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ORIGINAL ARTICLE

Functional and Structural Alterations of Cardiac and Skeletal Muscle Mitochondria in Heart Failure Patients

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Background and Aims. The fundamental mechanisms involved in the genesis and progression of heart failure are not clearly understood. The present study was conducted to analyze the cardiac mitochondrial involvement in heart failure, the possible parallelism between cardiac and skeletal muscle and if there is a link between clinical symptoms and mitochondrial damage.

Methods. Left ventricle and pectoral biopsies were obtained from patients with heart failure (*n*: 21) and patients with inter-auricular communication as the unique diagnosis for surgery (*n*: 6). Mitochondria were isolated from these tissues and studied through electron microscopy, spectrophotometry to measure the activity of respiratory complex III and immunohistochemistry to determine the presence of reactive oxygen species.

Results. More than 90% of cardiac and skeletal muscle mitochondria presented structural and functional alterations in relation to an increment in the reactive oxygen species production, even in patients without the presence of any clinical Framingham criteria.

Conclusions. We demonstrated some parallelism between cardiac and skeletal muscle mitochondrial alterations in patients with heart failure and that these alterations begin before the major clinical Framingham criteria are installed, pointing to mitochondria as one of the possibly responsible factors for the evolution of cardiac disease.

Key Words: Heart failure, Cardiac and skeletal muscle, Mitochondria.

Introduction

Heart failure is a very important cause of morbidity and mortality throughout the world (1,2). Even though there have been several studies performed on the subject, the fundamental mechanisms involved in the genesis and progression of left ventricular failure are not clearly understood (3,4). The development of heart failure involves abnormalities at tissue and cellular levels such as reduction in myocardial contractility as a consequence of cardiac ischemia, deficit in the capacity to respond to reactive oxygen species (ROS), changes in ionic fluxes, electrophysiological alterations and fibrosis and cardiac remodeling (4,5), leading to cardiomyocyte loss and modifications in the ability to produce and metabolize energy (5). Several studies have proposed that cardiac cell response to oxidative stress causes progressive cellular changes that target mitochondria (3,6–8). It has been demonstrated that oxidative stress triggers the mitochondrial apoptotic pathway, which would be involved in the pathophysiology of ischemic heart disease and heart failure (6).

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The heart is an organ with great energy demand; mitochondria represent 30% of the total volume of the myocytes and provide 90% of the cardiac energy, fatty acids being the primary energy substrates for cardiac muscle ATP generation through the mitochondrial oxidative phosphorylation pathway.

Observations carried out in patients and animal models of heart failure propose that mitochondria would be the key to understand the beginning and progression of several heart diseases, such as dilated and hypertrophic cardiac disease, ischemic and alcoholic cardiopathy, electrical disturbances and myocarditis, among others (5,9).

Taking into consideration that heart failure represents a major public health problem, largely preventable through blood pressure controls and the reduction of other vascular risk factors that affect the heart, we conducted this study in order to establish the mitochondrial involvement in heart failure, the possible parallelism between cardiac and skeletal muscle and if there is a link between clinical symptoms and mitochondrial damage.

Improved understanding of the impact of cardiac and skeletal muscle mitochondrial alterations in cardiac diseases will improve and facilitate their diagnosis and prognosis, and increase the possibility of modifying the natural evolution of heart disease.

Subjects and Methods

Patients

Twenty seven patients who underwent cardiovascular surgery for different reasons and accepted to participate in this study were included.

Inclusion criteria for control group (*n*: 6) were patients from either gender with normal left ventricular ejection fraction (LVEF) (>60%), without rheumatologic disease, diabetes, hypertension, dyslipidemia, artery obstruction and with inter-auricular communication as the unique diagnosis for surgery.

Inclusion criteria for the group with chronic heart failure (*n*: 21) were patients with chronic heart failure as a result of dilated cardiomyopathy, with left ventricular ejection fraction <60%, without rheumatologic or immunologic disease. They all belonged to functional classification II of the New York Heart Association because their cardiac compromise was compensated before surgery.

This study complies with the Declaration of Helsinki and was approved by the ethics committee of the Allende Clinic, Córdoba, and INCOR, La Rioja, Argentina.

A detailed clinical analysis was obtained from each patient recording age, heart failure etiology, risk factors, electrocardiography, echocardiography, coronary catheterization and medication.

Muscle Biopsy

Cardiac and skeletal muscle biopsies of about 1 mm^2 (from the left ventricle and from the pectoral muscle, which contains predominantly type I fibers because individuals included in this study were sedentary) were obtained from patients during surgery. Each sample was divided into two sections: one was examined under a Zeiss electron microscope and the other was used to study the functional activity of the mitochondrial respiratory chain enzymes or to carry out the immunohistochemical analysis.

Electron Microscopy Studies

A 0.5-mm^2 section from either cardiac or skeletal muscle samples (*n*: 10 from heart failure group and three from control group) was fixed immediately after extraction in a Karnovsky solution (4% formaldehyde and 1.5% glutaraldehyde in 0.1 M cacodylate buffer) for at least 2 h at room temperature. The tissues were then washed three times in cacodylate buffer and postfixed in 1% osmium tetroxide for 1–2 h. After dehydration in a graded acetone solution (50, 70 and 90%), the samples were included in a mixture of araldite 506 epoxy composite (48.5%), dodecenylsuccinic anhydride (48.5%), dibutylphthalate (0.5%) and dimethylaminobenzene accelerator (2.5%). Ultrathin cuts were stained with uranyl acetate and lead citrate and examined under a Zeiss electron microscope.

Mitochondrial diameter and area were analyzed using Axiovision 4.8. In order to evaluate the changes on mitochondrial morphology observed in either group of patients (five micrographs for each patient as done previously) (10–12), a 4 degree classification was used:

Grade 0: normal structure
Grade I: normal size, dilated cristae
Grade II: normal size and/or altered shape; intact membrane with few cristae
Grade III: mitochondrial swelling

Three-dimensional studies were carried out using the Femtoscan program.

Mitochondria Isolation

Cardiac and skeletal muscle sections were washed and suspended in ice-cold isolation buffer (5 mM HEPES, pH 7.2 containing 210 mM mannitol, 70 mM sucrose, 1 mmol EGTA, and 0.5% BSA [fatty acid-free], tissue/buffer ratio, 1:10 w/v) and immediately homogenized. Homogenates were centrifuged at 1500 *g*, 4°C for 20 min and the supernatant transferred to a new tube and resuspended in isolation buffer, homogenized, and centrifuged again at 10,000 *g*, 4°C for 5 min. The supernatant was rejected and the pellet resuspended in buffer and centrifuged at

Table 1. Clinical characteristics of the patients studied

	Heart failure group (n = 21)	Control group (n = 6)
Age (years)	57.7 ± 1.77	49.5 ± 3.64
Diabetes	3 (14.3%)	0 (0%)
Hypertension	10 (47.6%)	0 (0%)
Dyslipidemia	8 (38.1%)	0 (0%)
Two or more factors combined	16 (76.2%)	0 (0%)
LVEF (%)	51 ± 2.1%	66 ± 3.5%
Electrocardiogram alterations	100%	40%
Coronary arteriography		
No obstructed artery	5 (23.8%)	6 (100%)
One obstructed artery	2 (9.5%)	0 (0%)
Two or three obstructed arteries	5 (23.8%)	0 (0%)
More than three obstructed arteries	9 (42.8%)	0 (0%)
Medications		
Calcium channel blockers	3 (14.2%)	0 (0%)
Diuretic drugs	4 (19.04%)	0 (0%)
Nitrates	2 (9.52%)	0 (0%)
Digoxin	1 (4.76%)	0 (0%)
ACE inhibitors	10 (47.61%)	0 (0%)
Beta blockers	7 (33.33%)	0 (0%)
Acetylsalicylic acid	6 (28.57%)	0 (0%)
Amiodarone	1 (4.76%)	0 (0%)
Acenocoumarol	0 (0%)	0 (0%)
Statins	4 (19.04%)	0 (0%)
Antidepressants	3 (14.28%)	0 (0%)
Antibiotics (in the last 20 days)	1 (4.76%)	0 (0%)

LVEF, left ventricular ejection fraction; ACE, angiotensin-converting enzyme.
Data of age are mean ± standard error.

10,000 *g* 4°C for 10 min (twice = purification). The mitochondrial pellet was resuspended in isolation buffer (tissue/buffer, 1:1 ratio, w/v), and the aliquots stored at −80°C.

Immunohistochemical Analysis

Protein expression of the inducible nitric oxide synthase (iNOS) was analyzed using polyclonal IgG antibodies against the iNOS amino terminal end (Sc8310, Santa Cruz Biotechnology, Santa Cruz, CA).

The paraffinized sections from cardiac and skeletal muscles (n: 4 from heart failure group and n: 2 from the control group) were deparaffinized using xylol and rehydrated with alcohol (100°, 96°, 70° and 50°), and finally with PBS 1X, pH 7.3. Endogenous peroxidase activity was blocked using 3% of hydrogen peroxide in PBS for 15 min. Specific immunoglobulin sites were blocked with 5% of SBF (GIBCO BRL, Grand Island, NY) diluted in PBS, and finally washed three times.

The sections were incubated with the rabbit anti- iNOS primary antibody (diluted 1:200) overnight at 4°C. Sections were then incubated with the secondary antibodies and PBS at room temperature for 30 min, washed three times in PBS and dried. Sections were exposed to peroxidase conjugated with streptavidin for 20 min, washed and re-incubated in 3,3'diaminobenzidine in hydrogen peroxide, 0.3% H_2O_2 in buffer Tris 0,05 M, pH 7.6 until staining. A total of 15 slices from each group were analyzed with a 40x objective. The detection of iNOS was classified as (+ + +) intense, (+ +) moderate and (+) mild.

Nitrotyrosine Analysis

Nirotyrosine analysis was carried out in slices of cardiac and skeletal muscle of 5 μm (n: 4 from heart failure group and n: 2 from control group). The technique was performed as explained in the preceding paragraph for the determination of iNOS, however, here the primary antibody was a monoclonal antinitrotyrosine (1/100). The sections were then incubated with the secondary multilinker and

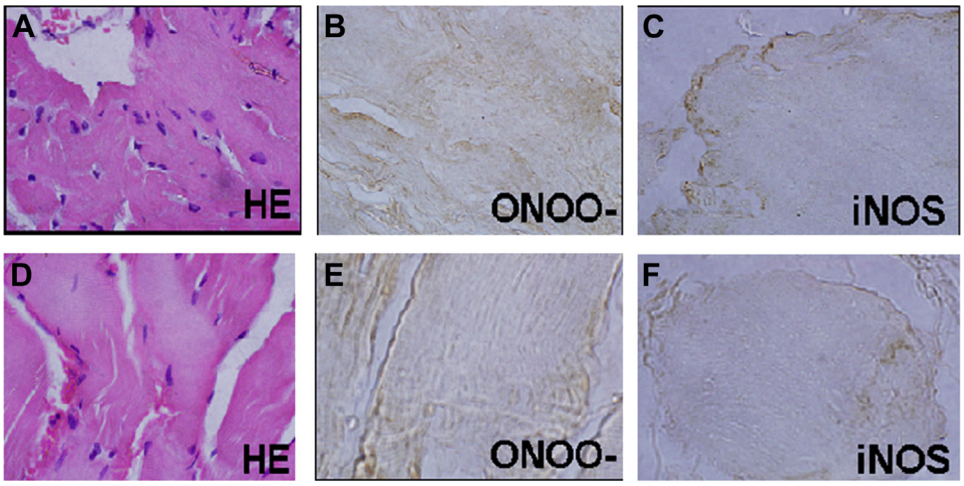

Figure 1. Presence of inducible nitric oxide synthase (iNOS) and nitrotyrosine ($ONOO^-$) in tissues from control group. (A) Cardiac sample stained with hematoxylin-eosin. (B) Cardiac sample showing $ONOO^-$ distribution. (C) Cardiac sample showing iNOS location in the cells. (D) Skeletal muscle sample stained with hematoxylin-eosin. (E) Skeletal muscle sample showing $ONOO^-$ distribution. (F) Skeletal muscle sample showing iNOS distribution. (A color figure can be found in the online version of this article.)

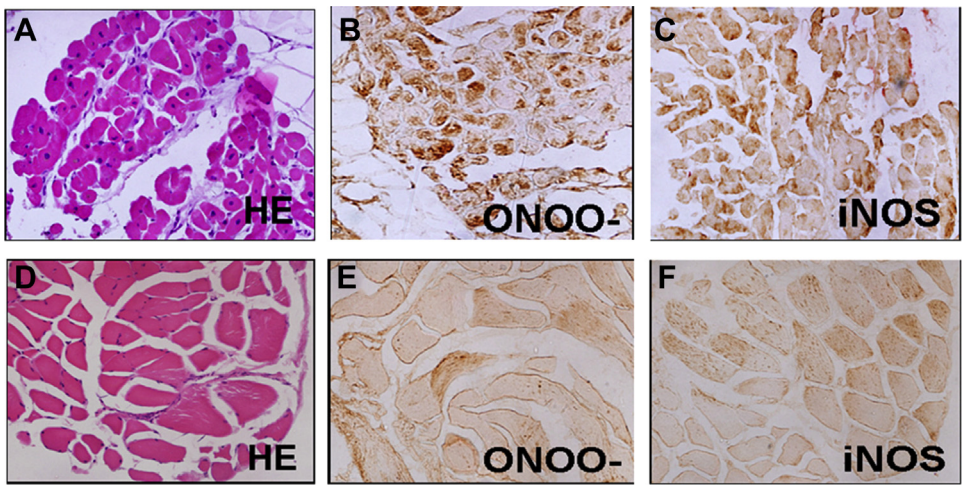

Figure 2. Presence of inducible nitric oxide synthase (iNOS) and nitrotyrosine ($ONOO^-$) in tissues from heart failure patients. (A) Cardiac sample stained with hematoxylin-eosin. (B) Cardiac sample showing $ONOO^-$ distribution. (C) Cardiac sample showing iNOS location in the cells. (D) Skeletal muscle sample stained with hematoxylin-eosin. (E) Skeletal muscle sample showing $ONOO^-$ distribution. (F) Skeletal muscle sample showing iNOS distribution. (A color figure can be found in the online version of this article.)

continued as described until staining. A total of 15 slices from each group were analyzed with a 40x objective. The detection of nitrotyrosine was classified as (+ + +) intense, (+ +) moderate and (+) mild.

Mitochondrial Respiratory Complex III Activity

Included were *n*: 8 from heart failure group and *n*: 4 from control group. The activity of the respiratory complex III (CIII) was monitored by spectrophotometry as previously described (13–15) with slight modifications as follows. CIII (ubiquinol-cytochrome c oxidoreductase): mitochondria were suspended in 50 mmol Tris-HCl buffer, pH 7.4 containing 1 mmol EDTA, 250 mM sucrose, 2 mM KCN, and 50 μM oxidized cytochrome c. After the addition of 80 μM reduced DB (DBH_2), reduction of cytochrome c was measured at 550 nm (ε 19.0 mol^{-1} cm^{-1}). Protein concentrations were calculated by the Bradford assay (16). We only studied the activity of CIII because this is the complex most affected during heart failure (5).

Statistical Analysis

Results are shown as mean ± standard error. The obtained data were analyzed by ANOVA and multiple comparisons by Fisher's exact test and χ^2 test for categorical variables. Significance level was set at $p < 0.05$.

Results

Ages from control group (49.5 ± 3.64) and heart failure patients (57.7 ± 1.77) did not show statistical differences. The clinical features presented by the 21 patients from the heart

Table 2. Immunohistochemistry analysis of inducible nitric oxide synthase (iNOS) and nitrotirosine ($ONOO^-$) in cardiac and skeletal muscle biopsies obtained from patients with heart failure and from the control group

	Heart failure group (n = 4)				Control group (n = 2)			
	CM		SM		CM		SM	
	iNOS	$ONOO^-$	iNOS	$ONOO^-$	iNOS	$ONOO^-$	iNOS	$ONOO^-$
Intensity	+++ (33.6%) ++ (58.3%) + (7.6%)	+++ (23.8%) ++ (55.6%) + (20.5%)	+++ (0%) ++ (60.4%) + (39.1%)	+++ (0%) ++ (56.9%) + (43.4%)	+++ (0%) ++ (30%) + (70%)	+++ (0%) ++ (27%) + (73%)	+++ (0%) ++ (25%) + (75%)	+++ (0%) ++ (20%) + (80%)
Distribution	100% Diffuse	100% Diffuse	100% Diffuse	100% Diffuse	100% Diffuse	82% Diffuse 18% Focal	100% Diffuse	100% Diffuse
Location	Membrane Cytoplasm	Cytoplasm	Membrane Cytoplasm	Cytoplasm	Membrane Cytoplasm	Cytoplasm	Membrane Cytoplasm	Cytoplasm

CM: cardiac muscle; SM: skeletal muscle +++ Intense, ++ moderate, + mild; $p < 0.05$ for when compared iNOS and $ONOO^-$ between control and cardiac muscle from heart failure patients. $p < 0.05$ for when compared iNOS and $ONOO^-$ between control and skeletal muscle from heart failure patients. χ^2 and Fisher tests were used.

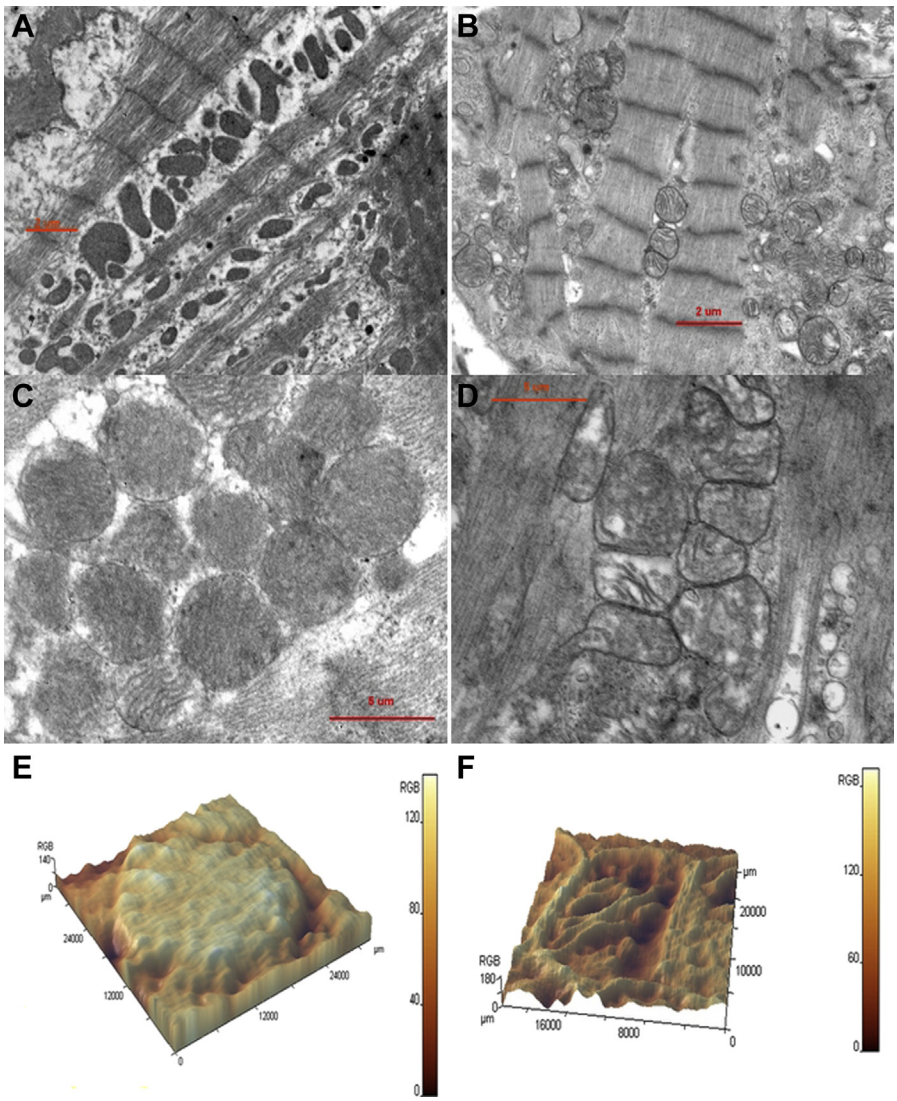

Figure 3. Cardiac mitochondria ultrastructural analysis. (A) Cardiac ultrastructure from control patients (*n*: 3) where sarcomere and mitochondria can be observed, x10,000. (B) Cardiac ultrastructure from heart failure patients (*n*:10) where mitochondria with dilated cristae can be observed, x10,000. (C) Cardiac ultrastructure from control patients where mitochondria with a conserved structure can be observed, x27,800. (D) Ultrastructure of cardiac mitochondria from heart failure patients showing separate cristae with increase of matrix volume, x27,800. (E) Three-dimensional reconstruction of cardiac mitochondria from control patients. (F) Three-dimensional reconstruction of cardiac mitochondria from heart failure patients showing a serious lack of substance in the matrix area and dilated cristae. (A color figure can be found in the online version of this article.)

failure group and the six patients included in the control group are shown in Table 1. The etiology of heart failure was dilated cardiomyopathy in all patients because 76% had at least one obstructed coronary artery and the remainder had valvulopathy. Most of the patients were hypertensive and the rest presented diabetes, dyslipidemia or at least two risk factors combined; the average left ventricular ejection fraction of the patients included in the heart failure group was 51 $\pm$ 2.1%. These results allowed us to classify these patients as belonging to the heart failure group with preserved left ventricular ejection fraction (LVEF).

Immunohistochemical Analysis

In order to evaluate ROS levels in the studied groups, we analyzed the presence of iNOS and membrane lipid peroxidation determining the presence of the peroxide nitrite ion ($ONOO^-$) in heart and skeletal muscle samples from patients from the control (*n*: 2) (Figure 1) and heart failure (*n*:4) groups (Figure 2). For both iNOS and $ONOO^-$, an intense or moderate reaction was found with a diffuse distribution in $>80\%$ of the slices of cardiac muscle obtained from the patients with heart failure. This percentage was significantly higher than the one presented by the control

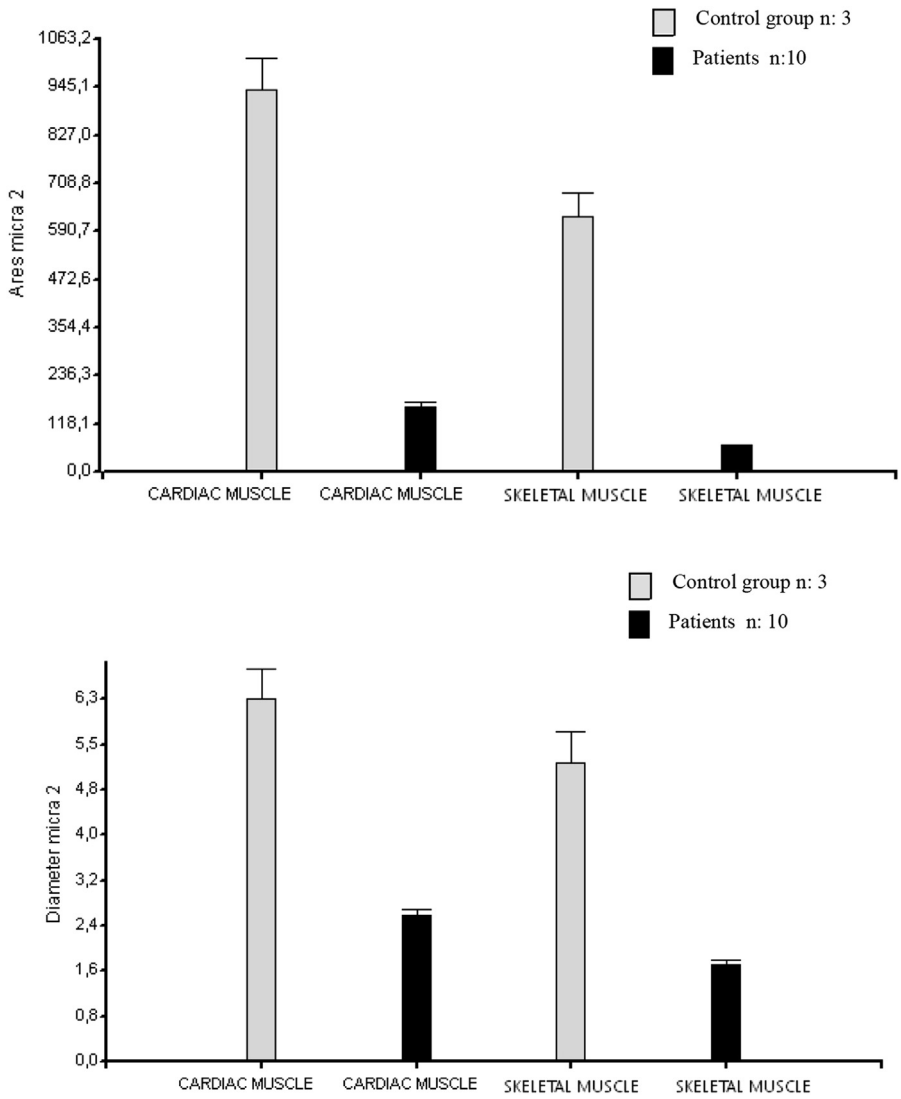

Figure 4. Cardiac and skeletal muscle mitochondrial areas and diameters from control and heart failure patients. Results are shown as mean ± SE. Both parameters were calculated with Axiovision 4.8 program and the statistical analyses were obtained by ANOVA test. $p < 0.01$ between control and patients for both types of muscle.

group ($p < 0.05$) that showed the majority of the slices with a mild reaction (Table 2). Inducible NOS was detected both in the plasma membrane and the cytoplasm, whereas nitrotyrosine was detected only in the cytoplasm (Table 2).

Similarly, the presence of nitrotyrosine and iNOS in skeletal muscle samples from patients with heart failure (Figure 2) was fundamentally moderate with a diffuse distribution, significantly higher than in the control group

Table 3. Results of the ultrastructural analysis of cardiac mitochondria of control group (n=5 micrographs/patient mouse) and heart failure group (n = 5 micrographs/patient)

	Cardiac muscle			Skeletal muscle		
	Heart failure group (n = 10)	Control group	p	Heart failure group	Control group	p
Total area occupied by mitochondria (μm2)	16839.29	55271.51	<0.001	6521.3	23819.56	<0.001
Average area mitochondria (μm2)	160.37 ± 9.87	936.81 ± 78.48	<0.001	61.52 ± 5,39	626.83 ± 57,8	<0.001
Average mitocondrial diameter (μm)	2.57 ± 0.10	6.35 ± 0.52	<0.001	1.69 ± 0.09	5.21 ± 0.56	<0.001
Average number of mitochondria	74 ± 15.88	45 ± 1		61 ± 10.92	75 ± 15.35	

Results are expressed as mean ± standard error.

Table 4. Degree of cardiac and skeletal muscle mitochondrial alteration in relation to major Framingham clinical criteria and New York Heart Association (NYHA) classification

	Degree of mitochondrial alteration							
	Grade 0		Grade I		Grade II		Grade III	
	CM	SM	CM	SM	CM	SM	CM	SM
Control group (n = 3)	70%	81%	32%	14%	0%	1%	0%	0%
None Framingham clinical criteria (n = 4) Grade II NYHA	1%	10%	48%	28%	30%	48%	15%	14%
1 - 3 major Framingham clinical criteria (n = 2) Grade II NYHA	8%	30%	56%	39%	24%	17%	7%	15%
4 - 6 major Framingham clinical criteria (n = 4) Grade II NYHA	5%	17%	40%	28%	48%	40%	12%	22%

CM, cardiac muscle; SM, skeletal muscle.
Chi square and Fisher tests were used.

(Figure 1; $p < 0.05$) and with a similar distribution as in the heart (Table 2).

Cardiac Mitochondrial Ultrastructural Analysis

The myocardium and mitochondria from the control group (Figures 3A, 3C and 3E) presented normal ultrastructure. On the other hand, mitochondria from the patients with heart failure (Figures 3B, 3D and 3F) showed an increment in their matrix with a reduction in the number of cristae and structural disorganization, as clearly seen in the three-dimensional analysis (Figures 3E and 3F). The area occupied by mitochondria was reduced in 78% when compared with the control ($p < 0.001$); additionally, the diameter of these organelles was significantly reduced ($p < 0.001$; Figure 4 and Table 3). Between 90 and 99% of these mitochondria presented at least one of the following alterations: increment in their matrix, disorganized cristae, or swelling or membrane disruption. In relation to the Framingham clinical criteria, even patients without any Framingham major criteria presented 48% of their cardiac mitochondria with grade I alterations, 30% with grade II and 15% with alterations compatible with grade III (Table 4).

Skeletal Muscle Mitochondrial Ultrastructural Analysis

Mitochondria from the skeletal muscle samples from the control group presented a normal ultrastructure (Figures 5A, 5C and 5E), whereas those obtained from the patients with heart failure (Figures 5B, 5D and 5F) were smaller, presented hydropic degeneration and membrane disruption. These characteristics were similar to those described for the cardiac biopsies from the same patients. Additionally, the area occupied by mitochondria was reduced in 72% when compared to the control group ($p < 0.001$) and the diameters were also significantly reduced when compared to those from the control group ($p < 0.001$) (Figure 4 and Table 3). When the skeletal muscle mitochondrial alterations were analyzed taking into consideration the Framingham clinical criteria (Table 4), we found that when the cardiac mitochondria were damaged, the skeletal muscle were also altered. These alterations, as the ones in the cardiac mitochondria, appeared even in patients without the presence of any Framingham major criteria.

Cardiac and Skeletal Muscle Mitochondrial Respiratory CIII Activity

In order to evaluate mitochondrial function, we analyzed the activity of CIII of the respiratory chain (situated in the cristae) in mitochondria obtained from cardiac and skeletal muscle samples from patients from the heart failure (n: 8) and control groups (n: 4). CIII activity was significantly diminished in the myocardium from patients with heart failure ($p < 0.01$). CIII activity was also reduced in the skeletal muscle samples from this group, but we could not demonstrate significant differences when compared to the control group (Figure 6).

Discussion

Many observations have drawn attention to mitochondria as one of the factors to be considered in the physiopathology of a failing heart and that their alterations are involved in the progression of heart failure (5,17).

Some studies have shown that patients with heart failure have a diminished activity of the mitochondrial respiratory enzymes (5,15) and a 25−30% reduction in ATP levels (18,19). In the present study, cardiac mitochondria from patients with heart failure clearly demonstrated a significantly reduced CIII enzyme activity ($p < 0.01$) related to the reduced number of cristae and the disorganization observed, the lesser area occupied by mitochondria and their reduced diameter (Figure 3), which suggest not only structural alterations but also functional modifications with difficulty to maintain an effective membrane potential due to changes in transmembrane gradients. The lower ability

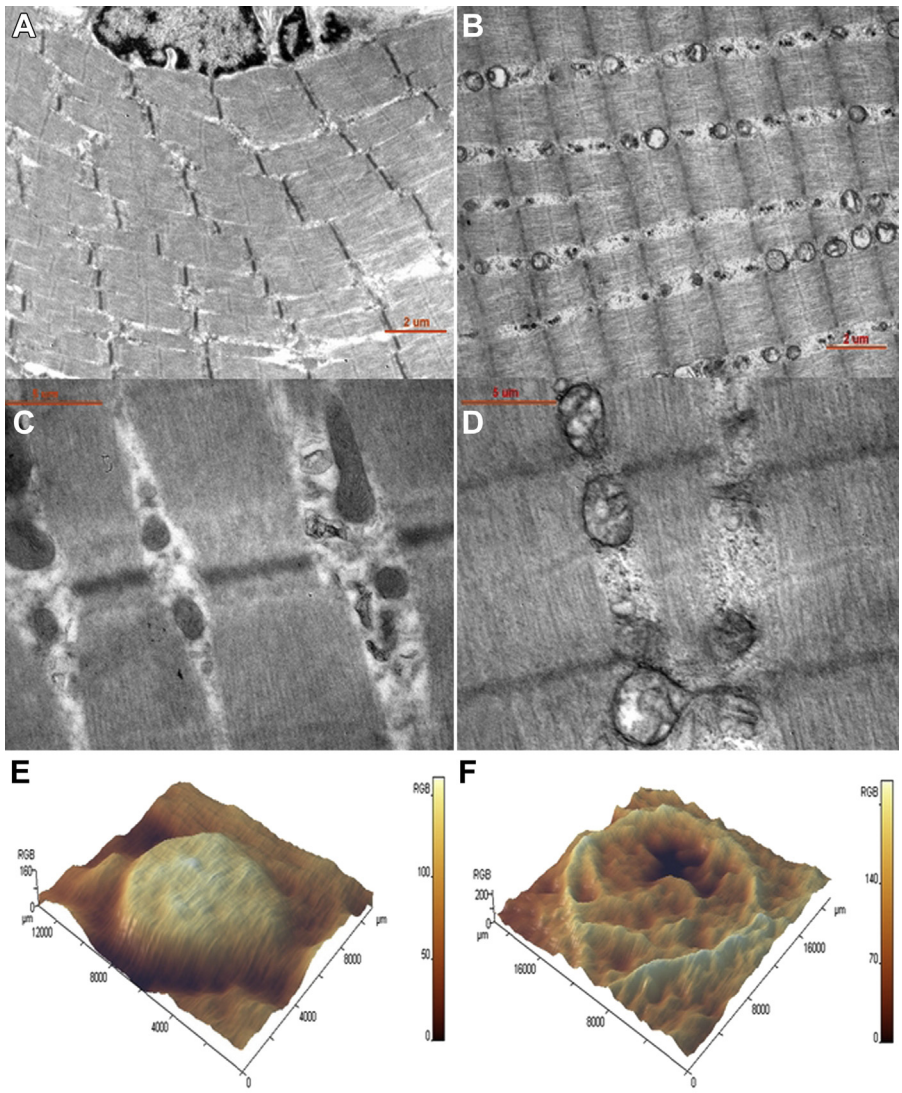

Figure 5. Skeletal muscle mitochondria ultrastructural analysis. (A) Skeletal muscle ultrastructure from control patients (*n*: 3): sarcomere and mitochondria can be observed, x10,000. (B) Skeletal muscle ultrastructure from heart failure patients (*n*: 10) where mitochondria can be seen, x14,600. (C) Skeletal muscle ultrastructure from control patients: mitochondria with normal structure can be observed, x27,800. (D) Ultrastructure of skeletal muscle mitochondria from heart failure patients showing diminished number, separate cristae and electron lucid structures, x27,800. (E) Three-dimensional reconstruction of skeletal muscle mitochondria from control patients. (F) Three-dimensional reconstruction of skeletal muscle mitochondria from heart failure patients showing dilated cristae and a serious lack of substance in the matrix area. (A color figure can be found in the online version of this article.)

to produce energy can be proposed as a consequence of the diminished and disorganized cristae (5).

The present results also demonstrate similar ultrastructural alterations in skeletal muscle mitochondria when compared with the cardiac ones; the reduction in cardiac and skeletal muscle mitochondrial diameter was~67% in heart failure patients when compared to the control group; both tissues presented a reduction of ~75% in the total area occupied by mitochondria.

These results demonstrate that, in heart failure, cardiac and skeletal muscle mitochondria are involved in a similar manner, showing comparable structural alterations. However, we did not find modifications in CIII enzyme activity in samples from skeletal muscle as shown for cardiac samples. Marked reductions in the complexes of the respiratory chain have been described for both cardiac and skeletal muscles in canine and rat heart failure models (20).

Some authors (5) propose that mitochondrial contribution to heart failure is related to ROS production because the majority of intracellular ROS comes from mitochondrial metabolism.

Every cell type, including cardiomyocytes, is capable of generating ROS, with mitochondria, xanthine oxidase and NADPH oxidase being their major sources. Under pathological conditions such as heart failure, ROS levels are increased in the heart as demonstrated here by the iNOS and nitrotyrosine analysis and as has also been described by other authors (3,5,7). We also demonstrated an

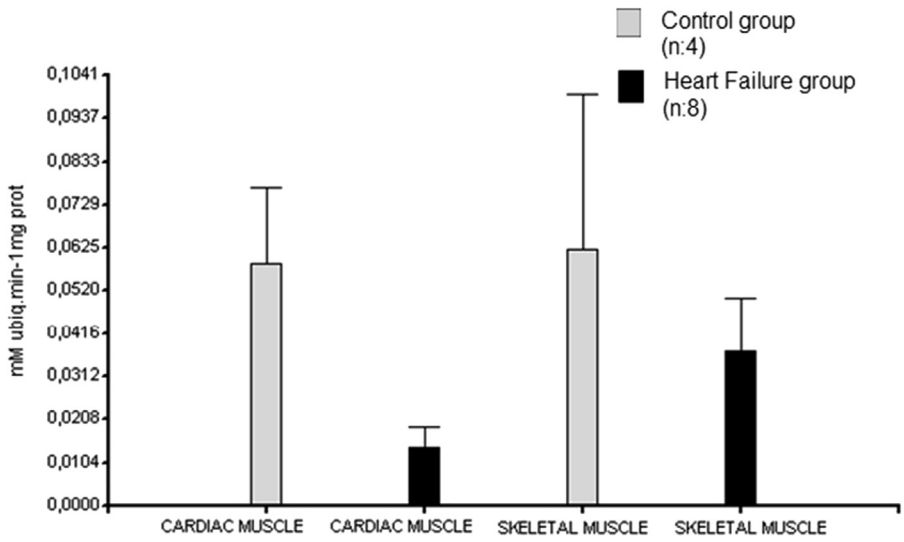

Figure 6. Enzymatic activity of CIII from cardiac and skeletal mitochondria (n: 8 from heart failure group, n: 4 from control group). Results are shown as mean ± SE and statistical analyses were performed by ANOVA test; $p < 0.01$ when compared CIII activity from mitochondria from control and heart failure groups.

increment in ROS levels in skeletal muscle with less intensity than in cardiac tissue, but significantly higher than the control group ($p < 0.05$).

Mitochondria from cardiomyocytes can neutralize ROS by forming antioxidant enzymes. In chronic diseases, however, such as heart failure, the homeostatic control becomes insufficient, which would be worsened by alterations in mitochondrial structure and function such as those found in the present results.

When the patients were grouped using the major clinical Framingham criteria, we demonstrated that even those patients without any major criteria presented severely damaged mitochondria (both from cardiac and skeletal muscles). Fatigue and dyspnea frequently present in patients with heart failure (20) seems to be related to increased oxidative stress (21), not only in the heart tissue but also in the skeletal muscle (22), which would be explained by the results found in the present study regarding the mitochondrial alterations in the later tissue. Other authors have demonstrated augmented ROS levels in the skeletal muscle from mice with experimental heart failure and that this increment is originated in the mitochondria (23). Depressed mitochondrial function in cardiac and skeletal muscles during heart failure in mice has also been found to be linked to altered expression of mitochondrial protein genes (24).

The present results demonstrate that ROS levels are augmented in both cardiac and skeletal muscles and mitochondria from both tissues are compromised in patients with heart failure. Heart failure is not only characterized by hemodynamic adaptations but also by sympathetic overdrive, activation of immune response with systemic inflammation and a catabolic state; consequently enzymatic and mitochondria abnormalities are produced in skeletal muscle that provoke a limitation of exercise in these patients (25,26).

In summary, we found some parallelism between cardiac and skeletal muscle mitochondrial alterations, and these mitochondrial alterations begin before the major clinical Framingham criteria are installed, pointing to mitochondria as one of the possible factors responsible for the evolution of the cardiac disease.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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