Mol Cell Biochem (2013) 373:161–170
DOI 10.1007/s11010-012-1485-6

Skeletal muscle mitochondrial dysfunction precedes right ventricular impairment in experimental pulmonary hypertension

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Received: 25 June 2012 / Accepted: 17 October 2012 / Published online: 26 October 2012

Abstract We assessed the time courses of mitochondrial biogenesis factors and respiration in the right ventricle (RV), gastrocnemius (GAS), and left ventricle (LV) in a model of pulmonary-hypertensive rats. Monocrotaline (MT) rats and controls were studied 2 and 4 weeks after injection. Compensated and decompensated heart failure stages were defined according to obvious congestion signs. mRNA expression and protein level of peroxisome proliferator activated receptor gamma co-activator 1α (PGC-1α), citrate synthase (CS) mRNA and activity, and mitochondrial respiration were investigated. In addition, mRNA expression of sirtuin1, nuclear respiratory factor 1, and mitochondrial transcription factor A were studied. As early as 2 weeks, the expression of the studied genes was decreased in the MT GAS. At 4 weeks, the MT GAS and MT RV showed decreased mRNA levels whatever the stage of disease, but PGC-1α protein and CS activity were significantly reduced only at the decompensated stage. The functional result was a significant fall in mitochondrial respiration at the decompensated stage in the RV and GAS. The mRNA expression and mitochondrial respiration were not significantly modified in the MT LV. MT rats demonstrated an early decrease in expression of genes involved in mitochondrial biogenesis in a skeletal muscle, whereas reduced protein expression, and the resulting mitochondrial respiratory dysfunction appeared only in rats with overt heart failure, in the GAS and RV. Dissociations between mRNA and protein levels at the compensated stage deserve to be further studied.

Keywords Pulmonary hypertension · Right ventricular failure · Mitochondria · Monocrotaline · PGC-1α

Abbreviations

CL	Control rats
CS	Citrate synthase
GAS	Gastrocnemius muscle
LV	Left ventricle
MT	Monocrotaline
MT2	Monocrotaline rats at 2 weeks after injection
MT4c	Monocrotaline rats at 4 weeks after injection, in compensated stage of heart failure
MT4d	Monocrotaline rats at 4 weeks after injection, in decompensated stage of heart failure
NRF1	Nuclear respiratory factor 1
PGC-1α	Peroxisome proliferators-activated receptor gamma co-activator 1α
PH	Pulmonary hypertension
RV	Right ventricle
SIRT1	Sirtuin 1
TFAm	Mitochondrial transcription factor A
TMPD	*N,N,N',N'*-Tetramethyl-*p*-phenylenediamine

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Introduction

Pulmonary hypertension (PH) is a condition characterized by vascular remodeling and vasoconstriction, resulting in increased pulmonary vascular resistance and subsequent right ventricular (RV) hypertrophy. This initial ventricular adaptive response is followed by progressive contractile dysfunction and RV failure, defined by the inability of the heart to supply sufficient blood flow to meet the needs of the body, and leading to fluid retention and organs congestion.

The patient's prognosis is mainly determined by the progression of RV dysfunction [1]. PH therapies target pulmonary vascular remodeling and vasoconstriction, but treatments aimed at maintaining RV contractility are lacking. Energy metabolism disorders, including reduced ATP production by the mitochondria, have emerged as important factors contributing to left cardiac failure development [2–4]. A strong relationship has been described between cardiac oxygen consumption and cardiac work [5, 6] and preserving cardiac oxidative phosphorylation is regarded as a potential therapeutic target. In keeping with this concept, restoring the transcriptional activity of the main genes involved in mitochondrial biogenesis, especially peroxisome proliferator activated receptor gamma co-activator 1α (PGC-1α) activity which is reduced in left heart failure, appears to be a promising strategy [7, 8]. Knowledge of the time course of these metabolic disorders is thus necessary, in order to determine the appropriate moment to intervene. In right-sided heart failure, which differs greatly from left-sided diseases by its pathophysiology and prognosis [9], little is known about the mitochondrial ATP-producing ability of the RV during the progression of right ventricular disease [10]. Very few experiments provided data from rats with compensated and rats with decompensated heart failure, data that may help understanding the transition mechanisms from one stage to another [11]. To our knowledge, mitochondrial biogenesis has not been investigated in PH. Eventually, nothing is known about mitochondrial respiration and biogenesis in the left ventricle (LV) of PH rats or human, which however demonstrates histological as well as contractile abnormalities [12–14].

Besides impairment of RV performance, the skeletal muscle dysfunction of PH patients contributes to the decreased exercise capacity [15–17] and worsens the patient's prognosis [18]. In experimental models and in patients [19], muscle atrophy, transition from slow oxidative to fast anaerobic fibers, reduced capillarity, and decreased muscle oxidative capacities have been described [20–22]. It has been shown in several chronic diseases, such as chronic obstructive pulmonary disease, that exercise training can improve exercise capacity and quality of life [3, 23–25]. Skeletal muscle alterations, including reduction of mitochondrial respiratory capacity are reversible by training [26]. Traditionally, in PH, it was recommended to limit physical activity which was thought to aggravate the clinical condition, particularly through worsening of right heart dysfunction [27]. Today, a few studies are in progress to determine safe exercise conditions for PH patients [28, 29]. However, exercise training will be efficient and safe if skeletal muscle dysfunction precedes the inability of the RV to respond to an increased demand in energy. Whether impairment of mitochondrial function in the skeletal muscle, which is submitted to PH-induced systemic disorders, precedes that developing in the RV, which in addition fights against pressure overload, deserves to be elucidated.

We designed this study to describe the time courses of mitochondrial biogenesis alterations and maximal muscle oxidative capacity in parallel in the RV, the LV, and the gastrocnemius (GAS) of PH rats. Measurements were performed in monocrotaline-treated (MT) rats at two weeks, early in the development of the disease, and at 4 weeks, in compensated and decompensated heart failure rats.

Methods

Animal model

Experiments were performed on adult male Wistar rats (Depré, Saint-Doulchard, France) weighing 250 ± 35 g. PH was induced by a single intra-peritoneal injection (60 mg/kg body weight) of monocrotaline (Sigma Chemicals, St Louis, MO, USA). Control rats were injected with the same volume of saline solution. Animals were housed in a neutral temperature environment (22 ± 2 °C) on a 12-h light–dark cycle, and received food and water ad libitum.

Experimental design

Animals were randomly assigned to one of the four studied groups: control and monocrotaline-injected at 2 weeks (CL2 and MT2, respectively) and at 4 weeks (CL4 and MT4) after injection. All animals were observed for general appearance including signs of respiratory distress and behavior. In the monocrotaline-injected rat model, the three consecutive stages of RV disease (RV hypertrophy, RV dilation, and RV failure) have been well characterized [30]. Disease progression can be properly assessed using anatomical data, right heart catheterization, echocardiography or magnetic resonance imaging parameters, which have been shown to correlate strongly with one another [31–34]. In our study, decompensated right heart failure was defined

as development of peritoneal and pleural effusions, and hemorrhage lungs at sacrifice and animals which showed these signs at 4 weeks after MT injection were included in the decompensated sub-group (MT4d). As expected [35], these animals presented with strong tachypnea, lack of spontaneous activity, piloerection and cold extremities before sacrifice. Rats which did not developed signs of decompensation 4 weeks after monocrotaline injection were included in the compensated group (MT4c).

At appropriate timings, the animals were anesthetized with isoflurane (3, 5 % in 1:1 O_2/air mix) and the superficial parts of glycolytic gastrocnemius muscles (GAS), the RV and the LV were isolated. RV and LV were weighed. The rats were euthanized by exsanguination (under isoflurane). The ratio of right ventricle (RV) weight on the LV plus septum weight (RV/LV + septum) was used as an index of RV hypertrophy and of PH severity. The RV and LV weight/body weight ratios were calculated. Body weight is a strong predictor of heart weight [36, 37], allowing the use of RV or LV weight/body weight ratio to compare PH rats to controls. However, because of PH-induced weight loss, this index may overestimate cardiac hypertrophy when comparing MT4 to MT2 rats. Left muscle GAS was immediately used for mitochondrial respiration (see below) and right muscle GAS was rapidly frozen at −80° for q-RT-PCR (see below).

Procedures were conducted in accordance with the US National Institutes of Health guidelines and approved by the Ethics Committee of the University of Strasbourg (Reference number: AL/02/13/07/09).

Quantitative real-time polymerase chain reaction (q-RT-PCR)

Quantification of gene expression of peroxisome PGC-1α, citrate synthase (CS), sirtuin1 (SIRT1), nuclear respiratory factor 1 (NRF1), and mitochondrial transcription factor A (TFAm) were performed. Total RNA was obtained from muscles using Trizol reagent™ (Invitrogen Life Technologies, Rockville, MD, USA) following manufacturer indications. RNA was stored at −70 °C until to the reverse transcription reaction being performed. cDNA was synthetized from total RNA (2 μg) with the SuperScript First-Strand Synthesis System (Invitrogen) and random hexamer primers. To perform the real-time PCR reaction, 2.5 μL of cDNA was used in a final volume of 8 μL, containing 10 μM of each primer (sense and antisense), and SYBR green (Invitrogen Life Technologies, Rockville, MD, USA) as fluorescent dye and H_2O. The real-time PCR measurement of individual cDNAs was performed in triplicate using SYBR green dye to measure duplex DNA formation with the LightCycler System (Roche Diagnostics, Meylan, France). The sequences of the primers were designed using information contained in the public database GeneBank of the National Center for Biotechnology Information (NCBI). The sequences of the primer sets used are listed on Table 1. Quantification of gene expression was carried out by the method described by [38] using glucocerebroside (GCB) gene as inner control, which is the most stable gene for real time RT-PCR measurements in muscles. Amplification efficiency of each sample was calculated as described by [39].

Relative mRNA levels were calculated using the $\Delta\Delta CT$ method between CL2 and MT2 groups and between CL4 and MT4 groups [40].

Table 1 Primer sequences used for quantitative real-time PCR amplification

Target gene	Forward primer 5′ → 3′ Reverse primer 5′ → 3′	Accession number
SIRT1	AGCATCACACGCAAGCTCTA GTGCCAATCATGAGGTGTTG	NM 001107627
PGC-1α	CACCAAACCCACAGAGAACAG GCAGTTCCAGAGAGTTCCACA	NM 031347
NRF1	GGCCCTTAACAGTGAAGCTG CATCTGGGCCATTAGCATCT	NM 001100708
TFAm	GAAAGCACAAATCAAGAGGAG CTGCTTTTCATCATGAGACAG	NM 031326
Citrate synthase	TATGGCATGACGGAGATGAA CATGGACTTGGGCCTTTCTA	DQ 403126
GCB	GCACAACTTCAGCCTCCCAGA CTTCCCATTCACCGCTCCATT	BC 166732

SIRT1 sirtuin 1, *PGC-1α* peroxisome proliferators-activated receptor gamma co-activator 1α, *NRF1* nuclear respiratory factor 1, *TFAm* mitochondrial transcription factor A, *GCB* glucocerebroside gene

PGC-1α immunoassay

The quantitative determination of rat PGC-1α concentration in muscles extracts was assayed by Enzyme-Linked Immunosorbent assay (ELISA, USCN Life Science Inc, China). Tissue assay procedure was carried out following the manufacturer's instructions. Briefly, the RV, LV and GAS muscles were pulverized using Sartorius (Mikrodismembor, Germany), homogenized and then centrifuged 5 min at 5,000×*g* in ice-cold PBS (0.02 M/pH ~ 7.0–7.2).The supernatant was removed and the protein content determined by BioRad protein assay using BSA as standard. The supernatants were then stored at −80 °C until used. The amount of protein in each well was 15 μg for PGC-1α. The concentration of PGC-1α (ng/mg of protein) detected in each sample was compared to their own standard curve.

CS activity measurement

Frozen tissue samples were weighed, homogenized into ice-cold buffer (30 g/mL) containing (in mM) 5 HEPES, 1 EGTA, 5MgCl2, 1DTT, and Triton X-100 (0.1 %), pH 8.7, and incubated for 60 min at 0 °C to ensure complete enzyme extraction. CS activity was assessed by standard spectrophotometric methods as previously described [3].

Muscle mitochondrial respiration

The relative contribution of the respiratory chain complexes I, II, and IV to the global mitochondrial respiratory rate of the saponin-skinned fibers was evaluated as previously described [4]. Measurements of oxygen consumption were performed at 22.1 °C using a Clark-type electrode (Strathkelvin Instruments, Glasgow, UK).

The substrates used in the respiration experiments were glutamate (5 mmol/L) and malate (2 mmol/L), which are substrates for the complex I. Maximal respiration (V_{max}) evaluated the functionality of complexes I, III, and IV. Complex I was blocked with amital (0.02 mmol/L) and complex II was then stimulated with succinate (25 mmol/L). The complex II, III and IV activities were measured in these conditions (V_{succ}). The functionality of complex IV ($V_{TMPD/asc}$) was determined by adding ascorbate (0.5 mmol/L) and *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD, 0.5 mmol/L) which are artificial electron donors to complex IV. Two measurements were made for each muscle.

After the experiments, fibers were harvested and dried for 15 min at 150 °C, and the respiration rates were expressed as μmol O_2/min/g dry weight.

Statistics

Data are expressed as mean ± standard error mean. Data were analyzed using two-way ANOVA followed by Neuman–Keuls post-hoc test. Values of $p < 0.05$ were considered as significant.

Results

Animal model characteristics

At 2 and 4 weeks, 15 and 12 MT rats were studied, respectively. At 4 weeks, 7 rats had compensated disease (MT4c sub-group) and 5 showed signs of decompensated heart failure (MT4d sub-group). 11 and 10 control rats were studied at 2 and 4 weeks, respectively.

The time course of changes in body, right and LV weights of the rats are shown in Table 2. The weight gain of the MT rats was lower than that of the CL at 2 and 4 weeks, and this, despite the presence of pleural effusion and ascites in MT4d. The RV weight, RV/body mass ratio, and RV/LV + septum ratio were higher in MT2 and MT4c compared to their respective controls, and more pronounced in MT4d.

Factors associated with mitochondrial biogenesis and function

At 2 weeks, lower mRNA expressions of SIRT1, PGC-1α, NRF1, TFAm (Fig. 1) and CS (Fig. 2) was observed only in the GAS. PGC-1α protein level as well as CS activity did not differ in the GAS, RV and LV between CL2 and MT2 (Figs. 3, 4).

At 4 weeks, compared to CL4, we found that mRNA expression of SIRT1, PGC-1α, and CS were decreased in the GAS and RV of MT4c rats, while TFAm was decreased only in the GAS. These changes in mRNA expression in MT4c were not associated with a significant change in PGC-1 protein level and CS activity in the GAS and the RV. In the LV, no difference between CL4 and MT4c mRNA and protein level or activity was observed. At 4 weeks, in MT4d rats, mRNA expression of SIRT1, PGC-1α, TFAm, and CS were decreased in both GAS and RV,

Table 2 Anatomic parameters of control and monocrotaline rats

n:	CL2 11	MT2 15	CL4 10	MT4c 7	MT4d 5
Weight gain, g	90.45 ± 5.27	60.0 ± 4.95***	132.20 ± 10.34	73.86 ± 18.65*	58.40 ± 21.76*
RV, g	0.16 ± 0.01	0.18 ± 0.01*	0.18 ± 0.01	0.24 ± 0.02**	0.39 ± 0.02***§
LV + S, g	0.61 ± 0.01	0.60 ± 0.03	0.68 ± 0.02	0.68 ± 0.04	0.60 ± 0.03
RV/LV + S, g/g	0.26 ± 0.01	0.31 ± 0.02*	0.26 ± 0.02	0.36 ± 0.04*	0.65 ± 0.06***§
RV/body mass, mg/g	0.51 ± 0.03	0.63 ± 0.04*	0.46 ± 0.02	0.71 ± 0.05**	1.22 ± 0.07***§
LV + S/body mass, mg/g	1.96 ± 0.03	2.03 ± 0.05	1.77 ± 0.03	1.99 ± 0.13	1.91 ± 0.12

n number of rats,*CL2* and *CL4* controls at 2 and 4 weeks, respectively,*MT2* monocrotaline rats at 2 weeks, *MT4c* and *MT4d* monocrotaline rats with compensated and decompensated disease, respectively, *RV* right ventricle, *LV* left ventricle, *S* septum. Results were expressed as mean ± SEM

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus CL; $^{\S}p < 0.001$ versus MT4c

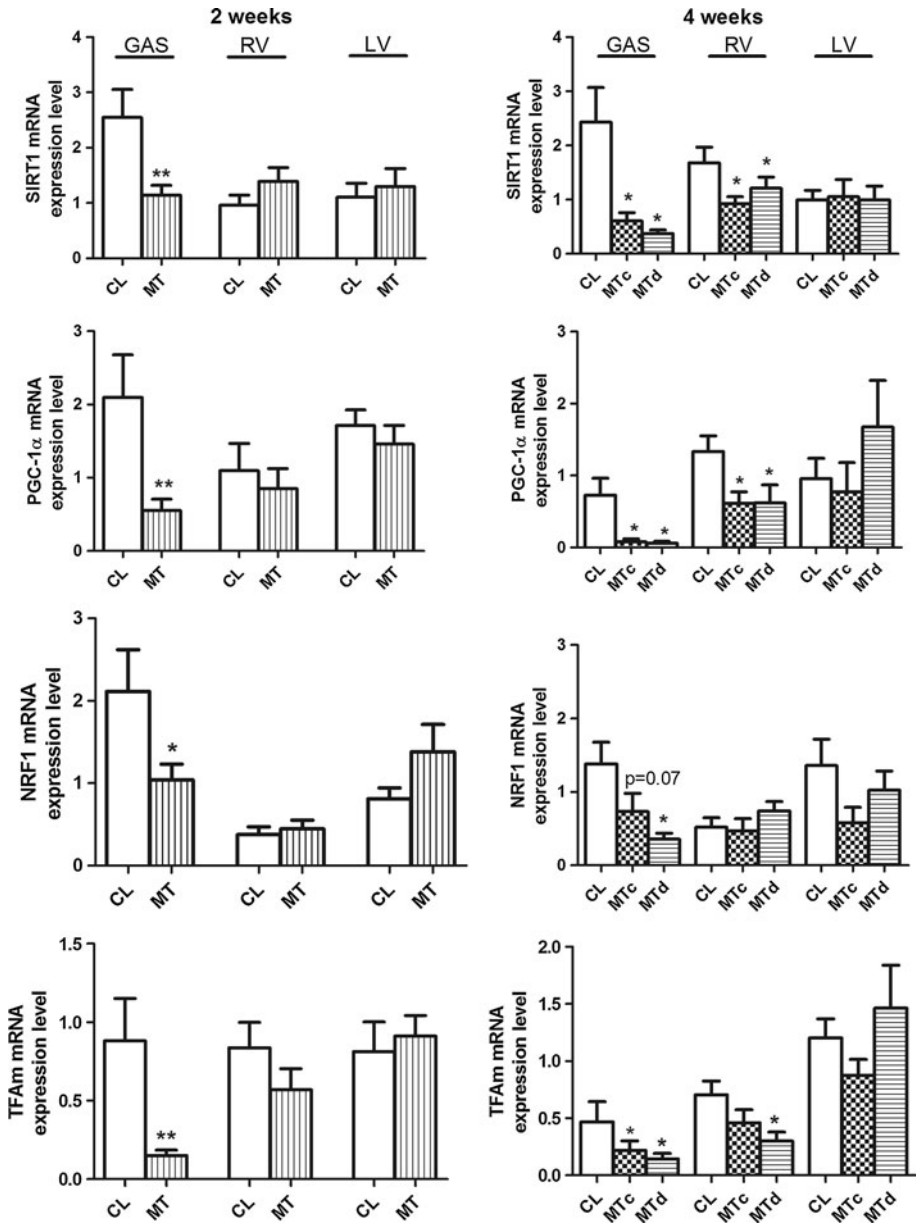

Fig. 1 Relative mRNA expression level of sirtuin1 (SIRT1), peroxisome proliferators-activated receptor gamma co-activator 1α (PGC-1α), nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAm) in the gastrocnemius (GAS), right ventricle (RV), and left ventricle (LV). *CL* control rats, *MT* monocrotaline rats, *MTc and MT4d* monocrotaline rats with compensated and decompensated disease, respectively. Results were expressed as mean ± SEM. $*P < 0.05$, $**P < 0.01$, MT versus CL

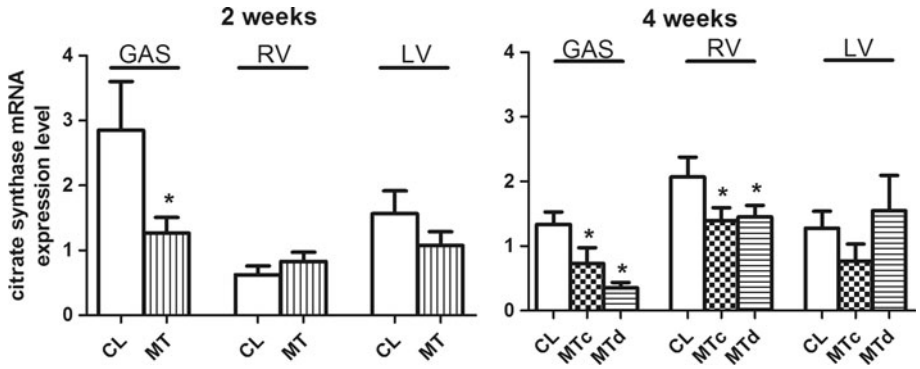

Fig. 2 Relative mRNA expression level of citrate synthase in the gastrocnemius (GAS), right ventricle (RV), and left ventricle (LV). *CL* control rats, *MT* monocrotaline rats, *MTc and MT4d* monocrotaline rats with compensated and decompensated disease, respectively. Results were expressed as mean ± SEM. $*P < 0.05$, MT versus CL

while NRF1 fell only in the GAS. In the LV, no difference between CL4, MT4c, and MT4d mRNA expression was observed. PGC-1α protein level was lower in MT4d compared to MT4c in all three muscle types. CS activity was lower in MT4d compared to CL4 in RV, GAS, and LV.

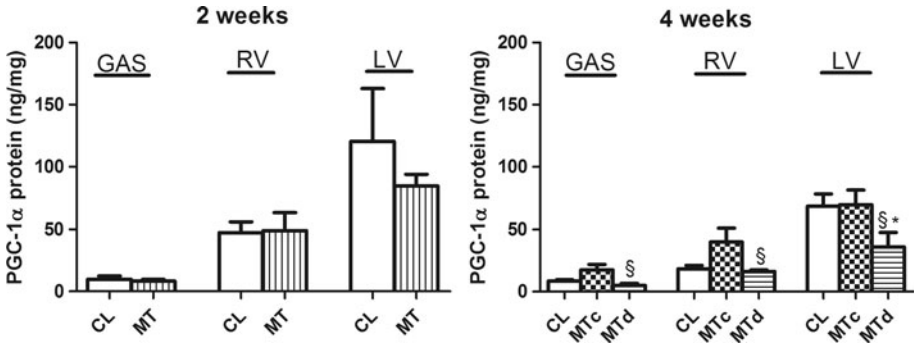

Fig. 3 PGC-1α protein level in the gastrocnemius (GAS), right ventricle (RV), and left ventricle (LV). *CL* control rats, *MT* monocrotaline rats, *MTc and MT4d* monocrotaline rats with compensated and decompensated disease, respectively. Results were expressed as mean ± SEM $^{*}P < 0.05$, MTd versus CL; $^{\S}P < 0.05$, MTd versus MTc

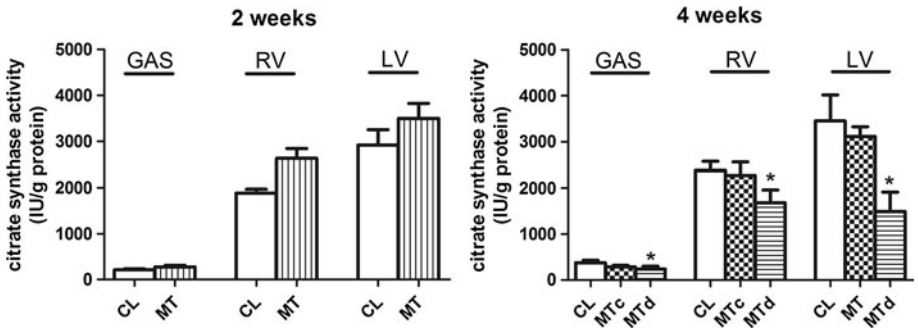

Fig. 4 Citrate synthase activity in the gastrocnemius (GAS), right ventricle (RV), and left ventricle (LV). *CL* control rats, *MT* monocrotaline rats, *MTc and MT4d* monocrotaline rats with compensated and decompensated disease, respectively. Results were expressed as mean ± SEM $^{*}P < 0.05$, MTd versus CL

Mitochondrial respiratory chain complex activity

At 2 weeks, mitochondrial respiration was similar in CL and MT for the GAS, RV, and LV (Fig. 5).

At 4 weeks, in the GAS, the maximal mitochondrial respiration (V_{max}) was decreased in MT4d compared to CL4. The RV V_{max} was significantly lower at 4 weeks in MT4d than in MT4c and CL4. The RV Complex IV mitochondrial respiration ($V_{TMPD/asc}$) was reduced in MT4d compared to CL4. Mitochondrial respiration was not significantly different in LV of CL4, MT4c, and MT4d.

Discussion

This study provided data on the time course of mitochondrial biogenesis and respiration, evaluated concomitantly in right and left ventricles and skeletal muscle in a well-characterized model of PH. The results showed that (1) reduction of SIRT1, PGC-1α, NRF1, TFAm, and citrate-synthase mRNA transcripts appeared early, at 2 weeks, in the GAS. (2) At 4 weeks, the GAS and RV of rats with compensated heart failure showed decreased mRNA expression for these factors. (3) These GAS and RV mRNA changes were associated with reduced CS activity and PGC-1α protein level, and with a decreased mitochondrial oxidative capacity in rats with overt right ventricular failure only. (4) The LV mRNA expression of these genes and LV oxidative capacity were not significantly altered, whatever the stage of disease.

Mitochondrial alterations at 2 weeks

The only alteration we observed at 2 weeks in MT rats was a decreased transcription of genes implicated in mitochondrial biogenesis and function in the GAS. Glycolytic muscles show usually larger changes than oxidative muscles in heart failure. These abnormalities include pronounced apoptosis and atrophy, inhibition of muscle protein synthesis, and impaired mitochondrial function and have been reported at an advanced stage of disease [18, 19, 21, 22, 41]. In this series, we report tiny but very early alterations in a glycolytic muscle.

By contrast with the GAS, no changes were observed in the RV. At 2 weeks, the MT rats were free of heart failure signs but showed slowdown of weight gain and slightly increased RV weight/body weight ratio, indicating progression of PH. Since the RV is directly affected by pressure overload, abnormalities could be expected to develop earlier in the RV than in a skeletal muscle. The reason why mitochondrial biogenesis of skeletal muscles is impaired earlier than that of the myocardium remains to be determined. The myocardium, which is a vital tissue, may have better protection mechanisms, as illustrated in a recent study [42] performed in a model of statin toxicity. It has been hypothesized that the insufficient reactive oxygen species (ROS)-detoxifying constituents of the skeletal muscle could explain the observed mitochondrial dysfunctions and down-regulated biogenesis. The better ROS-detoxifying constituents of the LV may have resulted in milder oxidative stress and protective mitochondrial

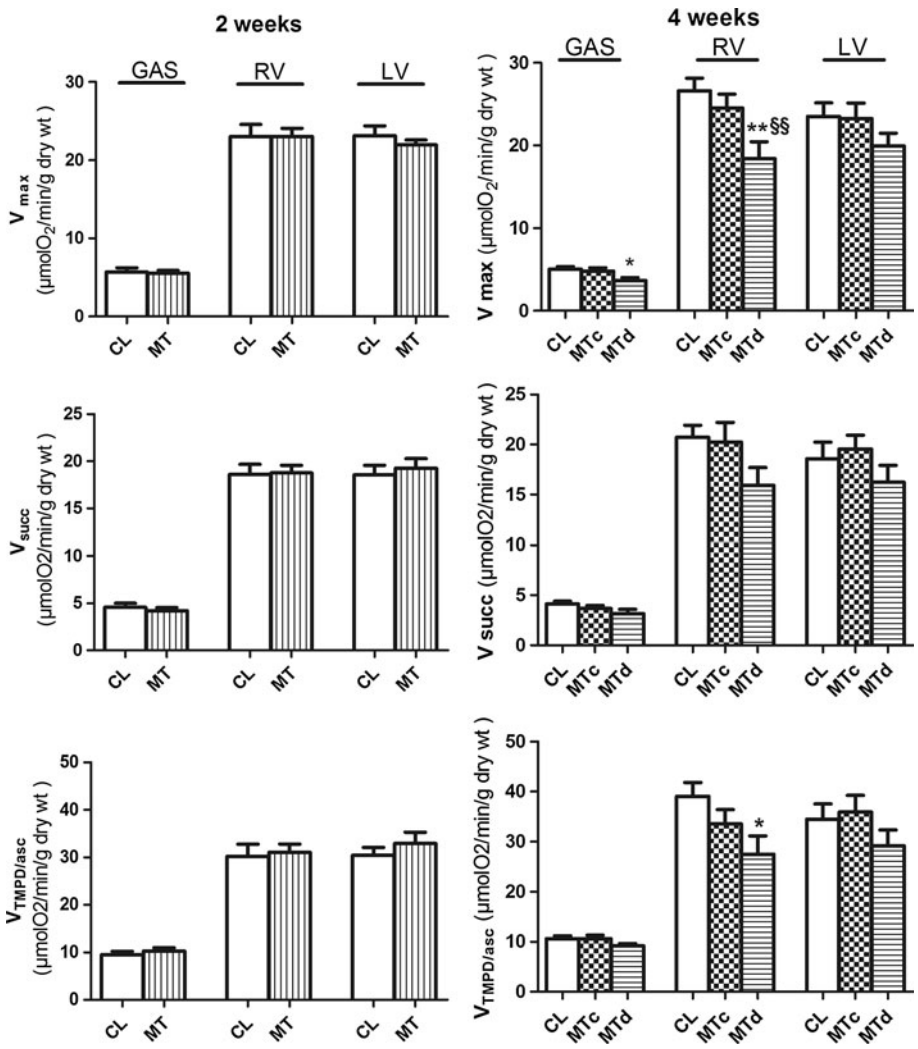

Fig. 5 Mitochondrial oxygen consumption rate measured with glutamate/malate/ADP (V_{max}), succinate (V_{succ}), and ascorbate/TMPD ($V_{TMPD/asc}$) as substrate, in skinned fibers of gastrocnemius (GAS), right ventricle (RV), and left ventricle (LV). *CL* control rats, *MT* monocrotaline rats, *MTc and MT4d* monocrotaline rats with compensated and decompensated disease, respectively. Results were expressed as mean ± SEM. $^{*}P < 0.05$, MTd versus CL at 4 weeks; $^{**}P < 0.01$, MTd versus CL at 4 weeks; $^{\S\S}P < 0.01$, MTd versus MTc at 4 weeks

biogenesis [42]. On the other hand, the goal of this mitochondrial biogenesis impairment may be the protection of the skeletal muscle itself. Indeed, it has been hypothesized that fiber atrophy and reduced mitochondrial volume density may help protecting against the development of low intracellular oxygen pressure in PH [18].

The mechanisms leading to alterations of mitochondrial biogenesis in skeletal muscles of MT rats have been described only in advanced PH. The responsibility of MT toxicity beyond 2 weeks is unlikely [18, 43]. PH is a systemic disease and the role of systemic factors is more likely. In the model elaborated by Vescovo et al. [44] PGC-1α had a central position and linked inflammatory and hormonal stimulations with mitochondrial biogenesis. Increased levels of TNF-α, interleukine 1, angiotensin II, and noradrenaline lowered PGC-1α through MAP kinase [45, 46]. In another potential signaling cascade developed in this model, resistance to growth hormone also led to low PGC-1α expression [44].

Besides inflammatory and hormonal factors, deconditioning and muscle fiber type shift may contribute to altering skeletal muscle mitochondrial biogenesis, although the timing of these changes remain to be assessed in the monocrotaline model. A substantial part of the mitochondrial alterations may be secondary to a physical inactivity induced by PH. Indeed, in humans, taking precisely into account patients and controls level of activity, it has been argued that deconditioning is a major contributor to skeletal muscle dysfunction in heart failure [47, 48]. Eventually, transition from slow oxidative to fast anaerobic fibers has been described in monocrotaline rats [18, 44]. Mitochondrial volume density as well as several key functions have been shown to differ according to the fiber type [49, 50]. Fiber type shift may partly explain the mitochondrial biogenesis alterations we observed in our experiment, since mitochondrial function and protein expression are one of the most rapidly adaptable features during fiber type alterations.

Mitochondrial alterations at 4 weeks

At a compensated stage of right heart failure, the GAS and RV of MT rats showed reduced SIRT1, PGC-1α, and citrate-synthase mRNA transcripts. CS activity, PGC-1α protein level, and mitochondrial respiration were not altered. This suggests that efficient post-transcriptional mechanisms or decreased degradation of the mitochondrial proteins and enzymes allowed preserving the respiratory chain complexes synthesis and/or activity during the compensated heart failure period. Another hypothesis may be that mRNA production level, even though lowered, remained sufficient to maintain mitochondrial proteins synthesis. Interestingly, in a recent study performed in human failing LV, the PGC-1α protein level was significantly increased whereas mRNA level was unchanged [51], suggesting as well that post-transcriptional mechanisms may be playing a role. In contrast, in keeping with the concept of a link between altered mitochondrial function and cardiac pump failure, we found concomitant reductions in mRNA level, PGC-1α protein level, CS activity, and mitochondrial respiration in the RV of rats with overt heart failure. Similar mitochondrial abnormalities were found in the GAS at the same time. These last results extend those showing a reduction of mitochondrial respiration due to oxygen utilization [22].

At 4 weeks, the transition from a compensated to a decompensated heart failure stage can occur rapidly in the MT model we used, and the decompensated stage is very quickly followed by the death of the animal. As a result, very few experiments provided data on both stages achieved in animals from the same series, as done in this study. We found that patterns of mRNA expression, protein level and mitochondrial respiration differed according to the stage of disease. Studying the mechanisms leading to the dissociation between transcriptional activity and maintenance of protein level may help understanding the transition to overt heart failure.

Results obtained in the LV contrast with those observed in the RV and GAS. Even though the LV is not affected by pressure overload in PH, an intrinsic left ventricular myocardiopathy has been described [13, 14, 52] and attributed to PH-induced neurohumoral activation [12–14]. In our experiment, we did not find any significant changes in expression of genes implicated in the mitochondrial biogenesis, whatever the stage of disease. Our findings are consistent with those of a study of the myocardium proteome showing a significant decrease in several proteins involved in the synthesis of ATP in the RV, but not in the LV of MT rats. Rather, the LV showed an increase in a few proteins contributing to ATP synthesis (ATP synthase alpha chain, acetyl-coenzyme A dehydrogenase) [53]. In our MT rats with overt heart failure, the decrease in the LV mitochondrial oxidative capacity was small compared to that of the RV and GAS (15 vs. ~30 %) and did not reach significance, even at the decompensated stage of the disease. This contrasts with the decrease in CS activity observed in the LV of our MT4d rats. However, it has been shown that CS activity is not a limiting factor for mitochondrial respiration [54]. Moreover, supporting our results, a study reported neither increase in oxidative stress nor changes in the activity of the respiratory chain complex II of the LV in overt RV failure [55]. Taken together, these results suggest that, compared to skeletal muscles, which are also exposed to systemic inflammation and neurohumoral activation, the LV is able to better preserve mitochondrial activities till an advanced stage of the disease. Again, this may be explained by the intrinsic characteristics of the cardiac muscle, which may have better defenses against aggressions than the skeletal muscle [42].

In conclusion, this study showed that the magnitude and time course of the mitochondrial activity and biogenesis alterations differ greatly according to the muscle phenotype in PH rats. The earliest alterations occurred in a skeletal muscle, and not in the RV. A concomitant decrease in mRNA expression, protein levels, and mitochondrial respiration characterized overt heart failure, whereas dissociations between changes in mRNA expression and protein levels were found at the compensated stage of the disease. Taking into account these time-related alterations should help elaborating treatments aimed at maintaining cardiac and skeletal muscle energy metabolism in PH.

Conflict of interest None.

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