ml between the Q_C responses to exercise (calculated as submaximal exercise Q_C – resting Q_C) measured in both body positions.

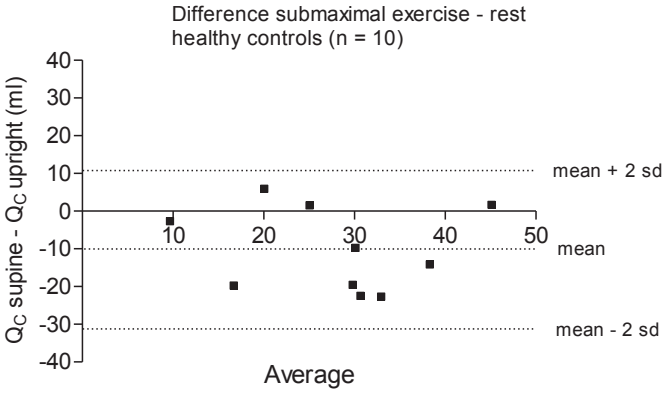

Figure 2.2 *Bland Altman plot from Q_C responses to exercise in supine and upright position (mean ± 2sd difference versus average).*

Intra-breath versus MRI

The Bland Altman plot in Figure 2.3 shows a statistically non significant mean difference of 0.05 ± 9 ml between the exercise responses of Q_C supine and SV_{MRI} in the healthy control group.

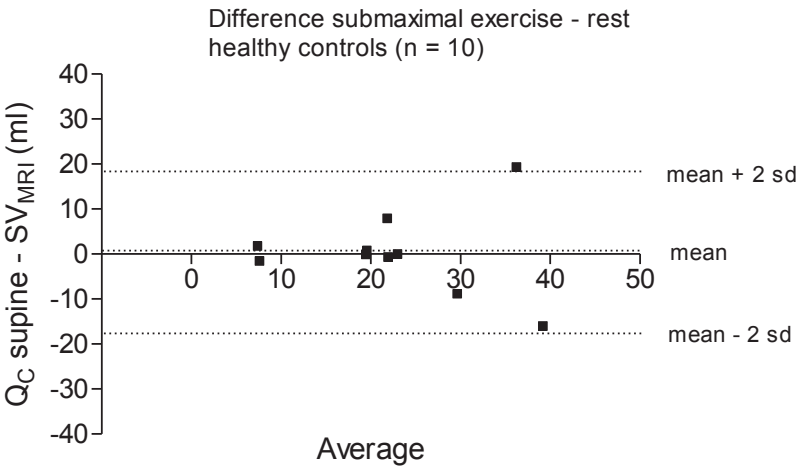

Figure 2.3. *Bland Altman plot of Q_C supine response to exercise versus SV_{MRI} (mean ± 2sd difference versus average).*

Figure 2.4 shows that in both controls and patients, the exercise response of Q_C (assessed in the upright position) was significantly greater than the exercise response in SV_{MRI} (assessed in supine position). While the absolute exercise response was considerably smaller in patients than in healthy subjects, the difference in responses between the measurements in the two body positions was quite similar.

iPAH versus healthy

In the iPAH patients resting HR is significantly higher compared to the healthy controls (86 ± 13 vs. 71 ± 9 beats/min) as well as the HR during submaximal exercise (117 ± 12 vs. 109 ± 10 beats/min). Figure 2.5 shows that the Q_C response to exercise measured by the intra-breath method was significantly smaller in the iPAH patients compared to the healthy controls (11 ± 17 ml vs. 33 ± 12 ml, respectively, both in upright position), whereas SV_{MRI} augmentation to exercise was – 0.6 ± 8 ml vs. 23 ± 12 ml respectively (both supine).

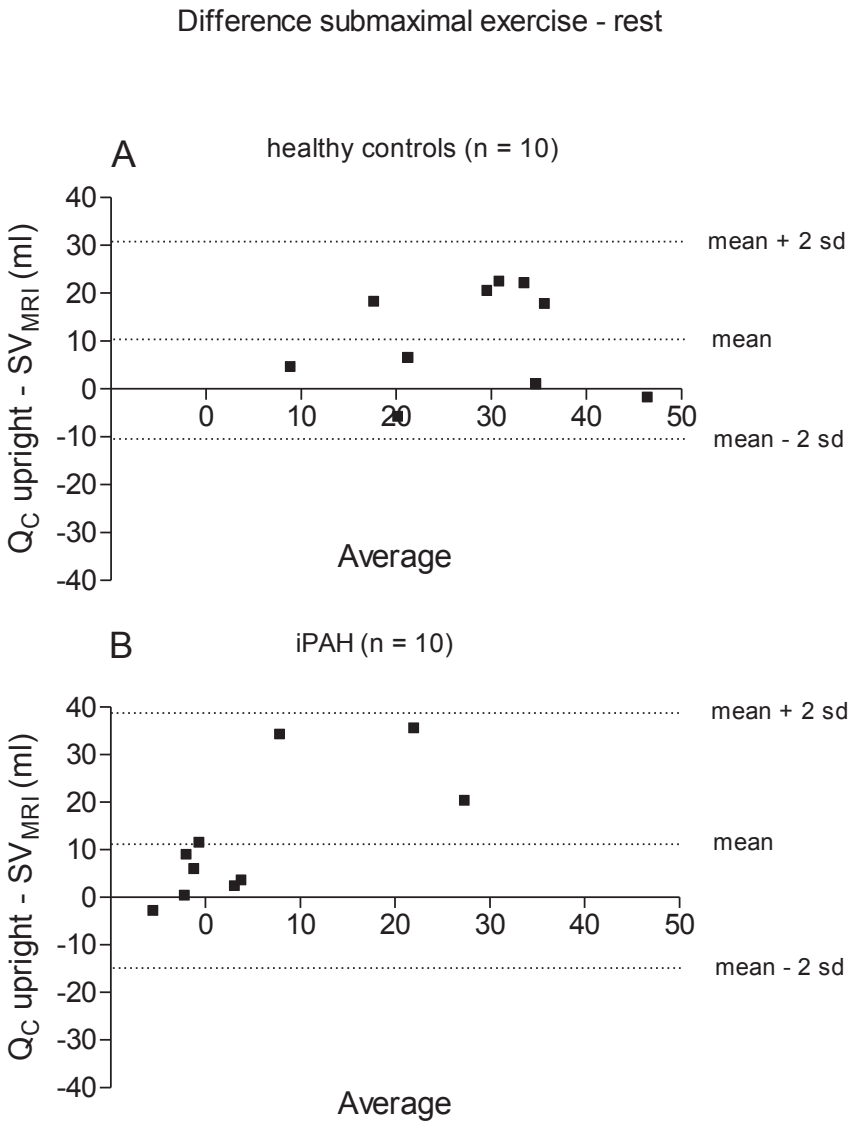

Figure 2.4 *Bland Altman plots from the response to exercise of QC upright versus SVMRI in healthy controls (A) and iPAH patients (B) (mean ± 2sd difference versus average).*

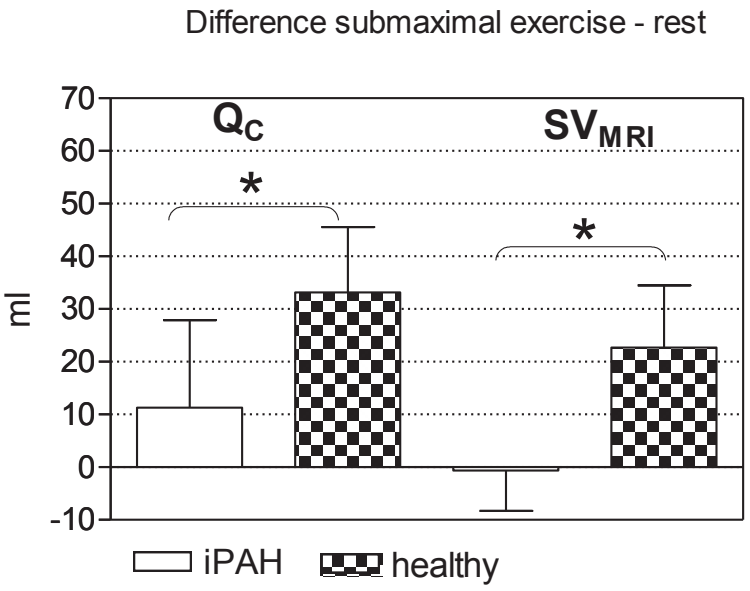

Figure 2.5
Absolute changes to exercise in pulmonary blood flow by heart beat (Q_C) measured by intra-breath acetylene absorption and stroke volume by MRI (SV_{MRI}) in iPAH patients and healthy controls. (mean ± sd) = $p < 0.05$.*

DISCUSSION

The present study shows that non-invasive measurement of Q_C at rest and during exercise by intra-breath acetylene absorption is feasible in iPAH patients and healthy controls and can be of use as an estimate of SV augmentation during an exercise protocol.

The intra-breath method was originally developed to calculate the TLCO during a constant exhalation (Huang, et al; 2002). The advantage of such a technique is that it can be performed during exercise. Earlier studies have already shown that by using acetylene pulmonary blood flow can be measured in resting conditions (Ramage, Jr. et al., 1987;Johnson et al., 2000;Johnson, Jr. et al., 1960;Spicer, Jr. et al., 1962). For this reason it was investigated whether the intra-breath method measuring the absorption of acetylene can be used to measure SV during exercise. To validate the intra-breath method we used MRI to measure SV since this method is non-invasive, clinically accepted, and can be used during exercise (Firmin et al., 1987;Kondo et al., 1991;Rebergen et al., 1993).
Q_C and SV_{MRI} values were closely related in the healthy controls. In this group intra-breath measurements in the upright position showed an increased Q_C response to exercise compared to the supine stroke volume response (SV_{MRI} or Q_C). From the literature it is well known that the absolute stroke volume response to exercise depends on the initial stroke volume at rest (Wang et al., 1960). Q_C and SV_{MRI} measurements were not measured simultaneously. The Q_C measurements were performed on a different day in a different room, and a maximal inspiration was required, which is known to cause a lowered pulmonary blood flow. Therefore, the measured systematic differences between both methods could probably be explained by

different circumstances especially at rest.
MRI measurements were performed in the supine position whereas the intra-breath maneuver is most easily performed in the upright position. Our results performed in the healthy subjects showed that the mean Q_C response to exercise in the upright position was higher than in supine position. This is in agreement with literature that shows that supine stroke volume is higher due to an increased venous return (Bestler et al., 1992;Parker & Thadani, 1979). Despite these absolute differences, the responses in both positions were related to SV responses measured by MRI. However, the small sample size to validate the Q_C response to exercise in different body positions is a limitation of this study.
The clinical significance of the intra-breath method is demonstrated in the iPAH patients, who were unable to augment SV during exercise, which was reflected by a significantly smaller Q_C response compared to healthy controls. The variation in SV responses within the group of iPAH patients was probably caused by the baseline characteristics of the iPAH patients showing a widely spread pulmonary vascular resistance caused by a significant variation in pulmonary vascular damage. Those 3 patients with a preserved SV response had a milder elevation of right ventricular afterload compared to the other iPAH patients. The iPAH patients were symptom limited in their maximal exercise tolerance as indicated by the significantly reduced maximal HR during CPET compared to the healthy controls. Hence, iPAH patients were measured at a lower submaximal exercise tolerance compared to the healthy controls because intra-breath measurements were performed at 40% of maximal workload, determined during CPET. It is unlikely that this has influenced the outcomes of the study since the O_2-pulse as an indirect measure of stroke volume did not increase in the iPAH patients after reaching their submaximal exercise level. This is in agreement with a recent study showing that iPAH patients have a more rapid heart rate response during exercise than healthy subjects in order to compensate for their inability to raise SV (Sun et al., 2001). The differences found between the iPAH patients and healthy subjects illustrate that the intra-breath technique can be helpful as a simple non-invasive tool during CPET. The advantage of the intra-breath method over the O_2-pulse method is that it provides an absolute value for SV, and is independent from peripheral factors such as muscle oxygen extraction. Earlier studies demonstrated that the rise in SV occurs in the first part of the exercise till 50% of the maximal aerobic capacity is reached and remains stable thereafter to the point of subject exhaustion (Spina et al., 1992;Jones et al., 1998). Therefore, a maximal exercise test is not needed to test the stroke volume response to exercise. Another advantage is the inexpensiveness of the measurement compared to the mass spectrometer and inhalation gas needed for the acetylene rebreathing technique. Furthermore, it can be performed with commercially available standard pulmonary function equipment. The test gas is similar to the gas used during standard TLCO measurements (0.3% CO, 0.3% CH_4, 21% O_2, rest N_2) when completed with 0.3% acetylene.
In conclusion, this study showed that the intra-breath method might be of value as a non-invasive tool to assess the stroke volume response to exercise and can detect differences in stroke volume responses between iPAH patients and healthy subjects.

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3 Exercise Stroke Volume and Heart Rate Response Differ in Right and Left Heart Failure

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ABSTRACT

Aims

In pulmonary arterial hypertension (PAH) the exercise induced increase of stroke volume is limited by the increase in pulmonary artery pressure. In left heart failure (LHF) systemic arterial pressure increases little during exercise and the stroke volume increase is limited by the left ventricle itself. These differences might be reflected in a dissimilar exercise response in stroke volume (SV) and heart rate (HR), which could have important clinical therapeutic implications, such a beta blockade therapy. Therefore, we tested the hypothesis that SV and HR responses during exercise are different between PAH and LHF patients.

Methods

We included 28 PAH and 18 LHF patients (recruited from the heart failure unit) matched on a maximal oxygen uptake less than 15 $ml*kg^{-1}*min^{-1}$ referred to our pulmonary function department between 2000 and 2008 for a maximal cardio-pulmonary exercise test. Only patients not exposed to beta-blockers were included.

Results

PAH and LHF patient groups showed equal impaired exercise tolerance (about 42 % of predicted) with a maximal oxygen uptake of 0.80 ± 0.29 and 0.86 ± 0.19 $l*min^{-1}$. The response to exercise in peak SV, was significantly lower in PAH (-14 ml, $p = 0.01$) compensated by a steeper slope of HR relating to oxygen uptake (+ 0.03 beats $*ml^{-1}$, $p = 0.001$)

Conclusion

We conclude that PAH patients have a smaller SV response, but larger HR response than LHF patients.

INTRODUCTION

Patients with pulmonary arterial hypertension (PAH) as well as patients with left heart failure (LHF) show impaired exercise tolerance due to circulatory limitations.(1) In both patient groups this circulatory limitation is reflected by a smaller increase in stroke volume (SV) and heart rate (HR) response to exercise, in relation to oxygen uptake (VO_2), as compared to healthy subjects.(2-5) In addition, for both diseases the chronotropic response is positively related to 6-min walk distance, a benchmark for disease severity, therapy and progression. (6;7) Despite these similarities there are also differences.

In PAH the exercise induced increase in cardiac output will bring about an increase in pulmonary artery pressure, thereby limiting the right ventricular SV increase and demanding a larger HR increase.(5;8) In LHF arterial pressure increases little during exercise resulting in a decreased systemic vascular resistance and therefore the increase in SV is less limited than in PAH.(9)

In a study to estimate differences in exercise testing variables, Deboeck et al. found a lower oxygen pulse, as a marker of a more impaired SV, in PAH compared to LHF patients, matched for functional class (NYHA II–IV).(2) However, since most of the LHF patients (84%) in that study used beta blockers; limiting the heart rate response, it is not clear whether the observed differences in oxygen pulses, as measure of SV, reflects a different exercise physiology between both diseases.

A more impaired SV in PAH compared to LHF will make exercise induced augmentation of cardiac output more dependent on HR, leading to a steeper HR response with exercise. Therefore, we conducted this study to test the hypothesis that SV and HR responses during exercise are different between PAH and LHF patients not exposed to beta blockers.

METHODS

Patients

We included 28 PAH and 18 LHF moderate to severely impaired patients (matched on maximal oxygen uptake (peak VO_2) of <15mL*kg^{-1}*min^{-1}) with objective evidence of cardiac systolic or diastolic dysfunction at rest or during exercise referred to the pulmonary function department at our institute for a maximal cardio-pulmonary exercise test (CPET) between 2000 and 2008.

PAH was defined as a mean pulmonary pressure of > 25 mmHg and a pulmonary capillary wedge pressure < 15 mmHg obtained by right heart catheterization in the absence of left sided heart disease, a disorder of the respiratory system and/or hypoxia, chronic thrombotic and/or embolic disease or disorder directly affecting the pulmonary vasculature.(10) The cause of pulmonary hypertension classified according to the Venice criteria(11) was idiopathic pulmonary hypertension in 14 patients, collagen vascular disease in 13 patients and drugs and toxins in one patient. None of the PAH patients had a history of cardiovascular or significant pulmonary parenchyma disease on high resolution computed tomography.

LHF patients in this study were recruited from the heart failure unit. Patients showed no pulmonary function disorder reflected by the normal spirometric values in both groups. LHF-

was a clinical diagnosis according to European Society of Cardiology guidelines and required the presence of typical signs and symptoms of heart failure in combination with objective evidence of structural or functional abnormality of the left ventricle on echocardiography. (12) In twelve patients, LHF was caused by ischaemic heart disease and in six patients by dilated cardiomyopathy. Because all the included LHF patients were new patients none of them were yet exposed to beta blockers. The study protocol was approved by the VU University Medical Center Institutional Review Board and informed consent was waived due to the retrospective nature of this study.

Pulmonary function and Cardio Pulmonary Exercise Test (CPET)

All patients were subjected to a CPET. After a spirometric pulmonary function test at rest, physician-supervised maximal CPET was performed on an electromagnetically braked cycle ergometer (Rehcor, Lode Groningen, The Netherlands) adhering to American Thoracic Society guidelines.(4)

Three minutes of upright rest were followed by three minutes of unloaded pedaling (0 W) and subsequently the workload was progressively increased (5-20 W min^{-1}) to maximum tolerance. The rate of workload increase was empirically determined by the supervising physician, based on the medical history and clinical data. Test duration was between 8 and 12 minutes (from unloaded pedaling to peak exercise) in all patients.

Minute ventilation (VE), VO_2, and carbon dioxide output (VCO_2) were measured breath by breath using a metabolic cart (Vmax 229, Viasys, Yorba Linda, USA) and analyzed as 20 second averages. Peak respiratory exchange ratio (RER), as a measure of maximal metabolic effort, was calculated by dividing VCO_2 by VO_2 values at peak exercise (20 seconds average). Anaerobic threshold (AT) was determined by the V-slope method.(13) Oxygen saturation of arterial blood (SaO_2) was measured by pulse-oximetry (9600, Nonin, Plymouth, USA). Hemoglobin was measured by finger tip blood puncture at rest before CPET by the hemocue 201$^+$ (Hemocue, Angelholm, Sweden). No patients were using supplemental oxygen during CPET.

Before each test the equipment was calibrated according to manufacturer's specifications. Briefly, the flow sensor was calibrated and verified by a calibrated three liter syringe. Gas analyzers were calibrated by a two point calibration (0% CO_2, 26% O_2 and 4% CO_2, 16% O_2, respectively) and ambient air (0% CO_2, 20.9% O_2).

HR was measured by electrocardiography (Eagle 4000, Marquette). O_2pulse was calculated as VO_2 divided by HR. Arterial (CaO_2) in ml*100ml^{-1} was calculated as hemoglobin (gr*dl^{-1}) times 1.34 times SaO_2.

According to the method described by Agostoni et al. and Stringer et al.,(14;15) SV was estimated from the oxygen pulse divided by peripheral extraction ratios taken from the literature: 6% at rest, and 12 .%, for exercise. These values have been validated in patients using invasive measurements at rest and during maximal exercise.(9;16)

Statistical analysis

Statistical analysis was performed with the SPSS 14.0 package (SPSSinc, Chicago, USA). All data are expressed as mean values ± standard deviations. The unpaired student's t test was used to check for differences in age, gender, body mass index, pulmonary function and CPET characteristics between PAH and LHF.
Linear regression analysis (appendix 1.) was used to check and correct for confounding between LHF versus PAH for peak SV, peak HR, and the slope relating HR to VO_2.

RESULTS

The PAH and LHF patients showed normal spirometric values (vital capacity: 94 ± 26; 91 ± 19, and forced expiratory volume in one second: 79 ± 21; 82 ± 15, respectively as percentage of predicted).
Table 3.1 shows that PAH patients and LHF patients were similar in terms BMI, and hemoglobin, but differed in the sense that the PAH group existed of more women and were significantly younger compared with the LHF patients.
At rest HR, SV, O2pulse, and VO_2 were identical in both groups. Although SaO_2 was significantly lower in the PAH patients arterial oxygen content was in both group was similar. (Table 3.2)

Cardio-pulmonary exercise test

From table 3.2 it can be seen that, although SaO_2 at anaerobic threshold (AT) and maximal exercise are lower in the PAH group, CaO_2 is similar. Both groups showed equally impaired exercise tolerance. PAH as well as LHF patients reached maximal workload by the use of an equal peak VO_2 (0.80 ± 0.29 l*min^{-1}, and 0.86 ± 0.19 l*min^{-1}, p = 0.43, respectively) (Table 3.2 and Figure 3.1A.) However, PAH compared to LHF patients reached their AT at a significantly lower exercise level (Table 3.2).
RER values above one indicate that maximal aerobic capacity in both groups was reached (Table 3.2). O_2pulse at AT and maximal exercise in PAH was significantly lower compared to LHF. (Table 3.2) Peak SV in PAH compared to LHF was significantly lower (Table 3.2 and Figure 3.1B).While HR at AT and maximal exercise (Table 3.2 and Figure 3.1C) was larger and the slope relating HR to VO_2 (0.08 ± 0.03 beats * ml^{-1}, 0.05 ± 0.03 beats * ml^{-1}, p = 0.001, respectively, Figure 3.1D) steeper.

Demographic values

	PAH (n=28)	LHF (n=18)	*p*-value
Gender, % female	75	39	< 0.05
Age, yr	53 ± 16	67 ± 13	< 0.01
BMI	25 ± 6	26 ± 5	ns
Hemoglobin, mmol*L^{-1}	8.8 ± 1.3	8.4 ± 1.0	ns

Table 3.1 *PAH: pulmonary arterial hypertension; LHF: left heart failure; BMI: body mass index.*

Cardio pulmonary exercise variables

	PAH (n=28)			LHF (n=18)		
	Rest	**AT**	**max.**	**Rest**	**AT**	**max**
SaO_2, %	95±4	91±5	90±5	97±2*	96±3*	96±3*
CaO_2, ml/dl	18.2±2.6	17.6±2.6	17.3±2.6	17.5 ± 2	17.3±2	17.3±2
HR, beats*min^{-1}	91±16	117±18	135±21	82 ± 16	104±22*	115±23*
O_2p, ml*$beat^{-1}$	2.9±1.1	5.4±2.0	5.9±1.9	3.1 ±0.7	7.0±1.4*	7.7±1.8*
SV, ml	49±18		49±15	52±12		64±15*
VO_2, ml*kg^{-1}*min^{-1}	3.7±0.9	8.6±2.0	11.2± 3	3.3±0.6	9.4±2.0	11.3 ± 2
Work, watt		27±19	56 ± 26		41±20*	58 ± 27
Work, % ref.		20±13	42 ± 16		29±14*	42 ± 18
RER		1.00±0.01	1.17± 0.14		0.96±0.04*	1.07 ± 0.11*

Table 3.2 *PAH: pulmonary arterial hypertension; LHF: left heart failure; AT: anaerobic threshold; max: maximal exercise; SaO_2: arterial oxygen saturation measured by pulse oximetry; CaO_2: Arterial oxygen content; HR: heart rate; O_2p: oxygen uptake divided by HR; VO_2: oxygen uptake; % ref.: percentage of predicted; RER: respiratory exchange ratio; ns: not significant. * Significant different from PAH.*

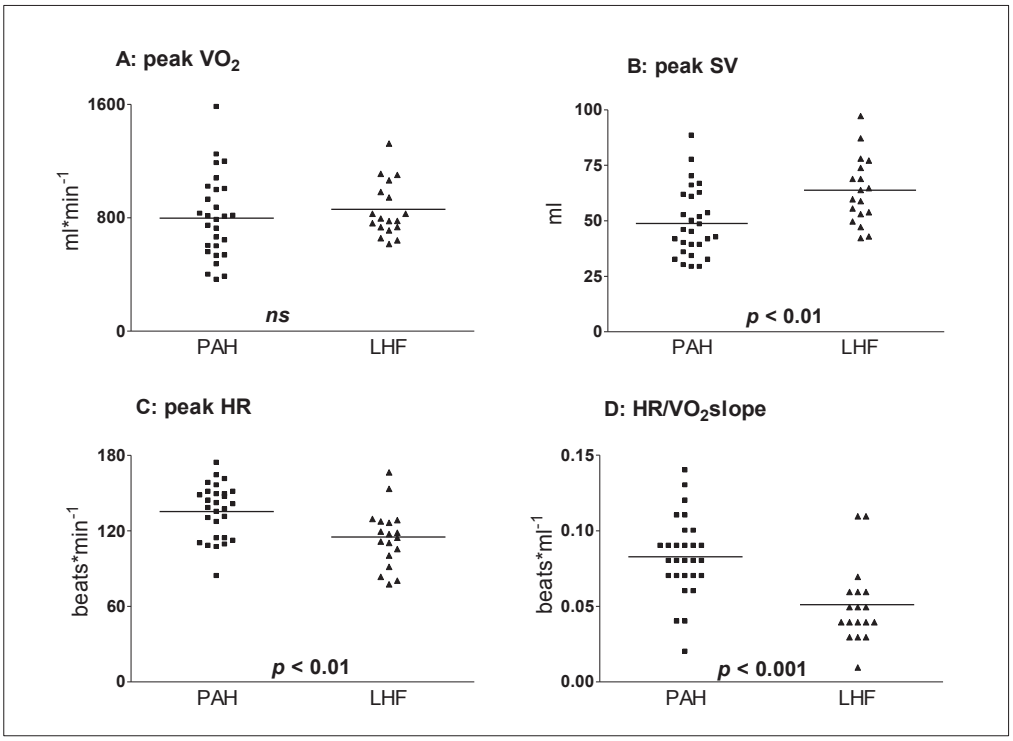

Figure 3.1 *A: peak oxygen uptake (VO_2) , B: peak stroke volume (SV) , C: peak heart rate (HR) and D: The slope relating HR to VO_2 during exercise of PAH and LHF. ns: not significant.*

Confounders

Age was marked as confounder for peak SV and HR. After correction by linear regression peak SV remained significantly different between patient groups. Even so, a trend towards a higher peak HR remained in the PAH group ($p = 0.05$). (Table 3.3) More importantly, no influence of confounding due to age was found for the slope relating HR to VO_2.

DISCUSSION

In this study we demonstrate that for similar maximal exercise levels, with identical oxygen demand, and normal pulmonary function, PAH patients have, compared to LHF patients, a smaller SV response, which is, compensated by a larger rise in HR.

Cardio circulatory response to exercise

PAH and LHF patients show no difference in SV at rest. Although in LHF an impaired SV response to exercise is common (9) our data show that the SV response in PAH is even smaller. This result is in keeping with differences in cardiac response between functional class matched PAH and LHF patients as reported by Deboeck et al.(2)

The main goal of our study was to compare cardiac response in both patient groups matched for an equal oxygen uptake. Therefore we used a study protocol which differs from the protocol used by Deboeck et al., where matching on functional class was used and patients with beta-blockers were not excluded.

The most likely explanation for the more impaired SV response in PAH compared to LHF patients is increased afterload pressure. Holverda et al. showed equal to our results, an almost absent SV response in a group of PAH patients as a consequence of an increased afterload. (17)

The influence of the pulmonary vascular resistance as estimate of afterload on SV response has already been described by Sun et al. showing that increased severity of pulmonary hypertension is accompanied by a larger pulmonary vascular resistance and a decrease in peak O_2 pulse during exercise.(5) Recently we confirmed their findings by showing an association between O_2 pulse response and PVR.(18) In contrast to PAH, and similar to our results, Weber et al showed a modest SV response in LHF patients with peak VO_2 values between 10 – 15 $ml*kg^{-1}*min^{-1}$.(9)

Because in PAH, HR is the main mechanism to increase cardiac output, these patients compensate the inability to augment SV during exercise by an increased HR, probably by sympathetic drive, with, as a consequence a significantly steeper slope relating HR to VO_2 during exercise.(19)

Arterial oxygen saturation at rest and at maximal exercise was significantly lower in PAH compared to LHF patients. However, oxygen delivery estimated by the arterial oxygen content was equal in both patient groups at rest and during exercise, respectively.

A limitation of this study is the non-invasive method to estimate SV using an average PE at rest of 6 vol.% and during exercise of 12 vol.%, according invasive data reported in the literature,(9;16) rather than individually determined PE.

Dantzker al. found extremely low mixed venous O_2 saturation values during exercise in pul-

monary hypertension patients. (20) Therefore, a decreased PE in this patient group is unlikely. Additionally, Tolle et al. showed invasively that PE is only minimally lower at maximum exercise in PAH when compared to the invasively measured PE found by Weber et al. in LHF patients (11.4 vs. 12.0 vol.%).(9;21) The effect of these differences on SV can be estimated by rearrangement of the Fick equation ("SV= O_2 pulse /PE"). For example, the mean peak O_2 pulse of 5.9 ml*beat^{-1} reached by the PAH patients in this study, with a maximal extraction of 11.4 vol.%, gives a SV of 51 ml while an extraction of 12 vol.% gives a SV of 49 ml. Hence, a disparity of only 0.6 vol.% of PE will bring about a difference of less than three ml in SV. Consequently, it is not likely that differences in PE between both study groups did influence the main finding of this study. Consequently, the additional value of O_2 pulse differences between these VO_2 matched groups is limited because it mirrors the different response of HR. Furthermore, based on the lowered anaerobic threshold and increased maximal RER values, in PAH (Table 3.1) it can be speculated that compared to LHF, PAH patients do not completely compensate the impaired SV increase by their HR response resulting in lowered cardiac output and therefore smaller oxygen delivery. This finding may, in part, result from down-regulation of the chronotropic response of the heart, as a result of the increased sympathetic drive found in PAH.(19)
The findings of this study may imply that therapeutic interventions aimed to reduce HR response by e.g. beta blockers will influence exercise tolerance in PAH more than in LHF. Indeed, this is in line with clinical observations, showing that the chronotropic response in PAH was independently associated with exercise tolerance and withdrawal of beta blockade therapy was related with an increase in exercise tolerance in patients with pulmonary hypertension. (7;22)

Since PAH is usually diagnosed late in the disease, peakVO_2 is already significantly impaired at time of diagnosis.(5) Mean peakVO_2 in the PAH patient group was identical to the value found in a large group of mild to severe primary PH patients described by Sun et al.(5) Since we matched LHF patients to this PAH group on peakVO_2, our LHF patients can be classified as having moderate to severe LHF .(9) Whether or not our results hold in patients with peakVO_2 above 15 ml*kg^{-1}*min^{-1} is not well possible, since these PAH patients are rare, since they can only be found if referred earlier in the course of the disease.
Still, these study results have a direct and clinical value, and confirm our hypothesis that in PAH the HR increase is the main mechanism to increase cardiac output while in LHF both increased SV and HR can contribute to increase cardiac output during exercise.
Although age and gender were different in both patient groups only age was marked as a significant confounder. However, after correction for confounding by age, the main outcome parameters continued to be significantly different between the study groups. Therefore we conclude that the main outcome results are not influenced by age difference.
In summary, our results show that for similar maximal exercise level, with identical oxygen demand, PAH patients have a smaller SV response, which is compensated by an increased HR response, compared with LHF patients.

Conflict of interest

The results of the present study do not constitute endorsement by American College of Sports Medicine. None of the authors had any financial associations that might pose a conflict of interest in connection with the submitted manuscript. Only A. Vonk-Noordegraaf was, in part, supported by the Netherlands Organisation for Scientific Research (NWO), VIDI grant.

APPENDIX

We used linear regression for the main outcome results of this study (maximal HR, SV and HR/VO2 slope) to check and correct for confounding by age and gender as stated in the statistics section.

The first step was creation of a linear prediction model (model 1.) for peak heart rate (HR) by patient group (with LHF (=1) vs. PAH (=0)): From these results the linear regression coefficient (RC) for patient group (13 beats*min^{-1}, p = 0.002) shows the mean difference between PAH versus LHF in peak HR. (Table 3.3)
The second step is adding age to the linear prediction model (model 2.). Resulting in a change of RC coefficient for patient group (8 beats*min^{-1}, p = 0.050) from model 1 to model 2 of more than 10% (which is the arbitrary threshold for significant confounding). Maximal HR is 8 beats*min^{-1} higher in the PAH compared to LHF for a mean aged patient. (Table 3.3)
The third and last step was adding gender to the linear prediction model (model 3.): Resulting in no change of RC for patient group (8 beats*min^{-1}, p = 0.057) from model 2 to model 3. Therefore, gender was not a significant confounder for HR. Consequently, maximal HR stays 8 beats*min^{-1} higher in PAH compared to LHF for a mean aged and gender patient. (Table 3.3)

An identical statistic procedure was performed for maximal stroke volume (SV) (Table 3.4)

Linear regression model for peak HR

Model		RC	SD	*P*-value
1	patient group	13	4	0,002
2	patient group: age	8	4	0,050
3	patient group: age, gender	8	4	0,057

Table 3.3 *RC: regression coefficient; sd: standard error*

Linear regression model for peak SV

Model		RC	SD	*P*-value
1	patient group	-13	3	0,000
2	patient group: age	-14	3	0,000
3	patient group: age, gender	-14	3	0,000

Table 3.4 *RC: regression coefficient; sd: standard error*

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Exercise testing to estimate survival in pulmonary hypertension

4

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ABSTRACT

Background

The six minute walk distance (6MWD) predicts survival in pulmonary hypertension (PH). The peak oxygen consumption (peakVO_2) measured during a cardiopulmonary exercise test (CPET) also relates to survival in PH, and it is unknown how the prognostic information from measurements of ventilatory responses and gas exchange during CPET compares to the prognostic information obtained by the 6MWD alone. The aims of our study were to compare prognostic values of different exercise parameters in PH and to assess whether CPET adds prognostic value to the information from the 6MWD.

Methods

After baseline right heart catheterisation and exercise testing, survival was assessed in a cohort of 115 PH patients.

Results

During four years of follow-up 18 patients died. At baseline, pulmonary arterial pressure was 49±17 mmHg, the slope relating minute ventilation to carbon dioxide output (VE/VCO_2slope) 45±11, peakVO_2 15±6 ml*kg^{-1}*min^{-1}, increase in O_2 pulse from rest to peak exercise (ΔO_2 pulse) 5±2 ml*$beat^{-1}$ and 6MWD 445±128m. For the prediction of mortality, the areas under the receiver-operating curves were very similar for the different parameters and ranged from 0.69 to 0.74. Patients with a VE/VCO_2slope < 48, peakVO2 > 13.2 ml*kg^{-1}*min^{-1} , ΔO_2 pulse > 3.3 ml*$beat^{-1}$or a 6MWD > 399m had a higher cumulative survival ($p < 0.05$). Multivariable Cox regression with a forward selection procedure showed that only ΔO_2 pulse improved the univariate 6MWD prediction model significantly ($p < 0.05$).

Conclusion

CPET parameters predict survival in PH patients and add marginally to the prognostic value of the 6MWD.

Introduction

Pulmonary hypertension (PH) is a life threatening disease characterized by an increased pulmonary vascular resistance, leading to right ventricular failure, exercise limitation and, ultimately, death. During the past 20 years improvements in treatment have prolonged survival and improved quality of life, but there is currently no cure.(11) The median survival from the time of diagnosis varies between three and ten years.(6;16)
Mortality in PH is largely associated with right ventricular dysfunction,(16) but accurate determination of hemodynamic parameters requires right heart catheterisation. Non-invasive markers predicting survival in PH could be helpful to monitor patients and to guide treatment. Variables derived during exercise testing have been proposed for this purpose.(19) The six minute walk test is relatively simple to perform and the total distance walked in this test (6MWD) predicts survival in PH.(12) However, the strength of the prediction is modest and the 6MWD is only weakly correlated to hemodynamic parameters.(12)

In heart failure from other causes, prognostic information is obtained through comprehensive cardiopulmonary exercise testing (CPET) with measurements of ventilatory efficiency and peak oxygen consumption(VO_2). (7;14;20) The latter parameter has also been shown to predict survival in PH(24), but it remains unproven if CPET yields additional prognostic information after determination of the 6MWD. Therefore we conducted a study to determine the additional prognostic value of CPET to 6MWD in a cohort of pulmonary hypertension patients.

METHODS

Patients

We performed a retrospective analysis of all patients referred to our institute between June 2002 and June 2007, meeting the following criteria: 1) diagnosis of pulmonary arterial hypertension or chronic thrombo-embolic pulmonary hypertension; 2) age <70 years; 3) mean pulmonary arterial pressure (mPAP) > 20 mmHg and a pulmonary capillary wedge pressure < 15 mmHg on baseline right heart catheterization; 4) ability to perform exercise testing. 127 patients fulfilled these criteria, their classification according to Venice criteria (17) is shown in table 4.1.

Classification	N
Idiopathic or familial	54
Associated with collagen vascular disease	18
Congenital systematic to pulmonary shunts	8
Porto-pulmonary hypertension	5
Associated with HIV infection	5
Associated with drugs or toxins	9
Chronic thrombo-embolic disease	28

Table 4.1 *PH cohort, classification according to Venice criteria.[9]*

Treatment strategies were standardized. Medications included anticoagulants and oxygen supplementation when necessary in all patients. Patients referred to our institution in 2002 and subsequent years were treated with prostacyclin analogs (epoprostenol and treprostenil) and oral compounds such as bosentan, sildenafil and more recently sitaxentan. The usual practice was to start with an oral compound and to add a prostacyclin analog in case of clinical worsening. The study protocol was approved by the VU University Medical Center Institutional Review Board and informed consent was waived due to the retrospective nature of this study.

Hemodynamic Studies

Baseline hemodynamic parameters were measured in all patients by diagnostic right heart catheterization and included right arterial pressure (RAP), mPAP, total pulmonary vascular resistance (TPVR), wedge pressure and cardiac output (CO). CO was measured by the direct Fick method. TPVR was calculated by dividing mPAP by CO. CO was indexed (C^I) by dividing it by body surface area.

Six-minute Walk Distance

6MWD was measured in all patients according to a standardized protocol that follows the American Thoracic Society guidelines.(9) Patients were instructed to walk as far as they could during six minutes at a pace that was comfortable to them. During the six minutes they stopped and rested when necessary. The elapsed time was read to the patient every two minutes. No other encouragement or conversation was permitted.

Cardiopulmonary Exercise testing

One day before the baseline right heart catheterisation, a physician-supervised cardiopulmonary exercise test (CPET) was performed on an electromagnetically braked cycle ergometer (Rehcor, Lode Groningen, The Netherlands) adhering to American Thoracic Society guidelines.(1) Three minutes of upright rest were followed by three minutes of unloaded pedalling (0 W) and subsequently a progressive increase in workload (5-20 W min^{-1}) to maximum tolerance. The rate of workload increase was empirically determined by the supervising physician, based on the medical history, clinical data and the result of a previous 6MWD, if available. Test duration was between 8 and 12 minutes (from unloaded pedaling to peak exercise) in all patients.

Minute ventilation (VE), VO_2 and carbon dioxide output (VCO_2) were measured breath by breath using a metabolic cart (Vmax 229, Viasys, Yorba Linda, USA) and analyzed as 20 seconds averages. Peak respiratory exchange ratio (RER) as a measurement of maximal metabolic effort was calculated by dividing VCO_2 by VO_2 values at peak exercise (20 seconds average). Anaerobic threshold (AT) was determined by the V-slope method.(4) Oxygen saturation of arterial blood (SaO_2) was measured by pulse-oximetry (9600, Nonin, Plymouth, USA). No patients were using supplemental oxygen during CPET.

Before each test the equipment was calibrated according the manufactory's specifications. Briefly, the flow sensor was calibrated and verified by a calibrated three litre syringe. Gas analysers were calibrated by a two point calibration (0% CO_2, 26% O_2 and 4% CO_2, 16% O_2, respectively) and ambient air (0% CO_2, 20.9% O_2). Heart rate (HR) was measured by electro-cardiography (Eagle 4000, Marquette). O_2 pulse was calculated as VO_2 divided by heart rate. CPET was measured on a separate day, but within one week of the 6MWD.

Survival Estimates

At least every three months, all patients were seen in the outpatient clinic or contacted by telephone by our PH nurse. Follow-up data were available from all patients. From our initial cohort of 127 patients with 23 non-survivors we excluded 8 patients with congenital heart disease (see figure 4.1). From the 119 remaining patients 21 patients had died on June 30th 2007, 18 of whom from cardiopulmonary causes. The other 3 deaths were not included in the analyses. Finally, one patient was excluded since there was no baseline 6 MWD available.

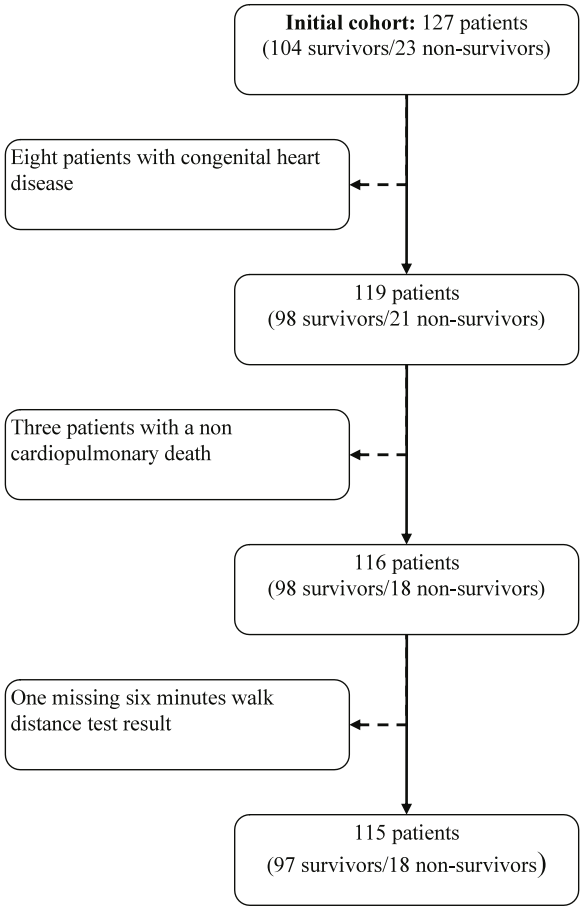

Figure 4.1 *Patients flow chart from initial cohort with in- and excluded patients.*

Statistical Analysis

Statistical analysis was performed with the SPSS 14.0 package (SPSSinc, Chicago, USA). All data are expressed as mean values ± standard deviations. The unpaired student's t test was used to check for differences in age, body mass index, RAP, mPAP, CO, C^I, TPVR, CPET and 6MWD characteristics.

The CPET characteristics that were evaluated were: peak RER, VO_2, and HR. ΔO_2 pulse (calculated as the change in VO_2/HR from rest to maximal exercise), ventilatory equivalents for O_2, end-tidal CO_2 ($PetCO_2$) and SaO_2 at rest and during maximal exercise. Additionally, the ventilatory response to exercise was quantified in five ways: 1) The slope of the plot describing the relationship between VE and VCO_2 from rest till peak exercise (VE/VCO_2slope); 2) The VE/VCO_2 slope calculated from rest till the AT (VE/VCO_2slopeAT); 3) The difference between these two slopes ($\Delta VE/VCO_2$slope); 4) the nadir in the plot describing the ratio of VE to VCO_2 vs. time ($VEVCO_2$nadir) and 5) the VE/VCO_2 ratio at peak exercise ($VEVCO_2$peak).

Pearson correlations were calculated to check for linear associations between non-invasive diagnostic tests (6MWD and CPET characteristics) and invasive hemodynamic parameters (RAP, mPAP, C^I and TPVR. For the variables that were significantly different between survivors and non-survivors, optimal cut-off points for predicting survival were identified by determining the receiver operating characteristic (ROC) based on the highest sum of sensitivity and (1-specificity) values. Areas under the curve (AUC) are presented with a 95% confidence interval (CI).

For those tests with an AUC significantly different from 0.5, univariate Kaplan-Meier survival curves were calculated. For the continuous values of the different diagnostic tests, hazard ratios (HR) and their 95% CI were calculated by univariate Cox proportional risk analysis. Multivariate Cox-regression with a forward selection procedure was used to check for significant additional predictive value of survival from CPET parameters to the 6MWD. In all analyses, values below 0.05 (2–tailed) were considered significant.

RESULTS

Survival

No patients were scheduled for transplantation (lung or heart-lung) or pulmonary end-arterectomy during the follow-up period. 18 out of 115 patients died after a mean follow-up of 846 ± 461 days. Mean follow-up time of the 97 survivors was 907 ± 462 days (range 31 -1465). The cumulative proportional survival of all patients was 93±2% at 1 year, 84±4% at 2 years, 81±4% at 3 years and 78±5% at 4 years.

No significant differences in age, body mass index, RAP, mPAP, C^I and TPVR were found between survivors and non-survivors. CO was significantly lower in the non-survival group. Based on peak RER (table 4.2), patients in this study showed maximal (metabolic) effort during the CPET. PeakVO_2, $PetCO_2$ at AT, ΔO_2 pulse and 6MWD were significantly higher, whereas VE/VCO_2slope, VE/VCO_2slopeAT, $VEVCO_2$nadir, and $VEVCO_2$peak were significantly lower in survivors as compared to non-survivors (Table 4.2). Peak HR, ventilatory equivalents for O_2,

SaO_2 and $PetCO_2$ at rest were not predictive of survival and are therefore not included in the subsequent sections.

Relations between hemodynamic with 6MWD and CPET parameters.

Table 4.3 shows that most 6MWD and CPET parameters correlated modestly with resting hemodynamic parameters. $VEVCO_2$nadir had the strongest correlations with the clinically important variables of RAP, C^I and TPVR, but the maximum variance that could be explained was still only 25%.

	All patients	Survivors	Non-survivors
Number of patients	115	97	18
Female/male	80/35	66/31	14/4
NYHA I	n = 2	n = 2	n = 0
NYHA II	n = 16	n = 15	n = 1
NYHA III	n = 89	n = 74	n = 15
NYHA IV	n = 8	n = 6	n = 2
Age (years)	48 ± 13	48 ± 14	52 ± 10
BMI (kg/m^2)	26 ± 5	26 ± 5	24 ± 4
RAP (mmHg)	7 ± 6	7 ± 6	10 ± 7
mPAP (mmHg)	49 ± 17	49 ± 17	47 ± 13
CO (l/min)	5.2 ± 1.5	5.3 ± 1.6	4.5 ± 1.3*
C^I ($l/min/m^2$)	2.8 ± 0.8	2.9 ± 0.8	2.6 ± 0.6
TPVR ($dyne.sec.cm^{-5}$)	720 ± 390	715 ± 412	749 ± 245
Peak RER	1,09 ± 0.09	1,10 ± 0.10	1,07 ± 0.06
$PeakVO_2$ ($ml*kg^{-1}*min^{-1}$)	15.3 ± 5.6	15.8 ± 5.8	12.3 ± 3.2#
Peak HR ($beats*min^{-1}$)	136 ± 23	136 ± 24	134 ± 22
VE/VCO_2slope	48.5 ± 12.6	47.0 ± 11.6	56.9 ± 14.4#
VE/VCO_2slopeAT	44.6 ± 11.3	43.3 ± 10.8	51.5 ± 11.3#
$\Delta VE/VCO_2$slope	4.0 ± 5.2	3.7 ± 4.7	5.5 ± 7.2
$VEVCO_2$nadir	47.3 ± 11.1	45.9 ± 10.2	54.7 ± 12.5#
$VEVCO_2$peak	52.7 ± 12.8	51.2 ± 12.0	60.9 ± 14.1#
$PetCO_2$rest (mmHg)	27.1 ± 5.0	27.4 ± 4.8	25.4 ± 5.7
$PetCO_2$AT (mmHg)	27.3 ± 6.0	27.8 ± 5.7	24.6 ± 7.3*
ΔO_2 pulse ($ml*beat^{-1}$)	4.6 ± 2.3	4.9 ± 2.3	3.1 ± 1.6#
SaO_2rest	95 ± 4	96 ± 4	95 ± 3
SaO_2peak	92 ± 6	92 ± 5	89 ± 8
6MWD (m)	445 ± 128	458 ± 129	370 ± 93#

Table 4.2 *Summary of baseline demographics, hemodynamics, cardiopulmonary exercise test variables and six minutes walk distance*
- *NYHA: New York Heart Association; BMI: body mass index; RAP: right arterial pressure; mPAP: mean pulmonary arterial pressure; CO: cardiac output; C^I: cardiac index;*
- *TPVR: pulmonary vascular resistance; Peak RER: respiratory exchange ratio at peak exercise;*
- *$PeakVO_2$: oxygen consumption during peak exercise per kilogram;*
- *Peak HR: Hart rate at peak exercise;*
- *VE/VCO_2slope: mean regression slope relating minute ventilation to carbon dioxide production till maximum exercise;*
- *VE/VCO_2slopeAT: mean regression slope relating minute ventilation to carbon dioxide production before anaerobic threshold;*
- *$\Delta VE/VCO_2$slope: difference between mean regression slope relating minute ventilation to carbon dioxide production till peak exercise and till anaerobic threshold;*
- *$VEVCO_2$nadir: the nadir of VE/VCO_2 curve;*
- *$VEVCO_2$peak: VE/VCO_2 ratio at peak exercise;*
- *$PetCO_2$rest: end-tidal CO_2 at rest;*
- *$PetCO_2$AT: en-tidal CO_2 at anaerobic threshold; ΔO_2 pulse: VO_2 difference from rest to maximum exercise per heartbeat; -*
- *SaO_2rest: pulse oximetry value at rest;*
- *SaO_2peak: pulse oximetry value during peak exercise;*
- *6MWD: six minute walk distance; *: $p<0.05$ for survivors versus non-survivors; #: $p<0.01$ for survivors versus non-survivors.*

Receiver Operating Characteristics

Receiver operating characteristic (ROC) curves from resting hemodynamic parameters and 6MWD and CPET characteristics showed that only VE/VCO_2slope, $VEVCO_2$nadir, $VEVCO_2$peak, peakVO_2, ΔO_2 pulse and 6MWD were accurate predictors of 4-year survival in our cohort (table 4.4). The optimal cut-off point was determined by the largest sum of Sensitivity and (1-Specificity) from the ROC plots of the VE/VCO_2slope, $VEVCO_2$nadir, $VEVCO_2$ peak, peakVO_2, ΔO_2 pulse and 6MWD (≥48, ≥52, ≥49, ≤13.2 ml*kg^{-1}*min^{-1}, ≤3.3 ml*beat^{-1} and ≤ 399m, respectively).

Correlation matrix between hemodynamic, 6MWD and CPET parameters.

	mPAP	C^I	TPVR	PeakVO2	VE/VCO2s	VEVCO2n	VEVCO2p	$PetCO_2f$	$PetCO_2A$	ΔO2pulse	SaO2peak	6MWD
RAP	0,24#	-0.27#	0.27#	-0.26#	0.24*	0.27#	0.23*	-0.12	-0.22*	-0.17	-0.07	-0,34#
mPAP		-0,35#	0,70#	-0.36#	0.33#	0.41#	0.34#	-0.44#	-0.44#	-0.30#	-0.16	-0.16
C^I			-0.72#	0.30#	-0.29#	-0.32#	-0.31#	0.21*	0.28#	0.15	0.10	0.20*
TPVR				-0.44#	0.43#	0.50#	0.45#	-0.43#	-0.46#	-0.37#	-0.21*	-0.23*
$PeakVO_2$					-0.44#	-0.46#	-0.40#	0.16	0.32#	0.78#	0.18	0.63#
VE/VCO_2slope						0.92#	0.96#	-0.64#	-0.80#	-0.40#	-0.35#	-0.37#
$VEVCO_2$nadir							0.92#	-0.75#	-0.86#	-0.40#	-0.40#	-0.40#
$VEVCO_2$peak								-0.67#	-0.81#	-0.38#	-0.34#	-0.33#
$PetCO_2$rest									0.90#	0.14	0.28#	0.06
$PetCO_2$AT										0.26#	0.31#	0.19*
ΔO_2pulse											0.15	0.46#
SaO_2peak												0.14
6MWD												

Table 4.3 *RAP: right arterial pressure; mPAP: mean pulmonary arterial pressure; CI: cardiac index; TPVR: total pulmonary vascular resistance; PeakVO2: oxygen consumption during peak exercise per kilogram; VE/VCO2slope: mean regression slope relating minute ventilation to carbon dioxide production; VEVCO2nadir: the nadir of VE/VCO2 curve; VEVCO2peak: VE/VCO2 ratio at peak exercise; PetCO2rest: end-tidal CO2 at rest; PetCO2AT: en-tidal CO2 at anaerobic threshold; ΔO2pulse: VO2 difference from rest to maximum exercise per heartbeat; SaO2peak: pulse oximetry value during peak exercise; 6MWD: six minute walk distance; *: p<0.05; #: p<0.01.*

Kaplan Meier Survival Analysis

Pulmonary hypertension patients with a VE/VCO_2slope < 48 had a significantly better prognosis (p<0.05) at 4 years (cumulative survival 90%; 95% CI 81 to 98) compared with pulmonary hypertension patients with a slope of ≥ 48 (cumulative survival 68%; 95% CI 53 to 83). Pulmonary hypertension patients with a $VEVCO_2$nadir < 52 had a significantly better prognosis (p<0.01) at 4 years (cumulative survival 89%; 95% CI 82 to 97) compared with pulmonary hypertension patients with a nadir of ≥ 52 (cumulative survival 58%; 95% CI 38 to 77). Pulmonary hypertension patients with a $VEVCO_2$peak < 49 showed no significantly better prognosis (p= 0.33) at 4 years (cumulative survival 88%; 95% CI 78 to 98) compared with pulmonary hypertension patients with a $VEVCO_2$peak of ≥ 49 (cumulative survival 73%; 95% CI 60 to 87). Pulmonary hypertension patients with a $peakVO_2$ > 13.2 ml*kg^{-1}*min^{-1} had a significantly better prognosis (p<0.05) at 4 years (cumulative survival 83%; 95% CI 66 to 99) compared with pulmonary hypertension patients with a $peakVO_2$ of ≤ 13.2 ml*kg^{-1}*min^{-1} (cumulative survival 71%; 95% CI 56 to 85). Pulmonary hypertension patients with a ΔO_2 pulse > 3.3 ml*beat^{-1} had a significantly better prognosis (p<0.01) at 4 years (cumulative survival 88%; 95% CI 76 to 99) compared with pulmonary hypertension patients with a

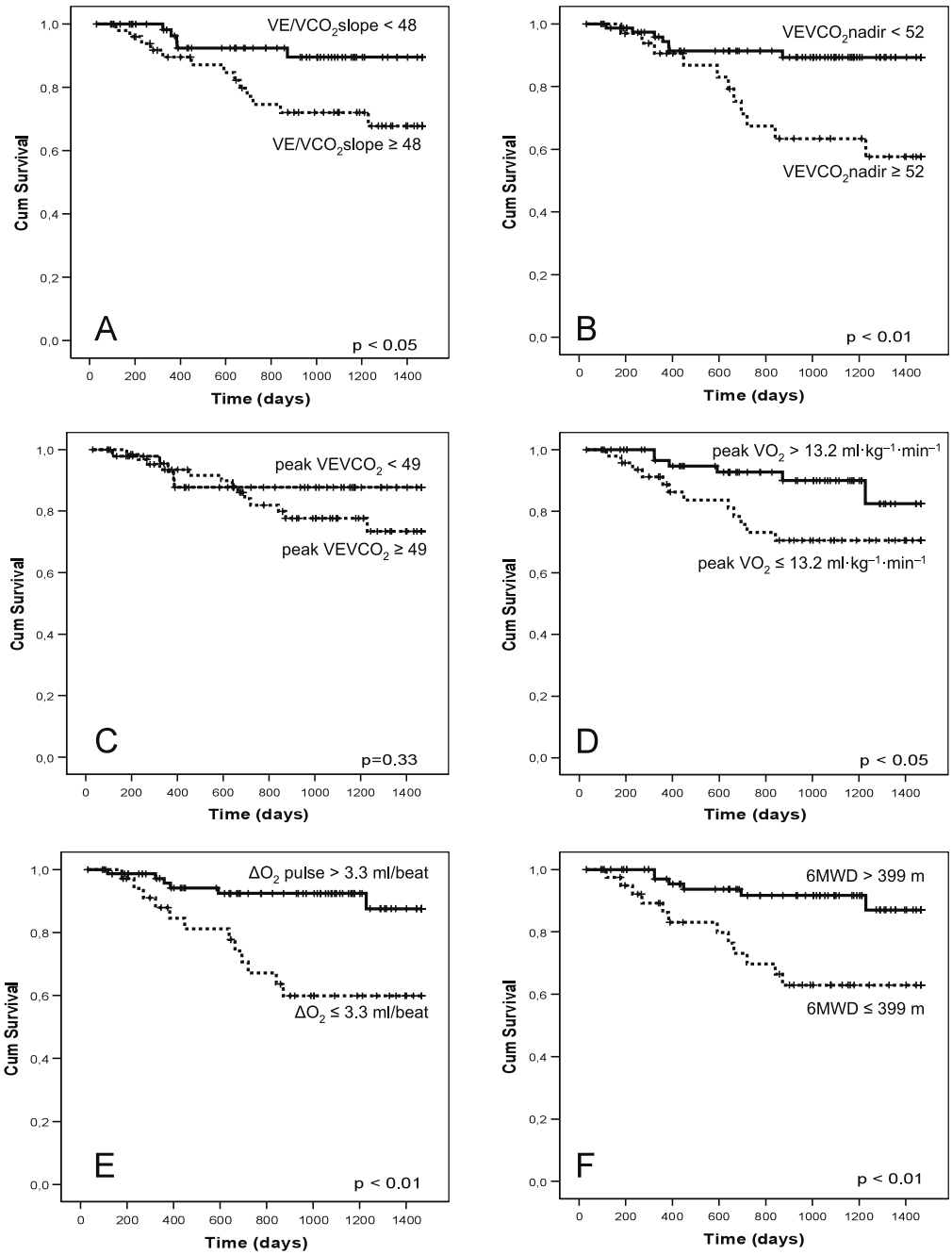

Figure 4.2 *Kaplan-Meier cumulative survival curves for (A) mean regression slope relating minute ventilation to carbon dioxide production till maximum exercise(VE/VCO_2slope), (B) the nadir in the relationship between the VE/VCO_2 ratio and time ($VEVCO_2$nadir), (c) VE/VCO_2 ratio at peak exercise ($VEVCO_2$peak), (D) peak oxygen consumption (peakVO_2), (E) VO_2 difference from rest to maximum exercise per heartbeat (ΔO_2 pulse) and (E) six minute walk distance (6MWD).*

ΔO_2 pulse of ≤ 3.3 ml*beat^{-1} (cumulative survival 60%; 95% CI 42 to 78). Pulmonary hypertension patients with a 6MWD > 399 m had a significantly better prognosis (p<0.01) at 4 years (cumulative survival 87%; 95% CI 76 to 98) compared with pulmonary hypertension patients with a 6MWD ≤ 399 m (cumulative survival 63%; 95% CI 46 to 80, see figure 4.2).

Cox Proportional Hazard Analysis

Table 4.5 shows that by univariate Cox proportional hazard analysis only the non-invasive exercise parameters VE/VCO_2slope, $VEVCO_2$nadir, $VEVCO_2$peak, peakVO_2, ΔO_2 pulse and 6MWD were significant predictors of impaired survival (here shown as continuous variables). Multivariable Cox regression with a forward selection procedure, forcing 6MWD as the first predictor, showed that from all separate included CPET parameters only ΔO_2 pulse significantly improved the univariate 6MWD prediction model (p < 0.05)(table 4.6).

DISCUSSION

In this study we demonstrate that ventilatory and gas exchange CPET parameters predict survival in PH patients. However, from all CPET parameters only ΔO_2 pulse added a significant, but modest, prognostic value to the 6MWD. All the non-invasive CPET parameters as well as 6MWD showed a significant area under the curve to estimate an optimal cut-off point for making the results dichotomous (to predict survivors versus non-survivors) by ROC analysis (table 4.4). We found by Kaplan Meier Survival Analysis
a significantly better prognosis for survival at 4 years for patients with a VE/VCO_2slope < 48 or a $VEVCO_2$nadir < 52 or a peakVO_2 > 13.2 ml*kg^{-1}*min^{-1} or ΔO_2 pulse > 3.3 ml*beat^{-1} or 6MWD > 399 m (figure 4.2).

	AUC	95% CI	P value
RAP	0.59	0.43 – 0.75	0.24
mPAP	0.47	0.35 – 0.59	0.67
C^I	0.60	0.47 – 0.74	0.17
TPVR	0.56	0.45 – 0.68	0.40
VE/VCO_2slope	0.68	0.54 – 0.82	0.02
$VEVCO_2$nadir	0.69	0.55 – 0.82	0.02
$VEVCO_2$peak	0.67	0.53 – 0.82	0.03
$PetCO_2AT$	0.65	0.50 – 0.80	0.06
PeakVO_2	0.66	0.54 – 0.78	0.03
ΔO_2 pulse	0.72	0.58 – 0.86	0.01
6MWD	0.70	0.60 – 0.81	0.01

Table 4.4 *Receiver Operating Characteristics.*
- *AUC: area under the curve of receiver operating characteristic;*
- *RAP: right arterial pressure; mPAP: mean pulmonary arterial pressure;*
- *C^I: cardiac index; TPVR: pulmonary vascular resistance;*
- *VE/VCO_2slope: the mean regression slope relating minute ventilation to carbon dioxide production till maximum exercise;*
- *$VEVCO_2$nadir: the nadir of VE/VCO_2 curve; $VEVCO_2$peak: VE/VCO_2 ratio at peak exercise;*
- *$PetCO_2AT$: end-tidal CO2 at anaerobic threshold;*
- *peakVO_2: oxygen consumption during peak exercise per kilogram;*
- *ΔO_2 pulse: VO_2 difference from rest to maximum exercise per heartbeat;*
- *6MWD: six minute walk distance.*

Univariate Cox proportional hazard analysis showed that all included non-invasive exercise parameters (as continuous variables) were significant predictors of impaired survival (table 4.5). Furthermore, from the CPET parameters only ΔO_2 pulse improved the univariate 6MWD prediction model significantly (Table 4.6).
Exercise capacity, whether assessed during a walk test or during CPET (6MWD and peak VO_2, respectively), is a well established predictor of survival in heart failure and PAH.(5;8;15;24) The fact that a low ΔO_2 pulse was also a strong predictor in our PAH cohort, confirms earlier findings of a relationship between a reduced stroke volume response and exercise intolerance in these patients.(10) Rearrangement of the Fick equation (VO_2 = CO x arterial and mixed venous blood oxygen content differences ($C(a\text{-}vO_2)$) shows that O_2 pulse (VO_2 /HR) is equal to the product of stroke volume and $C(a\text{-}vO_2)$. Agostoni et al. showed that in chronic heart failure patients, $C(a\text{-}vO_2)$ reaches normal values at maximal exercise.(2) Patients in this study showed no severe desaturation and we can therefore assume their peak exercise $C(a\text{-}vO_2)$ was normal as well and that their low peak O_2 pulse indicate a low stroke volume at maximal exercise (18;23). In the multivariate analysis, a low increase in O_2 pulse and a low 6MWD were independent predictors of mortality. This could indicate that the 6MWD in these patients is not determined by the stroke volume or cardiac output response only. The relative limited number of events in our cohort prevents us from drawing firm conclusions, however.

In addition to exercise capacity and the stroke volume response, excessive ventilation (out of proportion of the metabolic rate) predicted survival in our cohort of PH patients. We quantified the ventilatory response during exercise using five different methodologies, which theoretically have different predictive values.(3) The VE/VCO_2slope is the mean regression slope in the relationship between VE (y-axis) and VCO_2 (x-axis). In accordance with other investigators and for the sake of methodological simplicity and a better prognostic value (20), we first included all the data till peak exercise in the regression analysis. Our second approach was to only include the linear data that are obtained during the first part of the exercise test, until the AT. Since the VE increases out of proportion of the VCO_2 after the AT, the first method yields higher slopes than the second. The difference between these two slopes ($\Delta VE/VCO_2$), has been described as a predictor by itself (3). This was not the case in our cohort, however (see table 4.2). We subsequently used two alternative quantifications of the ventilatory response to exercise, the VE/VCO_2nadir and the peak VE/VCO_2 peak, and obtained very similar results. VE/VCO_2nadir, which is the nadir in the relationship between the VE/VCO_2 ratio and time, is virtually equal to the VE/VCO_2 ratio at AT and both have been proposed as prognostic tools in PH.(19)
Excessive ventilation in PH is either due to increased dead space ventilation (inadequate perfusion of ventilated lung areas) or alveolar hyperventilation due to a decreased $PaCO_2$ set-point.(19) This set-point is affected by: a) metabolic acidosis due to an impaired stroke volume response to exercise;(10;22) b) exercise induced hypoxemia; c) sympathetic over stimulation;(21) and d) increased stimulation of blood-pressure responsive receptors in the pulmonary vasculature.

	HR	95% CI	P value
RAP	1.046	0.981 - 1.115	0.17
mPAP	0.985	0.965 - 1.016	0.34
C^I	0.713	0.368 - 1.379	0.32
TPVR	1.000	0.999 - 1.001	0.81
VE/VCO_2slope	1.039	1.010 - 1.069	0.01
$VEVCO_2$nadir	1.042	1.011 - 1.074	0.01
$VEVCO_2$peak	1.038	1.008 - 1.068	0.01
$PetCO_2AT$	0.927	0.850 - 1.011	0.09
$PeakVO_2$	0.872	0.772 - 0.985	0.03
ΔO_2 pulse	0.635	0.457 - 0.881	0.01
6MWD	0.995	0.991 - 0.999	0.01

Table 4.5 *Univariate Cox hazard analysis*
HR: Hazard Ratio from Cox regression analysis; RAP: right arterial pressure; mPAP: mean pulmonary arterial pressure; C^I: cardiac index; TPVR: pulmonary vascular resistance;VE/VCO_2slope: the mean regression slope relating minute ventilation to carbon dioxide production till maximum exercise; $VEVCO_2$nadir: the nadir of VE/VCO_2 curve; $VEVCO_2$peak: VE/VCO_2 ratio at peak exercise; $PetCO_2AT$: end-tidal CO2 at anaerobic threshold; $PeakVO_2$: peak oxygen consumption; ΔO_2 pulse; VO_2 difference from rest to maximum exercise per heartbeat; 6MWD: six minute walk distance.

		Wald	HR	Δ Chi-square	*p*-value
Step 1	6MWD	7.219	0.995	7.447	0.01
Step 2	6MWD	3.991	0.996	2.435	0.12
	VE/VCO2slope	2.672	1.026		
Step 3	6MWD	3.963	0.996	2.251	0.13
	$VEVCO_2$nadir	2.501	1.028		
Step 4	6MWD	4.541	0.996	2.421	0.12
	$VEVCO_2$peak	2.636	1.026		
Step 5	6MWD	1.874	0.996	0.835	0.36
	$peakVO_2$	0.752	0.936		
Step 6	6MWD	2.308	0.997	4.988	0.03
	ΔO_2 pulse	4.200	0.689		

Table. 4.6 *Multivariate Forward Cox regression each step starting with 6MWD*
Wald: Wald statistic, HR; Hazard Ratio, Δ Chi-square: Change in chi-square from previous step; 6MWD: six minute walk distance; VE/VCO_2slope: the mean regression slope relating minute ventilation to carbon dioxide production till maximum exercise; $VEVCO_2$nadir: the nadir of VE/VCO_2 curve; $PeakVO_2$: oxygen consumption during peak exercise per kilogram; ΔO_2 pulse: VO_2 difference from rest to maximum exercise per heartbeat.

Our data provide no insight into the relative contributions of these different factors in the excessive exercise ventilation in PH.

Surprisingly, the RAP C^I and CO did not predict survival in our cohort, which in contrasted with previous studies.(6;16;24). We speculate that the main explanation for this contrasting finding is our high survival rate compared to the rates that were found in the reports from Sandoval and Wensel (12;16;24). These studies included only idiopathic pulmonary hypertension patients and anticoagulant therapy were not used as a routine(16). Furthermore, our patients seemed in a relatively better clinical condition, as evidenced by a higher mean peakVO_2 and a lower mPAP and TPVR at baseline. Wensel et al. proposed systolic blood pressure at peak exercise as an additional independent predictor of survival in pulmonary hypertension.(24) We did not include blood pressure measurements in our study protocol because accurate non-invasive determination of this parameter is frequently not feasible during routine clinical exercise testing.

No significant differences in baseline hemodynamics were found between survivors and non-survivors except for CO which was marginally higher in survivors. There was insufficient follow-up data in our cohort to determine the predictive value of subsequent repeat-catheterizations. It is possible that changes in hemodynamics would have had predictive value. In agreement with previous studies, the 6MWD, VE/VCO_2slope and peakVO2 were significantly correlated with right ventricular function.(12;19;24) However, the rather weak correlations show that these parameters are not exclusively determined by the pulmonary vascular resistance. Treatment strategies in our hospital are highly standardized; baseline CPET characteristics and 6MWD do not determine the choice of initial treatment, which usually consists of an oral single agent (sildenafil, bosentan or sitaxentan). It is therefore unlikely that the predictive values of baseline peakVO_2, ΔO_2 pulse, $VEVCO_2$nadir, VE/VCO_2slope and 6MWD are explained by differences in treatment strategies between survivors and non-survivors. A change in 6 MWD after follow-up may lead to a change in treatment, but the limited size of our cohort prohibits assessment of the prognostic value of changes in exercise data over time.

Smoking could have influenced VE/VCO_2slope by increasing dead space ventilation. In our cohort there very few current smokers and pulmonary function testing and high resolution computed tomography scanning (which are both routinely performed in all patients) did not suggest emphysema in any of our patients. Therefore, effects of smoking or associated chronic obstructive pulmonary disease should have been negligible.

The six minute walk test is much easier to perform than CPET with ventilatory measurements because it is less technically demanding and less time consuming. The test is therefore frequently used as a primary end-point for clinical studies.(13) From our data, derived from a cohort of 115 patients with only 18 deaths, which is a limitation of this study, it is difficult to determine which of the five non-invasive parameters (peakVO_2, ΔO_2 pulse, $VEVCO_2$nadir, VE/VCO_2slope and 6MWD) provides the strongest prediction of survival.

We conclude that ventilatory and gas exchange parameters measured during maximal CPET predict survival in PH patients with moderate hemodynamic abnormalities. However, the additional prognostic value of CPET in pulmonary hypertension patients that performed a 6MWD previously is only marginal.

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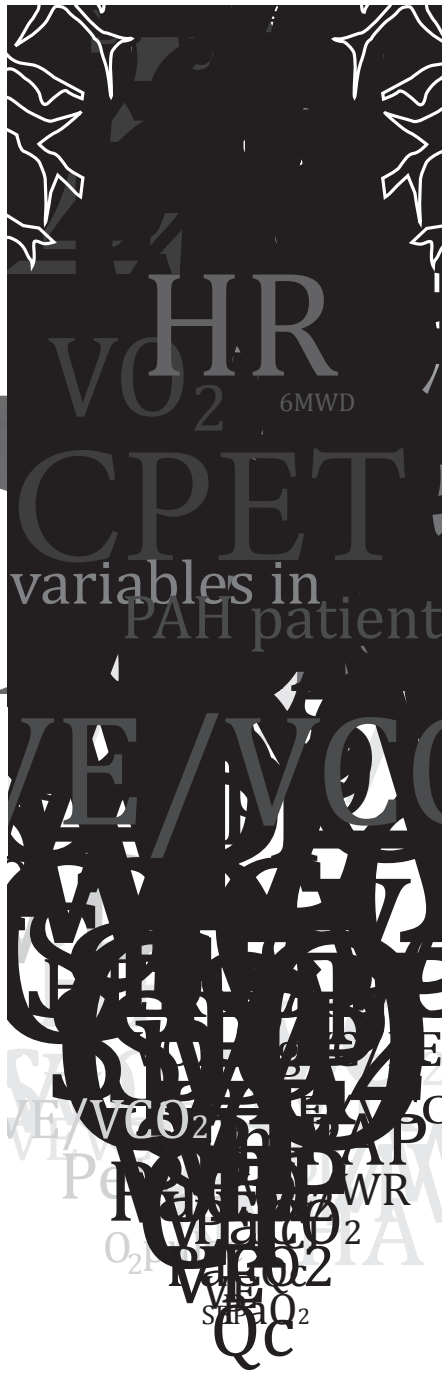
5 Prognostic relevance of changes in exercise parameters in pulmonary hypertension



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ABSTRACT

Introduction

Cardio pulmonary exercise test (CPET) variables measured at baseline predict survival in pulmonary arterial hypertension (PAH), but whether these variables have prognostic value when measured as changes over time is unknown. The aim of this study was to determine changes in CPET variables in PAH patients treated with specific therapy and to relate these changes to survival.

Methods

Baseline CPET variables were available from 65 idiopathic PAH patients (50 females; mean age 45±2 yrs). The same variables were determined one year later in a sub group of 39 patients (31 female; mean age 45±2 yrs). Using Kaplan-Meier analysis, survival was related to baseline CPET variables (whole group; mean survival 58±5 months) and to changes in CPET variables (mean survival 52±5 months in the subgroup of patients with available follow-up CPET data).

Results

From baseline till end of the study period (< 90months), two patients underwent a lung transplantation and 13 patients died. Follow-up CPET data were available from the two transplanted patients and from seven of the deceased patients. Survival analysis showed that from all CPET variables studied at baseline, only maximal heart rate ($p<0.0001$) and the slope relating ventilation with carbon dioxide production ($p<0.05$) were significant predictors of survival. In the subgroup with follow-up CPET data, only the change in oxygen uptake and oxygen pulse predicted survival ($p<0.001$).

Conclusion

CPET variables which have prognostic significance when determined at baseline are not necessarily predictive for survival when measured as changes over time. A change in maximal aerobic capacity does predict subsequent survival, but with current PAH specific therapeutic interventions, changes in other CPET variables do not have prognostic significance.

INTRODUCTION

Patients with pulmonary arterial hypertension (PAH) show specific patterns of gas exchange abnormalities during a cardio pulmonary exercise test (CPET) (18). For that reason, international guidelines promote CPET to determine exercise capacity and prognosis in PAH (6, 8). CPET variables affected by pulmonary hypertension reflect an impaired cardiocirculatory function and a decreased ventilatory efficiency, and predict subsequent survival when measured at the time of diagnosis (7, 22). A low maximal oxygen uptake (VO_2), a low oxygen pulse (O_2pulse), a high linear regression slope relating ventilation to carbon dioxide production (VE/VCO_2) and a lowered maximal heart rate (HR) all predict subsequent death in PAH (7, 22). It was recently reported that in PAH, important prognostic information is yielded by the responses of several established predictors of outcome during the disease (10). That changes in 6MWD are predictive of subsequent survival was already suggested in several clinical PAH trials which used exercise time and six minute walk distance (6MWD) as clinical end-points (9, 14, 15). Together, these studies suggest that changes in exercise responses over time, either resulting from disease progression or from a response to PAH specific therapy, are predictive of subsequent survival. However, specific knowledge about the prognostic significance of changes in different CPET variables is lacking. To determine the relevance of changes in CPET variables to prognosis, we analyzed the results of CPET at baseline and after one year of PAH specific treatment in a cohort of patients with idiopathic and familial PAH.

METHODS

Patients

All idiopathic and heritable PAH patients referred to the VU University Medical Centre in Amsterdam between December 2000 till December 2011 who underwent CPET were included in this retrospective analysis.

At the time of diagnosis, hemodynamic parameters were measured in all patients by a diagnostic right heart catheterization and included right arterial pressure, mean pulmonary arterial pressure, pulmonary vascular resistance, wedge pressure and cardiac output.

Pulmonary hypertension was diagnosed by a mean pulmonary arterial pressure > 25 mmHg and a mean pulmonary capillary wedge pressure <15 mmHg measured at rest during right heart catheterisation. Idiopathic PAH was diagnosed when all other causes of pulmonary hypertension were excluded. Heritable PAH was diagnosed in patients with a known family history of PAH.

Patients were treated by a standardized treatment strategy, as reported before (7). All included patients visited the outpatient clinic at least once every three months, according to our institutional protocol. Results were available from CPET performed at the time of PAH diagnosis prior to the start of PAH specific treatment in 65 patients (50 females; mean age 45 ± 2 yrs). A follow-up CPET was performed within 24 months of the initial test in 39 patients (31 females; mean age 45 ± 2 yrs).

The study protocol was approved by the VU University Medical Centre Institutional Review Board and informed consent was waived due to the retrospective nature of this study.

Cardio pulmonary exercise test

CPET was performed on an electromagnetically braked cycle ergometer (Lode bv, Groningen, The Netherlands) according to international guidelines (16). Briefly, three minutes of upright rest were followed by three minutes of unloaded pedalling (0 W) and subsequently a progressive increase in workload (5-20 W min^{-1}) to maximum tolerance. The mean exercise test duration was 10 minutes (from unloaded pedaling to peak exercise) in all patients.
All ventilatory parameters, calculated from the breath by breath measured flow, inspiratory and expiratory gas fraction at the mouth were calculated by a commercial available metabolic computer (Vmax 229, Viasys, Yorba Linda, USA) and analyzed as 20 seconds averages. Oxygen pulse (O_2pulse) was calculated as VO_2/HR. VE/VCO_2 was derived from the first linear part of the curve, from unloaded work till the anaerobic threshold (19). The anaerobic threshold was determined by the combination of the V-slope method and the lowest respiratory equivalent for oxygen (3). Oxygen saturation of arterial blood (SaO_2) was determined by pulse-oximetry (9600, Nonin, Plymouth, USA). No patients were using supplemental oxygen during CPET. Heart rate (HR) was measured by electro-cardiography (Eagle 4000, Marquette).

Statistical Analysis

CPET variables included in baseline and follow-up analyses were maximal VO_2, HR, O_2pulse, SaO_2 and VE/VCO_2. Baseline values and changes over time in CPET variables were related to survival, which was 58±5 months in the whole group and 52±5 months in the subgroup with available follow-up data. Statistical analysis was performed with the SPSS-15 package (SPSSinc, Chicago, USA). All data were expressed as mean values ± standard error of the mean. The unpaired student's t test was used to check for differences between survivors and non-survivors. Receiver operating characteristics (ROC) were determined to identify optimal cut-off points for predicting survival based on the highest sum of sensitivity and specificity values. Areas under the ROC curve are presented with a 95% confidence interval (CI). Based on these optimal cut-off points, univariate Kaplan-Meier survival curves were calculated. In all analyses, p-values below 0.05 (2–tailed) were considered significant.

RESULTS

From the initial group of 65 patients, 13 died and two underwent a lung transplantation (<90 months). Both patients receiving a lung transplant and seven deceased patients were included in the subgroup analysis with follow-up CPET data (n=39).

	ALL	Survivors	Non-Survivors	*p*
n	65	50	15	
Female (n, %)	50 (77%)	42 (84%)	8 (53%)	<0.05
Age, yr	45 ± 2	44 ± 2	50 ± 4	0.13
Height, cm	167 ± 1	167 ± 1	167 ± 2	0.76
Weight, kg	75 ± 2	76 ± 2	73 ± 4	0.62
Mean PAP, mmHg	55 ± 2	55 ± 3	56 ± 4	0.83
CO, L*min^{-1}	4.7 ± 0.2	4.7 ± 0.2	5.0 ± 0.4	0.49
PVR, dynes*s*cm^{-5}	881± 55	877 ± 66	893 ± 103	0.90
RAP, mmHg	8.1 ± 0.6	8.0 ± 0.7	8.4 ± 1.2	0.79
SVO_2,%	64 ± 1	65 ± 1	60 ± 2	0.06

Table 5.1 *Baseline demographic and hemodynamic characteristics at time of diagnosis. PAP: pulmonary arterial pressure, CO: cardiac output, PVR; pulmonary vascular resistance, RAP; right atrial pressure, SVO_2: venous oxygen saturation. (mean ± SE)*

BASELINE VALUES

As expected in idiopathic PAH, a high percentage of female patients was included. Demographic and resting hemodynamic variables were not different between survivors and non-survivors (Table 5.1). Out of all analysed baseline CPET variables, only maximal HR was significantly higher and the VE/VCO2 slope significantly lower in the survivors (table 5.2).

	ALL	Survivors	Non-Survivors	*p*
VO_2max, ml*kg^{-1}	14.3 ± 0.7	14.7 ± 0.8	13.2 ± 1.0	0.36
ΔVO_2max, ml*kg^{-1}	1.7 ± 0.6	2.3 ± 0.6	-0.8 ± 0.9	0.02
HR, beats*min^{-1}	143 ± 3	147 ± 3	129 ± 5	0.01
ΔHR, beats*min^{-1}	-1 ± 3	0 ± 4	-5 ± 8	0.56
O_2pmax, ml*beat^{-1}	7.4 ± 0.3	7.4 ± 0.4	7.5 ± 0.7	0.87
ΔO_2pmax, ml*beat^{-1}	0.8 ± 0.2	1.1 ± 03	- 0.2 ± 0.5	0.03
SaO2max, %	91 ± 1	92 ± 1	90 ± 2	0.42
ΔSaO2max,%	0 ± 1	1 ± 1	-2 ± 1	0.05
VE/VCO_2	46 ± 2	44 ± 2	54 ± 4	0.01
ΔVE/VCO_2	-5 ± 2	-6 ± 2	-2 ± 5	0.38

Table 5.2 *Baseline (n=65) and change (Δ) at follow-up (n=39) of CPET parameters VO2max: peak oxygen uptake, HRmax: peak heart rate, O_2pmax: Oxygen pulse (=VO_2max/HRmax), SaO_2max: oxygen saturation at maximal exercise. VE/VCO_2: slope relating ventilation to carbon dioxide production. (mean ± SE)*

CHANGE AT FOLLOW-UP

Due to clinical and logistical reasons 26 out of 65 patients did not perform a follow up CPET within 24 months of the initial test. The subgroup with available follow-up CPET data had identical demographic, hemodynamic and CPET characteristics as the whole initial cohort (data shown in table 5.4).

After a mean follow up of one year, subsequent survivors showed a significantly greater change in maximal VO_2 and O_2pulse than subsequent non-survivors. Changes in maximal HR, SaO_2 and VE/VCO_2 slope were not significantly different between survivors and non-survivors (table 5.2).

SURVIVAL ANALYSIS

The cumulative proportion of survival was 97 ± 2 % at 1 yr, 91± 4 % at 2 yr, 84 ± 5% at 3 yr, 78 ± 6% at 5 yr, 68 ± 7% at 7 yr and 64 ± 8% at 9 yr. ROC analysis of initial CPET parameters identified significant areas under the curve of only maximal HR and VE/VCO_2 slope (Table 5.3). These two parameters were the only significant predictors of survival as determined by Kaplan-Meier analysis (figure 5.1).

The cumulative proportion of survival of the subgroup of patients with available follow up CPET data was 89 ± 5 % at 1 yr, 83± 6 % at 2 yr, 79 ± 7% at 3 yr, 75 ± 8% at 4 yr and 69 ± 9% at 5 yr.

Only the change in maximal VO_2 and the change in maximal O_2pulse showed significant areas under the curve by ROC analysis (table 5.3). Change in maximal VO_2 and change in maximal O_2pulse were predictive of survival as determined Kaplan-Meier analysis (figure 5.1).

	Area	95% CI	*p*
VO_2max, ml*kg^{-1}	0.57	(0.42 - 0.72)	0.42
ΔVO_2max, ml*kg^{-1}	0.77	(0.60 - 0.95)	0.02
HRmax, beats*min^{-1}	0.76	(0.61 - 0.90)	0.00
ΔHRmax, beats*min^{-1}	0.54	(0.32 - 0.76)	0.74
O_2pmax, ml*beat^{-1}	0.50	(0.34 - 0.66)	0.96
ΔO_2pmax, ml*beat^{-1}	0.73	(0.55 - 0.92)	0.04
SaO2max, %	0.56	(0.39 - 0.72)	0.50
ΔSaO2max,%	0.70	(0.50 - 0.89)	0.08
VE/VCO_2slope	0.71	(0.56 - 0.86)	0.01
ΔVE/VCO_2slope	0.51	(0.30 - 0.72)	0.95

Table 5.3 *Receiver operating characteristics of baseline and change (Δ) at follow-up of CPET parameters*
CI : confidence interval, VO2: oxygen uptake, HR; heart rate, VE/VCO2: slope relating ventilation to carbon dioxide production

DISCUSSION

This study shows that in patients with PAH, baseline CPET variables which predict survival are not equally prognostic when measured as changes over time. In this study, the chronotropic response and ventilatory efficiency at baseline were significant predictors of long time survival. When analysing changes in CPET variables after one year of treatment, a change in maximal aerobic capacity turned out to be the only predictor of subsequent survival.

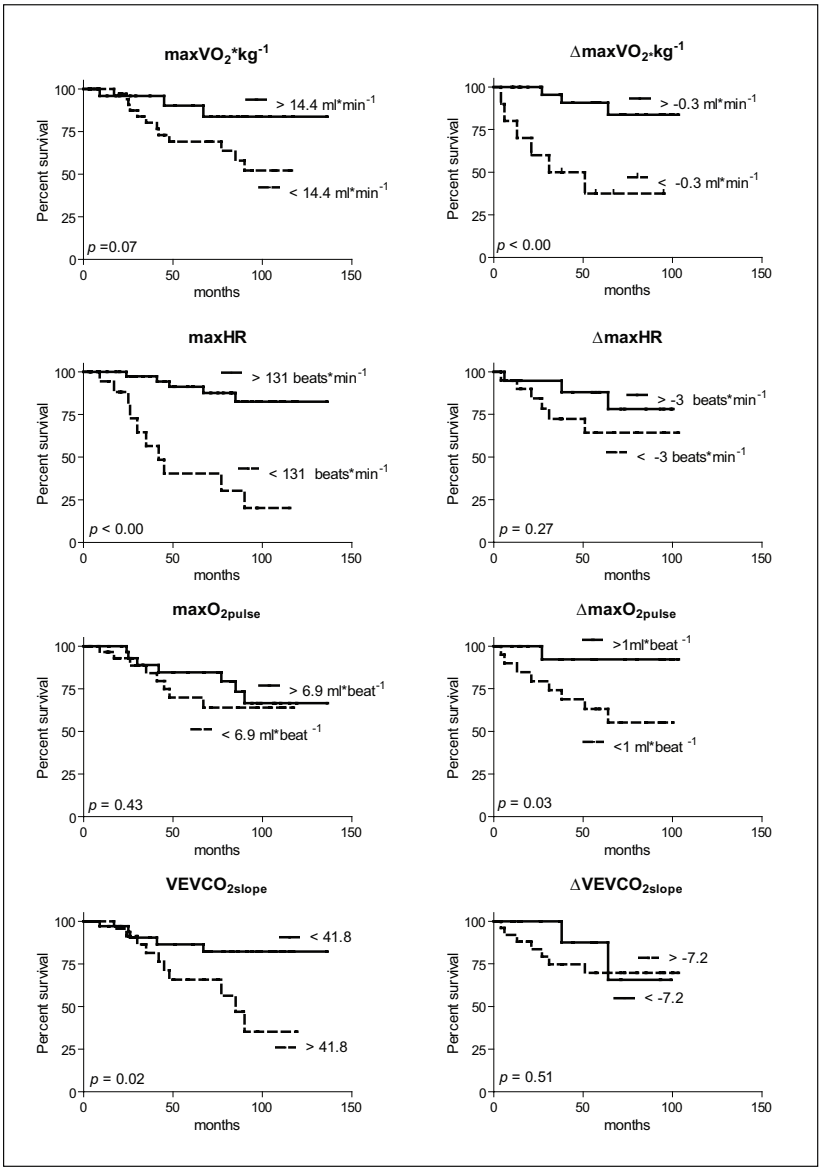

Figure 5.1 *Kaplan-Meier curve's of baseline (left-panels) and change (Δ) (right-panels) at follow-up of CPET parameters. VO_2: oxygen uptake, HR; heart rate, VE/VCO_2: slope relating ventilation to carbon dioxide production.*

INITIAL VALUES AT TIME OF DIAGNOSIS

Survivors and non-survivors were, except for gender, demographically and hemo-dynamically well matched at baseline (table 5.1). We found no significant difference in maximal aerobic capacity between survivors and non-survivors and although there was a trend (p=0.07) survival estimated by Kaplan-Meier analysis was not significantly related to aerobic capacity as measured during the initial CPET. This finding contrasted with earlier research (7, 22) and although under-powering cannot completely be ruled out, it is likely that this difference is caused by differences in disease severity and patients characteristics between our patients and those in earlier studies. Wensel et al. included more severely affected patients, as reflected by a 20% lower maximal aerobic capacity and a much higher pulmonary vascular resistance (22). We studied the prognostic value of exercise testing in an earlier study, but then included in addition to patients with idiopathic and heritable PAH, also patients with chronic trombo-embolic pulmonary hypertension (7). We report here in agreement with Wensel et al. (22), that in a group with a less severe exercise intolerance, maximal heart rate during the initial CPET was higher in survivors. Chromotropic incompetence during exercise in severe PAH patients was also recognized by Provencher et al. (13).
An increased ventilatory response for a given degree of CO_2 production may be caused by over activation of the sympathetic nervous system (20), which by itself is also associated with subsequent mortality in PAH (5). Hence, the worse prognosis of patients with a decreased ventilatory efficiency at the time of diagnosis is in agreement with earlier and very recent literature (5, 7, 17, 22).

CHANGES AFTER FOLLOW - UP

Because a comprehensive CPET is a demanding test for patients and technicians, the relatively simple 6MWD is usually preferred as clinical end-point (12). CPET was used in only a few clinical trials to estimate differences in exercise responses upon treatment (1, 11). In the randomized controlled study STRIDE -1, CPET was deemed unuseful because no changes in maximal VO_2 were found after therapy despite improvements in 6MWD (1). This discrepancy between the two estimations of exercise tolerance was probably due to technical failure in some of the collaborating exercise laboratories (12).
A clinical study by Oudiz et al. showed significant improvements in aerobic capacity and ventilatory efficiency in treated patients compared to non-treated controls, but the association between changes in CPET variables over time and subsequent survival was not analyzed (11). This is the first clinical PAH study in which changes in CPET variables are related to subsequent survival. After one year of standard PAH treatment, only improvements in maximal aerobic power and maximal O_2pulse were predictive of a better survival rate. An improved aerobic capacity after therapy in surviving PAH patients was also found by McLaughlin et al. after 3 years of therapy (9). In an early study using CPET and 6MWD to estimate exercise performance after 20 months of infusion with Prostacyclin, VO_2 and 6MWD improved (and correlated) in a group of 16 primary pulmonary hypertension patients (21). We could not confirm the data by Provencher et al., who showed that a change in the chronotropic res-

ponse during exercise after treatment was related to survival (14). In this study, no changes in maximal HR or maximal SaO_2 were found after therapy, despite improvements in exercise tolerance, which is in agreement with the results of a very early study by Barst et al.(2).
Because changes in maximal HR were similar in survivors and non-survivors, the increased VO_2 max and O_2pulse yielded very similar results. When maximal HR does not change despite an increase in VO_2 (= increased O_2pulse), patients have likely improved their stroke volume. An improved stroke volume has been shown to predict survival after PAH specific therapy (2, 14). Despite differences in ventilatory efficiency at the time of diagnosis, changes in VE/VCO_2 over time were not significantly different between subsequent survivors and non-survivors. This is in line with previous observations that PAH specific therapies improve aerobic capacity but not ventilatory efficiency (1, 2, 9, 12, 14, 15, 21). It could be speculated that the current PAH therapies are mainly improving cardio-circulatory function (VO_2) and do not affect the sympathetic nerve system (VE/VCO_2). At the time of diagnosis, the sympathetic nerve system is hyper activated in PAH (5). Here we show that after one year of PAH specific therapy, VE/VCO_2 doesn't improve, which finding agrees with the fact that exercise hyper-ventilation due to increased peripheral chemoreceptor activation is sustained even after heart transplantation in left heart failure patients (4).
In conclusion, CPET variables which have prognostic significance when determined at baseline are not necessarily predictive for survival when measured as changes over time. A change in maximal aerobic capacity predicts subsequent survival, but with current PAH specific therapeutic interventions, changes in other CPET variables have no prognostic significance.

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	ALL	Survivors	Non-Survivors	*p*
n	39	30	9	
Female (n, %)	31 (79%)	25 (83%)	6 (67%)	> 0.05
Age, yr	44 ± 2	43 ± 3	49 ± 4	0.29
Height, cm	167 ± 1	168 ± 1	167 ± 2	0.73
Weight, kg	75 ± 3	76 ± 3	72 ± 6	0.48
Mean PAP, mmHG	54 ± 2	55 ± 3	51 ± 3	0.46
CO, $L*min^{-1}$	4.6 ± 0.2	4.5 ± 1.3	4.8 ± 1.1	0.55
PVR, $dynes*s*cm^{-5}$	913 ± 62	948 ± 76	803 ± 94	0.32
RAP, mmHg	8.6 ± 0.8	8.5 ± 1.0	9.0 ± 1.7	0.78
SVO_2 ,%	63 ± 1	64 ± 2	60 ± 3	0.27
VO_2, $ml*kg^{-1}$	13.7 ± 0.8	13.7 ± 1.0	13.7 ± 1.0	0.98
HR, $beat*min^{-1}$	144 ± 3	147 ± 3	132 ± 6	0.04
SaO_2, %	91 ± 1	92 ± 1	90 ± 3	0.42
VE/VCO_2	49 ± 2	47 ± 3	54 ± 5	0.20

TABLE 5.4 *Baseline demographic, hemodynamic and CPET characteristics of follow-up coort at time of diagnosis. CPET: cardiopulmonary exercise test, PAP: pulmonary arterial pressure, CO: cardiac output, PVR: pulmonary vascular resistance, RAP: right arterial pressure, SVO_2: venous oxygen saturation, VO_2: maximaloxygen uptake, HR; maximal heart rate, SaO_2 oxygen saturation measured by pulse oximetry. VE/VCO_2; linear regression slope of ventilation te carbom dioxide production*

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Effects of exercise training in patients with idiopathic pulmonary arterial hypertension

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ABSTRACT

Objective

Determine the physiological effects of exercise training on exercise capacity and quadriceps muscle function in patients with idiopathic pulmonary arterial hypertension (iPAH).

Methods

Nineteen clinically stable iPAH-patients (NYHA II-III) underwent a supervised exercise training program for the duration of 12 weeks. Maximal capacity, endurance capacity, and quadriceps function were assessed at baseline and after twelve weeks. In 12 patients, serial quadriceps muscle biopsies were obtained.

Results

Six minute walk distance and peak exercise capacity did not change after training. However, endurance capacity improved significantly after training, demonstrated by a shift of the anaerobic threshold to a higher workload (from 32±5 to 46±6 Watt; $p=0.003$) together with an increase in exercise endurance time ($p<0.001$). Moreover, exercise training increased quadriceps strength with 13% ($p=0.005$) and quadriceps endurance with 34% ($p=0.001$).
Training enhanced aerobic capacity of the quadriceps, by increasing capillarization (1.36±0.10 to 1.78±0.13 capillaries per muscle fiber; $p<0.001$) and oxidative enzyme activity, especially of the type I (slow) muscle fibers. No changes were found in cross sectional area and fiber type distribution.

Conclusions

Exercise training in iPAH improves exercise endurance and quadriceps muscle function, which is also reflected by structural changes of the quadriceps.

INTRODUCTION

Idiopathic pulmonary arterial hypertension (iPAH) is a life threatening disease, which eventually leads to right heart failure. A high pulmonary vascular resistance and right ventricular dysfunction impair stroke volume, thereby limiting oxygen supply to the working skeletal muscles, especially during exercise, resulting in lactic acidosis at low work rates and impaired functional capacity[1,2].

Traditionally, exercise training in iPAH-patients was contra-indicated due to the risk of sudden cardiac death[3]. However, with the increase in medical treatment options in the last decennium, prognosis has improved significantly, and the role of exercise training in patients with iPAH was reconsidered[3]. Recently, the first clinical trial on exercise training in patients with pulmonary arterial hypertension reported promising results of improved exercise capacity and quality of life[4].

Exercise training is a well-established adjunct therapy in several chronic diseases such as COPD and congestive heart failure[5,6]. In patients with these chronic diseases, skeletal muscle dysfunction contributes to exercise intolerance[7-9]. The beneficial effects of exercise training in COPD and congestive heart failure are partially attributed to improved skeletal muscle efficiency[10,11] and increased capillary density and oxidative enzyme activity in quadriceps muscle biopsies[11-13].

Also in iPAH-patients, skeletal muscle dysfunction has been reported. Respiratory muscle dysfunction was found in two studies, both by voluntary and non-voluntary techniques[14,15]. More recently, forearm muscle dysfunction has been reported in these patients[16]. In a pilot study, examining voluntary strength of the respiratory, forearm and quadriceps muscles in iPAH-patients, we previously found specific quadriceps muscle dysfunction[17]. However, these studies focused on muscle function only, without giving more insight in the roles of muscle atrophy, fiber type switching, decreased oxidative enzyme activity, or reduced capillary density, as potential underlying mechanisms of skeletal muscle dysfunction[18].

We hypothesize that exercise training in iPAH-patients improves exercise capacity and diminishes quadriceps muscle dysfunction by counteracting these structural muscle alterations. We therefore assessed the effects of an outpatient exercise training program on exercise capacity, quadriceps function and quadriceps structure.

METHODS

Study population

In total, 19 patients were recruited from the VU University Medical Center Amsterdam between 2006 and 2008 and met the following criteria: 1) Diagnosed with iPAH according to WHO-criteria[19] established by right heart catheterization; 2) Stable clinical condition, defined as a change in six minute walk distance (6MWD) of less than 10% in three consecutive measurements prior to inclusion (over a period of minimally one year), and no change in medical therapy for at least three months; 3) Eighteen years or older; 4) Living within five kilometers of a rehabilitation center associated with this study.

The Institutional Review Board on Research Involving Human Subjects approved the protocol. Informed consent was obtained from all subjects.

Study protocol

Patients were evaluated at baseline and after twelve weeks, on two consecutive days. On day 1: a cardio-pulmonary exercise test (CPET), quadriceps function tests and pulmonary function tests[20] were performed, and NT-proBNP was determined. On day 2: an endurance exercise test was performed and the 6MWD determined. Twelve of nineteen patients underwent a quadriceps muscle biopsy on the second day of evaluation before and after training.
All patients attended an exercise-training program of three times a week in a rehabilitation center for a period of 12 weeks. The training program was performed in rehabilitation centers nearby, according to usual clinical care. The standardized exercise protocol was adopted from the American Heart Association guidelines for rehabilitation of chronic heart failure patients[5]. The exercise training consisted of cycle and quadriceps muscle training (Table 6.1). For safety reasons, physiotherapists, who recorded heart rate and oxygen saturation, always accompanied the patients. When oxygen saturation dropped below 85%, or heart rate exceeded 120 bpm, the training session was paused or terminated earlier. Rehabilitation physicians or pulmonologists were on site and directly available for consultation. Attendance was recorded as indicator for patient's compliance.

Exercise testing

6MWD test was performed according to ATS guidelines[21]. Maximal capacity was determined during CPET as described before[22]. During CPET heart frequency, pulse oximetry and gas exchange (breath-by-breath) were recorded. Anaerobic threshold was determined by the V-slope method[23].
Endurance capacity was evaluated by a submaximal exercise test performed at a constant load of 75% of baseline peak-workload[24]. After 3 minutes of rest and 3 minutes of unloaded cycling, the patients had to exercise at 75% of baseline peak-workload for as long as possible or the observer terminated the test after 15 minutes. During the submaximal test heart frequency, pulse oximetry and gas exchange (breath-by-breath) were recorded.

Quadriceps muscle function and biopsy

Quadriceps function was assessed with a hydraulic dynamometer, as previously described[25]. In addition, twelve of nineteen patients gave informed consent for a quadriceps muscle microbiopsy at baseline and after training, to analyze the effects of training on structural changes of the quadriceps muscle. Circumference of the leg was measured. Under local anesthetics (2% lidocaine), biopsies were taken from the vastus lateralis of the quadriceps muscle 10 cm above the patella with a 16G spring-loaded biopsy needle (QC-16-15.0-10T, Cook Medical, Limerick, Ireland). The biopsy was immediately evaluated under the microscope, embedded in 15% gelatin in Tyrode's solution containing 20mM butanedione monoxime and frozen in liquid nitrogen. Serial cryosections were cut at -20ºC and collected on slides coated with Vectabond.

Week	Intensity	Exercise	Rest time	Sets
Cycle exercise				
1-3	50% VO_{2max}	2 min	2 min	10
4-6	50% VO_{2max}	3 min	2 min	7
7-9	75% VO_{2max}	4 min	2 min	6
10-12	75% VO_{2max}	5 min	2 min	5
Quadriceps strength				
1-3	50% ORM	12 rep	1 min	3
4-6	50% ORM	13 rep	1 min	3
7-9	75% ORM	14 rep	1 min	3
10-12	75% ORM	15 rep	1 min	3
Quadriceps endurance				
1-3	30% ORM	30 rep	1 min	3
4-6	30% ORM	40 rep	1 min	4
7-9	40% ORM	50 rep	1 min	5
10-12	40% ORM	60 rep	1 min	6

Table 6.1 *Twelve weeks supervised exercise training protocol*
The exercise training consisted of cycle training (based on VO_{2max} assessed at baseline measurements) and quadriceps training (based on one-repetition-maximum assessed at first day of training). Abbreviations: VO_{2max}: maximal oxygen consumption determined during CPET; ORM: one-repetition-maximum; rep: repetition

Number of capillaries in quadriceps muscle

Capillarization of the quadriceps muscle was determined by quantitative immunofluorescence microscopy. Briefly, quadriceps cryosections (5 µm) were incubated for 60 min with primary CD31-antibody (1:100; sc-1506-R, Santa Cruz Biotechnology), followed by secondary antibody staining with anti-rabbit Alexa fluor 488, as well as WGA (glycocalyx) and DAPI (nuclei) counterstaining. Image acquisition was performed on a Marianas digital imaging microscopy workstation (Intelligent Imaging Innovations (3i), Denver CO) using multiple fluorescence channels. SlideBook imaging analysis software (SlideBook 4.2, 3i) was used to semi-automatically quantify the images. Capillarization was expressed as the number of capillaries per quadriceps myocyte measured in the whole section.

Fiber type distribution, oxidative enzyme activity, and cross sectional area

Cross sectional area (CSA), fiber type distribution and oxidative enzyme activity were determined as previously described[26,27]. In detail, in 10 randomly selected type I and 20 randomly selected type II cells (identified by serial sections stained for myofibrillar ATPase), oxidative enzyme activity was analyzed by measuring succinate dehydrogenase (SDH) absorbance at 660 nm (10 µm sections incubated for 20 min at 37 ºC). For the same cells, CSA were measured. Fiber type distribution was analyzed by counting of all type I and type II muscle fibers in the biopsy. Images were analyzed using ImageJ imaging analysis software (ImageJ for Windows 1.39a, NIH, Besthesda MD).

Statistics

All data are presented as mean±SEM, unless stated otherwise. A p-value <0.05 was considered statistically significant. The effects of training on exercise capacity, quadriceps function and quadriceps capillarization were assed by a paired t-test. Changes in fiber type distribution were assessed by two-way repeated measures ANOVA. Oxidative enzyme activity and muscle fiber CSA were analyzed by multi-level analyses, to correct for the non-independence of successive measurements per patient[28]. Regression analyses were performed to study the association between changes in quadriceps endurance and aerobic capacity. Analyses were performed with SPSS 16.0 or MLwiN 2.02 software.

RESULTS

Nineteen patients were recruited for this study and their baseline characteristics are presented in Table 6.2. The patient cohort was typical iPAH, with a female predominance, median NYHA class III, and a mean age of 42 years. In addition, the last known catheterization data (< 1 year) prior to the rehabilitation are given in Table 6.2.

The training program was well tolerated by all patients and no adverse events were observed. The compliance to the exercise training program was 91±2%. Only two patients required a minor training adjustment, because of complaints of dizziness during the quadriceps exercise. Training did not elevate NT-proBNP levels (947±429 to 1043±462 pg/ml; n.s.) and did not change pulmonary function (data not shown).

	Study population (n=19)
Gender male/female	4/15
Age (yr)	42±13
Height (m)	1.70±0.09
Weight (kg)	72±14
NYHA class II/III	3/16
NT-proBNP (pg/ml)	947±1606
Hemodynamics	
mPAP (mmHg)	53±19
PVR (dynes.s/cm^5)	845±460
Cardiac index (l/min/m^2)	2.7±0.9
Right atrial pressure (mmHg)	7±4
PCWP (mmHg)	5.7±2.4
Exercise capacity	
6 minute walk distance (m)	496±108
Peak work (Watt)	78±42
Peak VO_2 (ml/kg/min)	15±4
Peak VE (l/min)	63±30
Peak $VECO_2$	51±13
Medication	
Single / combination treatment	8/11
Treatment duration (months)	20±16

Table 6.2 *Patient characteristics at baseline*
Data are presented as mean±SD. BSA: body surface area; NYHA class: New York Heart Association functional class; pred: predicted value; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; PCWP: pulmonary capillary wedge pressure

Exercise training improved endurance

The CPET revealed no significant improvements of maximal capacity and gas-exchange after training. Moreover, exercise training did not improve 6MWD (Fig.6.1A). However, endurance exercise characteristics improved significantly after training, demonstrated by a shift of anaerobic threshold to a higher workload (from 32±5 to 46±6 Watt; p=0.003). In addition, exercise endurance time increased with 89% after training (Fig.6.1B; p<0.001).

Exercise training improved quadriceps function and aerobic capacity

Quadriceps muscle strength improved modestly with 13% after training (Fig.6.1C; from 94±7 to 106±8 Nm; p=0.005). Quadriceps endurance improved markedly with 34% after training (Fig.6.1D; from 136±10 to 181±18 s; p=0.001).

Leg circumference did not change after training (from 43±1 to 44±1 cm). No increase in CSA was found in both type I (from 4224±363 to 4877±325 μm^2) and type II (from 3676±347 to 4235±462 μm^2) muscle fibers (Fig.6.2A). Fiber type distribution did not change after training (Fig.6.2B; Type I: from 35±3 to 38±3%; Type II: from 65±3 to 62±3%)

However, training increased the number of capillaries per myocyte by 30% (Fig.6.3; from 1.36±0.10 to 1.78±0.13; p<0.001). SDH absorbance increased with 39% in type I (slow) muscle fibers (Fig.6.4; from 0.161±0.011 to 0.216±0.020; p<0.001) whereas it increased with 30% in the type II (fast) muscle fibers (from 0.105±0.009 to 0.133±0.010; p=0.05).

The change in SDH absorbance of the type I (slow) muscle fibers and the change in the number of capillaries were highly associated to the improvements of quadriceps endurance (R^2=0.73; p<0.001).

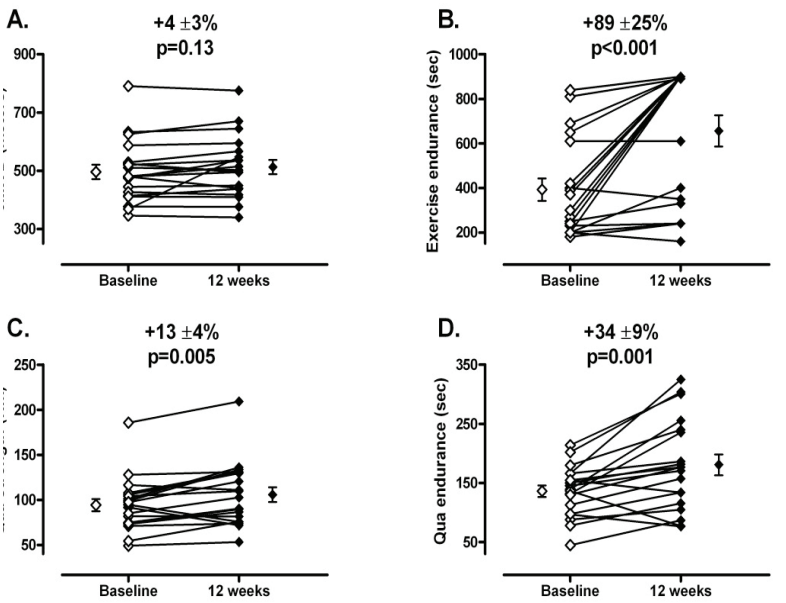

Figure 6.1 *Functional effects of exercise training Effect of a 12 week exercise training program on six minute walk distance (A), exercise endurance (B), quadriceps strength (C) and quadriceps endurance (D).*

Data presented as mean ±SEM. Open diamonds: baseline values, closed diamonds: values after training of individual patients.

DISCUSSION

To our best knowledge, this is the first study that evaluates the effect of exercise training on quadriceps function and morphology in iPAH-patients. We have demonstrated that a twelve weeks out-patient training protocol increases endurance capacity, without an improvement of maximal capacity. The same phenomenon was seen on quadriceps function: a large improvement in quadriceps endurance was found, and a small (but significant) increase in quadriceps strength. Histological analyses revealed improved aerobic capacity by increased quadriceps capillarization and oxidative enzyme activity, without hypertrophy or fiber type switch. Finally, these morphological changes were strongly correlated with an improvement in quadriceps endurance.

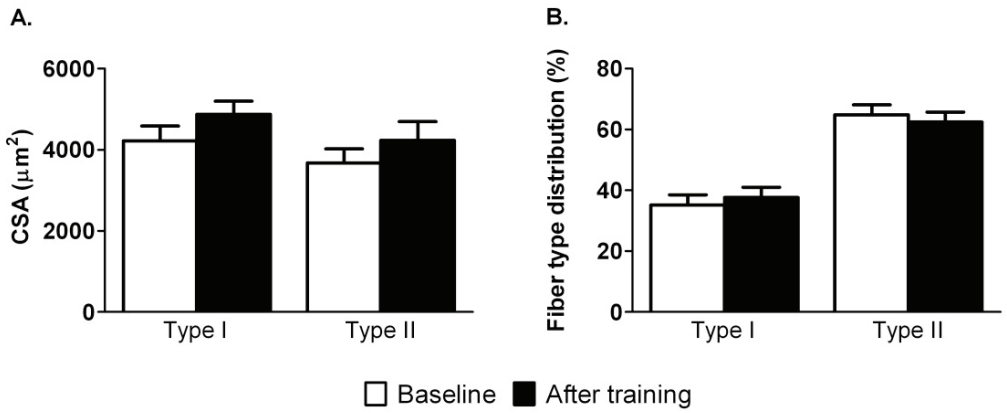

Figure 6.2 *No changes in cross sectional area and fibre distribution after training*
Cross sectional area (CSA) and fiber type distribution after twelve weeks of training in both type I (slow) as in type II (fast) muscle fibers. Data are presented as mean±SEM. White bars: baseline values, black bars: values after training.

Training improved endurance capacity

In the first clinical trial by Mereles et al, investigating the effect of training in pulmonary hypertension, a major improvement was found in the 6MWD, which was even greater than any medical intervention had achieved previously[4]. They also reported improvements in both maximal as well as sub-maximal exercise capacity. However, our study shows improvement in endurance capacity only. This can be explained by differences in training modalities and study population. Firstly, our training schedule consisted of cycle and quadriceps training, whereas the training schedule of Mereles et al. consisted of cycling and walking training. Furthermore, our training protocol was mainly focused on improving endurance capacity, which might explain the absence of improved maximal capacity. Secondly, baseline 6MWD of our patient population were slightly higher (496±108 m in our study vs. 439±82 m in the study of Mereles et al.) which may imply that our patients had a smaller window of improvement in 6MWD. In addition to Mereles et al. we were able to confirm the improvement of endurance capacity with an endurance exercise test, where we found an improvement

in endurance time of 89%. Moreover, we observed the same phenomenon on quadriceps function: quadriceps strength was only modestly improved, whereas quadriceps endurance improved significantly by 34%.

Recently, Sitbon and co-workers presented preliminary results from their 12 weeks outpatient rehabilitation program in patients with iPAH[29]. Interestingly, they also found only improvements of endurance but not in maximal capacity, which is in line with our findings.

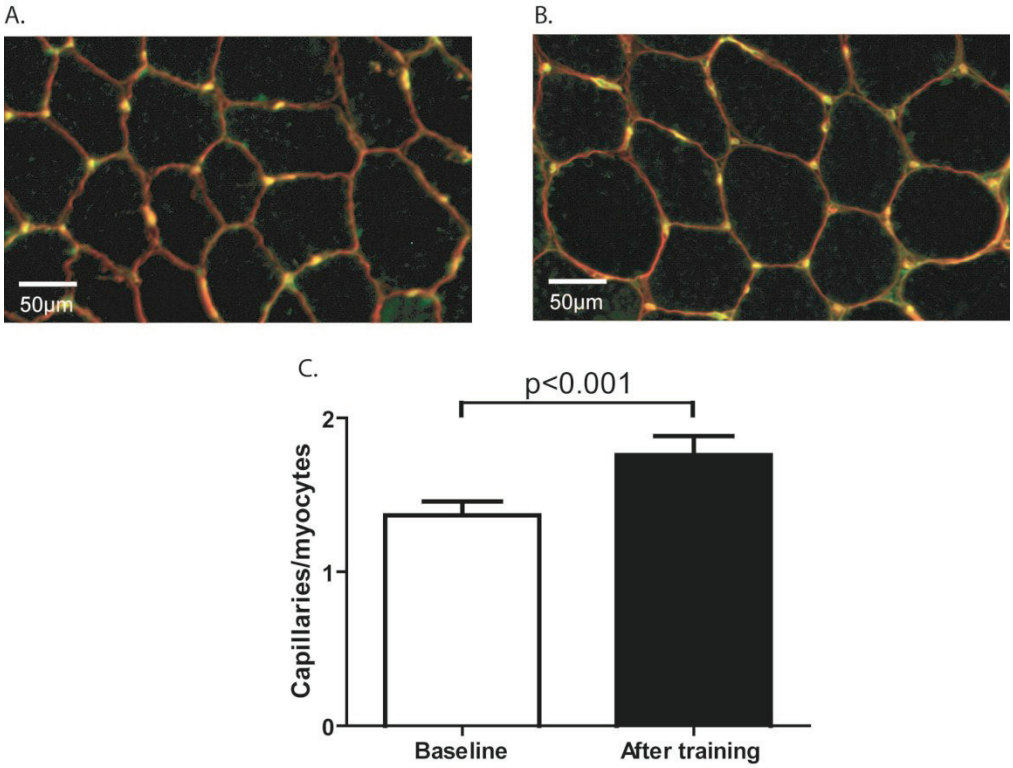

Figure 6.3 *Training increased quadriceps capillarisation*
Typical examples of quadriceps capillarization in one patient before (A) and after (B) training (100x magnification, red=cell membrane of the quadriceps myocytes, yellow=capillaries). Number of capillaries per quadriceps myocyte at baseline and after training (C). Data are presented as mean±SEM.

Improved quadriceps endurance is associated with increased aerobic capacity

Quadriceps dysfunction is often observed in several chronic diseases such as COPD and chronic heart failure. More recently, several reports suggested muscle dysfunction in patients with pulmonary arterial hypertension[14-16]. Mechanisms are still unclear, but it can be speculated that inactivity of the skeletal muscles, together with a decreased cardiac output leading to a reduced oxygen transport to the skeletal muscles, trigger morphological changes, such as muscle atrophy, fiber type switching, and reduced aerobic capacity[18].

In patients with COPD or heart failure, prolonged exercise training is an effective tool to reverse changes in mitochondria, key metabolic enzymes, capillarization and - to a lesser extent - changes in fiber type composition[13]. These findings are in line with our findings of improved oxidative enzyme activity and number of capillaries. However, we did not find changes in CSA or fiber type distribution, which might be a consequence of the relative short exercise period at a low intensity[12].

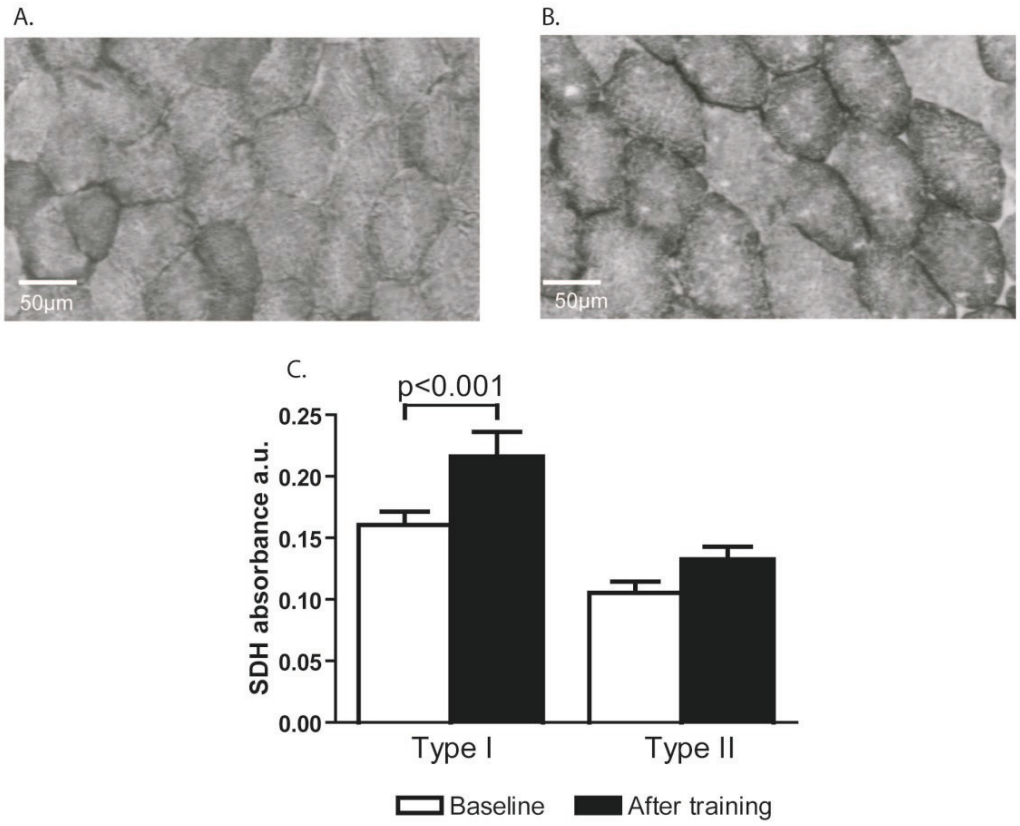

Figure 6.4 *Training increased oxidative enzyme capacity of type 1 fibers*
Typical examples of oxidative enzyme activity in one patient before (A) and after (B) training (dark cells indicates high oxidative enzyme activity, light cells indicates low oxidative enzyme activity). Training increased oxidative enzyme activity especially in the type I (slow) muscle fibers (C). Data are presented as mean±SEM; White bars: baseline values, black bars: values after training. Abbreviations: SDHabsorbance: absorbance due to succinate dehydrogenase activity.

Limitations

To limit the burden of the study for the patients, we limited the number of tests and the invasiveness of the measurements. We were therefore not able to assess the effect of training on quadriceps muscle function with electrical stimulation. A potential bias due to differenc-

es in motivation before and after training, may have overestimated the observed improvements in quadriceps function. However, as we found a strong association between quadriceps function and biopsy data, motivation differences may have had only a minor effect on outcome. Moreover, to limit the number of measurements, we did not measure quadriceps muscle mass by magnetic resonance imaging. Based on the measurements of leg circumference and CSA of the individual muscle fibers, changes in muscle mass are unlikely. To decrease the invasiveness of a muscle biopsy, we used a microbiopsy technique instead of the Bergström method[30,31]. As a consequence, protein analyses were not performed. However, we used well-validated histological techniques. For instance, capillary density was semi-automatically quantified with a CD31-antibody, which is a standard method when investigating angiogenesis [9,32].

Clinical relevance

This study confirms that iPAH is not only associated with compromised cardiopulmonary function, but also with impaired skeletal muscle function. We found that skeletal muscle dysfunction could partially be reversed by exercise training in iPAH. Moreover, our training program improved quadriceps endurance, more than quadriceps strength, even though both aspects were implemented in the training protocol. This implies that future training protocols should focus on enhancing endurance capacity rather than maximal capacity. Although, we have found an overall beneficial effect of exercise training we can not generally recommend exercise training for all PAH-patients yet. For instance, we were not able to rule out repercussions on cardiac function and hemodynamics, although after training NT-proBNP levels remained unaltered. Moreover, not all patients seemed to benefit from the exercise training therapy (Fig.6.1B). However, the relative small number of patients did not allow us to discriminate responders from non-responders at baseline. Future studies and clinical trials should asses the effects of training on right ventricular remodeling and function, and determine factors that can predict which patients can benefit most of exercise training.

Conclusion

Exercise training improves endurance and quadriceps muscle function, which is also reflected by structural changes of the quadriceps muscle. Our present study supports the potential role of exercise training as an adjunct therapy in stable iPAH-patients.

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Cardiopulmonary exercise test characteristics in COPD patients with associated pulmonary hypertension

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ABSTRACT

Background

Pulmonary hypertension (PH) is a well-known complication of COPD. It remains unclear whether exercise parameters can be used to discriminate between COPD patients with associated PH (COPD-PH) and COPD patients without associated PH (COPD-nonPH).

Objective

To study whether the existence of pulmonary hypertension in COPD is related to characteristic findings in gas exchange and circulatory parameters during cardiopulmonary exercise testing (CPET).

Methods

We retrospectively analysed CPET data in 25 COPD patients in whom right heart catheterization had been performed. Differences were assessed between COPD-PH and COPD-nonPH patients in peak oxygen uptake (VO2 peak), ventilatory efficiency (VE/VCO2), oxygen pulse (O2-pulse), peak ventilation and pulse oximetry (SpO2).

Results

PH was found in 10 out of 25 patients (mPpa = 33±7 mmHg), in 15 patients mPpa was below 25 mmHg (18±3 mmHg). CPET in COPD-PH was characterized by a higher VE/VCO2 at nadir, a higher VE/VCO2 slope, and a lower SpO2 at rest and during exercise, but values in both groups were overlapping considerably. In the whole group mPpa was associated with resting PaO2 ($r = -0.70$, $p<0.001$), VECO2 nadir ($r = 0.43$, $p<0.05$), and inversely related to SpO2 at rest and during exercise ($r = -0.58$ and $r = -0.64$, $p<0.01$ respectively).

Conclusion: Although CPET characteristics showed a large overlap in both groups, the existence of PH in COPD is associated with a significantly reduced ventilatory efficiency during CPET. However, a low SpO2 at rest and a further decrease during exercise similarly suggest the presence of PH in COPD.

INTRODUCTION

Pulmonary hypertension (PH) is a well-known complication of chronic obstructive pulmonary disease (COPD), with a reported prevalence of 20 to 90 % [1-5]. Recent studies indicate that the direct effects of tobacco smoke might contribute to the development of PH in COPD through effects on the intrapulmonary vessels with abnormal production of mediators that control vasoconstriction, vasodilatation, and vascular cell proliferation [6]. However, alveolar hypoxia has been identified as the main cause of PH in COPD: acute hypoxia causes pulmonary vascoconstriction and long-term hypoxia induces pulmonary vascular remodelling [7]. Scharf and colleagues demonstrated that mPpa correlates negatively with partial arterial oxygen pressure, but also indicated that chronic hypoxia is not the only factor contributing to the development of PH. [4;8]. Arterial oxygen saturation has been shown to correlate closely with mPpa [1].

The degree of PH in COPD is usually mild to moderate, with resting mean pulmonary artery pressure (mPpa) in a stable state of the disease ranging between 20 and 35 mmHg [9]. Exercise in these patients may be associated with further marked increases in Ppa [10]. It has therefore been suggested that exercise testing may be useful in early diagnosis of PH.[11;12] Validated methods to establish the diagnosis and to grade the severity of PH are cardiac catheterization and echocardiography. While the clinical value of cardiopulmonary exercise testing (CPET) to non-invasively analyse exercise limitation in COPD is well recognized, it is not known whether the presence of PH in COPD leads to changes in gas exchange characteristics during exercise. Studies performed in idiopathic PH revealed that the most consistent and characteristic findings in these patients were: reductions in oxygen uptake (VO_2) at the anaerobic threshold and at peak exercise, a reduction in peak oxygen pulse (O_2-pulse) and a diminished ventilatory efficiency [13]. It remains unclear whether these parameters are different between COPD patients with associated PH (COPD-PH) and COPD patients without associated PH (COPD-nonPH). The objective of the present study was to verify whether the existence of pulmonary hypertension (PH) in COPD is related to characteristic CPET findings. In addition, we investigated whether gas exchange measurements during CPET lead to a better recognition of PH in COPD than exercise pulse oximetry.

PATIENTS AND METHODS

Subjects and Pulmonary Function Testing

This study is part of a larger research project investigating the effects of treatment of pulmonary hypertension in COPD patients. This study requires right heart catheterization and exercise testing in all patients. The VU University medical ethics committee approved the study, and written informed consent was obtained from all subjects. Twenty-five patients with moderate to severe COPD and increasingly symptomatic during daily activities despite stable pulmonary function were retrospectively included in this study. Selection of patients was based on the following criteria: 1. Clinical diagnosis of COPD (GOLD criteria); 2. No clinical evidence of cardiovascular disease (including arterial hypertension and previous myocardial infaction), and a normal left ventricular function on echocardiography; 3. No

Characteristics	COPD-nonPH (*n = 15*)	COPD-PH (*n = 10*)
Male / female	7 / 8	5 / 5
Age, yr	66 ± 10 (48-78)	64 ± 11 (46-80)
BMI, kg/m^2	25 ± 3 (20-33)	22 ± 4 16-28)
VC, % predicted	101 ± 14 (73-126)	101 ± 26 (65-144)
FEV_1, % predicted	49 ± 19 (26-80)	62 ± 31 (21-116)
FEV_1/VC, %	39 ± 15 (20-65)	47 ± 16 (24-77)
TLC, % predicted	126 ± 16 (101-151)	115 ± 19 (80-146)
FRC, % predicted	169 ± 27 (129-214)	145 ± 37 (92-207)
DLCO, % predicted	50 ± 13 (33-85)	37 ± 14 (21-55)
PaO_2, kPa	9.9 ± 1.4 (7.0-12.4)	7.6 ± 2.0 (4.8-10.5)*
$PaCO_2$, kPa	5.4 ± 0.6 (4.1-6.7)	5.0 ± 1.3 (3.5-7.6)
mPpa, mm Hg	18 ± 3 (13-22)	33 ± 6 (26-45)**
PVR, $dyne \cdot s \cdot cm^{-5}$	208 ± 93 (51-411)	393 ± 170 (200-713)**
$mvSpO_2$, %	70 ± 6 (60-83)	68 ± 6 (56-75)

Table 7.1 *Demographics, respiratory function and hemodynamic characteristics of COPD-nonPH and COPD-PH patients BMI = body mass index; COPD = chronic obstructive pulmonary disease; DLCO = carbon monoxide transfer coefficient; FEV_1 = forced expiratory volume in one second; FEV_1/VC = Tiffeneau index; FRC = functional residual capacity; mPpa = mean pulmonary artery pressure; $mvSpO_2$ = mixed venous oxygen saturation; PVR = pulmonary vascular resistance. Values are mean ± standard deviation. * p < 0.05, versus COPD-nonPH, ** p < 0.01, versus COPD-nonPH*

pathology possibly interfering with the ability to perform exercise. Medical histories were checked and all patients underwent physical examination, and EKG. The group was divided into a COPD-nonPH and a COPD-PH subgroup. The diagnosis of PH was based on right heart catheterisation. A mPpa > 25 mmHg with a wedge pressure below 15 mmHg confirmed the diagnose of PH secondary to COPD [14]. Just before CPET studies, pulmonary function was evaluated by standard spirometry, including determination of transfer factor for carbon monoxide (DLCO), and measurement of functional residual capacity (FRC) and total lung capacity (TLC) according to ERS/ATS guidelines [15-17]. Arterial blood was obtained at rest

in all patients for determination of PaO_2 and $PaCO_2$ and calculation of the alveolar-arterial oxygen pressure difference ($D(A\text{-}a)PO_2$) using the alveolar gas equation. General characteristics and pulmonary function data are shown in Table 7.1.

CardioPulmonary Exercise Testing

Each patient performed a standard, incremental exercise test on an electronically braked cycle ergometer (Lode, Groningen, The Netherlands). Measurements of VO_2, carbon dioxide output (VCO_2), minute ventilation (V_E) and tidal volume (V_T) were made breath-by-breath (Vmax229, Sensormedics, Yorba Linda, California, USA). Calculations were made of ventilatory equivalents for oxygen and carbon dioxide (V_E/VO_2 and V_E/VCO_2, respectively). The slope of V_E versus VCO_2 was determined and V_E/VCO_2 nadir was defined as the lowest point on the V_E/VCO_2 curve. Pulse oximetry, heart rate and gas exchange were recorded and monitored during 3 minutes of rest, 3 minutes of unloaded cycling at 60 rpm followed by a progressively increasing work rate to maximum tolerance, and 3 minutes of recovery [18]. The oxygen pulse (O_2- pulse), i.e. oxygen uptake divided by heart rate was used as an estimator of stroke volume. In the majority of the studied COPD patients the anaerobic threshold could not be identified, therefore values of exercise parameters measured at anaerobic threshold are not provided.

Right Heart Catheterization

All patients underwent diagnostic right heart catheterization at rest with a 7F Swan-Ganz catheter (131HF7; Baxter Healthcare Corp; Irvine, CA) to assess pulmonary artery pressure (Ppa) within 1 week of CPET.

Statistics

Data are presented as mean ± SD. SPSS 14.0 software package was used for statistical analyses and a value of $p < 0.05$ was considered significant. The Mann Whitney test was applied for between-group analyses. Differences between resting and exercise values within subjects were assessed by the Wilcoxon signed rank test. Pearson correlation analyses were calculated to determine the correlations between hemodynamic and CPET data.

RESULTS

Subject Characteristics

Patients were classified as having moderate (stage II) to very severe (stage IV) COPD according to GOLD criteria [19]. Patient characteristics and pulmonary function data are shown in Table 7.1. Although COPD-PH patients tended to have less severe airflow obstruction, reflected by a lower FEV1 (% predicted, $p = 0.39$), and a reduced DLCO (% predicted, $p = 0.07$) compared with COPD-nonPH patients, differences in pulmonary function between COPD-nonPH and COPD-Ph the patients groups were not statistically significant. PaO_2 was reduced in COPD-PH. Furthermore, COPD-PH patients showed a significantly larger $D(A\text{-}a)PO_2$ at rest (6.9 ± 1.9 (range: 4.0-9.4 kPa) versus 3.2 ± 2.2 (range: (0.5-7.0 kPa), $p < 0.001$).

	COPD-nonPH (*n = 15)*	COPD-PH (*n = 10)*
Peak workload, W	65 ± 25 (20-100)	47 ± 20 (16-77)
Peak Workload, % predicted	50 ± 21 (20-96)	39 ± 23 (15-88)
Peak VO_2, ml/kg/min	13.0 ± 3.8 (5.8-18.8)	13.2 ± 5.1 (6.8-22.7)
Peak VO_2, % predicted	55 ± 14 (29-87)	56 ± 21 (22-87)
Peak heart rate, bpm	119 ± 19 (84-149)	126 ± 21 (91-160)
Peak Heart rate, % predicted	77 ± 11 (55-96)	81 ± 12 (65-99)
Peak O_2-pulse, ml/beat	7.6 ± 1.6 (4.7-9.6)	6.6 ± 2.4 (2.3-9.3)
Peak O_2-pulse, % predicted	62 ± 17 (36-85)	54 ± 21 (21-96)
Peak $P_{ET}CO_2$, kPa	4.6 ± 1.0 (2.4-6.4)	3.8 ± 1.8 (1.5-7.0)
Peak V_E, l/min	37 ± 10 (19-51)	46 ± 24 (19-86)
Peak V_E, % predicted	66 ± 16 (47-99)	68 ± 26 (43-129)
Peak V_E/VO_2	39 ± 7 (29-54)	56 ± 16 (27-80)**
Slope V_E/VCO_2	36 ± 11 (20-65)	51 ± 23 (24-103)*
V_E/VCO_2 nadir	41 ± 9 (25-63)	55 ± 15 (38-76)**
$\Delta VO_2/\Delta$ Workload	8.3 ± 2.3 (2.0-11.0)	8.0 ± 3.0 (3.5-11.7)
Desaturation, %	-4 ± 3 (-11-0)	-8 ± 5 (-16-(-1))*

Table 7.2 *CPET characteristics of COPD-nonPH and COPD-PH patients*
*COPD = chronic obstructive pulmonary disease; O_2-pulse = oxygen pulse; $P_{ET}CO_2$ = end-tidal CO_2 pressure; V_E = minute ventilation ; V_E/VCO_2 = ventilatory equivalent for CO_2; V_E/VO_2 = ventilatory equivalent for O_2; VO_2 = oxygen uptake. Values are mean ± standard deviation (range). * $p < 0.05$, versus COPD-nonPH, ** $p < 0.01$, versus COPD-nonPH .*

Right Heart Catheterization

Elevated Ppa was found in 10 out of 25 COPD patients. By definition, right heart catheterization yielded a significantly higher mPpa and pulmonary vascular resistance (PVR) in COPD-PH compared with COPD-nonPH (Table 7.1).

CardioPulmonary Exercise Testing

CPET characteristics of both groups are presented in Table 7.2. Peak workload and peak VO_2 as percentage of predicted were comparable in COPD-PH and COPD-nonPH patients. The individual values of peak VO_2 (% predicted), increase in oxygen uptake relative to workrate increase (Δ VO_2 / Δ Workload), Ve/VCO_2 nadir and maximum O_2 pulse are shown in Figure 7.1. COPD-nonPH patients showed more efficient ventilation during exercise, reflected by a significantly lower V_E/VCO_2 nadir ($p < 0.01$). Exercise capacity and VO2 and V_E at peak exercise were not different between both groups. O_2 pulse at maximal exercise was comparable in both groups.

Figure 7.2 shows that arterial oxygen saturation (SpO_2) was lower in COPD-PH both at rest (96 ± 3 (range: 88-99 %) versus 91 ± 4 (range: 85-99 %), $p < 0.01$) and at peak exercise (92 ± 5 (range: 84-99 %) versus 83 ± 7 (range: 72-98 %), $p < 0.01$). The extent of desaturation from rest to exercise was larger in COPD-PH patients (Table 7.2).

Pulmonary Artery Pressure and CPET characteristics

As shown in Figure 7.3, in the whole group resting mPpa was inversely related to resting PaO_2 and DLCO ($r = -0.70$, $p < 0.001$ and $r = - 0.58$, $p < 0.01$, respectively), as well as to SpO_2 at rest and during peak exercise ($r = -0.58$ and $r = -0.63$, $p < 0.01$, respectively). Furthermore, our data showed a correlation between mPpa and exercise ventilatory efficiency ($V_E/CO2$ nadir, $r = 0.43$, $p < 0.05$).

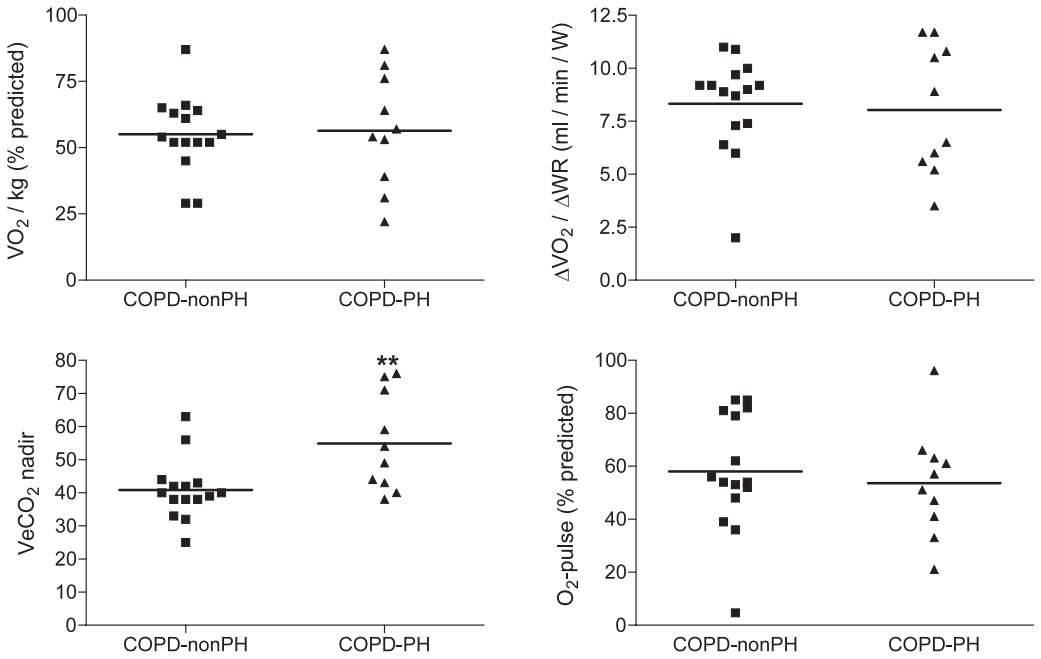

Figure 7.1 *Individual values of CPET characteristics in COPD-nonPH and COPD-PH. No significant difference in peak oxygen uptake per kilogram bodyweight (top left), increase in oxygen uptake relative to workrate increase (top right) and maximal oxygen pulse (bottom right) was observed between both groups. The ventilatory equivalent for CO_2 (V_E/VCO_2) at nadir was significantly higher in COPD-PH patients (bottom left). * $p < 0.05$, versus COPD-nonPH.*

DISCUSSION

The present study sought to investigate whether the presence of PH in COPD is reflected by specific exercise gas exchange characteristics. Characteristic findings in idiopathic PH are a reduced peak work capacity, reductions in VO_2 at the anaerobic threshold and at peak exercise, a diminished peak oxygen pulse (O_2-pulse), a diminished ventilatory efficiency and a gradual decrease in SpO_2 [13]. Here we show that the presence of PH in COPD only results in a few alterations in CPET patterns, with considerable overlap between PH and non-PH patients. PH was associated with a reduced SpO_2 at baseline, a further reduction in SpO_2 during exercise and an increased V_E/VCO_2 nadir (Table 7.2). These parameters were related to mPpa measured at rest. COPD-PH patients demonstrated a reduced PaO_2 and a trend to a lower diffusion capacity as compared to the COPD-nonPH patients. Furthermore, a weak inverse correlation of mPpa with PaO_2 at rest and diffusion capacity was shown. Most gas exchange variables showed a large overlap in both groups.

The patients we studied were part of a cohort of COPD patients screened for PH. In this cohort, right heart catheterization is performed in case of increasing symptoms on exertion

despite stable pulmonary function tests. We included all patients who had undergone both CPET and right heart catheterization. The separation of COPD with and without associated PH resulted in 2 groups with a reduced (not significant) airflow obstruction in COPD-nonPH patients, and on average a significantly lower PaO_2 in COPD-PH patients. 2 Out of 10 COPD-PH patients were normoxic (PaO_2 > 10 kPa) and 2 patients only showed mild hypoxemia, as shown by a PaO_2 > 9 kPa. Moreover, 7 out of 15 COPD-nonPH patients had a PaO2 < 10 kPa, suggesting that hypoxemia is not the only factor leading to PH in COPD. COPD-PH patients had moderate PH (mPpa = 33 ± 6 mmHg), although 2 patients had a mPpa of more than 40 mm Hg.. The latter may be explained by a selection bias in the present study; we specifically included patients with progressive exercise intolerance despite stable pulmonary function tests. The 40% prevalence of PH in our COPD patients is comparable to that in other studied COPD populations [4;5;9].

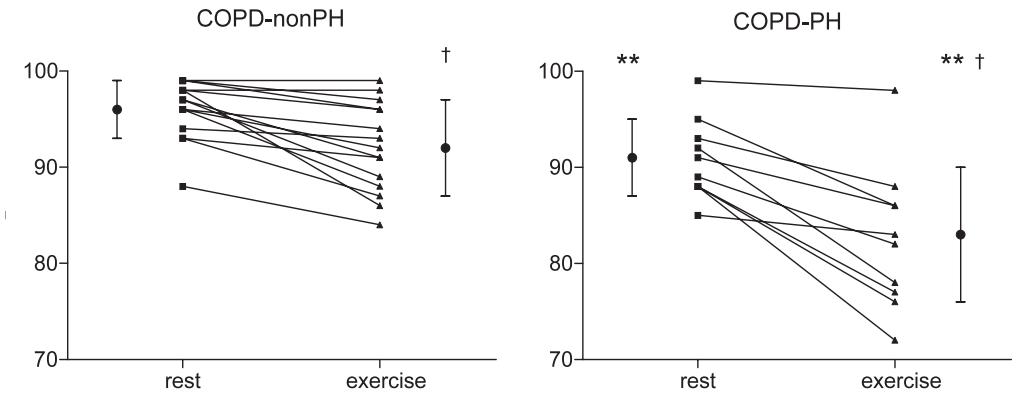

Figure 7.2 *Arterial oxygen saturation (SpO_2) at rest and during maximal exercise in COPD-nonPH (left) and COPD-PH patients (right). COPD-PH patients showed a significantly reduced SpO_2 at rest and during exercise. Note that during maximal exercise all but one COPD-PH patients showed a SpO_2 below 90%. * p < 0.01, versus COPD-nonPH, † p < 0.01, versus rest.*

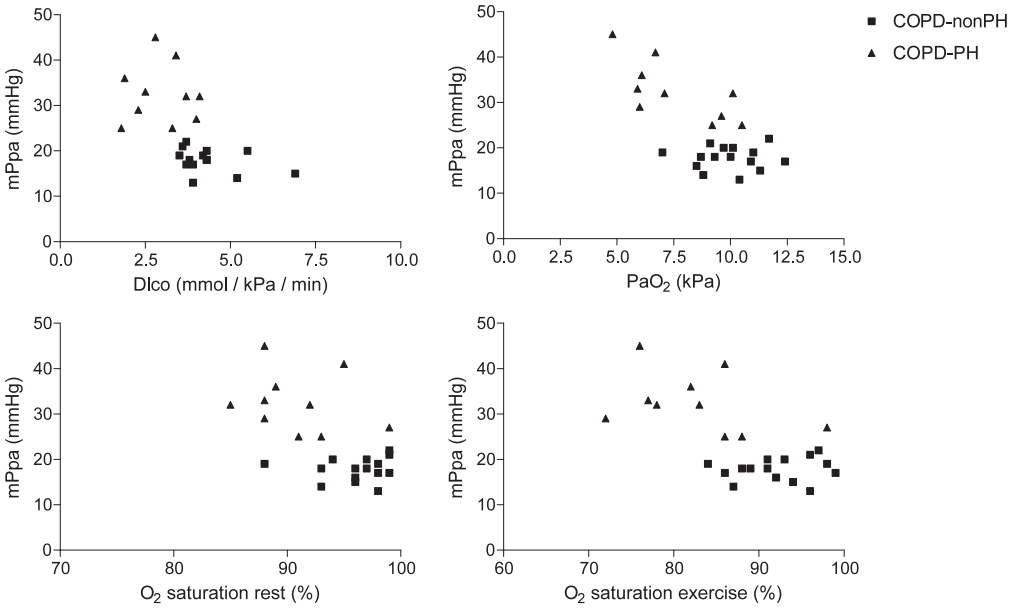

Figure 7.3 *In the whole group group resting mPpa was inversely related to diffusion capacity (DLCO, top left) and resting PaO_2 (r = - 0.58, p < 0.01 and r = -0.70, p < 0.001, top left and top right, respectively). Furhtermore, mPpa showed a correlation with arterial oxygen saturation (SpO_2) at rest and during peak exercise (r = -0.58 and r = -0.63, p < 0.01, bottom left and bottom right, respectively). * p < 0.01, versus COPD-nonPH.*

Gas exchange measurements

It is known that exercise often induces an abnormal rise in mPpa in COPD patients[10;20], which may in part explain that, in the present study, only V_E/VCO_2 nadir and slope were found to differ between COPD-nonPH and COPD-PH patients. An increased nadir of the V_E/VCO_2 curve has been proposed as an index of ventilatory inefficiency [21]. The index is known to be consistently elevated in idiopathic PH [13]. An important determinant of this nadir is the degree of dead space ventilation due to loss of pulmonary vascular bed. A decreased $PaCO_2$ set point (i.e., the ventilatory center regulates $PaCO_2$ at a lower value), such as in chronic psychogenic hyperventilation and metabolic acidosis, will also result in a higher V_ECO_2 nadir. The weak correlation between resting mPpa and V_E/VCO_2 nadir in this study suggests that loss of pulmonary vascular bed only partially explains the inefficient ventilation in these patients. The decreased ventilatory efficiency at peak exercise in COPD-PH patients might also explain their comparable aerobic capacity, despite lower peak workloads, through an increased work of breathing. Exercise characteristics at the anaerobic threshold (AT) were not provided since AT, assessed by the V-slope method [22], could not be determined, i.e. no VO_2 level at which VCO_2 began to increase with an inflection could be determined in almost half of the patients. Our findings confirm previous studies that showed that in patients with moderate to severe COPD the AT is not reached in approximately half of the patients [23;24].

Absence of the AT in COPD patients can indicate that exercise was terminated before significant metabolic acidosis occurred. In addition, in COPD gas exchange methods for determination of the anaerobic threshold do not agree very well with methods that are based on the assessment of anaerobic metabolism in venous blood [25].
It is well known that an increased right ventricular afterload leads to an impaired stroke volume response (SV) to exercise [26]. Accordingly, Sun and co-workers [13] found a reduced peak O_2-pulse in PH patients. The O_2-pulse can be used as an estimator of the SV response to exercise on the assumption that O_2 extraction is unaltered [18]. In COPD patients, peak O_2-pulse is usually reduced and related to exercise capacity [27;28]. Hypoxic pulmonary vasoconstriction [29] and a reduced pulmonary capillary reserve capacity result in increased right ventricular afterload during exercise, impairing SV. This increase in afterload is augmented by increased intrathoracic pressure swings, which have also been shown to be related to peak O_2 pulse[27;28]. Although functional residual capacity was not different at rest between both groups, we have no data on dynamic hyperinflation during exercise. Our study confirmed findings of a reduced O_2 pulse, but could not demonstrate that the presence of PH in COPD is associated with a further decrease in O_2-pulse at maximal exercise.

Pulse oximetry

SpO_2 at rest was reduced in COPD-PH patients, and showed a further significant decrease during exercise; SpO_2 dropped below 90% in all but one COPD-PH patient. Furthermore, in accordance with previous studies[1], SpO_2 and PH were closely associated. Although this study does not clarify whether hypoxemia is cause or consequence of PH, hypoxemia should be considered as the main cause for the progression and development of PH in COPD [2]. Ventilation/perfusion inequality results in a low SpO_2 during exercise in COPD [30], and these factors were likely to be more impaired in the COPD-PH group, given a lower DLCO and larger $D(A\text{-}a)O_2$ at rest in these patients.

Clinical implications

In general, the increase in Ppa in COPD patients tends to be modest (mPpa, 20-35 mmHg), and the progression of PH in COPD is slow [4]. The relevance of PH in COPD was emphasised by Kessler and coworkers [12] who showed that the presence of PH in COPD is associated with an increased risk of hospitalisation. It has furthermore been shown that in COPD, PH results in shorter survival [1;9]. Hence, simple noninvasive tools pointing to the presence of PH in COPD patients are warranted. Gas exchange measurements during exercise may be useful in the recognition of PH [11]. Surprisingly, COPD-PH patients in our study had a similar exercise capacity compared to COPD-nonPH, and showed only a reduced ventilatory efficiency during exercise. However, the individual values in both groups showed a large overlap. Therefore, gas exchange measurements during CPET do not seem to have an additive value over exercise pulse oximetry. The latter can e.g. easily be performed during a six minute walk test.

Study limitations

A larger number of patients may have resulted in significant differences between both groups. However, our results clearly show that there is a considerable overlap between the parameters in the nonPH and PH group, demonstrating the heterogeneity within COPD patients It is therefore unlikely that a larger sample size in our study would have shown more diagnostic relevancy of CPET in the diagnosis of COPD related PH. In our study, we compared patient groups with different degrees of airway obstruction, as COPD patients with associated PH showed less sever airway obstruction. Althoug one could argue that it is an unjustified comparison, it followed automatically from our stratification of COPD patients according to presence or absence of PH. A relatively preserved FEV_1 in COPD patients with PH has also been found by other investigators [31;32]. Thabut et al. (2005) identified a subgroup of COPD patients characterized by an elevated pulmonary artery pressure and a marked hypoxemia, contrasting with moderate bronchus obstruction [5]. The observation that patients with PH showed less severe bronchus obstruction, but comparable peak VO2, suggests that in these patients exercise is not impaired by airflow limitation only. If the patients in both groups would have shown similar degrees of airflow obstruction, differences in peak VO_2, VE/VCO_2 and SpO_2 would possibly have been larger between the two groups. It is very likely, however, that still there would have been a considerable overlap of these parameters in patients with and without PH.

CONCLUSION

During exercise in this cohort of COPD patients, the existence of PH was associated with hypoxemia and a reduced ventilatory efficiency. Gas exchange parameters measured during CPET showed a large overlap between COPD patients with and without PH. We therefore conclude that to detect PH in COPD, gas exchange measurements during CPET have no additive value to exercise pulse oximetry.

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Exercise pathophysiology in patients with chronic mountain sickness

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ABSTRACT

Background

Chronic mountain sickness is characterized by a combination of excessive erythrocytosis, severe hypoxemia and pulmonary hypertension, all of which affect exercise capacity.

Methods

Thirteen chronic mountain sickness patients and 15 healthy highlander and 15 newcomer lowlander controls were investigated at an altitude of 4350m (Cerro de Pasco). All of them underwent measurements of lung diffusing capacity for nitric oxide and carbon monoxide at rest, echocardiography for estimation of mean pulmonary arterial pressure and cardiac output at rest and at exercise, and an incremental cycle ergometer cardiopulmonary exercise test.

Results

The chronic mountain sickness patients, the healthy highlanders and the newcomer lowlanders reached a similar maximal oxygen uptake, at 32±1, 32±2 and 33±2 $ml.min^{-1}.kg^{-1}$ respectively, mean ± SE, p=0.8, with ventilatory equivalents for CO_2 versus end-tidal PCO_2, measured at the anaerobic threshold, of 0.9±0.1, 1.2±0.1 and 1.4±0.1 $mmHg^{-1}$, p<0.001, arterial O_2 content of 26±1, 21±2 and 16±1 $ml.dl^{-1}$, p<0.001, diffusing capacity for carbon monoxide corrected for alveolar volume of 155±4, 150±5 and 120±3% predicted, p<0.001, with diffusing capacity for nitric oxide and carbon monoxide ratios of 4.7±0.1 at sea-level decreased to 3.6±0.1, 3.7±0.1 and 3.9±0.1, p<0.05 and a maximal exercise mean pulmonary arterial pressure at 56±4, 42±3, and 31±2 mmHg, p<0.001.

Conclusions

The aerobic exercise capacity of chronic mountain sickness patients is preserved in spite of severe pulmonary hypertension and relative hypoventilation, probably by a combination of increased oxygen carrying capacity of the blood and lung diffusion, the latter being predominantly due to an increased capillary blood volume.

INTRODUCTION

Chronic mountain sickness (CMS) is a syndrome characterized by symptomatic excessive erythrocytosis, hypoxemia, pulmonary hypertension and eventual heart failure, which is observed in long-term residents above 2500 m (1). The condition has been initially described in high altitude dwellers on the South American altiplano, but has also been reported in Colorado and in the Himalayas (though not in Tibetan natives). The prevalence of CMS varies from 1 to 30% depending on altitude, ethnicity and overlap with chronic lung diseases, and is thus an important public health issue (1, 2). Patients with CMS present with pulmonary hypertension in proportion to decreased arterial oxygenation (2). The primary determinant of CMS is generally thought to be a failure of ventilatory adaptation to hypoxia (1-3).

Patients with CMS are intolerant to exercise (1, 2). However, little is known about exercise physiology in CMS, in relation with common belief among local health care providers that strenuous exercise is contra-indicated in these patients. Exercise capacity in CMS could be decreased because of pulmonary hypertension and hypoxemia, but maintained because of increased hemoglobin concentrations and lung diffusing capacity (4). High altitude dwellers have been shown to present markedly increased lung diffusing capacity, which allows for maintained gas exchange at lower levels of ventilation during exercise (5).

In the present study, we measured lung diffusing capacity, pulmonary hemodynamics at rest and exercise, and exercise capacity as determined by a cardiopulmonary exercise test (CPET) in patients with CMS as compared to healthy highlanders and newcomer lowlanders. We hypothesized that healthy highlanders would have a preserved exercise capacity as compared to lowlanders, with similar increase in pulmonary artery pressure, lower ventilatory responses and higher lung diffusing capacity, but that exercise capacity would be altered in CMS patients because of more severe pulmonary hypertension and hypoxemia.

MATERIALS AND METHODS

Subjects

Thirteen CMS patients, 15 highlanders and 15 lowlanders gave informed consent to the study which had been approved by the institutional review boards of Erasme University Hospital (Brussels, Belgium) and Universidad Cayetano Heredia (Lima, Peru). All the highlanders were born and living at 4350m (Cerro de Pasco). The lowlanders were born and living at sea-level. The lowlanders were selected such as to match as closely as possible the age and body dimensions of the highlanders. None of the participants were smokers.
The diagnosis of CMS was based on excessive erythrocytosis, defined by excessive hemoglobin concentration (> 19 g/dl in women, > 21 g/dl in men) with variable combinations of dyspnea, exercise limitation, palpitations, insomnia, headache, confusion, anorexia, altered concentration and memory impairment symptomatologies, and absence of cardiac or lung-diseases (1). The severity of CMS was evaluated by a specific scoring system, the "Qinghai score" (1).

Study design

All the subjects were investigated at the altitude of 4350m in Cerro de Pasco. The lowlanders were also investigated at sea-level a week before they travelled to Peru. The lowlander measurements at altitude were performed after four nights at altitude (two at 3000m followed by 4350m). The subjects underwent sequentially a clinical assessment, lung-function tests, exercise stress echocardiography and CPET.

Clinical assessment

Clinical assessment included a standard history and examination, including Hb (Hemocue 201+, AB, Angelhulm, Sweden) and a "Lake Louise" scoring of acute mountain sickness (6). Arterial oxygen content (CaO_2) was calculated as $Hb*SpO_2*1.34$, where SpO_2 is pulse oximetry oxygen saturation.

Pulmonary function measurements

Lung diffusing capacities for NO (DLNO) and CO (DLCO) were measured (Hyp'Air Compact, Medisoft, Dinant, Belgium) with corrections for Hb and inspired PO_2 as previously reported (7, 8) in keeping with American Thoracic Society (ATS) / European Respiratory Society (ERS) guidelines (9). Previously reported European reference equations were used to calculate predicted values (10).

Exercise Stress Echocardiography

Exercise stress echocardiography examination was performed with a Cx50 echocardiographic system (Philips Medical System, Andover, MA) on a semi-recumbent cycle ergometer (Ergoline, model 900 EL, Bilz, Germany), with the exercise table tilted laterally by 20 to 30 degrees as previously described (11). The workload was increased by 20 W every 2 min until the maximum tolerated because of dyspnea and/or leg pain. Measurements of pulmonary arterial pressure, cardiac output (CO), heart rate (HR) and left atrial pressure were taken

during the last minute of each workload. The mean pulmonary arterial pressure (Ppa_m)-indexed CO (CI) relationships were analyzed qualitatively and quantitatively before and after applying Poon's technique using pooled multiple subject data to estimate the underlying physiological relation ship in the absence of inter subject variability (12). A distensibility coefficient a of the pulmonary circulation was calculated using a distensibility model of the pulmonary circulation (11, 13).

Cardiopulmonary exercise testing

The CPET was performed on a cycle ergometer (Monark, Ergomedic 818E, Vansbro, Sweden) with measurements of ventilation (VE), O_2-uptake (VO_2), CO_2-output (VCO_2), HR and pulse-oximetry (SpO_2) using a metabolic system (Oxycon Mobile, Jaeger, Hochberg, Germany), as previously reported (14). The work-rate was increased by 15-30 W/min until exhaustion. The anaerobic threshold (AT) was estimated by the V-slope method (15). Blood pressure (sphygmomanometry) was recorded at baseline and during the last 15s of each workload. Before starting CPET, two maximum voluntary ventilation (MVV) measurements were performed according to ATS/ERS guidelines (16). Previously reported equations were used to calculate predicted values based on height, age and gender (17).

Characteristics	Patient with CMS	Highlanders	Lowlanders	
			Altitude	Sea level
N	13	15	15	
m/f, n	13/0$^{\$,*}$	9/6	9/6	
Age, yr	50 ± 3$^{\$,*}$	41 ± 2	35 ± 3	
Height, cm	166 ± 2$^{\$,*}$	159 ± 2*	176 ± 2	
Weight, kg	71 ± 2$^{\$}$	62 ± 2*	73 ± 4	
BMI, kg.m^{-2}	26 ± 1	24 ± 1	23 ± 1	
Hb, gr.dl^{-1}	24 ± 1$^{\$,*}$	18 ± 1*	15 ± 1$^{\#}$	14 ± 1
SpO_2,%	84 ± 2$^{\$,*}$	90 ± 1	89 ± 1$^{\#}$	99 ± 1
CaO_2, ml.dl^{-1}	27 ± 1$^{\$,*}$	21 ± 1*	18 ± 1	19 ± 1
SBP, mmHg	136 ± 9	118 ± 3	130 ± 4$^{\#}$	123 ± 3
DBP, mmHg	79 ± 4	75 ± 3	77 ± 3	73 ± 2

Table 8.1 *Demographic- and clinical values*
*Values expressed as mean ± SE or otherwise as stated. Definition of abbreviations: CMS: chronic mountain sickness patients, BMI: body mass index, Hb: hemoglobin, SpO_2: pulse oximetry oxygen saturation, CaO_2: arterial oxygen content, SBP: systolic blood pressure, DBP: diastolic blood pressure. $p < 0.05$: $^{\$}$ vs healthy highlanders, * vs lowlanders at altitude, $^{\#}$ vs lowlanders at sea level*

Statistics

Results are presented as mean ± SE. Differences between the three study groups at high altitude were analyzed by analysis of variance (SPSS-15). When the F ratio of the analysis of variance reached the level of significance ($p < 0.05$), modified t-test was applied as post hoc test. Paired t-tests were used to compare the results of the lowlanders at sea level and high altitude.

RESULTS

Subjects

Table 1 depicts the clinical characteristics of the three study groups. The CMS patients were older than the healthy highlander and lowlander groups, and in contrast were only male. There were differences in height and weight. However, body mass indexes were not different between the groups. The CMS patients had higher Hb and lower SpO_2, but higher CaO_2 compared to the two other groups. The healthy highlanders had similar SpO_2 but higher Hb and CaO_2 compared to lowlanders. Systemic blood pressures were not different between the groups.

Altitude exposure in the lowlanders increased Hb and decreased SpO_2, so that CaO_2 was unchanged. The Lake Louise score of the lowlanders at altitude was 2.6±0.6 indicating absence of acute mountain sickness. The CMS score in the CMS patients was 10.1±3.1 (mean±SD), corresponding to mild to moderate severity of the disease, but limited to 1.5±1.6 in the healthy highlanders and on average 0.5 in the newcomer lowlanders.

One CMS patient was not able to understand the instructions of the diffusing capacity measurements. The echocardiography measurements were of insufficient quality in five lowlanders.

	CMS	Highlanders	Lowlanders High altitude	Lowlanders Sea level
DLCO, $mL.min^{-1}$ $mmHg^{-1}$	**64 ± 3***	**55 ± 3**	**51 ± 2#**	**33 ± 6**
DLCO,% predicted	**198 ± 8***	**181 ± 8***	**143 ± 5#**	**93 ± 3**
DLCO/VA, % predicted	**155 ± 4***	**150 ± 4***	**120 ± 3#**	**103 ± 3**
DLNO, $mL.min^{-1}$. $mmHg^{-1}$	**229 ± 9**	**204 ± 11**	**198 ± 7#**	**155 ± 8**
DLNO, % predicted	**150 ± 7***	**143 ± 7***	**119 ± 3#**	**92 ± 3**
DLNO/DLCO	**3.6 ± 0.1***	**3.7 ± 0.1**	**3.9 ± 0.1#**	**4.7 ± 0.1**

Table 8.2 *Diffusion variables Values expressed as mean ± SE. Abbreviations: CMS: chronic mountain sickness patients, DLCO: diffusing capacity of the lung for carbon monoxide, VA: alveolar volume, DLNO: diffusion capacity of the lung for nitric oxide. $p < 0.05$: * vs lowlanders at high altitude, $p < 0.01$ # vs lowlanders at sea level*

Diffusion

As shown in Table 8.2, DLCO was markedly increased in the study groups, with CMS. and healthy highlanders achieving higher values than lowlanders. After corrections for alveolar volume, and expressed in % predicted, both CMS patients and healthy highlanders had a similar increase, while the increase was less in the lowlanders. The DLNO showed a proportional increase, but the DLNO/DLCO ratio decreased with altitude exposure in lowlanders, and was further decreased in highlanders, especially those with CMS.

	CMS		Highlanders		Lowlanders at high altitude		Lowlanders at sea level	
	Rest	Exercise	Rest	Exercise	Rest	Exercise	Rest	Exercise
Work, W		117 ± 4		102 ± 8		115 ± 12$^{\#}$		187 ± 13
HR, beat.min^{-1}	78 ± 2	127 ± 6*	75 ± 4	135 ± 6	75 ± 2$^{\#}$	145 ± 3$^{\#}$	68 ± 3	163 ± 4
CO, L.min^{-1}	5.3 ± 0.2	12.4 ± 0.5	4.7 ± 0.3*	11.5 ± 0.7	5.9 ± 0.4	13.7 ± 1.2$^{\#}$	5.8 ± 0.5	17.8 ± 1.3
CI, L.min^{-1} .m^{-2}	2.9 ± 0.1	6.9 ± 0.3	2.9 ± 0.2	6.9 ± 0.5	3.1 ± 0.1	7.2 ± 0.5$^{\#}$	3.0 ± 0.1	9.3 ± 0.5
Ppa_m, mmHg	26 ± 2*	56 ± 4$^{\$,*}$	23 ± 1*	42 ± 3*	20 ± 1$^{\#}$	31 ± 2	16 ± 1	32 ± 2

Table 8.3 *Echocardiographic measurements*
*Values expressed as mean ± SE or otherwise as stated. Definition of abbreviations: CMS: chronic mountain sickness patients, W: watt, HR: heart rate, CO: cardiac output, CI: cardiac index, Ppa_m: mean pulmonary arterial pressure. $p < 0.05$: $^{\$}$ vs HH, * vs LL HA, $^{\#}$ vs LL SL.*

Stress echocardiography

As shown in Table 8.3, altitude in the lowlanders increased resting Ppa_m, and decreased maximal workload, HR, and CI, while maximal Ppa_m was unchanged. In the study groups at altitude, resting CI and HR were the same, while resting Ppa_m of highlanders was higher. Exercise in highlanders was associated with similarly decreased maximal workload and CI as lowlanders at altitude. HR increased least in CMS patients, while Ppa_m increased in healthy highlanders and more so in CMS patients. Altitude exposure was associated with an increase in Ppa_m-CI relationships, with the largest in CMS, followed by healthy highlanders and lowlanders (Figure 1, Table 4). Individual Ppa_m-CI relationships with distensibility model fitting are shown in Figure 2. Altitude was associated with a decreased a, with lowest values at maximal exercise in CMS, followed by healthy highlanders and lowlanders (Table 8.4).

Cardiopulmonary exercise test

As shown in Table 5, altitude exposure in lowlanders was associated with decreased maximal workload, HR, respiratory exchange ratio (RER), O_2pulse and VO_2max, while resting and maximal VE, VE/VCO_2 slope or ventilatory equivalent for CO_2 ($VEVCO_2$) at the AT were

	CMS	Highlanders	Lowlanders at high altitude	Lowlanders at sea level
α	**0.003 ± 0.003**	**0.007 ± 0.003**	**0.013 ± 0.005**	**0.013 ± 0.007**
α_{rest}	**0.007 ± 0.003**	**0.009 ± 0.004**	**0.014 ± 0.006**	**0.017 ± 0.006**
α_{peak}	**0.002 ± 0.003**	**0.006 ± 0.003**	**0.012 ± 0.006**	**0.013 ± 0.006**
Slope of Ppa vs. CI	**7.68 ± 3.88**	**4.58 ± 1.33**	**2.88 ± 1.62**	**2.36 ± 0.66**
Poon adjusted slope	**7.32**	**4.66**	**2.83**	**2.41**

Table 8.4 *Alpha (a) calculated from whole pressure-flow curve and from just the point at rest (a_{rest}) and at peak exercise (a_{peak}). Including the average slopes of a linear fit to the Ppa vs CI as well as the Poon adjusted Ppam vs CI curves. CMS: chronic mountain sickness patients, Ppa: mean pulmonary artery pressure, CI: cardiac index.*

increased (Figure 3). Maximal workload was lower in CMS and in healthy highlanders compared to lowlanders, but maximal RER and VO_2max were the same. The CMS patients had a lower HR and increased O_2pulse at maximal exercise. Maximal SpO_2 and CaO_2 were lowest in lowlanders, maximal CaO_2 and SpO_2 were higher and lower respectively in CMS patients as compared to healthy highlanders. Systemic blood pressure increased similar in all the groups. At high altitude, the VE/VCO_2 slope was the highest in the lowlanders and lowest in the CMS patients. As illustrated in Figure 3 by plots of $VEVCO_2$ at AT as a function of end-tidal CO_2-pressure ($PetCO_2$), chemosensitivity increased markedly in lowlanders, was much lower in healthy highlanders, and lowest in the CMS patients at high altitude ($p<0.001$).

	CMS	Highlanders	Lowlanders at high altitude	Lowlanders at sea level
Power, W	137 ± 7*	119 ± 9*	183 ± 14#	255 ± 22
Power, % reference	81 ± 4	92 ± 8	90 ± 6#	123 ± 8
VO_2, % reference	102 ± 6	117 ± 13	87 ± 4#	107 ± 6
VO_2, $ml.min^{-1}.kg^{-1}$	32 ± 1	32 ± 2	33 ± 2#	41 ± 2
RER	1.13 ± 0.02	1.13 ± 0.01	1.14 ± 0.02#	1.28 ± 0.02
$VE/VCO_{2\ slope}$	32 ± 1$^{\$,*}$	36 ± 1*	40 ± 1#	26 ± 1
VE, $L.min^{-1}$	105 ± 8*	106 ± 8 *	145 ± 11#	130 ± 9
VE/MVV, %	70 ± 5	73 ± 6	77 ± 4	83 ± 5
HR, $beat.min^{-1}$	145 ± 5$^{\$,*}$	160 ± 4	165 ± 4#	173 ± 5
HR, % reference	87 ± 3	93 ± 2	93 ± 2#	98 ± 3
O_2pulse, $ml.beat^{-1}$	16 ± 1$^{\$}$	12 ± 1*	15 ± 1#	17± 1
O_2pulse, % reference	80 ± 3	77 ± 5	69 ± 3#	80 ± 3
SpO_2, %	82 ± 1$^{\$}$	88 ± 1*	78 ± 1#	98 ± 1
CaO_2, $ml.dl^{-1}$	26 ± 1$^{\$,*}$	21 ± 1*	16 ± 1#	19 ± 1
SBP, mmHg	153 ± 6	148 ± 4	164 ± 7	153 ± 5
DBP, mmHg	85 ± 3	83 ± 2	90 ± 5#	74 ± 2

Table 8.5 *Maximal cardiopulmonary exercise results. Values expressed as mean ± SE. Abbreviations: CMS: chronic mountain sickness patients, VO_2: oxygen uptake, RER: respiratory exchange ratio, VE: ventilation, MVV: maximal voluntary ventilation, VCO_2: CO_2 output, HR: heart rate, O_2pulse: VO_2/HR, SpO_2; pulse oximetry O_2 saturation, CaO_2: arterial oxygen content. SBP: systolic blood pressure, DBP: diastolic blood pressure. $p < 0.05$: $^{\$}$ vs highlanders, * vs vs lowlanders at altitude, : $p < 0.01$ $^{\#}$ vs lowlanders at sea level.*

DISCUSSION

The present results show that aerobic exercise capacity is similarly decreased at high altitude in patients with CMS, in healthy highlanders and in newcomer lowlanders. Exercise pathophysiology in CMS is characterized by a combination of severe pulmonary hypertension, relative hypoventilation, exaggerated hypoxemia, increased CaO_2 and improved lung diffusing capacity. These changes resemble those observed in healthy highlander controls, but are more marked, except for lung diffusing capacity, which appears already maximally increased.

Diffusion

Lung diffusing capacity was measured by the simultaneous transfers of CO and NO. Because the affinity of NO for hemoglobin is much higher than that of CO, DLNO predominantly reflects the membrane component (Dm) of the alveolo-capillary transfer of gases (18).

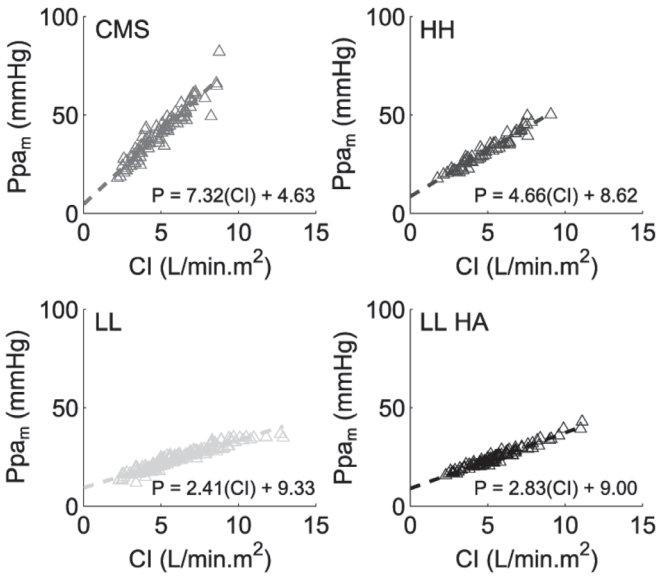

Figure 8.1 *Poon-adjusted mean pulmonary artery pressure (Ppa_m) as a function of cardiac index (CI) measurements at rest and at progressively increased workloads in chronic mountain sickness (CMS), healthy highlander (HH), recently acclimatized lowlander (LL HA) and sea level lowlander (SL) subjects.*

In the present study, lung diffusing capacity was increased in the patients with CMS, to the same extent as in healthy highlanders, with however a more pronounced decrease in the DLNO/DLCO, indicating a relatively more important contribution of capillary blood volume (Vc) with respect to Dm. These results are in keeping with a previous report, studied with the same methods in Oruro, on Bolivians, at the altitude of 4000m (7). In high altitude residents, Dm and Vc have both been reported to be increased, with either parallel changes (19, 20) or predominant increase of Dm (21) or Vc (7). Increased Vc has been speculated to be related to hypoxia-induced angiogenesis next to distension and recruitment of the pulmonary capillaries (7)

An unexpected finding was the marked increase in diffusing capacity in the high altitude newcomers, with, like in the high altitude inhabitants, a fall in the DLNO/DLCO ratio. Diffusing capacity in high altitude newcomers has been reported to be unchanged in the majority of studies (5, 8, 19, 21, 22) but some report a decrease (7, 20, 22) and others an increase (23, 24). An increase in diffusing capacity may be explained by capillary recruitment (4, 23, 24) and recovery from initial sub-clinical lung edema (23).

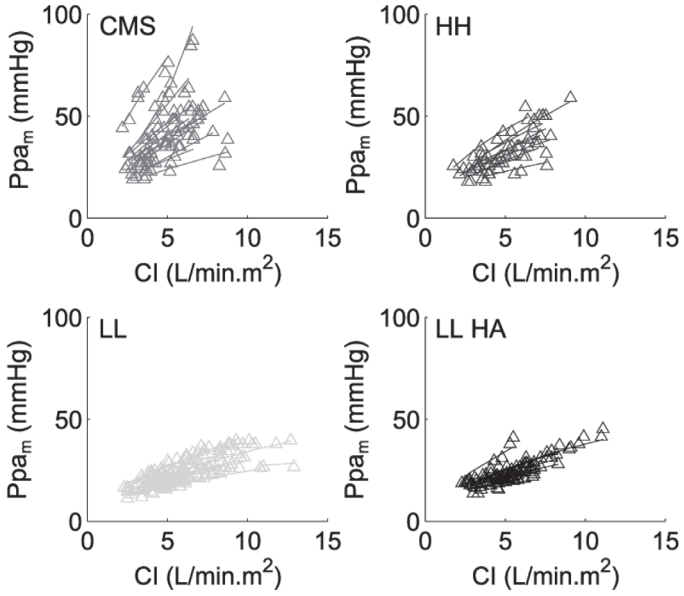

Figure 8.2 *Mean pulmonary artery pressure (Ppa$_m$) vs. cardiac index (CI) measurements at rest and at progressively increased workloads in CMS, HH, LL HA and LL SL subjects. By best fit to a simple model of pulmonary vascular distensibility, a slight curvilinearity with convexity to the pressure axis can be seen in Ppa$_m$ – CI relationships. Abbreviations see Fig 8.1.*

Pulmonary hemodynamics

Hypoxic pulmonary hypertension in humans shows variability, and is usually mild (25). Accordingly resting Ppa$_m$ was only slightly increased in both high altitude sojourners and residents. Some CMS patients had a more marked increase in resting Ppa$_m$, which is expected as these patients are chronically more hypoxemic (2). Exercise was associated with a steep increase Ppa$_m$, and this response was more marked in healthy highlanders compared to sojourners, but particularly prominent in CMS patients. A review of the literature of invasive and non invasive reports of Ppa$_m$ as a function of cardiac index (CI) at sea level and altitude, summarized in Fig 4, shows that high altitude residents have higher Ppa at any given level of cardiac output than sojourners, and that this tendency is more pronounced in CMS patients (11, 26-32). High altitude Tibetan residents are a remarkable exception, in relation with different ancestry and genetic predisposition (2, 28).

Pulmonary resistive vessels are distensible, so that inspection of multipoint Ppa$_m$-flow plots reveals a slight curvilinearity, allowing for the calculation of distensibility coefficient a (11, 13). In the present study, altitude exposure in lowlanders did not affect a, in keeping with

minimal increase in PVR (13, 33). However, a was decreased in healthy highlanders, and much more so in CMS patients, due probably to more extensive vascular remodelling (2). Hemoglobin was increased in healthy highlanders, and more so in CMS patients. Associated increase in hematocrit therefore contributed to increased PVR (34). However, in the absence of direct measurements, the respective contributions of hematocrit and vasculopathy to increased PVR is difficult to assess.

Cardiopulmonary exercise test

In the present study, aerobic exercise capacity at altitude was identical in newcomer lowlanders, healthy highlanders and CMS patients. This result contrasts with the widespread belief that high altitude natives have a higher aerobic exercise capacity than their acclimatized lowlander counterparts (2). Loss of aerobic exercise capacity at altitude depends on decreased ambient PO_2, but also on many other factors including age, gender, pre-existing fitness, body composition and acclimatization state. A recent review of the literature of aerobic exercise testing identified a slight but significant tendency to higher VO_2max in high altitude inhabitants, and underscored many uncertainties about adequate matching and possible selection biases (35). Some studies, like the present one, reported unremarkable aerobic exercise capacities in Andeans versus matched newcomer lowlander controls.

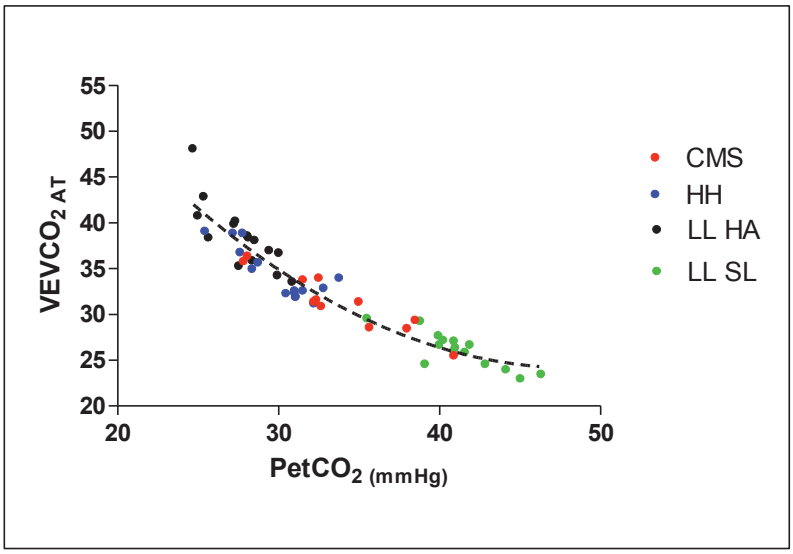

Figure 8.3 *The ventilatory response to exercise, expressed as ventilation per unit of carbon dioxide production ($VEVCO_{2AT}$) at anaerobic threshold as a function of respective end tidal carbon dioxide pressure ($PetCO_2$) in healthy highlanders, chronic mountain sickness patients, lowlanders at high altitude, and lowlanders seal level. Abbreviations see Fig 8.1.*

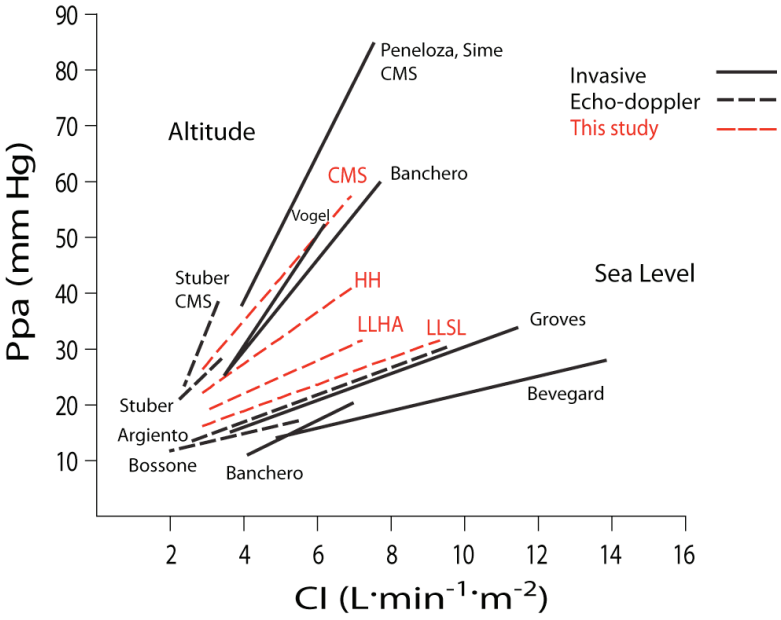

Figure 8.4 *Averaged mean pulmonary artery pressures (Ppa) versus indexed cardiac index (CI) plots measured invasively (cardiac catheterization, full lines) or non-invasively (echo-Doppler, stippled lines) in highlanders exercising at high altitude and in lowlanders exercising at sea level. From references (11, 26-32) with indications of names of first authors. With the exception of Tibetans in Lhasa, highlanders have higher resting Ppa and increased slopes of Ppa-CI. Slopes of Ppa-CI are particularly steep in patients with chronic mountain sickness (CMS), but there is overlap with those of healthy highlanders (HH). The present results are shown as red lines. Other abbreviations see Fig 8.1.*

An inverse correlation between Ppa_m and VO_2max has been reported in acutely hypoxic or recently acclimatized lowlanders (14, 36). No such correlation was found in healthy or CMS highlanders in the present study, suggesting that other adaptive mechanisms predominated to preserve exercise capacity.

The CPET profile of both healthy and CMS highlanders was characterized by a striking decrease in the ventilatory responses quantified as $VEVCO_2$ versus $P_{ET}CO_2$ relationships measured at the AT. These results are in keeping with the notion of a continuum of ventilatory responses from health to CMS during prolonged high altitude residence. The ventilatory response to hypoxia has been shown to be markedly increased in high altitude newcomers (4, 37) but blunted in CMS (3, 4, 38). The present results show decreased ventilatory responses that are more pronounced in CMS than in healthy highlander controls.
There has been some controversy as to whether decreased chemosensitivity explains all of the CMS symptomatology (1, 39, 40). Polycythemia could decrease ventilator responses through the Haldane effect, that is the increased CO_2 carrying capacity of high haemoglobin content blood, decreasing PCO_2 acting on chemoreceptors. This would partly explain the decreased ventilatory responses of the CMS patients in the present experiments. Another possibility would be a baroreflex-related decreased chemosensitivity caused by increased blood pressure, which may occur in CMS patients. In the present study, the CMS patients had normal blood pressures, excluding this mechanism.

CONCLUSION

High altitude residents demonstrate a unique CPET profile characterized by preserved aerobic exercise capacity in spite of pulmonary hypertension and relative hypoventilation, in relation to polycythemia and markedly increased lung diffusing capacity. These characteristics are exaggerated with the development of CMS, excepted for already maximally increased lung diffusing capacity.

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Summary, conclusions and future perspectives

SUMMARY AND CONCLUSIONS

The studies in this thesis aim to provide insight into the clinical relevance of different exercise profiles in pulmonary hypertension. Exercise testing in pulmonary arterial hypertension (PAH) is the main focus of this thesis. PAH is a disease state defined by an increased pulmonary arterial pressure and is characterized by a progressive increase in pulmonary vascular resistance leading to right ventricular failure, exercise intolerance and ultimately death (1). Even in advanced stages of the disease, PAH patients have no symptoms at rest. However, symptoms develop during exercise when pulmonary vascular resistance increases and cardiac output can not rise. As a consequence, gradually progressive exercise intolerance with symptoms of breathlessness and fatigue are common in PAH patients. The prognostic relevance of maximal functional capacity has been shown in several clinical trials in PAH patients (2, 7). The six minute walk test and /or maximal cardiopulmonary exercise test (CPET) are frequently chosen to estimate maximal functional capacity in PAH drug trials (3). The pathophysiological exercise profile of PAH patients measured by CPET is characterized by a low aerobic capacity and decreased ventilatory efficiency (5). In addition to PAH, my research has involved two other categories of PH with global clinical relevance: pulmonary hypertension associated with chronic obstructive pulmonary disease (COPD) and with chronic mountain sickness (CMS).

Chapter 1 starts with an overview of the exercise pathophysiology in PAH and CMS and continues by providing a rationale for exercise testing in the clinical setting. The chapter concludes with a brief introduction of the two most commonly used exercise tests in clinical trails in PAH: the six minute walk test and CPET.

Non-invasive determination of stroke volume during CPET can contribute to the detection of heart failure. However, none of the variables measured during CPET are direct measurements of cardiac output or stroke volume. In **chapter 2** we compared stroke volume measured by magnetic resonance imaging (MRI) to the data provided by an intra-breath technique measuring acetylene absorption, a technique which offers the possibility to determine the augmentation of pulmonary blood flow per heart beat. Both methods were compared at rest and during sub maximal exercise in healthy subjects (n=10) and PAH patients (n=10). The intra-breath technique showed that PAH patients have a smaller exercise induced change in stroke volume compared to healthy controls. These results were similar to the exercise induced changes of stroke volume measured by MRI. In conclusion, the intra-breath measurement of acetylene absorption can be of value to estimate the stroke volume response during exercise and can detect differences is stroke volume between PAH and healthy subjects.

A reduced exercise performance due to a reduced stroke volume response is also seen in chronic left heart failure (6). Dissimilar stroke volume and heart rate responses in PAH and left heart failure could have important therapeutic implications, for example in predicting the response of patients to beta-blocker therapy. In the study presented in **chapter 3**, exercise

oxygen pulse (which estimates stroke volume) and heart rate are compared between a group of PAH patients (n= 28) and left heart failure patients (n=18), matched for maximal aerobic capacity. I showed that patients with PAH demonstrate a smaller stroke volume response and a greater heart rate response during exercise compared to left heart failure patients.

Mortality in PAH patients is strongly associated with right ventricle dysfunction (4), but accurate determination of hemodynamic parameters requires an invasive right heart catheterisation. Exercise parameters could serve as good non-invasive alternatives. Although the correlations with hemodynamic parameters are weak, the 6MWD (2) and maximal oxygen uptake (VO_{2max}) measured during CPET (7) predict survival in PAH. CPET has the advantage over 6MWD to describe pathophysiological abnormalities characterizing the limitation of exercise (5). However, it remains unclear whether gas exchange parameters measured during CPET yield additional prognostic value after determination of the 6MWD, which is less technically demanding. This study question was answered in **chapter 4**. In this retrospective survival study we determined the additional prognostic value of different CPET parameters to 6MWD in a cohort of PAH patients (n=115). The conclusion of this study was that CPET parameters reflecting a low aerobic capacity or a decreased ventilatory efficiency predict survival in PAH. However, only the exercise induced change in oxygen pulse improved the univariate 6MWD prediction model significantly. As such, CPET variables predict survival in PAH, but add only marginally to the prognostic value of the 6MWD.

Although it is clear that several CPET variables have prognostic value when measured at baseline, it is unknown whether these variables have also prognostic value when measured as changes over time. The aim of the study described in **chapter 5** was to determine changes in CPET variables in PAH patients treated with specific therapy and to relate these changes to long term survival. Baseline CPET variables were available from 65 PAH patients. The same CPET variables were available one year later in a sub group of 39 patients. Survival analysis in this study showed that from all CPET variables studied at base line, only maximal heart rate and the slope relating ventilation to carbon dioxide production were significant predictors of survival. After follow–up, only the change in VO_{2max} and oxygen pulse predicted survival. It was concluded that CPET parameters with prognostic value at baseline are not necessarily predictive for survival when measured as changes over time.

Traditionally, exercise training was contraindicated in PAH due to the risk of sudden death. With the knowledge that an improved functional status improves prognosis, the role of exercise training in PAH is being reconsidered. In **chapter 6**, we assess the effects of 12 weeks of exercise training (3 times per week) in 19 stable PAH patients. Before and after the training program, measurements were made of exercise endurance, maximal exercise tolerance and quadriceps muscle strength and endurance. In a subset of 12 PAH patients we were able to obtain quadriceps muscle biopsies before and after training to analyse the effects of exercise training on skeletal muscle morphology. Our study revealed that exercise training improves exercise endurance and quadriceps muscle function in patients with

stable PAH. Enhanced quadriceps muscle function after exercise training was associated with improvements in oxygen handling of the quadriceps muscle fibres.

COPD patients may develop pulmonary hypertension during the progression of their disease. CPET could serve as an important tool in the early diagnosis of pulmonary hypertension in this patient group. The objective of the study described in **chapter 7** was to verify whether the existence of pulmonary hypertension in COPD was related to characteristic CPET findings. More specifically, we investigated the additional value of gas exchange parameters to pulse oximetry during exercise to recognize pulmonary hypertension in COPD patients. CPET data from 25 COPD patients were retrospectively analysed. Differences in gas exchange and pulse oximetry were assessed between COPD patients with associated pulmonary hypertension (n=10) and COPD patients without associated pulmonary hypertension (n=15). The patients with pulmonary hypertension showed a significantly lower maximal exercise tolerance accompanied by a lower ventilatory efficiency. Pulse oximetry was reduced at rest and during exercise in the COPD patients with associated pulmonary hypertension. Mean pulmonary arterial pressure at rest was inversely associated with oxygen saturation (at rest and during peak exercise) and exercise ventilatory efficiency. It can be concluded that COPD patients with associated pulmonary hypertension show a significantly decreased ventilatory efficiency. However, our results also show that a low saturation at rest and a further decrease in saturation during exercise suggest the existence of pulmonary hypertension in COPD patients. CPET gas exchange parameters showed a large overlap between COPD patient with and without associated pulmonary hypertension. We therefore conclude that to detect pulmonary hypertension in COPD, gas exchange measurements during CPET have no additive value over exercise pulse oximetry.

Like COPD patients, patients with chronic mountain sickness (CMS) may suffer from pulmonary hypertension. Pulmonary hypertension in CMS is caused by a reduced hypoxic ventilatory drive compensated by excessive erythrocytosis. The objective of the study presented in **chapter 8** was to improve the understanding of the exercise physiology of CMS patients. 13 CMS patients, 15 healthy highlanders and 15 newcomer lowlander controls were investigated at an altitude of 4350m in Peru. All included subjects performed single breath diffusion measurements corrected for haemoglobin concentration at rest. Echocardiography was used to estimate mean pulmonary arterial pressure and cardiac output values at rest and during exercise. CPET measurements were performed to measure gas exchange variables and arterial oxygen saturation. All three study groups reached a similar VO_{2max}. Both highlander groups showed an increased diffusion capacity. Due to the excessive erythrocytosis and despite a decreased arterial oxygen saturation, CMS patients had a significantly elevated arterial oxygen content (at rest and during exercise) compared to both other study groups. As hypothesized, the CMS patients showed the highest mean pulmonary arterial pressures at peak exercise and a reduced ventilatory drive, reflected by a decreased ventilatory equivalent for carbon dioxide at the anaerobic threshold. From these results we concluded that the aerobic capacity of CMS patients is preserved in spite of

severe pulmonary hypertension and relative hypoventilation, probably by a combination of an increased oxygen carrying capacity of the blood and an increased lung diffusion capacity.

FUTURE RESEARCH PERSPECTIVES

This thesis shows that patients with pulmonary hypertension have a decreased maximal exercise tolerance measured by 6MWD and CPET. The characteristic CPET profile of a decreased maximal aerobic capacity and reduced ventilatory efficiency has an important clinical value. Non-invasive estimates of cardiac output including the intra-breath acetylene absorption technique could have additional value, although the accuracy of these techniques remains questionable. As long as accurate non-invasive measurements of cardiac output and stroke volume during exercise remain unavailable, maximal aerobic capacity, oxygen pulse and estimates of ventilatory efficiency remain good alternatives. An important research question to answer in the near future is whether exercise stroke volume responses show additional clinical significance to the standard CPET measurement of oxygen pulse.

As was done in other papers, this thesis highlights a decreased ventilatory efficiency as an important clinical hallmark in PAH. Although this decreased ventilatory efficiency could be explained by increases in dead space ventilation and ventilatory drive, future research should be designed to address the question which mechanism is primarily responsible for an increased ventilatory drive. Moreover, it also remains to be determined whether the increased ventilatory requirement contributes to patients' symptoms and exercise intolerance.

In addition to estimations by 6MWD and CPET, functional status can be assessed by determining exercise endurance on a cycle ergometer. In the exercise training study, endurance had the highest sensitivity to detect changes in functional status over time. By using exercise endurance testing with additional gas exchange measurements, new surrogate end-points could be developed, to be used in clinical trials.

Our retrospective study showed that changes in functional capacity have additional prognostic value over baseline measurements. A prospective study in a bigger cohort would provide the necessary data to validate this concept.

Although exercise training improves functional status in PAH patients, it is currently unknown whether the improvements sustain after cessation of the rehabilitation program. Future research should address this question and should also be directed at further developing integrated training programs and optimizing schedules for training and maintaining physical fitness.

Although we showed that COPD patients with associated PH have a different ventilatory response than COPD patients without PH, the measurement of gas exchange did not provide additional diagnostic information to oxygen saturation measurements in the detection of PH. A simple six minute walk test combined with pulse oximetery measurements could be developed as a tool to detect PH in COPD patients. However, future studies also need to address the question whether COPD patients would benefit from PH detection, as treatment options for these patients are still very limited.

In the final study of this thesis we showed that CMS patients have a CPET profile characterized

by decreases in oxygen saturation and ventilatory drive. It is unknown whether CPET variables predict survival in CMS. An epidemiological study to determine the prognostic value of CPET in CMS could be of great importance. However, the prognostic value of a simple six minute walk test with additional measurements of HR and pulse oximetry could be of much greater relevance, because CPET is too demanding and expensive for most mountainous regions of the world.

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Samenvatting, conclusies en toekomst perspectief

SAMENVATTING EN CONCLUSIES

Dit proefschrift heeft als belangrijke doelstelling inzicht verkrijgen in de klinische relevantie van de verschillende inspanningstest parameters bij patiënten met een verhoogde bloeddruk in de longcirculatie (= pulmonale hypertensie). Pulmonale hypertensie kent meerdere oorzaken waaronder vernauwing van de longvaten, longziekten en linker hartfalen. De onderzoeken in dit proefschrift zijn voornamelijk uitgevoerd bij patiënten met pulmonale hypertensie als gevolg van een vernauwing van de longvaten, gedefinieerd als pulmonale arteriële hypertensie (PAH). Bij PAH gaat de pulmonale hypertensie gepaard met een progressief toenemende weerstand tegen de bloeddoorstroming door de longvaten resulterend in een verlaagde inspanningstolerantie en rechter hartfalen met als gevolg een vroegtijdig overlijden (1). In rust hebben PAH patiënten, tot in een vergevorderd stadium van de ziekte, weinig of geen klachten. Daarentegen tijdens inspanning, als gevolg van de toenemende vaatweerstand schiet de toename van het hart minuut volume tekort waardoor de klachten toenemen. Een geleidelijke afname van de inspanningstolerantie met klachten van kortademigheid en vermoeidheid is dan ook een veel voorkomend symptoom bij patiënten met PAH. De voorspellende waarde van de (maximale) inspanningscapaciteit voor overleving bij PAH patiënten is door middel van verschillende klinische onderzoeken herhaaldelijk aangetoond (2,7). De zes minuten looptest en de (meer uitgebreide) maximale fietstest met ventilatie metingen (CPET) zijn de meest gebruikte testen ter bepaling van het inspanningsvermogen in klinische studies bij PAH (3). Het patho-fysiologisch inspanningsprofiel van PAH patiënten zoals gemeten door middel van CPET wordt gekenmerkt door een afgenomen zuurstofopname capaciteit in combinatie met een verlaagde adem efficiëntie (5). Naast onderzoeksresultaten bij patiënten met PAH bevat dit proefschrift twee hoofdstukken met resultaten verkregen door middel van onderzoek bij andere wereldwijd relevante patiënten groepen waarbij mogelijk pulmonale hypertensie een belangrijke rol speelt: pulmonale hypertensie geassocieerd met chronisch obstructieve longziekte (COPD) en met chronisch hoogte ziekte (CMS).

Hoofdstuk 1 begint met een overzicht van de pathofysiologie tijdens inspanning in PAH en CMS waarna de beweegredenen worden gegeven voor het gebruik van inspanningsonderzoek in de klinische setting. Dit hoofdstuk sluit af met een korte introductie van de twee meest gebruikte inspanningstesten bij klinisch PAH onderzoek: De simpele zes minuten looptest en de veel uitgebreidere CPET.

Het non-invasief meten van het slagvolume van het hart tijdens CPET zou een belangrijke bijdrage kunnen leveren bij het vaststellen van hartfalen. Echter, geen van de gemeten CPET parameters is een directe meting van dit slagvolume. In **hoofdstuk 2** hebben we het slagvolume gemeten door middel van "magnetic resonance imaging" (MRI) in combinatie met slagvolume metingen door middel van een "intra-breath" ademtechniek gebruik makende van acetyleen opname. Deze "intra-breath" techniek maakt het mogelijk om de bloedstroom per hartslag door de longen te meten. Beide methoden zijn vergeleken in rust en tijdens een sub-maximale inspanning in een groep gezonde vrijwilligers (n=10) en bij PAH patiënten (n=10). De "intra-breath" techniek liet in de groep PAH patiënten een significant vermin-

derde toename van het slagvolume tijdens inspanning zien in vergelijking met de gezonde controle groep. Identiek aan de slagvolume veranderingen gemeten met de MRI. De conclusie van dit onderzoek was dan ook, dat de "intra-breath" techniek gebruik makende van acetyleen opname van waarde kan zijn voor het bepalen van de slagvolume response tijdens inspanning en bovendien is staat is het verschil in slagvolume response tijdens inspanning tussen PAH patiënten en gezonde proefpersonen vast te stellen.
Uit wetenschappelijk onderzoek blijkt dat patiënten lijdend aan chronisch linker hartfalen als gevolg van een verminderde slagvolume respons, net als PAH patiënten (= rechter hartfalen), ook een beperkte inspanningstolerantie hebben (6). Een verschil in slag volume - en hartfrequentie respons tijdens inspanning tussen linker hartfalen en PAH patiënten zou belangrijke therapeutische consequenties met zich mee kunnen brengen, zoals het voorspellen van de gevolgen van een bètablokker therapie. Het onderzoek beschreven in **hoofdstuk 3** vergelijkt de zuurstofopname per hartslag (= indirecte maat voor het slagvolume) en hartfrequentie tussen een groep linker hartfalen - (n=18) en PAH patiënten (n=28) voor een zelfde maximale zuurstof opname. In dit hoofdstuk toon ik aan dat de maximale slagvolume respons in de PAH groep kleiner is met als compensatie een toegenomen hartfrequentie respons in vergelijking tot de groep met linker hartfalen patiënten.

Mortaliteit bij PAH patiënten is sterk geassocieerd met een slecht functionerende rechter hartkamer (4), echter voor het nauwkeurig meten van de rechter kamer functie op basis van hemodynamische parameters is invasief onderzoek nodig, namelijk een rechterhartkatheterisatie. Parameters gemeten tijdens een inspanningstest zouden mogelijk als een goed niet invasief alternatief kunnen dienen. Ondanks dat de correlaties met de hemodynamische parameters zwak zijn, voorspellen zowel de zes minuten loopafstand (6MWD) als de tijdens CPET gemeten maximale zuurstofopname (VO_{2max}) overleving in PAH. Het voordeel van CPET boven de 6MWD is de mogelijkheid tot de beschrijving van de kenmerkende pathofysiologische afwijkingen van de inspanningslimitatie (5). Het is echter niet duidelijk of gaswisselingsparameters gemeten tijdens CPET van toegevoegde prognostische waarde zijn wanneer de, veel makkelijker te meten, 6MWD al bekend is. Deze onderzoeksvraag wordt in **hoofdstuk 4** beantwoord. In deze retrospectieve studie naar overleving werd de toegevoegde prognostische waarde van de verschillende CPET parameters op de 6MWD getoetst in een cohort van PAH patiënten (n=115). De conclusie van deze studie was dat CPET parameters gerelateerd aan een verlaagde zuurstofopname of verlaagde ademefficiëntie voorspellend zijn voor overleving in PAH. Echter, van al deze CPET parameters bleek alleen de inspanning geïnduceerde verandering in zuurstof opname per hartslag van toegevoegde waarde wanneer de 6MWD al bekend was.

Hoewel eerder onderzoek laat zien dat verschillende CPET parameters gemeten op baseline (= eerste test bij diagnose van de ziekte) voorspellend zijn, is niet duidelijk of een gelijke voorspellende waarde kan worden toegeschreven aan deze zelfde parameters wanneer gemeten als verandering in de tijd na een interventie en/of voortschrijding van het ziekteproces. Het doel van de studie zoals beschreven in **hoofdstuk 5** was het vaststellen van de

veranderingen van de verschillende CPET parameters in de tijd bij specifiek behandelde PAH patiënten om vervolgens deze veranderingen te relateren aan overleving. Baseline CPET parameters waren beschikbaar van 65 PAH patiënten. In 39 van deze patiënten was een jaar later een follow-up CPET gedaan. Overleving analyse in deze studie toonde aan dat van alle CPET parameters gemeten op baseline, alleen maximale hartfrequentie en de ventilatie in relatie tot de carbon dioxide productie significante voorspellers waren voor overleving. Na 1 jaar follow-up, bleken alleen de verandering VO_{2max} en de zuurstofopname per hartslag een voorspellende waarde te bezitten. De conclusie was dan ook dat CPET parameters met een voorspellende waarde voor overleving op baseline niet automatisch voorspellend zijn wanneer gemeten als verandering in de tijd.

Vanwege het risico van een plotselinge dood was PAH lange tijd een contra indicatie voor fysieke training. Echter met de wetenschap dat een verbeterde inspanningstolerantie de prognose van deze patiënten verbeterd werd fysieke training als therapie heroverwogen. In **hoofdstuk 6** worden de effecten van 12 weken intensieve training (3x/week) bij 19 stabiele PAH patiënten gepresenteerd. Voor en na het trainingsprogramma werd het algehele maximale uithoudings- en inspanningsvermogen en de lokale absolute spierkracht en uithoudingsvermogen van het bovenbeen gemeten. Om het effect van training op de morfologie van de skeletspier vast te stellen werd in een subgroep van 12 patiënten voor en na het trainingsprogramma een spierbiopt uit de 4 hoofdige bovenbeenspier afgenomen. Onze studie liet zien dat training een gunstig effect heeft op zowel het algehele uithoudingsvermogen als op de spierfunctie bij PAH patiënten. De verbeterde spierfunctie na training werd voornamelijk verklaard door een toegenomen zuurstof capaciteit van de spiervezels.

COPD patiënten kunnen tijdens hun ziekteprogressie ook pulmonale hypertensie ontwikkelen. Bij deze patiënten groep zou de uitgebreide CPET een belangrijke rol kunnen spelen in het vroegtijdig vaststellen van pulmonale hypertensie. Het doel van de studie zoals beschreven in **hoofdstuk 7** was vaststellen of de aanwezigheid van pulmonale hypertensie bij COPD patiënten een herkenbaar CPET profiel oplevert. Meer specifiek, we wilden onderzoeken wat de toegevoegde waarde was van de gaswisselingsparameters op de arteriële saturatie gemeten door middel van puls-oximetrie tijdens CPET voor het diagnosticeren van pulmonale hypertensie bij patiënten met COPD. Hiervoor hebben we retrospectief de waarden van 25 COPD patiënten geanalyseerd. In dit onderzoek ging het dus om de verschillen in gaswisseling- en puls-oximetrie parameters tussen COPD patiënten met (n=10) en zonder (n=15) pulmonale hypertensie. Bij de COPD patiënten met pulmonale hypertensie was het maximale inspanningsvermogen en de ademefficiëntie significant lager net als de arteriële saturatie zowel in rust als tijdens inspanning. De gemiddelde druk in de longslagader was omgekeerd evenredig gerelateerd met zowel de saturatie (rust en inspanning) als de ademefficiëntie. Op basis van deze resultaten kan geconcludeerd worden dat COPD patiënten met pulmonale hypertensie een lagere ademefficiëntie laten zien. Echter ook een verlaagde arteriële saturatie in rust, met een verdere afname tijdens inspanning, is een sterke aanwijzing voor het bestaan van pulmonale hypertensie bij patiënten met COPD. Daarentegen, er is veel overlap

tussen de gaswisselingsparameters gemeten tijdens CPET tussen COPD patiënten met - en zonder pulmonale hypertensie. Waardoor op basis van dit onderzoek geconcludeerd kon worden dat bij opsporing van pulmonale hypertensie bij COPD, CPET gaswisselingsparameters geen toegevoegde diagnostische waarde bezitten wanneer de arteriële saturatie al wordt gemeten.

Vergelijkbaar met COPD patiënten kunnen ook patiënten leidende aan chronisch hoogte ziekte (CMS) pulmonale hypertensie ontwikkelen. Pulmonale hypertensie bij CMS wordt veroorzaakt door een excessieve toename van de rode bloedcellen als gevolg van een verminderde ademrespons (normaal het belangrijkste adaptatie mechanisme) op de heersende lage zuurstofspanning op hoogte. Het voornaamste doel van het onderzoek beschreven in **hoofdstuk 8** was inzicht verkrijgen in de inspanningsfysiologie van CMS patiënten. Hiervoor werd onderzoek gedaan bij 13 CMS patiënten, 15 gezonde hooglanders en 15 (recent op hoogte gearriveerde) laaglanders op een hoogte van 4350 meter in Peru. Bij al deze deelnemers werd eerst in rust een diffusie capaciteit gemeten gecorrigeerd voor hemoglobine concentratie. De druk in de longslagader en het hartminuutvolume in rust en tijdens inspanning werden vervolgens gemeten door middel van echocardiografie. CPET werd gebruikt om gaswisselingsparameters en de arteriële saturatie te meten. Alle drie de studie groepen behaalden een vergelijkbare maximale zuurstofopname. De diffusie capaciteit van beide hooglander groepen was verhoogd. In vergelijking tot beide andere studie groepen, verklaard door de sterke toename in rode bloedlichaampjes, ondanks een afname in arteriële saturatie, hadden de CMS patiënten een significant hogere arteriële zuurstof opname capaciteit (zowel in rust als tijdens inspanning). Zoals vooraf verondersteld was de gemiddelde druk in de longslagader zowel in rust als tijdens maximale inspanning hoger en de “adem-drive” gemeten als ademequivalent voor carbon dioxide op de anaerobe drempel lager in de CMS studie groep. Op basis van deze resultaten kwamen we tot de conclusie dat CMS patiënten, ondanks de ernstige pulmonale hypertensie en relatieve hypoventilatie, een normale maximale zuurstof opname bezitten waarschijnlijk verklaard door de combinatie van een toegenomen diffusie – en zuurstof transport capaciteit van het bloed.

TOEKOMSTIG ONDERZOEK EN PERSPECTIEF

Dit proefschrift maakt duidelijk dat patiënten met pulmonale hypertensie zowel gemeten met 6MWD als CPET een verlaagde maximale inspanningstolerantie hebben. Het kenmerkende CPET profiel bestaande uit een afgenomen maximale zuurstofopname capaciteit en verlaagde ademefficiëntie is van significant klinische waarde. Niet invasieve hartminuutvolume metingen zoals de “intra-breath” acetyleen opname techniek zouden van een toegevoegde waarde kunnen zijn, de betrouwbaarheid van deze metingen blijft echter nu nog een probleem. Zolang het betrouwbaar niet invasief meten van zowel het hartminuutvolume - en dus ook het slagvolume tijdens inspanning niet mogelijk is, blijven de maximale zuurstofopname, zuurstofpuls en het meten van de ademefficiëntie een goed alternatief. Een belangrijke onderzoeksvraag om in de nabije toekomst te beantwoorden is dan ook of

er een significant toegevoegde klinische waarde is van een direct gemeten slagvolume respons op de zuurstofpuls respons zoals standaard gemeten met CPET tijdens inspanning. In overeenstemming met eerdere wetenschappelijke artikelen laat ook dit proefschrift zien dat een verlaagde ademefficiëntie een belangrijk klinisch kenmerk is van PAH. Al hoewel het voor de hand ligt om deze verlaagde ademefficiëntie te verklaren door een verhoogde dode ruimte ventilatie en/of ademdrive moet een toekomstig onderzoek worden bedacht waardoor antwoord kan worden gegeven welk mechanisme primair verantwoordelijk is voor de toegenomen ademdrive in PAH. Bovendien moet nog worden uitgezocht in welke mate deze verlaagde ademefficiëntie (=toegenomen ventilatie) bijdraagt aan de symptomen en inspanningsintolerantie van PAH patiënten. Net als met de 6MWD en CPET kan het inspanningsvermogen ook worden vastgesteld door middel van een uithoudingstest op een fiets ergometer. In de training studie, liet deze uithoudingstest de hoogste sensitiviteit voor het vaststellen van de veranderingen van het inspanningsvermogen in de tijd zien. Wanneer in de toekomst deze uithoudingstest in combinatie met gaswisselingsparameters gaat worden gebruikt dan zouden er nieuwe surrogaat eindpunten voor klinische studies ontwikkeld kunnen worden. Onze retrospectieve studie toonde aan dat veranderingen in maximaal inspanningsvermogen een toegevoegde waarde zouden kunnen hebben op de gemeten waarden op baseline. Een prospectieve studie gebruikmakende van een groter cohort zou de data kunnen leveren welke nodig is om dit concept te valideren. Alhoewel het inspanningsvermogen bij PAH patiënten kan worden verbeterd door middel van inspanningstraining is het onduidelijk of deze verbetering blijft bestaan na beëindiging van het revalidatie programma. Toekomstig onderzoek zou deze vraag moeten beantwoorden en zou bovendien gericht moeten zijn op het ontwikkelen van geïntrigeerde trainingsprogramma's en optimaliseren van de trainingsschema's voor het behoud van een optimale fysieke fitheid. Hoewel de ademrespons tijdens inspanning van COPD patiënten met pulmonale hypertensie afwijkt van de COPD patiënten zonder pulmonale hypertensie voegt het meten van de gaswisseling niets toe op het meten van de arteriële zuurstofspanning door middel van puls-oximetrie ter detectie van pulmonale hypertensie in COPD. Echter, onderzoek in de toekomst zou ook gericht moeten zijn op het beantwoorden van de vraag of COPD patiënten überhaupt voordeel hebben bij het vaststellen van pulmonale hypertensie, daar behandelopties gering zijn.

In de laatste studie van dit proefschrift lieten we zien dat CMS patiënten een CPET profiel gekenmerkt door een verlaagde zuurstof saturatie en ademdrive hebben. Het is echter onduidelijk of deze CPET parameters overleving voorspellen in CMS. Een epidemiologische studie naar de voorspellende waarde van deze parameters in CMS zou van grote waarde kunnen zijn. Echter, daar CPET een hoog opleidingsniveau vergt waardoor financieel moeilijk haalbaar in de hooggebergte regio's in de wereld zou onderzoek naar de voorspellende waarde van de makkelijker te meten 6MWD test met eventueel toevoeging van hartfrequentie en puls-oximetrie metingen wel eens van een veel grotere relevantie kunnen zijn.

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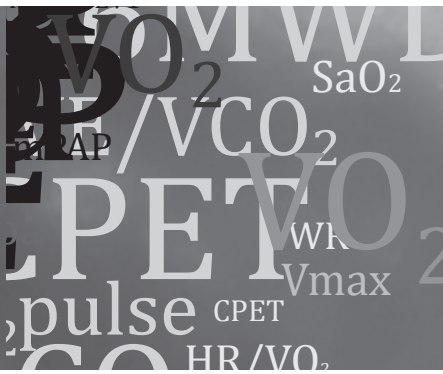
Dankwoord



Dankwoord

Het is begonnen met mijn aanstelling als hoofd van de functie afdeling longziekten in het VU medisch centrum (toentertijd nog gewoon “de VU”) . Na een jaar eerder nog te zijn afgewezen gunde prof. dr. Piet Postmus mij in 1993 gelukkig toch deze baan. Hoe belangrijk prof. Postmus uiteindelijk zou worden voor mijn wetenschappelijke “carrière” realiseerde ik mij toen nog niet. Sterker nog, juist werkzaam in de VU, begreep ik niets van al die promovendi die naast hun opleiding en drukke werkzaamheden nog de motivatie en tijd vonden om hun proefschrift af te ronden. Inmiddels weet ik beter.
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PUBLICATIONS

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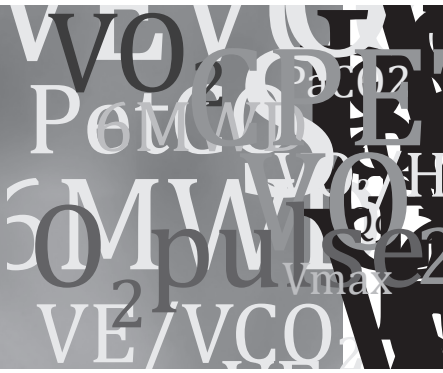
Abbreviations



ABBREVIATION

ANOVA	analysis of variance
AT	anaerobic threshold
ATP	adenosine triphosphate
AUC	area under the curve
BMI	body mass index
CaO_2	arterial oxygen content
CMS	chronic mountain sickness
CO	cardiac output
COPD	chronic pulmonary disease
CI (C^I; ch. 4)	cardiac index
CI	confidence interval (chapter 4.)
CPET	cardiopulmonary exercise testing
CSA	cross-sectional area
CTEPH	chronic thrombotic embolic pulmonary hypertension
$D(A\text{-}a)PO_2$	alveolar-arterial oxygen pressure difference
DBP	diastolic blood pressure
DLNO	lung diffusing capacity for nitric oxide
DLCO	lung diffusing capacity for carbon monoxide
Dm	membrane component of alveolar-capillary transfer of gases
ERS	European Respiratory Society
FEV1	forced expiratory volume in one second
HB	haemoglobin concentration
HC	healthy controls
HR	heart frequency
HRCT	high resolution computed tomography
iPAH	idiopathic pulmonary arterial hypertension
LHF	left heart failure
MPA	main pulmonary artery
mPpa	mean pulmonary artery pressure
MRI	magnetic resonance imaging
MVV	maximum voluntary ventilation
ns	not significant
NT-proBNP	N-terminal pro-B-type natriuretic peptide

NYHA	New York Heart Association
O_2pulse	oxygen pulse (= VO_2/HR)
PAH	pulmonary arterial hypertension
PCO_2	partial pressure of carbon dioxide
PE	peripheral extraction
$P_{ET}CO_2$	partial pressure of end-tidal carbon dioxide
PH	pulmonary hypertension
PaO_2	partial arterial oxygen pressure
PO_2	partial pressure of oxygen
Ppa_m	mean pulmonary arterial pressure
PVR	pulmonary vascular resistance
RAP	right arterial pressure
RC	linear regression coefficient
RER	respiratory exchange ratio
ROC	receiver operating characteristic
SaO_2	Oxygen saturation of arterial blood
SBP	systolic blood pressure
SD	standard deviation
SDH	succinate dehydrogenase
SpO_2	pulse oximetry oxygen saturation
SV	stroke volume
TLC	total lung capacity
TLCO	transfer factor for carbon monoxide (identical to DLCO)
TPVR	total pulmonary vascular resistance
VA	alveolar volume
Vc	capillary blood volume
VC	vital capacity
VCO_2	carbon dioxide output
VE	minute ventilation
$VEVCO_2$	ventilatory equivalent for carbon dioxide
VE/VCO_2slope	slope relating minute ventilation to carbon dioxide output
VO_2	oxygen uptake
Qc	pulmonary blood flow per heart beat
6MWD	six minute walk distance

About the author

ABOUT THE AUTHOR

Herman Groepenhoff (1963) is geboren te IJhorst in de provincie Overijssel. Tijdens zijn kleuterleeftijd verhuisde hij met zijn ouders, naar Badhoevedorp. Nog altijd woont Herman, nu samen met Carola Siebeling en hun twee kinderen: Floor (1994) en Kas (1997) in Badhoevedorp. Na het behalen van zijn HAVO diploma in 1980 ging hij Fysiotherapie studeren in Amsterdam. Omdat de arbeidsmarkt een vaste baan als fysiotherapeut in de weg stond besloot hij in 1986 de opleiding tot longfunctieassistent te gaan volgen in het Academisch Medisch Centrum (AMC) te Amsterdam. Na het behalen van dit diploma (1989) is hij als longfunctieassistent gaan werken in het Kennemer Gasthuis te Haarlem. In 1993 behaalde hij zijn management diploma voor ziekenhuizen en instellingen om vervolgens te solliciteren naar de vacature: hoofd van de functie afdeling longziekten in het VU medisch centrum (VUMC) in Amsterdam. Deze functie heeft hij gecombineerd met de studie bewegingswetenschappen (richting: systemen) aan de Vrije Universiteit (VU), afgerond in 2006. In 2008, werd vervolgens ook de Masteropleiding Epidemiologie aan het EMGO instituut van de VU met een diploma afgerond. Binnen de Nederlandse Vereniging voor Longfunctie Analisten (NVLA) is Herman Groepenhoff al jaren actief als lid van de commissie bijscholing.

Herman Groepenhoff (1963) was born in IJhorst in the province of Overijssel in the Netherlands. During his infancy he moved with his parents to Badhoevedorp. Herman still lives, now together with Carola Siebeling and their two children: Floor (1994) and Kas (1997) still in Badhoevedorp.
In 1980 after receiving his secondary school diploma (HAVO) he went to study Physiotherapy in Amsterdam. Due to poor labor for physiotherapists he decided in 1986 to start the training to become a pulmonary function technician at the Academic Medical Center (AMC) in Amsterdam. After earning this degree (1989) he started working as an technician in the Kennemer Gasthuis in Haarlem. In 1993 after he obtained his degree in management for hospitals and institutions Herman applied for the job: Head of the pulmonary function department at the VU University Medical Center (VUMC) in Amsterdam.
He combined this job with the study of kinesiology (direction: systems) at the VU university, which he completed in 2006. In 2008, he obtained a Master of Science degree in Epidemiology at the EMGO institute of the VUMC. For years now, Herman has been an active member of the committee training within the Dutch Association for Lung Function Analysts (NVLA).