Oxidative stress of myosin contributes to skeletal muscle dysfunction in rats with chronic heart failure

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EXERCISE INTOLERANCE and muscle fatigue are main factors restricting the daily life of patients with chronic heart failure (CHF). Numerous studies have reported intrinsic muscle abnormalities affecting skeletal muscles (38) and, in particular, diaphragm muscle in patients and animal models of CHF (24, 32). Cellular and molecular bases contributing to intrinsic muscle alterations in CHF include altered energy metabolism (50), impaired calcium homeostasis (52), changes in contractile protein isoforms (31, 47), and muscle atrophy (27).

Myosin represents the molecular motor of force generation, as it produces a power stroke that drives the myosin molecule along the actin filaments (19). Structural modifications of the myosin head are known to modulate muscle contractile properties. Using Huxley’s mathematical model of muscle contraction (19), we have previously reported that the force produced per cross-bridge interaction is reduced in diaphragm muscle of CHF (24), suggesting that actomyosin dysfunction may contribute to muscle weakness in CHF. Cross-bridge kinetics in whole diaphragm muscle are difficult to interpret given that modulation of actomyosin interactions in heart failure may relate to several factors, including delayed sarcoplasmic Ca2+ movements (52), abnormal mitochondrial regulation (50), and/or changes in regulatory protein isoforms (47). However, there is increasing evidence supporting intrinsic actomyosin alterations in CHF skeletal muscle. Indeed, an increased amount of proapoptotic proteins, including activated caspase-3, a pivotal protease involved in cell destruction, has been reported in skeletal muscle of animals with CHF (26). Activated caspase-3 has been reported to cleave several myofibrillar proteins, including cardiac myosin light chain (34), and thus might contribute to actomyosin dysfunction. Oxidative stress has also been reported in skeletal muscle during CHF (16, 48, 50), and contractile proteins have been identified as highly sensitive targets of oxidation (3, 10, 33). In addition to the multifaceted relationship with contractile function regulation, increased production of reactive oxygen species (ROS) may induce irreversible modification of contractile proteins such as protein carbonylation (3, 10), nitrotyrosylation (22, 33), and the formation of lipid peroxidation products (6). In skeletal muscles, formation of reactive carbonyl groups is the more general and by far the most widely used marker of protein oxidation (3, 9, 10).

The first aim of our study was to determine whether intrinsic functional abnormalities in myosin contributed to skeletal muscle dysfunction in CHF. In vitro motility assays with purified myosin molecules from diaphragm, the main inspiratory muscle, were performed in a rat model of CHF induced by chronic pressure overload. Although skeletal muscle weakness during CHF is part of a generalized myopathy (4), respiratory and limb skeletal muscles are not necessarily affected in the same way or to the same extent (7). Deconditioning is a potential factor that may contribute to limb muscle weakness in CHF. In contrast, the diaphragm muscle contracts continuously along with the heart, maintaining an almost continual activity that allows it to keep providing mechanical work throughout the day (41).
muscle strip per rat was carefully dissected in situ. Each muscle was from the soleus muscles as previously described (7). In brief, one on muscle strips from the ventral part of the costal diaphragm and used for the mechanical muscle study. The remaining diaphragm and tissue limitation. Thus, at the moment of killing, 10 rats/groups were included isolated muscles, in vitro motility, myosin isoforms, West- rats. Although, ideally, all biochemical and functional experiments, was excluded from the study. Another one died just after surgery. mean VW/BW ratio in the C group (normalized VW/BW ratio). One C group and expressed as a percentage. Cardiac hypertrophy was mined by dividing the LV mass by the mean value of LV mass in the different cardiac cycles (11). The normalized LV mass was deter- moment of death. Internal end-systolic (ESLVD) and end-diastolic (Acuson 128XP; Acuson, Mountain View, CA) and papillary muscle were assessed in C and CHF rats by means of Doppler echography without placement of the clip (C group, (12). Age-matched control animals underwent the same procedure (21, 42, 51). In a subgroup of experiments, the functional consequences of myosin oxidation were analyzed. To this end, myosins from C diaphragm and soleus muscles were treated with peroxynitrite (ONOO-) (Cayman, Ann Arbor, MI). ONOO- concentrations were determined by spectrophotometry (€302 nm = 1,670 mol·1·cm-1) and then diluted to 100, 200, or 500 nmol of ONOO- in myosin samples immediately before infusion. Because ONOO- was diluted in NaOH (final myosin buffer concentration 3 mmol), a baseline motility group was created in which myosin was incubated with 3 mmol of NaOH to discriminate between the effects of ONOO- and the effects of NaOH. ONOO- induced myosin oxidation was assessed by measuring protein carbonyls detected by a reaction with 2,4-dinitrophenylhydrazine (DNPH) and the conversion to hydrazones (Oxyblot Kit, Intergen; see below) (3). The movement of actin filaments was observed under a Zeiss epifluorescence microscope (Axiovert 200, 100/1.30 lens, Jena) equipped with an intensified camera (Hamamatsu C 2400, Hamamatsu City, Japan) and recorded on videotape. The mean velocities of each filament were analyzed using N. J. Carter’s freeware RETRAC program. MHC and MLC isoform compositions. Myosin isoform composition was determined in purified myosin. SDS-PAGE minigel electrophoresis was performed in a Bio-Rad Mini-Protein II Dual slab cell system. MHCs were separated on 8% polyacrylamide gels containing 30% glycerol for 24 h at 4°C and 70 V (7, 35). MLCs were separated on 15% polyacrylamide gels containing 10% glycerol for 1 h at room temperature and 250 V (47). Gels were stained with Coomassie blue or silver. MLC isoforms were identified on the basis of known MLC migration order (45), Western blot with specific anti-MLC1 (clone F 109 17A5, dilution 1/2,500; BioCytex) and anti-MLC2 (clone F 109 3E1, dilution 1/10,000; BioCytex) antibodies, and their comigration with commercial MLCs (Sigma, M8981). Myosin isoforms were quantified using Image Gauge software. Western blotting. Western blotting was performed on total protein extracts and myosin fractions obtained from muscle fragments. Ten micrograms of muscle protein per lane were loaded on 7.5, 12, or 15% SDS-polyacrylamide resolving gels. Selective antibodies were used to detect caspase activation and MLC cleavage in cytosolic protein fraction (13) and myofibrillar (46) extracts, respectively. These included anti-caspase-3, active (C8487, dilution 1/1,000; Sigma), anti-MLC1 (clone F 109 17A5, dilution 1/2,500; BioCytex); and anti-
Table 1. Characteristics of animals and cardiac function

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>CHF</th>
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<tr>
<td>Animal characteristics</td>
<td>(n=20)</td>
<td>(n=20)</td>
</tr>
<tr>
<td>BW, g</td>
<td>399±6</td>
<td>351±9*</td>
</tr>
<tr>
<td>VW, mg</td>
<td>949±5</td>
<td>1,956±98*</td>
</tr>
<tr>
<td>Normalized VW/BW ratio, %</td>
<td>100±2</td>
<td>217±10*</td>
</tr>
<tr>
<td>Lung weight, g</td>
<td>1.4±0.1</td>
<td>3.5±0.3*</td>
</tr>
<tr>
<td>Echocardiographic characteristics</td>
<td>(n=10)</td>
<td>(n=10)</td>
</tr>
<tr>
<td>Vmax, Lo/s</td>
<td>2.6±0.1</td>
<td>1.0±0.1*</td>
</tr>
<tr>
<td>P0, mN/mm²</td>
<td>40±3</td>
<td>21±3*</td>
</tr>
<tr>
<td>+dP/dtmax, mN/mm²</td>
<td>316.8±27.8</td>
<td>134.0±24.9*</td>
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<tr>
<td>−dP/dtmax, mN/mm²</td>
<td>124.1±15.0</td>
<td>77±16.2*</td>
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<tr>
<td>Fractional LV area shortening</td>
<td>0.43±0.02</td>
<td>0.32±0.03*</td>
</tr>
<tr>
<td>LV mass, g</td>
<td>864±49</td>
<td>1,949±128*</td>
</tr>
<tr>
<td>Normalized LV mass, %</td>
<td>100±3</td>
<td>227±15*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. CHF, congestive heart failure; BW, body weight; VW, ventricle (left + right) weight; P0, total tension; Vmax, maximum unloaded shortening velocity; EDLVD, end-diastolic left ventricular (LV) diameter; ESLVD, end-systolic left ventricular diameter. *P < 0.05.

RESULTS

Assessment of CHF. CHF rats showed decreased body weight associated with a marked increase in absolute and relative weights of the right and left ventricles (Table 1). As a result, the VW/BW ratio was 2.2-fold higher in CHF than in C (Table 1). Lung weight was 2.5-fold higher in CHF than in C rats, strongly suggesting lung congestion in CHF (Table 1). Echocardiography showed marked cardiac hypertrophy associated with a significant decrease in the fractional left ventricular shortening area (Table 1). The mechanical performance of LV papillary muscle was markedly reduced in CHF compared with C (Table 1). Total isometric tension and maximum unloaded muscle shortening velocity were, respectively, 2.0- and 2.6-fold lower in CHF than in C (each P < 0.05). This was associated with a substantial reduction in both positive and negative peak force derivatives.

Diaphragm and soleus muscle mechanics. Mechanical performance of diaphragm and soleus muscles is illustrated in Fig. 1. In diaphragm and soleus muscles, peak tetanic tension in CHF fell by ~35% compared with C (each P < 0.05) (Fig. 1A). In diaphragm, Vmax was significantly slower in CHF than in C (P < 0.05), whereas in soleus muscle, Vmax did not significantly differ between groups (Fig. 1B). Twitch tension and twitch Vmax followed the same patterns of change as those reported in tetanus (data not shown).

Velocity of actin sliding over C and CHF myosin. To determine the effects of CHF on motor function, in vitro actin motility assays were performed using myosin isolated from diaphragm and soleus. Velocities of actin filament sliding over myosins are shown in Fig. 2. When compared with C diaphragm, velocity driven by myosin extracted from CHF diaphragm was shifted toward lower values, mean velocities being ~16% lower in CHF diaphragm than in C (P < 0.05) (Fig. 2A). Likewise, mean motility speed of actin propelled by CHF MLC2 (clone F 109.3E1, dilution 1/10,000; Biocytex). Human recombinant activated caspase-3 (BioMol, Quantzyme Assay Kit AK-703) was used as a positive control. Oxidized proteins were detected by analyzing protein carbonyls using the Oxyblot Kit (Intergen) according to the manufacturer’s instructions and as previously described (3). In brief, denatured protein samples were derivatized to 2,4-dinitrophenylhydrazone by reaction with 2,4-DNP and separated by electrophoresis. DNP-derivatized proteins were detected by using a polyclonal anti-DNP moiety (3). A protein carbonyl detection procedure without the derivatization step was used to evaluate the selectivity of carbonyl measurements (negative controls). Secondary anti-mouse or anti-rabbit horseradish peroxidase-labeled antibodies (Amersham, 1/5,000) were used for detection. The membranes were revealed with enhanced chemiluminescence (ECL) substrate (Amer- sham, 1/5,000) followed by fluorescein isothiocyanate (FITC)-conju- gated streptavidin (Amersham, 1/50). After washing, sections were mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) and observed with a DMRB Leica microscope (Leica-Microsystems, Rueil-Malmaison, France) equipped with epifluorescence optics. A minimum of 100 muscle fibers from each animal in each study group were counted using an arbitrary intensity scale (e.g., 0 = absence, 1 = mild, and 2 = high-intensity staining). Sections exposed only to secondary antibodies were used as negative controls and showed very low background staining.

Statistical analysis. Data are presented as means ± SE. Comparisons of mechanical parameters between groups were performed using Student’s unpaired t-test. A P value < 0.05 was considered significant, and all P values were two tailed.

Fig. 1. Mechanical parameters in sham (control) and heart failure (chronic heart failure; CHF) diaphragm and soleus muscles. Peak tension (A) and maximum unloaded shortening velocity (Vmax, B) obtained from tetanic contractions. Values are means ± SE (n = 10 animal/group). Lo is the initial muscle length at which active tension is maximum. *P < 0.05 compared with controls.
soleus myosin was significantly reduced compared with C values (*P < 0.05) (Fig. 2B).

**Myosin isoforms.** Myosin electrophoretic patterns for MHCs in diaphragm and soleus are illustrated in Fig. 3, A–D. Four MHC isoforms were detected in the C rat diaphragm, the proportion of slow MHC-I type and overall fast MHC isoforms being, respectively, 25% and 75% (Fig. 3, A and C). When compared with C, CHF diaphragms contained more IIX MHC isoforms (*P < 0.05), associated with a slight reduction in the proportion of type IIB MHC (nonsignificant). The ratio of slow-to-fast MHC isoform did not significantly differ between C and CHF diaphragms. In diaphragm, four MLC isoforms were identified as MLC1slow, MLC1fast, MLC2, and MLC3 (Fig. 3E), the relative proportions of which did not significantly differ between C and CHF diaphragms (Table 2).

In C soleus, slow MHC isoform accounted for ~95% of the total MHC, and no significant differences in MHC isoform composition were observed between C and CHF soleus (Fig. 3, B and D). MLCs of soleus were identified as MLC1slow and MLC2 (Fig. 3E). There was no significant difference in MLC isoform pattern or in the ratio of total MLC1 to total MLC2 content between C and CHF soleus (Table 2).

**Myofibrillar protein cleavage and caspase-3 activation.** Western blotting was used to determine whether MLC cleavage might contribute to contractile dysfunction (Fig. 4, A and B). In diaphragm and soleus, immunoblotting with anti-MLC1 (Fig. 4A) and anti-MLC2 (Fig. 4B) antibodies did not detect the presence of additional protein fragments or complexes. In addition, there was no indication of caspase-3 activation within the different groups (Fig. 4, C and D).

**Evidence for oxidative stress in diaphragm and soleus from CHF.** To determine whether oxidative damage of myosin contributed to reduced in vitro motility, we next analyzed protein carbonylation in myosin extracts from CHF (Fig. 5). In both diaphragm and soleus, there was one major band corresponding to oxidized MHC (Fig. 5, A and B). When compared

### Table 2. Myosin light chain isoform composition in diaphragm and soleus muscles

<table>
<thead>
<tr>
<th></th>
<th>LCI Fast, %</th>
<th>LCI Slow, %</th>
<th>LC2, %</th>
<th>LC3, %</th>
</tr>
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<tbody>
<tr>
<td>DIA control</td>
<td>31 ± 1</td>
<td>16 ± 1</td>
<td>48 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>DIA CHF</td>
<td>28 ± 1</td>
<td>13 ± 1</td>
<td>54 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>SOL control</td>
<td>67 ± 2</td>
<td>33 ± 3</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>SOL CHF</td>
<td>66 ± 2</td>
<td>34 ± 2</td>
<td>4 ± 2</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE (n, number of animals), expressed as a percentage of total myosin light chain (MLC) isoform; n = 9 in each group. DIA, diaphragm; SOL, soleus.
with controls, oxidized MHCs were nine- and fivefold higher in CHF diaphragm and soleus, respectively (Fig. 5, C and D, each \( P < 0.05 \)). In addition, we examined lipid peroxidation in the soleus and diaphragm muscles from CHF rats (Fig. 6). In both muscles, CHF caused a significant increase in HNE-positive fibers (staining intensities 1 and 2) compared with C muscle fibers, associated with a concomitant decrease in the percentage of negative fibers (staining intensity 0). Most of the HNE adduct signals were localized in the sarcolemma, although there was some intracellular localization.

**Effects of myosin oxidation on function.** The relationships between MHC oxidation levels and the degree of myosin dysfunction are shown in Figs. 7 and 8. In both diaphragm and soleus muscles, myosin exposure to increasing ONOO\(^-\) concentrations was associated with an increasing amount of oxidized MHC (Fig. 7). The effects of myosin carbonylation on sliding velocities were then determined (Fig. 8). Baseline motilities following vehicle control (NaOH, 3 mmol) were, respectively, 2.0 ± 0.1 and 0.6 ± 0.1 \( \mu \)m/s in diaphragm and soleus and were unaltered compared with C values (without NaOH). Myosin exposure to 200 and 500 nmol of ONOO\(^-\) was associated with a reduction in mean velocities of almost 20 and 30%, respectively (\( n = 100 \) filaments in each group, each \( P < 0.05 \)). In both muscles, there was a linear relationship between the quantity of oxidized MHC and the reduction in myosin sliding velocity (Fig. 8).

**DISCUSSION**

The principal findings of our study were as follows: 1) in rats with CHF, myosin function was impaired, and this may have contributed to depressed skeletal muscle performance; 2) myosin carbonylation was identified as a contributing factor in the reduced functionality of diaphragm and soleus muscle myosin in CHF; and 3) myosin isoform switching, cleavage, or degradation of MLC was unlikely to explain the impaired cross-bridge cycling in CHF muscle.

**Contractile dysfunction of diaphragm muscle in CHF.** Studies of diaphragm muscle function in CHF are relatively sparse. From a theoretical point of view, reduced contractile properties can arise from fiber atrophy, from variations in metabolic or ionic conditions inside the fiber, from differences in actomyosin interactions, or from variations in regulatory contractile proteins (41). Although fiber cross-sectional area tended to decrease in CHF, there was no significant difference between groups (data not shown), a finding consistent with that previously reported in the compensatory stages of CHF (50). To the best of our knowledge, our study is the first to report in vitro motility assays in diaphragm and other skeletal muscle from CHF. We analyzed both muscle mechanics and in vitro actin filament sliding so as to better understand the mechanisms involved in muscle weakness. According to Huxley’s theory (19), cross bridges act as independent force generators. Therefore, muscle force depends on both the elementary force produced per single cross bridge and the total number of cross bridges.
bridges (19). $V_{\text{max}}$ and/or in vitro sliding velocity are limited by the rate constant of cross-bridge dissociation at the end of its working stroke (i.e., $g_2$) (19). Therefore, changes in kinetic parameters do not necessarily involve a change in isometric tension. In addition, in vitro motility assays show that variations in filament velocities between C and CHF muscles are due to intrinsic variations in myosin molecules (23, 42), whereas changes in Ca$^{2+}$ movements and/or in regulatory protein isoforms may also modify muscle mechanical performance. In CHF soleus, we found reduced myosin sliding velocities with no change in the maximum shortening velocity of the muscle ($V_{\text{max}}$). The rate constant for ADP release from actomyosin is thought to limit cross-bridge detachment, muscle shortening velocity, and in vitro sliding velocity (44). Therefore, unloaded shortening velocity and in vitro sliding velocity generally correlate, unless there are fortuitously compensating changes in the rates of both association and dissociation of ADP from myofibrils (17). For example, it has been demonstrated that regulatory proteins modulate velocity either by accelerating the rate of MgADP release from acto-heavy meromyosin (HMM)-MgADP or slowing its binding to acto-HMM (17). Because the experimental conditions for the in vitro motility assays (purified myosin without regulatory proteins, zero Ca$^{2+}$, 2 mmol MgATP) were different from those used to measure the shortening velocity (intact isolated muscle with regulatory proteins, 5 mM extracellular Ca$^{2+}$, electrical stimulation), we cannot exclude the possibility that ADP release varied differently in isolated muscle and in vitro motility assays from CHF soleus. Importantly, in the present study, actin velocities were recorded after addition of unlabeled F-actin filaments. Subsequent blockade of nonfunctional myosins argues against the potential confusing effects of noncycling myosins known to decrease the sliding velocity generated by the cycling myosins (18, 42). Therefore, potential mechanisms contributing to the reduced filament velocity in skeletal myosin during CHF in rats involved myosin isoform diversity (4, 43) or structural and posttranslational changes in the myosin head itself (18, 29, 39).

Myosin isoform composition and MLC integrity. In mammals, slow fibers mainly contain the slow MHC isoform...
(MHC-I), whereas fast fibers can be separated into type IIA (expressing mainly IIA MHC), IIX (IIX MHC), and the fastest, type IIB (IIB MHC). In normal skeletal muscle and for a given experimental temperature, the observed filament velocity is largely dependent on MHC isoform expression, and it decreases as the proportion of slow MHC isoforms increases (40). Among fast myosins, both MHC and MLC isoforms help to modulate the velocity of actin translocation, although in a rather complex interplay (4, 30, 43). No consistent changes in fiber type isoform have been reported between respiratory and hindlimb muscles during CHF (reviewed in Ref. 28). Diaphragm muscle weakness during CHF has been associated with a fast-to-slow transformation (47). At variance with these results, transformation of slow muscle to a faster phenotype has been reported in hindlimb skeletal muscle as a potential cause of exercise intolerance during CHF (14, 31, 41, 43). We found an increased proportion of diaphragmatic IIX MHC in the CHF group associated with a slight but nonsignificant decrease in IIB MHC (Fig. 3A). However, a clear transition toward a slow type, required to explain the decrease in shortening velocity and actin filament sliding velocity (4, 43), was not observed in our study. Similarly, there was no evidence of any change in the profile of MLC isoform between C and CHF. We cannot totally exclude the possibility that myosin polymorphisms marked by coexpression of MHC and/or MLC isoforms affect sliding velocity. However, taken as a whole, our results suggest that the change in MHC prevalence is an unlikely explanation for the reduced V_{max} and in vitro sliding motility in skeletal muscle from CHF.

Proteolysis, caspase-3 activation, and heart failure. Several studies in humans (1, 15) and in animal models (27) suggest that CHF is associated with increased skeletal muscle apoptosis. Increased amounts of proapoptotic proteins such as activated caspase-3 have been reported in skeletal muscle from the monocrotaline-treated rat, an animal model of subacute severe right heart failure (26), and a correlation has been found with exercise intolerance (1). It has been suggested that activated caspase-3 might influence the contractile machinery of myocytes through cleavage of myofibrillar proteins including MLC1 (34). MLC1 cleavage in vivo results in an ~20-kDa NH_{2}-terminal fragment and is associated with altered sarcomere organization and mechanical dysfunction (34). To test this hypothesis, the expression of activated caspase-3 and the presence of fragments of MLC were assessed by electrophoresis and Western blotting. No additional ~20-kDa fragments were found in CHF muscles. Finally, no evidence was found of activated caspase-3. This result agrees with those of Persinger et al. (37) who found no evidence of pro- and anti-apoptotic gene expression in skeletal muscle from CHF. It was therefore very unlikely that MLC cleavage contributed to muscle weakness in CHF.

Oxidative stress and myosin carbonylation. Oxidative post-translational modifications of myofibrillar proteins have been associated with important structural and functional alterations (3, 20, 22). Oxidative stress impairs myoplasmic Ca^{2+} homeostasis (22) and inhibits oxidative energy production in the mitochondria (20, 50), both of which may contribute to muscle contractile dysfunction. Myosin has also been reported to be a particularly sensitive target of oxidative damage (33), although a direct link between oxidative stress and myosin dysfunction has yet to be established. Carbonylation assays are widely used for detecting and quantifying oxidative damage to proteins in pathological situations. One of the main advantages is the relative stability of protein carbonyls compared with other markers of oxidative stress (10). Our data provide the first evidence that carbonylation of myosin occurred in CHF (Fig. 5) and may reduce the sliding velocity of myosin (Fig. 8). These results strongly suggest that oxidative modifications of skeletal myosin occurred during CHF and may mediate mechanical dysfunction of the myosin molecule. Myosin is an abundant muscle protein, has a long half-life, and is located close to sites potentially generating ROS such as mitochondria. These facts may help explain, at least in part, why practically only myosin is oxidized. However, the molecular basis for the apparent sensitivity of some proteins to carbonylation is not understood, so that such a conclusion remains speculative. In addition, other widely used markers of oxidative stress, i.e., lipid peroxidation products, were elevated in CHF muscles (Fig. 6). It was thus possible that myosin carbonylation in CHF resulted from the reaction of lysine, cysteine, or histidine amino acids with α- and β-unsaturated aldehydes formed during the peroxidation of polyunsaturated fatty acids (10, 49), although other mechanisms leading to the production of myosin carbonyls could not be excluded (10). We should emphasize that myosin carbonylation formation does not exclude the oxidation of other cytosolic and mitochondrial proteins that may also contribute to contractile dysfunction in skeletal muscles from CHF. Similarly, we cannot exclude the possibility that other translational alterations of myosin related to the redox state of the cell contribute to functional impairment of myosin. However, our data support the hypothesis that oxidative damage causes myosin dysfunction and contributes, at least in part, to skeletal muscle dysfunction in heart failure.

In conclusion, in rat with CHF, diaphragm and soleus muscle weakness was associated with slowing in the movement of actin filaments on myosin heads in in vitro motility studies. While myosin isoform diversity and myosin cleavage were unlikely to explain the modifications in the kinetics of myosin molecular motors, our data demonstrated that oxidative damage was present in CHF skeletal muscles and that carbonylation of myosin induced marked deleterious effects on myosin mechanical function. These findings suggest that oxidative posttranslational modifications within the myosin head contribute to skeletal muscle dysfunction in CHF.

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