Respiratory Physiology & Neurobiology 264 (2019) 33–39

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Exercise energy expenditure in patients with idiopathic pulmonary arterial hypertension: Impact on clinical severity and survival

Gabriele Valli[a,*], Paolo Palange[b], Roberto Badagliacca[c], Silvia Papa[c], Roberto Poscia[c], Carmine Dario Vizza[c]

[a] Emergency Department, San Giovanni Addolorata Hospital, Rome, Italy
[b] Department of Public Health and Infectious Diseases, Sapienza University of Rome, Italy
[c] Department of Cardiovascular and Respiratory Sciences, Sapienza University of Rome, Italy

ARTICLE INFO

Keywords:
Pulmonary hypertension
Survival
Exercise test
Ergospirometry

ABSTRACT

Patient with idiopathic pulmonary hypertension (IPAH) develop peripheral inefficiency which could lead to an increase total energy expenditure and that could have a significant prognostic impact.

To test the hypothesis, fifty-five consecutive stable IPAH patients (mean age 51 ± 17 years) and 24 matched controls underwent an incremental exercise test and followed for a 5 years' period. Total energy expenditure was assessed as the ratio between total V_{O_2} uptake (during both effort and recovery) and total external work ($V_{O_2\,TOT}/W_{TOT}$).

Patients with IPAH had a lower exercise capacity and a significantly higher $V_{O_2\,TOT}/W_{TOT}$ than controls ($V_{O_2\,TOT}/W_{TOT}$ 0.33 ± 0.09 ml/j vs 0.22 ± 0.04 ml/j, $p < 0.0001$). Among patients, $V_{O_2\,TOT}/W_{TOT}$ was higher in whom died during follow up compared to survivors (0.41 ± 0.11 ml/j vs 0.30 ± 0.06 ml/j, $p < 0.0001$).

In multivariate Cox regression analysis $V_{O_2\,TOT}/W_{TOT}$, gender, $P_{ET}CO_{2\ peak}$, $\dot{V}_{O_{2\,peak}}$ were independent risk factors for death.

Patients with IPAH have shown a less efficient muscular oxygen utilization than controls. Notably the high energy expenditure has a relevant independent prognostic impact.

1. Introduction

Pulmonary arterial hypertension is a clinical syndrome characterized by a progressive increase of pulmonary vascular resistance ultimately leading to right heart failure and death if left untreated (Vonk-Noordegraaf et al., 2013).

Exercise intolerance is the hallmark of patients affected by idiopathic pulmonary arterial hypertension (IPAH). In IPAH exercise is limited by the onset of breathless and early fatigue; maximal exercise capacity was typically reduced compared to normal subjects, and it is an independent prognostic factor. The mechanisms of the impaired exercise capacity include a reduced capacity to increase cardiac output during exercise (Riley et al., 2000a), a low ventilatory efficiency due to the markedly increased death space ventilation (Riley et al., 2000a; D'Alonzo et al., 1987; Valli et al., 2008; Weatherald et al., 2018) or even exertional dyspnoea-related ventilatory mechanics anomalies (Laveneziana et al., 2013, 2014; Laveneziana et al., 2015). Peripheral skeletal muscle could also have a role in the reduced exercise performance in these patients (Mainguy et al., 2010; Bauer et al., 2007).

Interestingly the cost of exercise, defined as the amount of oxygen consumed to sustain an exercise load and usually calculated as the changes in oxygen uptake ($\dot{V}_{O_2}$) over workload $\Delta\dot{V}_{O_2}/\Delta W$, has been reported to be low in severe heart failure and IPAH (Hansen, 1987; Riley et al., 2000a). This appears as an unexplained increased in exercise efficiency that contrast to the histologic report of structural muscular changes.

The flattening $\Delta\dot{V}_{O_2}/\Delta W$ is mainly due to two different phenomena, both related to impairment of peripheral muscle oxygen uptake: a slower oxygen kinetics (Barbosa et al., 2011) and, the increased oxygen deficit ($\dot{V}_{O_{2\,def}}$) developed during the active phase of the exercise (Levy et al., 2004).

In the present paper, we wanted to investigate the total exercise energy expenditure in IPAH using an innovative approach that takes in consideration the O_2 uptake during the active phase of effort and the recovery ($V_{O_2\,TOT}$) and dividing it for the total external work done ($V_{O_2\,TOT}/W_{TOT}$), and we studied its determinants and clinical impact.

We hypothesized that a high energy expenditure (high $V_{O_2\,TOT}/W_{TOT}$) could reflect a more advanced muscular impairment during exercise

* Corresponding author at: C/O UOC PS e Breve Osservazione, A.O. San Giovanni - Addolorata, V.le Amba Aradam, 9, 00184 Rome, Italy.
E-mail address: gvalli@hsangiovanni.roma.it (G. Valli).

https://doi.org/10.1016/j.resp.2019.04.003
Received 11 January 2019; Received in revised form 1 April 2019; Accepted 4 April 2019
Available online 06 April 2019

which could have a prognostic relevance.

2. Materials and methods

2.1. Population and study protocol

The study population included fifty-five consecutive stable IPAH patients who were followed in our center for five years and 24 age-matched healthy subjects.

The diagnosis of IPAH was defined as precapillary pulmonary hypertension (PAP > 25 mmHg, pulmonary wedge pressure, PWP < 15 mmHg) unexplained by any secondary cause, based and a step-by-step approach including lung function tests, a computed tomography scan of the chest, a ventilation/perfusion scan, echocardiography and laboratory tests to exclude left heart conditions with increased pulmonary venous pressure, lung diseases, thromboembolism, connective tissue disease and other associated PAH conditions, following recently updated guidelines (Galiè et al., 2015).

The evaluation included clinical history, physical examination, chest X-ray, and echocardiography. None of the patients had a history of systemic arterial hypertension, valvular or ischemic heart disease.

All patients were in stable clinical conditions under stable treatment for the last 6 months. Recent right heart catheterization (less than 6 months) performed as part of routine follow-up was used for the analysis.

The 24 age-matched healthy subjects were recruited among the personal who worked in our department.

The protocol was approved by the Institutional Review Board for human studies of the Policlinico Umberto I – Sapienza University of Rome (Protocol no. 42412), and all the participants gave informed consent.

Patients underwent a baseline evaluation including clinical evaluation, six-minute walking test (6 MWT), cardiopulmonary exercise test (CPET). Thereafter all patients were prospectively followed-up with phone calls (every month) and clinical examinations (every 1–3 months) by two physicians (R.P and B.P.) blinded to the CPET results. The occurrence of a death during the following three years was considered as the primary end-point.

2.2. Right heart catheterization

Hemodynamic evaluation was made with standard technique. Invasive hemodynamic measurements included mean right atrial pressure (RAP), PAP, PWP, cardiac output (CO) determined by the thermodilution technique. Cardiac index (CI) was calculated as cardiac output divided by body surface area. Pulmonary vascular resistance (PVR) was calculated as (PAP-PWP)/CO and was expressed as Wood units (WU).

2.3. Six-minute walking-test

Exercise capacity was measured by the non-encouraged 6-minute walking-test (6 MWT) performed in a 25 m (m) of length corridor in the same environmental conditions and at about the same time of the day (± 2 h) (ATS, 2002).

2.4. Exercise testing

Both IPAH patients and controls performed a maximal incremental exercise protocol on a electromagnetically braked cycle ergometer (Bosch, ERG 551, Germany) consisting of (a) 3 min at rest; (b) 1 min unloaded warm-up cycling; (c) individually-tailored incremental phase (5–10 Watt/min); (d) a recovery period as described below. To calculate in a uniform way all the phases of the effort and to be sure of the load administrated to patients during warm-up, we standardize warm-up for 1 min at the minimal workload guaranteed by the cyclergometer, which was 15 W.

Participants were asked to cycle at a pedaling rate of 60 rpm; the incremental phase of the test was conducted until exhaustion Oxygen uptake ($\dot{V}_{O_2}$, STPD), carbon dioxide output ($\dot{V}_{CO_2}$, STPD), minute ventilation ($\dot{V}_E$, BTPS), tidal volume (VT), respiratory rate (RR) and end-tidal carbon dioxide partial pressure ($P_{ET}CO_2$) were measured breath-by-breath (COSMED Quark b^2, Rome, Italy) and averaged every 5 s for subsequent analysis. The heart rate (HR) was monitored via 12 leads ECG. Arterial oxygen saturation (SpO_2) was monitored using a pulse oximeter. Peak work rate (W_{max}), peak $\dot{V}_{O_2}$ and peak $\dot{V}_E$ were defined, respectively, as the highest level of exercise and the highest $\dot{V}_{O_2}$ and $\dot{V}_E$ that could be sustained for at least 15 s during the last stage of incremental exercise. Test was considered as maximal if peak respiratory exchange ratio (RER, $\dot{V}_{CO_2}/\dot{V}_{O_2}$) was greater than 1. To estimate breathing reserve, $\dot{V}_E$ was expressed also as % of maximal ventilatory capacity (V_E/MVC) (that was estimated by multiplying the measured FEV_1 by 40) (Campbell, 1982). The anaerobic threshold (AT) was detected by the V-slope method (Beaver et al., 1986) and verified against other points, i.e., the $\dot{V}_{O_2}$ at which the ventilatory equivalent for oxygen ($\dot{V}_E/\dot{V}_{O_2}$) begins to increase systematically without an increase in the ventilator equivalent for carbon dioxide ($\dot{V}_E/\dot{V}_{CO_2}$), and where end-tidal oxygen partial pressure ($P_{ET}O_2$) begins to increase without a decrease in $P_{ET}CO_2$ (Wasserman et al., 2005). The slope of $\dot{V}_E$ over $\dot{V}_{CO_2}$ ($\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$) during incremental test was measured from unloaded pedalling to the ventilatory compensation point (VCP) and, for patients who did not reached the VCP (63% of cases) it was measured from unloaded pedalling to peak exercise. The dead space volume of the facemask was subtracted from the total $\dot{V}_E$ before calculating individual $\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$. Two experts independently read each test and the results were averaged.

Metabolic gas exchange measurements were made for 3 min at rest, the duration of exercise, and for at least 5 min following the cessation of exercise until oxygen uptake values had returned to pre-test levels and no further changes in ventilator or oxygen uptake had occurred at least 30 s, whichever was the longest.

2.5. Workload and oxygen cost calculation

The calculation of oxygen uptake is illustrated in Fig. 1.

Total oxygen uptake ($VO_{2\ TOT}$) was calculated as sum of the total oxygen uptake during the active face of the effort (Area A in the figure) and the recovery (Area B in the figure). Area A ($V_{O_2\ exe}$) was the results of $\sum_{FW}^{PEAK}(\dot{V}_{O_2} - \dot{V}_{O_2\,Rest})\cdot dt$, where FW is the beginning of free willing exercise and area B (V_{O_2} rec) was the results of $\sum_{PEAK}^{REST}(\dot{V}_{O_2} - \dot{V}_{O_2\,Rest})\cdot d$ where REC was the end of the recovery and it was settled at the first

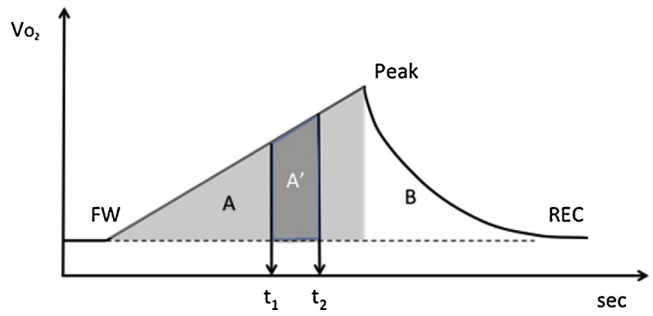

Fig. 1. Calculation of oxygen uptake: total oxygen uptake ($V_{O_2\,TOT}$) was calculates as sum of the oxygen uptake during the active face of the effort (Area A) and the recovery (Area B). In order to simplify the computing of the areas, values of effort and recovery V_{O_2} were approximated to the sum of the areas of the rectangular trapezes (A') with *d*t as base and ($V_{O_2\,t1}$ - $V_{O_2\,Rest}$) and ($V_{O_2\,t2}$ - $V_{O_2\,Rest}$) as heights, where t1 and t2 are the time when oxygen uptake was collected.
FW: beginning of free wiling exercise; REC: recovery.

value in which the difference ($\dot{V}_{O_2} - \dot{V}_{O_2\,Rest}$) was equal to 0(± 5% of $\dot{V}_{O_2\,Rest}$), after at least 5 min off recovery and followed by no changes for at least 30 s. In order to simplifying the computing of the areas described before, values of $V_{O_2\,exe}$ and $V_{O_2\,rec}$ were approximated to the sum of the areas of the rectangular trapezes (A') with *dt* as base and ($\dot{V}_{O_2\,t1} - \dot{V}_{O_2\,Rest}$) and ($\dot{V}_{O_2\,t2} - \dot{V}_{O_2\,rest}$) as heights, where t1 and t2 are the time when oxygen uptake were collected; as breath-by-breath values of the tests were mediated, dt was 5 s. Appling this approximation the formulas utilized were:

$$V_{O_2\,exe} = \sum_{FW}^{PEAK} \frac{(\dot{V}_{O_2\,t1} - \dot{V}_{O_2\,rest}) + (\dot{V}_{O_2\,t2} - \dot{V}_{O_2\,rest})}{2} \bullet dt,\ ml$$

$$V_{O_2\,rec} = \sum_{PEAK}^{REC} \frac{(\dot{V}_{O_2\,t1} - \dot{V}_{O_2\,rest}) + (\dot{V}_{O_2\,t2} - \dot{V}_{O_2\,rest})}{2} \bullet dt,\ ml$$

$$V_{O_2\,TOT} = V_{O_2\,exe} + V_{O_2\,rec}$$

Likewise, the values of total work load (W_{Tot}) was calculated as:

$$WTOT = \sum_{FW}^{PEAK} \dot{W} \bullet dt,\ J$$

The total exercise energy expenditure was defined as the oxygen cost of total work load:

$$V_{O_2\,TOT}\ T / WTOml \bullet J^{-1}$$

2.6. Statistical analysis

All statistical analysis was performed using SPSS software version 22.0.0 (SPSS Inc., Chicago, IL, USA); 55 PAH patients were included in the analysis and compared to 24 healthy age-matched patients. Time of origin was the date of exercise to date of death; the patient was censored if drop-out or at the end of the study (31.05.2015) if still alive.

Data are presented as mean ± standard deviation, and categorical data are expressed as counts and proportions. Two-group comparisons were done with unpaired or paired, two-tailed t tests for means if the data were normally distributed or with Wilcoxon's rank-sum tests if the data were not normally distributed. Chi square or Fisher's exact tests were used to analyse the categorical data.

Cox proportional hazards regression methods were used to determine predictors associated to survival. Hazard Ratio, 95% confidence intervals and p-values from the likelihood ratio test are given.

After all Cox univariate analyses were performed, a strict univariate p-value criterion (p 0.05) was used to select those variables that were initially entered in to the multivariate model. A p value of 0.10 was the criterion to remove covariates from the model until the most parsimonious model was found. Finally, two models were constructed: model-1 with baseline conventional clinical, hemodynamic and exercise parameters, and model-2 adding $V_{O_2\,TOT}$/ W_{TOT} to the model. The c statistic was calculated for each model and the comparison of the two values was tested by the method of DeLong et al (DeLong et al., 1988), to determine the incremental prognostic information of model-2. Multivariable Cox regression analysis with a backward selection procedure was used to determine independent predictors from the variable with $p < 0.10$ in model-2 univariate analysis.

For the variable that were predictive of survival, receiver operating characteristic (ROC) curves were plotted. The area under the curve (AUC) with 95% confidence interval and p-values was determined using non- parametric method. When the lowest 95% confidence interval was > 50% and p-values < 0.05, the optimal cut-off point for predicting survival was identified based on the highest sum of sensitivity and specify, and Kaplan-Meier graphs built for pattern above and below the threshold were used to describe survival rate. The log rank test was used to compare survival curves. The effect of the combination of the two main parameters predictive of survival ($\dot{V}_{O_2\,peak}$ and $V_{O_2\,TOT}$/ W_{TOT}) was estimated calculating odds of survival of the four groups obtained from cut-off values settled with ROC analysis. In all analyses, a p-values < 0.05 was considered significant

Table 1
Patients and controls characteristics and hemodynamics.

	IPAH	Controls	*P*
N.	55	24	
Age, yrs	51.2 ± 16.6	51.2 ± 16.0	*N.S.*
Height, cm	164 ± 9	174 ± 7	*< 0.0001*
Weight, Kg	67.8 ± 15.1	74.5 ± 10.3	*0.051*
BMI, Kg · m^{-2}	25.1 ± 4.4	24.6 ± 2.7	*N.S.*
NYHA	2.3 ± 0.5	–	–
6 MWT, m	314 ± 209	–	–
RAP, mmHg	5.1 ± 4.4		
PAP, mmHg	46.2 ± 17.6	–	–
PWP, mmHg	9 ± 3	–	–
CI, l · min^{-1} · m^{-2}	2.6 ± 0.9	–	–
PVR, wood units	11.7 ± 7.9	–	–
Follow-up, days	1982 ± 790	–	–

6 MWD: 6-min walkin test; BMI: body mass index; CI: Cardia Index; IPAH: idiopathic pulmonary arterial hypertension; N.S.: not significant; NYHA: New York Heart Association class; PAP: pulmonary arterial pressure; PVR: pulmonary vascular resistance; RAP: right atrial pressure; PWP: pulmonary capillary wedge pressure; WU: Wood Unit; yrs: years.

3. Results

3.1. Baseline

Table 1 shows the baseline demographic, clinical and hemodynamic characteristics of the study groups. Two patients were excluded because of poor signal to noise ratio and low RER values that suggests a submaximal effort.

There were no differences in age and gender distribution, controls showed a trend to have a weight and height higher than patients. Patients were in WHO class II (67%) or III (33%), had a shorter 6 MW distance and the hemodynamic evaluation showed a moderate-severe pulmonary hypertension with a preserved cardiac output and mild elevation of right atrial pressure.

Regarding the cardiopulmonary exercise test (Table 2) patients, compared to controls, had significantly lower exercise duration, peak oxygen uptake, maximal heart rate, HRR, $\dot{V}_{O_2\,AT}$, and peak oxygen pulse. No differences were found in peak RER suggesting that both groups performed an effort near their maximal capacity. Compared to control group peak ventilation and $\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$ were higher and $P_{ETCO_2\,peak}$ was significantly lower in patients as in condition of increased ventilatory request and hyperventilation.

Despite a significant flattering in $\Delta\dot{V}_{O_2}/\Delta W$ in IPAH patients, total exercise energy expenditure ($V_{O_2\,TOT}$/ W_{TOT}) was higher in patients compared to controls, this was mainly due to a significant increase in the proportion of oxygen uptake during recovery, as testify by the high $V_{O_2\,Rec}/V_{O_2\,Tot}$ compared to controls (Table 3).

3.2. Determinants of total energy expenditure

We did not find any significant correlation between VO_{2TOT}/ W_{TOT}, demographics, hemodynamics or ventilator parameters (nor if it was considered its absolute values, i.e. $\dot{V}_E$, neither if it was considered the values normalized to metabolic charge, i.e. $\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$). Only $\dot{V}_{O_2\,peak}$, and 6 MWD demonstrated a significant inverse correlation with $V_{O_2\,TOT}/\,WTOT$, but with a low R^2 (Fig. 2).

3.3. Prognostic impact of energy expenditure

During the five years of follow up 14 patients died (Fig. 3) and 11 patients were drop-out before the end of the follow-up (mean follow-up of the drop-out patients: 853 ± 187 days). In Table 4 are summarized

Table 2
Exercise test results.

	IPAH	Controls	*P*
W_{max}, W	67 ± 33	183 ± 62	*< 0.00001*
$\dot{V}_{O_2\,peak}$, ml · min^{-1} · kg^{-1}	16.7 ± 3.8	32.6 ± 12.2	*< 0.00001*
$\dot{V}_{CO_2\,peak}$, ml · min^{-1}	1175 ± 456	2708 ± 1037	*< 0.0001*
HR, beat · min^{-1}	129 ± 21	151 ± 37	*0.008*
$\dot{V}_{E\,peak}$, l · min^{-1}	56 ± 23	86 ± 33	*< 0.00001*
O_{2pulse}, ml · beat	8.9 ± 2.7	15.7 ± 3.4	*< 0.00001*
RER	1.02 ± 0.25	1.11 ± 0.09	*N.S.*
AT, ml · min^{-1}	759 ± 263	1106 ± 403	*0.004*
$\Delta\dot{V}_{O_2}/\Delta W$, ml · min^{-1} · W^{-1}	8.8 ± 1.3	10.7 ± 1.1	*< 0.001*
$\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$, l • $ml_{CO_2}^{-1}$	45 ± 12	28 ± 4	*< 0.00001*
$P_{ET\,CO_2\,peak}$, mmHg	24 ± 5	37 ± 5	*< 0.00001*
$\%\dot{V}_{O_2\,max}$, %	69 ± 26	99 ± 21	*0.001*
HRR, beat	40 ± 20	17 ± 29	*0.001*
$\dot{V}_{E\,peak}/MVV$, %	51 ± 18	60 ± 23	*N.S.*

HR: heart rate; HHR: heart rate reserve; IPAH: idiopathic pulmonary arterial hypertension; AT: anaerobic threshold; $\Delta\dot{V}_{O_2}/\Delta W$: oxygen uptake/watt slope; MVV: max voluntary ventilation; N.S.: not significant; $O_{2_{pulse}}$: oxygen pulse; $P_{ET\,CO_2\,peak}$: end tidal partial pressure of carbon dioxide at exercise peak; RER: respiratory exchange ratio; $\dot{V}_{CO_2\,peak}$: peak carbon dioxide production; $\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$: slope of $\dot{V}_E$ over $\dot{V}_{CO_2}$ during incremental exercise test; $\dot{V}_{E\,peak}$: ventilation at peak exercise; $\dot{V}_{O_2\,peak}$: peak oxygen uptake; W_{max}: maximum workload.

Table 3
Biomechanical efficiency evaluation.

	IPAH	Controls	*P*
Test duration, sec	712 ± 159	949 ± 215	*0.0001*
Effort duration, sec	419 ± 149	649 ± 161	*0.0001*
Recovery duration, sec	293 ± 42	300 ± 90	*N.S.*
$V_{O_2\,TOT}$, ml	5826 ± 3217	14569 ± 8061	*0.00001*
$V_{O_2\,REC}$, ml	1616 ± 837	2272 ± 1319	*0.03*
W_{TOT}, J	19193 ± 12793	63866 ± 28796	*0.0001*
$V_{O_2\,TOT}/W_{TOT}$, ml · J^{-1}	0.33 ± 0.09	0.22 ± 0.04	*0.00001*
$V_{O_2\,EXE}/W_{TOT}$, ml · J^{-1}	0.70 ± 0.11	0.84 ± 0.07	*0.00001*
$V_{O_2\,REC}/V_{O_2\,TOT}$	0.30 ± 0.10	0.16 ± 0.07	*0.02*

$V_{O_2\,TOT}$: total oxygen uptake; $V_{O_2\,EXE}$: oxygen uptake during exercise; $V_{O_2\,REC}$: oxygen uptake during recovery; W_{TOT}: total work.

the main characteristics and exercise variables of survivors compared to not survivors. Despite Not survivors had only a trend for a worst clinical status and hemodynamics, this group showed a significant impairment of effort capacity, ventilator efficiency and muscular efficiency compared to survivors.

At univariate Cox analysis (Table 5) gender, BMI, NYHA class, CI, $\dot{V}_{O_2\,Peak}$, $\dot{V}_{O_2\,AT}$, $\dot{V}_E/\dot{V}_{CO_2\,AT}$, $P_{ET}CO_{2\,peak}$, $\Delta\dot{V}_{O_2}/\Delta W$ were predictive of survival.

Cox regression model-1 for survival was constructed with those variables significantly resulting from univariate analysis excluding $V_{O_2\,TOT}/W_{TOT}$ (χ2 44.3, dF 4, $p < 0.0001$). Adding $V_{O_2\,TOT}/W_{Tot}$ model-2 was more predictive (χ2 41.6, dF 5, $p < 0.0001$). Using a backward multivariate Cox regression analysis, the final model included Gender, $\dot{V}_{O_2\,Peak}$, $P_{ET}CO_{2\,peak}$, $V_{O_2\,TOT}/W_{TOT}$ (χ2 49.5, dF 5, $p < 0.0001$).

Performing the receiver operating characteristics analysis, we found that the optimal cut-off values to predict survival for $\dot{V}_{O_2\,Peak}$ and $V_{O_2\,TOT}/W_{Tot}$ were respectively 15.0 ml· kg^{-1}· min^{-1} and 0.315 ml·J^{-1}.

In Table 6 and Fig. 3 are described the relative risk and survival curve according with these cut off values. Patients with $V_{O_2\,TOT}/W_{Tot}$ over 0.315 ml·J^{-1} have a risk of dead in the next 3 years 35 times higher than patients with lower values. $\dot{V}_{O_2\,Peak}$ higher than 15.0 ml· kg^{-1}· min^{-1} did not completely reduced the risk. Patients with

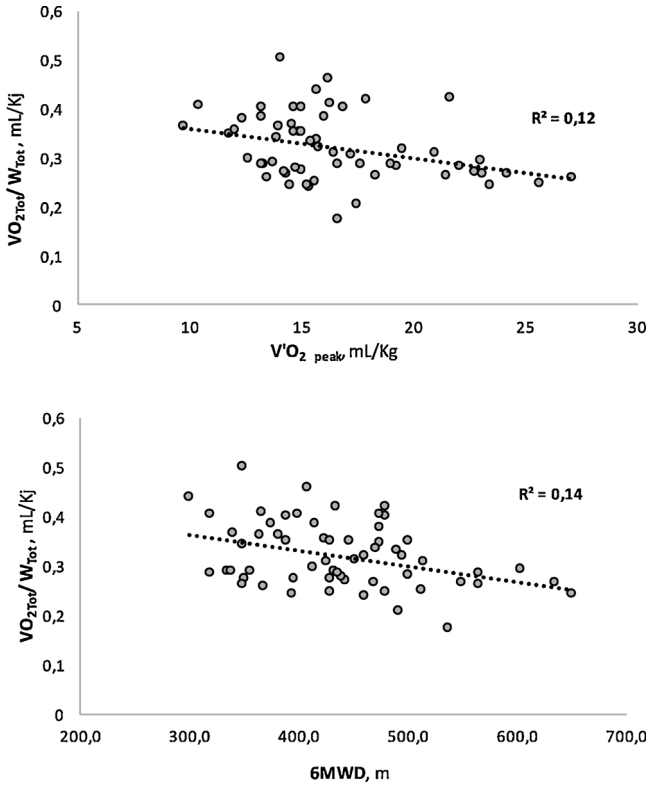

Fig. 2. Correlation between $\dot{V}_{O_2\,Peak}$, 6 MWD and $VO_{2\,TOT}/W_{TOT}$.

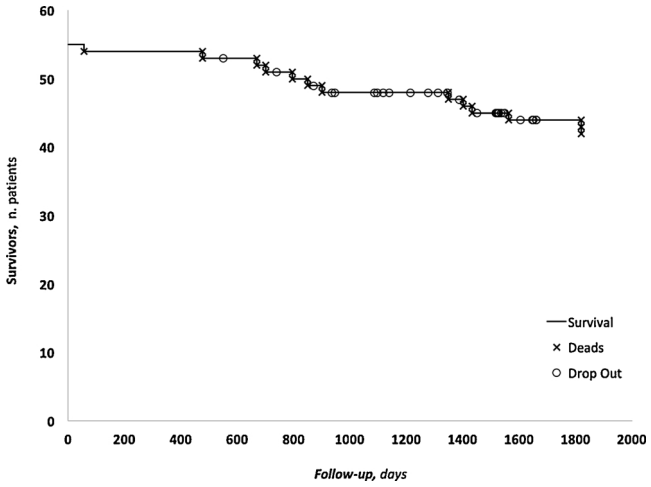

Fig. 3. Kaplan-Meier curve for cumulative event-free survival of study population.

$\dot{V}_{O_2\,Peak} > 15.0$ ml· kg^{-1}· min^{-1} and $V_{O_2\,TOT}/W_{Tot} > 0.315$ ml·J^{-1} still have a risk of death in the next three years 16.9 times higher than others (Fig. 4).

4. Discussion

Our data confirm that IPAH patients have a reduction in effort capacity and demonstrate that these patients have a markedly increased energy expenditure (higher oxygen requirement for a total given work) compared to age matched healthy subjects. The exercise energy expenditure is not related to the hemodynamic status measured at rest but has only a mild inverse relationship with effort capacity. Finally, we found its strong independent prognostic value.

Table 4
Characteristics of survivors.

	Survivors	Not Survivors	*P*
N.	43	13	
Follow-up, days	1574 ± 364	1084 ± 604	*0.0001*
Age, yrs	51 ± 17	52 ± 15	*N.S.*
Gender (F/M)	32/11	3/10	*0.01*
BMI, kg · m^{-2}	25 ± 5	24 ± 3	*N.S.*
NYHA	2.3 ± 0.5	2.5 ± 0.5	*N.S.*
6 MWT, m	444 ± 83	421 ± 58	*N.S.*
W_{max}, W	74 ± 34	47 ± 20	*0.008*
$\dot{V}_{O_2\,peak}$, ml · min^{-1} · kg^{-1}	17.6 ± 3.9	13.9 ± 1.9	*0.001*
$\%\dot{V}_{O_2\,max}$, %	74 ± 24	51 ± 14	*0.001*
$O_{2\,pulse}$, ml · beat	9.0 ± 2.8	8.2 ± 2.0	*N.S.*
$\Delta\dot{V}_{O_2}/\Delta W$, ml · min^{-1} · W^{-1}	8.8 ± 1.4	9.0 ± 1.1	*N.S.*
$\dot{V}_{E\,peak}/MVV$, %	54 ± 19	41 ± 11	*0.01*
$\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$, $l \bullet ml_{CO_2}^{-1}$	43 ± 11	50 ± 11	*0.05*
RAP, mmHg	6.4 ± 4.2	6.9 ± 3.0	*N.S.*
PAP, mmHg	43.8 ± 15.1	49.0 ± 22.0	*0.05*
PWP, mmHg	8.8 ± 2.8	9.6 ± 4.1	*N.S.*
PVR, Wood Unit	6.8 ± 1.0	7.8 ± 2.2	*N.S.*
CI, ml · min^{-1} · m^{-2}	2.7 ± 0.9	2.5 ± 0.7	*N.S.*
W_{TOT}, J	21707 ± 13335	11651 ± 70992	*0.001*
$V_{O_2\,TOT}/W_{TOT}$, ml · J^{-1}	0.30 ± 0.06	0.41 ± 0.11	*0.004*
$V_{O_2\,REC}$, ml	1551 ± 721	1808 ± 1128	*N.S*

Legend: see Table 1,2 and 3.

Table 5
Predictors of overall survival under univariate Cox proportional hazard regression.

	HR	95% CI	p-value
Subjects n	55		
Age	0.97	0.90-1.09	*0.9*
Sex Male	0.007	0.001-0.319	*0.003*
BMI	0.79	0.52-1.21	*0.3*
NYHA	30.2	1.8-574	*0.03*
6 MWD	1.038	1.002-1.075	*0.04*
CI	1.6	0.5-4.5	*0.4*
RAP	1.09	0.98-1.23	*0.1*
$\dot{V}_{O_2\,peak}$	0.45	0.26-0.78	*0.004*
$\dot{V}_{O_2\,AT}$	1.006	0.999-1.013	*0.05*
$\Delta\dot{V}_{O_2}/\Delta W$	1.88	0.48-7.32	*N.S.*
$\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$	0.83	0.64-1.08	*0.2*
$\dot{V}_E/\dot{V}_{CO_2\,AT}$	1.52	1.07-2.17	*0.02*
$P_{ET}CO_{2peak}$	1.93	1.08-3.45	*0.03*
$V_{O_2\,TOT}/W_{TOT}$	1.39	1.10-1.74	*0.005*

Legend: see Tables 1,2 and 3.

Table 6
Relative risk and survival curve according with cut off value of 15 ml·kg-1·min-1 for $\dot{V}_{O_2\,peak}$ and 0.315 ml•J-1 for $V_{O_2\,TOT}/W_{TOT}$.

	Odds Ratio	IC	*p*
$\dot{V}_{O_2\,peak} \leq 15$	6.6	18.2 – 24.2	*0.004*
$V_{O_2\,TOT}/W_{TOT} > 0.315$	41.3	5.0 – 341.8	*0.0006*
$V_{O_2\,TOT}/W_{TOT} > 0.315 \,\&\, \dot{V}_{O_2\,peak} > 15$	16.9	1.6 – 183.2	*0.002*
$V_{O_2\,TOT}/W_{TOT} > 0.315 \,\&\, \dot{V}_{O_2\,peak} \leq 15$	23.5	1.2 – 464.0	*0.04*

Legend: see Tables 1,2 and 3.

4.1. Total exercise energy expenditure and its determinants

This is the first study focused on total exercise cost in IPAH considering exercise recovery as informative as active exercise on total exercise capacity of the patient. We found that, taking in account all the exercise phases including recovery, patients have around 50% higher oxygen uptake for each joule worked than normal subjects. This denotes a great impairment of muscular energetics. The total increased oxygen cost for effort is manly driven by a slow recovery of oxygen uptake during recovery, as demonstrated by the higher $\frac{V_{O_2\,REC}}{V_{O_2\,TOT}}$ ratio.

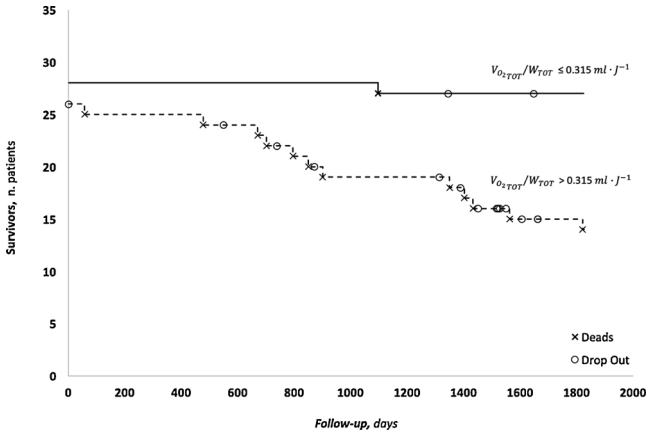

Fig. 4. Event-free survival curves according to cut off value fo $VO_{2\,TOT}/W_{TOT}$ (o.315 ml J^{-1}).
VO_{2TOT}/W_{TOT}: Total exercise energy expenditure.

In healthy subjects, after a symptom limited exercise test, recovery is fast and after few minutes $\dot{V}_{O_2}$ returns towards the resting value. In our IPAH population oxygen kinetics during the recovery is markedly slowed compared to control. This behaviour could be interpreted as an $O_{2\,deb}$ occurred during the exercise, a well-known phenomenon that justify the flat $\Delta\dot{V}_{O_2}/\Delta W$. during exercise (Whipp et al., 1970; Riley et al., 2000b; Mitchell et al., 2003), but our analysis allows us to calculate the actual O_2 uptake for each joule worked and makes evident how inefficient is the system.

This higher oxygen cost of the exercise could be accounted to several reasons: gas exchange inefficiency, reduced oxygen delivery and low peripheral muscle utilization with early onset of anaerobic metabolism. Which mechanism is the most important in IPAH is difficult to say. Our data are not conclusive but It is interesting to notice that we are not able to find any correlation between $VO_{2\,TOT}/W_{TOT}$, and basal hemodymanics, $\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$, $\dot{V}_E/\dot{V}_{CO_2\,AT}$ $P_{ET}CO_{2peak}$ suggesting that the low biomechanical efficiency is somewhat independent to the patient cardiovascular and lung performance. Some authors found that IPAH patients develop dyspnea-related ventilator mechanics anomalies during the exercise (Laveneziana et al., 2015) that could lead to an increase work of breathing. If the ventilator workload plays a role in the increased metabolic energy expenditure of these patients, this may be due to a reduced repiratory muscle perfusion it probably acts by subtracting a portion of cardiac output from peripheral muscles, leading to a less aerobic and more expensive muscular metabolism. Because we did not measure work of breathing and we are unable to evaluate the respiratory muscular contribution to total exercise energy expenditure this last consideration remains speculative.

On the other hand, it is possible that the reduced muscular aerobical capacity observed may be a consequence of changes in skeletal muscle ultrastructure. Skeletal muscle dysfunction in IPAH, with a decrease in oxidative type I fibers, abnormal intracellular calcium handling, slower restoration of muscle phosphocreatine stores after exercise has been documented in a well characterized population (Mainguy et al., 2010). More recently Batt et al. (2014) demonstrated that the loss of muscle in IPAH is accompanied by recruitment of cellular signaling networks that stimulate muscle proteolysis and down-regulate protein synthesis. Moreover, there is an alteration in a protein regulating sarcoplasmatic reticulum calcium sequestration, that could lead to impaired excitation-contraction coupling. (Batt J et al, 2014). Nerveless, a respiratory

muscles steals of cardiac output that lead to lower peripheral oxygen delivery cannot be completely excluded.

4.2. Prognostic impact of muscular efficiency

An important finding in our study is the clinical implication of an increase oxygen cost of exercise. In agreement with the literature we found in the univariate analysis a prognostic role of several meaningful clinical and hemodynamic variables as gender, functional class, effort capacity (Sitbon et al., 2006), ventilarory efficiency (Wensel et al., 2002; Ferrazza A et al, 2009; Deboeck et al., 2012). In the final model of the multivariate analysis Gender, $\dot{V}_{O_2\,Peak}$, $P_{ET}CO_{2peak}$, emerged as independent prognostic factors. Our study is not the only study in which $P_{ET}CO_{2rest}$ has demonstrated to have a predictive prognostic value (Wensel et al., 2002) while $P_{ET}CO_{2AT}$ has been utilized for IPAH severity stratification (Yasunobu et al., 2005) and, more recently $P_{ET}CO_{2PEAK}$ it has been found to correlate with PVR and CO (Yuan et al., 2017). In IPAH patients, which are presumed not to have any respiratory mechanical disturbance, $P_{ET}CO_2$ reflects the condition of hyperventilation almost invariably associated at the ventilator response to exercise.

The ROC analysis allows the identification of cut-off value for $\dot{V}_{O_2\,peak}$ and VO_{2Tot}/W_{Tot}. In this context VO_{2Tot}/W_{Tot} seems to provide additional prognostic information to the exercise tolerance, as shown by the increased risk of death in patients with a good exercise tolerance ($\dot{V}_{O_2\,Peak} > 15.0\ ml\cdot kg^{-1}\cdot min^{-1}$) but a decreased biomechanical efficiency ($VO_{2\,TOT}/W_{TOT} > 0.315\ ml\cdot J^{-1}$).

4.3. Study limitation

Some of the variables that traditionally were considered prognostic in IPAH (e.g., $\dot{V}_E/\dot{V}_{CO_2\,SLOPE}$), that significantly related with prognosis in the univariate analysis, then failed to be included in the multivariate analysis. It should be considered that the population is quite small with a wide range of disability; the follow-up period is relative short, not all the patients completed the five-years follow-up and the number of events is relative small. The Cox-stepwise analysis considers only the variables that improve the predictive ability of the equation, so it is possible that in a larger population with a longer period of follow-up, these variables could be included.

This precluded the inclusion of variables in the multivariate analysis, but did not weak the pathophysiological meaning of our study. Thus, the population seems adequate for this preliminary analysis.

5. Conclusion

Our data highlight the importance of the role of the peripheral muscular energetic in the clinical picture of IPAH as suggested by histological studies on muscular biopsies (Bauer et al., 2007; Mainguy et al., 2010) and the favourable effects of rehabilitation program in IPAH (Ehlken et al., 2016; Gerhardt et al., 2017).

In the light of the results of our study we can speculate that a significant increase of oxygen uptake during the daily activities combined to a reduction in nutrient absorption (liver and gut congestion) and reduction of nutritional intake (due to gastrointestinal side effect of IPAH drugs) could have impact in the development of cachexia in these patients. Further studies on the nutritional status and basal metabolism of these patients might clarify the role of nutritional strategies in the treatment of IPAH patients.

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