CONTROL BY CIRCULATING FACTORS OF MITOCHONDRIAL FUNCTION AND TRANSCRIPTION CASCADE IN HEART FAILURE. A ROLE FOR ENDOTHELIN-1 AND ANGIOTENSIN-II.

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ABSTRACT

**Background** - Evidence is emerging to support the concept that the failing heart is “energy-depleted” and that defects in energy metabolism are important determinant in the development and the progression of the disease. We have previously shown that depressed mitochondrial function in cardiac and skeletal muscles in chronic heart failure (CHF) is linked to decreased expression of the gene encoding transcriptional PPARγ coactivator-1alpha (PGC-1α), the inducible regulator of mitochondrial biogenesis and its transcription cascade, leading to altered expression of mitochondrial proteins. However, oxidative capacity of the myocardium of CHF treated patients and pathophysiological mechanisms of mitochondrial dysfunction are still largely unknown.

**Methods and Results** - In CHF patients treated with angiotensin-converting enzyme inhibition (ACEi), cardiac oxidative capacity, measured in saponin permeabilized fibers, was 25% lower and PGC-1α protein content was 34% lower compared with non-failing controls. In a rat model of myocardial infarction, ACEi therapy was only partially able to protect cardiac mitochondrial function and transcription cascade. Expression of PGC-1α and its transcription cascade were evaluated after a 48 hour exposure of cultured adult rat ventricular myocytes to endothelin-1, angiotensin II, aldosterone, phenylephrine, or isoprenaline. Endothelin-1 (-30%) and to a lesser degree angiotensin II (-20%) decreased PGC-1α mRNA content while other hormones had no effect (phenylephrine) or even increased it (aldosterone, isoprenaline).

**Conclusions** - Taken together, these results show that despite ACEi treatment, oxidative capacity is reduced in human and experimental heart failure and that endothelin and angiotensin could be involved in the down-regulation of the mitochondrial transcription cascade. **KEY WORDS:** angiotensin, endothelin, mitochondrial biogenesis, heart failure, PGC-1α.
INTRODUCTION

Chronic heart failure (CHF) is a syndrome resulting from multiple origins and, despite
significant advances in therapy it remains a leading cause of morbidity and mortality in
developed countries. Evidence is emerging to support the concept that the failing heart is
“energy-depleted” and that defects in energy metabolism are important determinants in the
development and the progression of the disease\textsuperscript{1-3}. This includes a shift in substrate utilization
from fatty acid to glucose, decreased oxidative and glycolytic capacity, and alterations in
energy transfer and utilization. Despite a renewed interest for the energetic side of heart
failure, our knowledge of the upstream events is still sparse.

Addressing the origin of decreased cardiac muscle oxidative capacity in the pathogenesis of
heart failure, we previously showed in a rat model of CHF induced by pressure overload that
the decrease in mitochondrial function in both cardiac and skeletal muscles is linked to altered
expression of mitochondrial proteins associated with decreased expression of the
transcriptional coactivator peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-
1\(\alpha\)), and its downstream transcription factors\textsuperscript{4}. PGC-1\(\alpha\) is a master regulator of energy
metabolism at the level of gene transcription. Through its interaction with multiple
transcription factors such as peroxisome proliferator-activated receptors (PPAR), estrogen
receptor related receptor (ERRs) or nuclear respiratory factor (NRFs), PGC-1\(\alpha\) enhances
mitochondrial capacity for fatty acid oxidation and oxidative phosphorylation (OXPHOS) and
triggers the coordinate expression of nuclear and mitochondrial encoded genes driving
mitochondrial biogenesis\textsuperscript{5-7}. Decreased PGC-1\(\alpha\) expression has been confirmed in other
experimental models of heart failure\textsuperscript{8-12} showing that deactivation of the PGC-1\(\alpha\) cascade
leads to decreased mitochondrial activity through the down-regulation of mitochondrial
protein expression, and contributes to the energetic failure of the failing heart. However,
oxidative capacity and the implication of this pathway in human heart failure need further investigation\textsuperscript{13}.

In contrast to experimental models\textsuperscript{4}, in patients with severe CHF under angiotensin-converting enzyme inhibition (ACEi) therapy, skeletal muscle mitochondrial oxidative capacity and OXPHOS protein expression are preserved despite lower exercise capacity\textsuperscript{14}. In line with this maintained function, no evidence for deactivation of PGC-1\textalpha{} regulatory cascade was found, although in healthy subjects, exercise performance is associated with improvement in mitochondrial function, PGC-1\textalpha{} activation and increased expression of mitochondrial proteins\textsuperscript{15}. This suggests a possible protective effect of ACEi on muscle energy metabolism. This appears to be true at least in skeletal muscle as in a rat model of myocardial infarction (MI), we showed that ACEi therapy could prevent the deleterious effects of CHF on skeletal muscle oxidative capacity through a preservation of the transcriptional PGC-1\textalpha{} regulatory cascade\textsuperscript{10}. However, inhibition of angiotensin receptors by losartan in experimental model of myocardial infarction led to contradictory results on cardiac PGC-1\textalpha{} expression\textsuperscript{11,12}. Thus, whether ACEi therapy is also protective on cardiac mitochondrial protein expression and function is still an open question.

The generalized character of the metabolic myopathy that affects both cardiac and skeletal muscles in CHF and the protective effect of ACEi therapy described earlier in skeletal muscles suggest that humoral systemic factors could be involved\textsuperscript{16}. In the progression from compensated hypertrophy to failure, there is a generalized hyperactivation of several neurohumoral systems, including renin-angiotensin-aldosterone axis, endothelin-1 and catecholamines and these humoral factors might be responsible for the deactivation of the PGC-1\textalpha{} regulatory circuit and thereby for energetic dysfunction.

The purpose of the present study was to determine whether ACEi treated patients exhibit decreased mitochondrial function, whether in experimental model of heart failure ACEi
therapy may be protective for the mitochondrial function associated with an activation of PGC-1α expression and finally to explore in isolated rat cardiomyocytes which hormonal signals can induce an altered mitochondrial function through PGC-1α cascade.
MATERIALS AND METHODS

Patient population

Left ventricular myocardium was obtained from terminally failing human hearts of 20 patients (18 male, 2 female; mean age 52±2 years) at the time of transplantation mainly due to coronary artery disease or non ischemic cardiomyopathy at the “Hôpitaux Universitaires de Strasbourg” (HUS) between 1998 and 2000. They had a mean ejection fraction of 24±2% (Table 1). All patients had medication with diuretics (furosemide) and angiotensin-converting enzyme (ACE) inhibitors at the time of heart transplantation. Twelve patients over 20 received beta-blockers in addition. As control, non-failing human myocardium (normal ejection fraction) was obtained from 9 donors who were brain dead as a result of traumatic injury and from 8 donors during open-heart surgery (16 males, 1 female). Plasma ET-1 was measured by radioimmunoassay (Amersham, Buckinghamshire, United Kingdom) as previously described17. The study of these human cardiac tissues had been approved by HUS’s ethics committee, the subjects or their families gave informed consent.

Rat model of CHF

We used a rat model of heart failure induced by ligation of the proximal left coronary artery as previously described10. Four months perindopril treatment (2mg.kg⁻¹.day⁻¹ in the drinking water) started one week after coronary artery ligation. Treated (MI-PE, n=8) and untreated (MI, n=7) animals were studied. Control rats were sham operated without coronary ligation and had no medication (SHAM, n=7). The viable part of the left ventricle was isolated, part of which was immediately used for mitochondrial function measurement and the remaining part was rapidly frozen and kept at −80° C for subsequent analysis.

Functional properties of mitochondria
Oxygen consumption measurements of fresh saponin-skinned fibers from rat left ventricle (LV) have been described previously\(^\text{18}\) and this protocol was slightly modified for human cardiac muscle fibers\(^\text{14}\). Basal (\(V_0\)) and maximal (\(V_{\text{max}}\)) respiration rates were expressed as \(\mu\text{mol} \text{O}_2\text{min}^{-1}\text{.g}^{-1}\) dry weight. For each human subject, the value is the mean of two to three separate measurements.

**Preparation of adult rat ventricular myocytes**

Adult rat ventricular myocytes (ARVM) were dissociated by retrograde perfusion of healthy isolated heart with collagenase as previously described\(^\text{19}\) with slight modifications. Freshly isolated cells were plated on laminin-coated culture dishes at a density of \(2.10^5\) to \(3.10^5\) cells per dish in minimal essential medium (M4780, Sigma) supplemented with 2.5% fetal bovine serum, penicillin (100 units.ml\(^{-1}\)), streptomycin (100 \(\mu\text{g}.\text{ml}^{-1}\)) and 2% HEPES (pH 7.4) for 1 hour, and switched to serum-free media for 12 hours. Cells were then incubated for 48 hours with endothelin-1 (ET-1, \(5.10^{-7}\)M), angiotensin II (AngII, \(10^{-6}\)M), aldosterone (Aldo, \(5.10^{-8}\)M), phenylephrine (Phe, \(5.10^{-5}\)M), isoprenaline (Iso, \(10^{-8}\)M), or their diluents (control). Five independent cultures were used for each condition.

**Metabolic enzyme activities**

Lactate dehydrogenase (LDH) activity was measured in cell culture medium to evaluate the cytotoxic effects of various drugs\(^\text{20}\). Rat and human frozen tissue samples were weighed and homogenized in ice-cold buffer and citrate synthase (CS), cytochrome-c oxidase (COX) and creatine kinase (CK) activities were determined using spectrophotometry at \(30^\circ\text{C}\) and pH7.5, as previously described\(^\text{4}\), and expressed per gram of total protein.

**Western blot analysis**
Proteins extracts (50μg) of human control and failing myocardium were loaded onto a 10% SDS-polyacrylamide gels. Blots were first incubated with a specific antibody for mouse PGC-1α that also reacts with human due to sequence homology (dilution 1:1000, CHEMICON International) and then with an anti-rabbit IgG, HRP-linked antibody (dilution 1:2500, Cell Signaling). PGC-1α protein was revealed with an enhanced chemoluminescent substrate (SuperSignal® West Dura, Pierce Biotechnology) and α-actin (dilution 1: 4000, Sigma) was used as reference protein.

**RT-PCR analysis and Mitochondrial DNA content**

Standard procedures were used for total RNA extraction from rat LV or ARVC and reverse transcription. Real-time PCR was performed using the SYBR®Green technology on a LightCycler rapid thermal cycler (Roche Diagnostics) as described4,15. Quantification results for each gene were normalized to glucocerebrosidase gene expression. Primers and PCR conditions are listed in Table 2. The mitochondrial DNA (mtDNA) content, expressed as the ratio of mtDNA to nuclear DNA (nDNA), was measured by a Southern blot analysis of DNA extracted from cultured myocytes4.

**Statistical analysis**

Data are expressed as means± standard error of the mean (SEM). Variables were checked for normal distribution. Significance was determined with Student’s t test for human study, or one-way ANOVA followed by Newman-Keuls test or Kruskal-Wallis non parametric test for animal studies when appropriate. For cell experiments, paired t-tests were used. A value of P<0.05 was considered to be significant.
RESULTS

Mitochondrial function and PGC-1α protein expression in human failing hearts

In the failing hearts of treated CHF patients, maximal mitochondrial oxidative capacity was significantly lower by 25% associated with 40% lower activities of CS, a Krebs cycle enzyme, and of COX, complex IV of the respiratory chain (Figure 1A and 1B), suggesting an overall decrease in mitochondrial activity compared to the group of healthy subjects. Energy transfer was impaired as indicated by a lower total CK activity (CT: 6582±1111 vs CHF: 3124±199 μmol.min⁻¹.g⁻¹ prot, p=0.004) and mitochondrial (mi-CK) and cytosolic (MM-CK) isoenzyme activities (Figure 1B). No significant differences with the group of healthy subjects were observed whether or not patients were treated with β-blockers in addition to ACEi (Table 3). A 34% lower PGC-1α protein content was observed in CHF patients (Figure 1C).

Effects of ACE inhibition on mitochondrial function and the transcriptional PGC-1α regulatory cascade in heart of infarcted rats

As previously described, rats with MI exhibited a LV remodeling attested by a significant dilation (+42% in LV end diastolic diameter) and a high score of akinesia (around 50% of the LV), as well as a marked reduction in cardiac contractility (fractional shortening, FS, -59%) compared to sham animals. ACE inhibition with perindopril improved survival and tended to increase FS (+36%)\(^{10}\). The effects of ACEi by perindopril treatment on cardiac energy metabolism were assessed in rats following myocardial infarction. Maximal oxidative capacity (Figure 2) and activities of CS, COX and mi-CK were lower by 20 to 30% in LV of MI rats (Table 4) compared to sham. These metabolic defects were associated with downregulation of gene expression of PGC-1α, NRF-2α and mtTFA and of COX subunits encoded by nuclear and mitochondrial genomes by 50% to 60% in failing hearts (Figure 2).
Perindopril treatment did not fully prevent the decrease in mitochondrial activity and expression of genes involved in the molecular control of mitochondrial protein expression, except for CS and mitochondrial CK activities and COXI gene expression (Table 4 and Figure 2) evidencing that ACEi therapy was not sufficient to preserve myocardial energetics.

**Effects of humoral factors involved in CHF on the transcriptional control of mitochondrial protein expression**

The next step was to identify the potential effects of circulating hormones on mitochondrial protein expression and transcription cascade in isolated adult rat ventricular myocytes. No effect of drug treatment was observed on cell viability (estimated by the release of LDH in the culture medium, results not shown). ANF gene expression was significantly higher with all hormones except AngII (Figure 3A). Activity of COX, the complex IV of the respiratory chain, was significantly lower by ET-1 (-40%), while AngII only tended to lower it (Figure 3B) compared to non-treated cells. ET-1 exposure also induced an alteration in mtDNA content (-20%) (Figure 3C). We then assessed the expression of PGC-1α and its transcription cascade involved in mitochondrial biogenesis. No significant effect was obtained with phenylephrine, whereas isoprenaline treatment induced a significant increase in PGC-1α and mtTFA mRNA levels (Figure 4). Effectors of the renin-angiotensin-aldosterone system seem to differentially regulate PGC-1α gene expression as we observed an upregulation by Aldo (+60%) and a downregulation by AngII (-20%) (Figure 4). A significant downregulation of PGC-1α (-30%), NRF-2α (-50%) and mtTFA (-20%) gene expression was observed with endothelin-1, associated with a lower COXI subunit mRNA level (-30%) (Figure 4).

Due to the major effects of endothelin on mitochondrial biogenesis, we then assessed in our experimental model of CHF, the status of the endothelin system. Endothelin converting enzyme (ECE-1) mRNA expression was higher in myocardium of MI rats compared to sham.
and ACEi therapy prevented the upregulation of cardiac ECE-1 (Table 4). Importantly, in patients, circulating ET-1 level was as high as 10.6±1.3 pg.ml⁻¹ (n=16).
DISCUSSION

In this study we investigated whether ACEi treated CHF patients exhibit decreased cardiac mitochondrial function, whether ACEi therapy may be protective for the mitochondrial function in association with activation of PGC-1α expression in experimental model of heart failure and which hormonal signals can induce an altered mitochondrial activity through PGC-1α transcription cascade. We show that 1) in CHF patients treated with ACEi, cardiac oxidative capacity and PGC-1α protein content are decreased, 2) in a rat model of myocardial infarction, ACEi therapy is only able to partially prevent mitochondrial function and mitochondrial transcription cascade, 3) in adult cardiomyocytes, endothelin-1 and to a minor degree angiotensin II can decrease the mitochondrial transcription cascade while other circulating hormones had either no (phenylephrine) or positive (aldosterone, isoprenaline) effects. Taken together, these results show that oxidative capacity is reduced in human and experimental heart failure despite ACEi treatment and that endothelin and angiotensin could be involved in the down-regulation of the mitochondrial transcription cascade in heart failure.

Mitochondrial function in human failing hearts

Oxidative capacity of the myocardial tissue has been assessed in situ by measuring oxygen consumption of permeabilized cardiac fibers of end-stage heart failure patients. Decreased oxidative capacity and metabolic enzymes together with decreased PGC-1α protein content clearly evidence an overall decrease in mitochondrial activity in CHF. Clinical studies of CHF have shown marked cardiac mitochondrial respiratory enzyme dysfunction, albeit variable in the extent and the specific enzymes affected21-24. Although a down-regulation of adult metabolic gene transcript profile has been observed in failing human heart25, another study found altered activity of respiratory chain complexes, without alteration in gene expression in terminally failing human myocardium, identifying beta-blockers as one putative protection
against this disturbance.\textsuperscript{24} Drug treatment of CHF protecting against mitochondrial damage in patients might be considered as explanations for this disparity. Although pharmacological therapy with ACE inhibitors has proved to be effective in patients with heart failure, the basis for these effects is still incomplete. In the present study, patients were all under ACEi therapy and still exhibited decreased oxidative capacity and mitochondrial enzymes with no difference whether or not they were additionally treated with \(\beta\)-blockers. Although clearly established in experimental heart failure (see\textsuperscript{26} for review), the issue to whether mitochondrial transcription cascade is altered in humans is still a matter of debate. Although it was found to be unchanged in terminally failing myocardium\textsuperscript{24}, in a very recent study, decreased gene expression of PGC-1\(\alpha\) and transcription cascade involved in mitochondrial biogenesis, fatty acid metabolism, and glucose transport was clearly observed in both dilated cardiomyopathy and ischemic heart disease patients\textsuperscript{27}. This last result agrees with the decrease in PGC-1\(\alpha\) protein content reported here. Thus despite recent improvement of CHF therapy, myocardium of CHF patients still exhibit defective mitochondrial function and biogenesis as well as energy transfer whose origin needs to be elucidated.

\textit{ACE inhibition and mitochondrial function and biogenesis in rats following myocardial infarction}

We showed previously that ACEi could totally prevent alterations in mitochondrial function and biogenesis in skeletal muscle of rats with myocardial infarction\textsuperscript{10}. In myocardium of Syrian myopathic hamsters with advanced heart failure, ACEi treatment increases cardiac performance and energy reserve via the creatine kinase reaction\textsuperscript{28}. Long-term ACEi therapy improves mitochondrial function in rats following myocardial infarction\textsuperscript{29}. To better understand the molecular and cellular effects of ACEi therapy on cardiac mitochondrial function and biogenesis, CHF was induced in rats by myocardial infarction (MI). Cardiac
muscle of MI rats showed a decrease in oxidative capacity together with alterations of mitochondrial protein expression and activity, associated with downregulated expression of PGC-1α and its transcription cascade. Whereas in skeletal muscles ACEi therapy totally prevented the adverse effects of MI on mitochondrial function and expression of genes involved in the molecular control of OXPHOS proteins10, this protective effect was only partial in cardiac muscle. Similarly, losartan, an angiotensin II type 1 (AT1) receptor antagonist, was shown to partially reverse the MI induced down-regulation of PGC-1α expression in male rats12, but not in female rats11. These partial cardiac and total “peripheral” beneficial effects of ACEi therapy on mitochondrial function and biogenesis could thus explain the different results observed in human failing skeletal14,15 and cardiac muscle (present results). Understanding the events mediating the decreased oxidative capacity in failing heart appears to be of major significance. Although ACE inhibitors exert their beneficial effects by reducing the synthesis of angiotensin II, there are clinical and experimental evidences that they also prevent the upregulation of other circulating factors like endothelin, cytokines, aldosterone and catecholamines30-33.

**Effects of humoral factors involved in CHF on the transcriptional control of mitochondrial protein expression**

We thus examined the effects of the main hormones known to be activated in heart failure on adult rat ventricular myocytes. Circulating catecholamines are elevated in heart failure, however, neither α or β-adrenergic stimulation led to decreased expression of PGC-1α transcription cascade. Indeed, the PGC-1α promoter contains a positive cyclic AMP (cAMP) response element (CRE)34 that could explain the increased expression of PGC-1α observed with isoprenaline incubation. It is well known that the renin/angiotensin/aldosterone (RAS) system is overactivated in heart failure. Interestingly, angiotensin II decreased PGC-1α
expression, while aldosterone had an opposite effect. Plasma levels of ET-1 are increased in heart failure patients and correlate with the severity of the disease\textsuperscript{35} and endothelin substantially contributes to left ventricular remodeling and progression of heart failure\textsuperscript{36}. We demonstrate that endothelin-1 also induced a down-regulation of PGC-1\textgreek{a} and its transcription cascade, NRF2\textgreek{a} and mtTFA. AngII and ET-1 were shown to play essentially different pathophysiological roles in states of chronic hypertrophy and subsequent CHF. The local ET-1 system remains in the basal state at the stage of compensatory hypertrophy and shows \textit{de novo} activation during the transition to CHF, while angiotensin II increases at the phase of compensated hypertrophy and remains stable thereafter\textsuperscript{37}. Local AngII and ET-1 systems participate in an autocrine/paracrine manner to their physiological effects. Cardiac ET-1 but also endothelin receptors and endothelin converting enzyme (ECE) are increased in heart failure\textsuperscript{33} and ACEi therapy induces a decrease in circulating and tissue endothelin-1 levels\textsuperscript{38,38-41}. ECE-1 expression was similarly upregulated in myocardium of MI rats, and reversed by PE treatment, showing a partial protective effect on the endothelin system\textsuperscript{33}. Taken together these results support a causal link between endothelin-1 and angiotensin II activation, and decreased PGC-1\textgreek{a} expression and oxidative capacity in cardiac muscle and suggest that ACEi could be only partially protective by a complex action on the RAS and endothelin systems.

\textit{Limitation of the study}

It was not possible to perform all biochemical and functional measurements for human studies on all subjects. Indeed, functional data need to have freshly collected biopsies. That was possible only on donors on open-heart surgery in the control group. In this group the small amount of collected tissue precluded from performing all the measurements on the same subjects. Another possible limitation is that the cause of heart failure is multiple in patients
with coronary artery disease and non obstructive cardiomyopathy being the main cause of heart failure. Heart failure in rats resulted from artificial coronary obstruction. In this model, the non ischemic part of the ventricle, remote from the ischemic region was used, and heart failure mainly resulted from increased workload on the viable part of the myocardium. Finally all patients were not under the same ACE inhibitor. However all of them have demonstrated a clear effect on mortality so that at least class effects are expected.

**Conclusion**

Interventions that modulate myocardial energy metabolism have been proposed as a new strategy for treatment of CHF and one possible way might be a direct stimulation of mitochondrial oxidative phosphorylation$^{3,26,42}$. In this way, moderately improving PGC-1$\alpha$ expression or activity _in vivo_ has been proposed to be a promising approach as a metabolic therapy in CHF$^{13,26,43,44}$. Long-term treatment with ET-1 receptor blockers improves survival and cardiac function and decreases LV hypertrophy and fibrosis$^{45}$. It also partially reverses myocardial dysfunction and specific mitochondrial enzyme deficiencies in pacing-induced CHF$^{46}$, underlining the importance of this hormone for the control of cardiac energy metabolism. In view of the synergistic and supplemental role of AngII and ET-1, long-term therapy with a combination of ACEi and ET antagonism could be beneficial for heart failure patients$^{37,47}$. Although promising in terms of improvement of heart failure and remodeling in several animal models, endothelin-1 receptor antagonists in CHF patients have shown neutral effects in terms of mortality and symptoms. However, selectivity towards endothelin receptors as well as time or duration of administration in these trials could be questioned$^{35,48}$ and their effects on energy metabolism and mitochondrial biogenesis need to be assessed.

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DISCLOSURES

We have no conflicts of interest to disclose.
REFERENCES


FIGURE LEGENDS

Figure 1. Mitochondrial function and PGC-1α protein expression in cardiac muscle of healthy subjects (CT) and patients with chronic heart failure (CHF).

A: Basal (V_0) and maximal respiration rates (V_max) were measured in saponin-skinned fibers (CT, n=8; CHF, n=20). B: Enzymatic activities of MM-CK and BB-CK, cytosolic creatine kinase (CK) isoenzymes; mi-CK, mitochondrial creatine kinase; CS, citrate synthase and COX, cytochrome c oxidase were assessed by spectrophotometry (CT, n=12; CHF, n=20). C: Western blot analysis of PGC-1α protein expression. Values are mean±SEM. *P<0.05 and **P<0.01 for difference from control values.

Figure 2. Mitochondrial oxidative capacity and transcriptional PGC-1α regulatory cascade in heart of sham-operated (SHAM), myocardial infarction rats with no medication (MI), or MI rats treated with perindopril (MI+PE).

Maximal respiration rates of saponin-skinned fibers (V_max), peroxisome proliferator activated receptor gamma co-activator 1α (PGC-1α), nuclear respiratory factor 2 DNA-binding subunit α (NRF-2α), mitochondrial transcription factor A (mtTFA), cytochrome c oxidase subunits encoded by nuclear (COXIV) or mitochondrial (COXI) genomes were measured. Values are mean±SEM (n=7-8). *P<0.05 and **P<0.01 for difference from SHAM group; §§P<0.01 for difference from MI group.

Figure 3. Effects of humoral factors on adult rat ventricular myocytes.

Cells were incubated for 48 h with endothelin-1 (ET-1, 5.10^{-7}M), angiotensin II (AngII, 10^{-6}M), aldosterone (Aldo, 5.10^{-8}M), phenylephrine (Phe, 5.10^{-5}M), or isoprenaline (Iso, 10^{-8}M). Atrial natriuretic factor (ANF) gene expression (A), cytochrome c oxidase (COX) activity
(B), and mitochondrial DNA content (C) were measured. Mitochondrial DNA (mtDNA) content was assessed by Southern blot analysis and expressed as the ratio of mtDNA to nuclear DNA (nDNA). Values are mean±SEM of five independent cultures per experimental condition and are expressed as percentage of control values. *P<0.05 and ***P<0.001 for difference from control values.

Figure 4. Neurohumoral regulation of the PGC-1α transcriptional cascade in adult rat ventricular myocytes.

Cardiac myocytes were exposed for 48h to phenylephrine (Phe, 5.10⁻⁵M), isoprenaline (Iso, 10⁻⁸M), aldosterone (Aldo, 5.10⁻⁸M), angiotensin II (AngII, 10⁻⁶M) or endothelin-1 (ET-1, 5.10⁻⁷M). mRNA expression of peroxisome proliferator activated receptor gamma co-activator 1α (PGC-1α), nuclear respiratory factor 2 DNA-binding subunit α (NRF-2α), mitochondrial transcription factor A (mtTFA), mitochondrial genome encoded cytochrome c oxidase subunit I (COXI) were measured. Values are mean±SEM of five independent cultures per experimental condition and are expressed as percentage of control values. *P<0.05, **P<0.01 and ***P<0.001 for difference from control values.
Table 1. Patient characteristics.

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<td>15</td>
<td>nd</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>48</td>
<td>Hdcm</td>
<td>Perindopril</td>
<td>Carvedilol</td>
<td>23</td>
<td>6.1</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>31</td>
<td>Nocm</td>
<td>Fosinopril</td>
<td>Carvedilol</td>
<td>17</td>
<td>12.5</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>61</td>
<td>Nocm</td>
<td>Ramipril</td>
<td>Carvedilol</td>
<td>15</td>
<td>8.0</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>56</td>
<td>Nocm</td>
<td>Ramipril</td>
<td>Carvedilol</td>
<td>35</td>
<td>6.5</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>64</td>
<td>Nocm</td>
<td>Ramipril</td>
<td>Carvedilol</td>
<td>30</td>
<td>6.1</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>32</td>
<td>familialcm</td>
<td>Ramipril</td>
<td>Carvedilol</td>
<td>24</td>
<td>8.4</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>60</td>
<td>Hcm</td>
<td>Ramipril</td>
<td></td>
<td>15</td>
<td>6.4</td>
</tr>
</tbody>
</table>

dcm: non obstructive cardiomyopathy; cad: coronary artery disease; hdcm: hypertrophic dilated cardiomyopathy; hcm : hypertrophic cardiomyopathy. LVEF : left ventricular ejection fraction ; ET-1 : plasma endothelin-1 level. nd: not determined.
Table 2. Primers and experimental conditions for real-time PCR amplification.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Acc GenBank</th>
<th>Forward primer (5’→3’)</th>
<th>Reverse primer</th>
<th>PCR product size (bp)</th>
<th>Annealing temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGC-1α</td>
<td>NM_031347</td>
<td>CACCAAAACCACAGAGAACAG</td>
<td>GCAGTTCCAGAGAGTCCACA</td>
<td>206</td>
<td>58</td>
</tr>
<tr>
<td>NRF-2α</td>
<td>XM_344002</td>
<td>CACCACACTCAACATTTCGG</td>
<td>CCTGGGGGACCTTTGAAC TT</td>
<td>244</td>
<td>58</td>
</tr>
<tr>
<td>mtTFA</td>
<td>NM_031326</td>
<td>GAAAGCACAAATCAAGGAGAG</td>
<td>CTGCTTTTCATCATGAGACAG</td>
<td>175</td>
<td>55</td>
</tr>
<tr>
<td>COX I</td>
<td>NC_001665</td>
<td>AGCAGGAATAGTAGGGACAGC</td>
<td>TGAGAGAAGTAGTGAGGACGGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COX IV</td>
<td>NM_017202</td>
<td>TGGGAGTGTTGTGAAGAGTGA</td>
<td>GCAGTGAAGCCGATGAAG AAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANF</td>
<td>NM_012612</td>
<td>GGGCTCCTTCTCCATCACCAA</td>
<td>CTTCATGCTGCTGCTGCTCA</td>
<td>203</td>
<td>60</td>
</tr>
<tr>
<td>ET-1</td>
<td>NM_012548</td>
<td>TGCTCTGCTCTCCCTGAGA</td>
<td>CTGGCATCTGTCCCTTGGT</td>
<td>177</td>
<td>65</td>
</tr>
<tr>
<td>ECE-1</td>
<td>NM_053596</td>
<td>TACTACTGGCCACCAAGAA</td>
<td>TCCCATCCTGTCATCTCCTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCB</td>
<td>NM_000157</td>
<td>GCACAACCTCAGCCTCCCAGA</td>
<td>CTCCCATTTACCGGTTCATT</td>
<td>151</td>
<td>60</td>
</tr>
</tbody>
</table>

PGC-1α: peroxisome proliferator activated receptor gamma co-activator 1α; NRF-2α: nuclear respiratory factor 2 DNA-binding subunit α; mtTFA: mitochondrial transcription factor A; COX I and COX IV: cytochrome-c oxidase subunits I and IV; ANF: atrial natriuretic factor; ET-1: endothelin-1; ECE-1: endothelin-converting enzyme-1; GCB: glucocerebrosidase.
Table 3. Effects of patient treatments on metabolic parameters.

<table>
<thead>
<tr>
<th></th>
<th>ACEi</th>
<th>ACEi + β blockers</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>11M/1F</td>
<td>7M/1F</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>50±4</td>
<td>55±2</td>
<td>0.277</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>22±3</td>
<td>27±4</td>
<td>0.437</td>
</tr>
<tr>
<td>V₀</td>
<td>2.5±0.2</td>
<td>2.3±0.4</td>
<td>0.695</td>
</tr>
<tr>
<td>Vmax</td>
<td>12.0±1.3</td>
<td>12.2±1.4</td>
<td>0.909</td>
</tr>
<tr>
<td>CK</td>
<td>3088±275</td>
<td>3179±299</td>
<td>0.824</td>
</tr>
<tr>
<td>CS</td>
<td>283±18</td>
<td>267±10</td>
<td>0.434</td>
</tr>
<tr>
<td>COX</td>
<td>171±20</td>
<td>139±32</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Values are means ± SEM. V₀: basal respiration rate and Vmax: maximal respiration rate are expressed in μmol O₂.min⁻¹.g dw⁻¹. LVEF: left ventricular ejection fraction. Activities of CK (creatine kinase), CS (citrate synthase) and COX (cytochrome oxidase) are expressed in μmole.min⁻¹.g prot⁻¹.
Table 4. Metabolic enzyme activities and mRNA expression in rat left ventricle.

<table>
<thead>
<tr>
<th></th>
<th>SHAM (n=7)</th>
<th>MI (n=7)</th>
<th>MI-PE (n=8)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzyme activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>502±44</td>
<td>398±20</td>
<td>588±47§§</td>
<td>0.004</td>
</tr>
<tr>
<td>Cytochrome c oxidase</td>
<td>1645±75</td>
<td>1198±126*</td>
<td>1733±277</td>
<td>0.033</td>
</tr>
<tr>
<td>Mitochondrial creatine kinase</td>
<td>528±66</td>
<td>363±45</td>
<td>538±121</td>
<td>0.219</td>
</tr>
<tr>
<td><strong>mRNA expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>0.96±0.06</td>
<td>1.14±0.05</td>
<td>1.17±0.09</td>
<td>0.093</td>
</tr>
<tr>
<td>Endothelin converting enzyme</td>
<td>0.85±0.05</td>
<td>1.09±0.10*</td>
<td>0.83±0.04§</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Values are means ± SEM and are expressed in μmole.min⁻¹.g prot⁻¹ for enzyme activity and in arbitrary units for mRNA expression. MI, myocardial infarction without treatment; MI-PE, myocardial infarction treated with perindopril. *P<0.05 for difference from SHAM group; §P<0.05 and §§P<0.01 for difference from MI group.
Figure 1

A

CT
CHF

(μmol O₂.min⁻¹.g⁻¹ dry wt)

0
5
10
15
20

V₀
V_max

B

CS
COX

MM-CK
BB-CK
mi-CK

(μmol.min⁻¹.g⁻¹ prot)

0
100
200
300
400
500
600
700

* *

C

PGC-1α protein

(μmol.min⁻¹.g⁻¹ prot)

0
100
200
300
400
500
600
700

(μmol.min⁻¹.g⁻¹ prot)

0
100
200
300
400
500
600
700
800

PGC-1α protein
Figure 2

**V\text{max}**

![Bar graph showing V\text{max} for SHAM, MI, and MI+PE groups.](image)

**PGC-1α**

![Bar graph showing PGC-1α mRNA levels for SHAM, MI, and MI+PE groups.](image)

**NRF-2α**

![Bar graph showing NRF-2α mRNA levels for SHAM, MI, and MI+PE groups.](image)

**mtTFA**

![Bar graph showing mtTFA mRNA levels for SHAM, MI, and MI+PE groups.](image)

**COX I**

![Bar graph showing COX I mRNA levels for SHAM, MI, and MI+PE groups.](image)

**COX IV**

![Bar graph showing COX IV mRNA levels for SHAM, MI, and MI+PE groups.](image)
Figure 3

A

**ANF mRNA**

![Graph showing ANF mRNA expression](image)

B

**COX activity**

![Graph showing COX activity](image)

C

**mDNA/nDNA**

![Graph showing mDNA/nDNA ratio](image)

Control ET-1 (5.10^{-7} M)
Figure 4

**Phe**

![Graph showing mRNA levels of PGC-1α, NRF-2α, and mtTFA under Phe treatment.](image)

**Iso**

![Graph showing mRNA levels of PGC-1α, NRF-2α, and mtTFA under Iso treatment.](image)

**Aldo**

![Graph showing mRNA levels of PGC-1α, NRF-2α, and mtTFA under Aldo treatment.](image)

**AngII**

![Graph showing mRNA levels of PGC-1α, NRF-2α, and mtTFA under AngII treatment.](image)

**ET-1**

![Graph showing mRNA levels of PGC-1α, NRF-2α, mtTFA, and COXI under ET-1 treatment.](image)
Control By Circulation Factors Of Mitochondrial Function And Transcription Cascade In Heart Failure: A Role For Endothelin-1 And Angiotensin-II

Anne Garnier, Joffrey Zoll, Dominique Fortin, Benoît N’Guessan, Florence Lefebvre, Bernard Geny, Bertrand Mettauer, Vladimir Veksler and Renée Ventura-Clapier

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