

Contraction and Intracellular Ca^{2+} Handling in Isolated Skeletal Muscle of Rats With Congestive Heart Failure

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Abstract—A decreased exercise tolerance is a common symptom in patients with congestive heart failure (CHF). This decrease has been suggested to be partly due to altered skeletal muscle function. Therefore, we have studied contractile function and cytoplasmic free Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$, measured with the fluorescent dye indo 1) in isolated muscles from rats in which CHF was induced by ligation of the left coronary artery. The results show no major changes of the contractile function and $[\text{Ca}^{2+}]_i$ handling in unfatigued intact fast-twitch fibers isolated from flexor digitorum brevis muscles of CHF rats, but these fibers were markedly more susceptible to damage during microdissection. Furthermore, CHF fibers displayed a marked increase of baseline $[\text{Ca}^{2+}]_i$ during fatigue. Isolated slow-twitch soleus muscles of CHF rats displayed slower twitch contraction and tetanic relaxation than did muscles from sham-operated rats; the slowing of relaxation became more pronounced during fatigue in CHF muscles. Immunoblot analyses of sarcoplasmic reticulum proteins and sarcolemma Na^+, K^+ -ATPase showed no difference in flexor digitorum brevis muscles of sham-operated versus CHF rats. In conclusion, functional impairments can be observed in limb muscle isolated from rats with CHF. These impairments seem to mainly involve structures surrounding the muscle cells and sarcoplasmic reticulum Ca^{2+} pumps, the dysfunction of which becomes obvious during fatigue. (*Circ Res.* 2001;88:1299-1305.)

Key Words: heart failure ■ skeletal muscle ■ fatigue ■ intracellular Ca^{2+} handling

Decreased fatigue resistance and skeletal muscle weakness are important symptoms in humans with congestive heart failure (CHF). The reason for the decreased fatigue resistance is not clear. Often, there is no clear correlation between the degree of heart dysfunction and the decrease in exercise tolerance.¹ This suggests that there could be functional impairments within the skeletal muscles that are due to, for instance, alterations in the local environment with restricted local blood flow.² In addition, numerous studies have been focused on possible abnormalities in skeletal muscle cells, and significant changes have been found both at the mRNA and protein levels. Generally observed changes in skeletal muscle cells in CHF include a shift of myosin heavy chain distribution toward more fast-type myosin heavy chain,³⁻⁵ altered expression of sarcoplasmic reticulum (SR) Ca^{2+} -ATPase (SERCA),^{6,7} and in later stages, decrements in mitochondrial enzymes and muscle cell atrophy.⁸ Functional studies of limb muscle function in CHF are more sparse. One study on bundles of muscle fibers from the fast-twitch extensor digitorum longus (EDL) muscles of rats showed marked dysfunction in CHF with $\approx 50\%$ reductions in tetanic Ca^{2+} and force and markedly accelerated fatigue development, which was not due to muscle cell atrophy.⁹ There seems to be a discrepancy between these very dramatic

functional changes and the relatively subtle changes of muscle protein levels observed in muscles from CHF subjects. In accordance, a more recent study showed more moderate and, in some instances, apparently opposite changes in contractile function and Ca^{2+} handling in skeletal muscle from rats with myocardial infarction.¹⁰ Having this in mind, we compared contractile function, intracellular Ca^{2+} handling, and fatigue resistance of intact, single, fast-twitch muscle fibers from CHF and sham-operated rats (CHF and sham fibers, respectively). The results show no major changes in the contractile function and Ca^{2+} handling of unfatigued fibers from CHF rats. The fatigue resistance of CHF fibers was similar to that of sham fibers, but the increase in baseline Ca^{2+} during fatigue was markedly larger in CHF fibers. Moreover, fibers from CHF rats were markedly more susceptible to damage during microdissection. We also studied contractions and fatigue in isolated slow-twitch soleus muscles. The most conspicuous result in soleus muscles from CHF rats was a pronounced slowing of relaxation and increase in baseline force during fatigue.

Materials and Methods

Animals

Experiments were performed on a total of 48 male Wistar rats: 25 CHF rats and 23 sham-operated control (sham) rats. The primary

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surgery was performed on 3-month-old rats (weight ≈ 300 g). These were anesthetized with 2.5% halothane in 30% O₂/70% N₂O. CHF was produced by ligating the left coronary artery.¹¹ Sham rats were subjected to the same surgical procedures, but the coronary artery was not ligated. During the surgery, transmitters recording body temperature, heart rate, ECG, and gross locomotor activity were placed in the peritoneal cavity of some animals.¹²

The secondary surgery was performed after 6 weeks by using the same anesthetic method. Systolic aortic pressure and left ventricular end-diastolic pressure (LVEDP) were measured by a micromanometer-tipped catheter (SPR-407, Millar Instruments) inserted through the right carotid artery. Soleus and flexor digitorum brevis (FDB) muscles were dissected out and kept in standard Tyrode's solution bubbled with 5% CO₂/95% O₂; $\approx 0.2\%$ FCS was added to the solution to improve the survival of single fibers.¹³ Experiments were performed at room temperature ($\approx 24^\circ\text{C}$). The present study was approved by the local ethics committee.

Experiments on Intact Single Fibers

Intact single fibers were dissected from FDB muscles by using dark-field illumination and $\times 80$ to $\times 120$ magnification.¹³ The isolated fiber was mounted between an Akers 801 force transducer (SenoNor) and an adjustable holder at the length giving maximum tetanic force. The fiber was continuously superfused by Tyrode's solution, and electrical stimulation was achieved by brief supramaximum current pulses delivered via platinum plate electrodes lying parallel to the fiber.¹³

In the majority of fibers, the free myoplasmic Ca²⁺ concentration ([Ca²⁺]_i) was measured with the fluorescent Ca²⁺ indicator indo 1 (Molecular Probes). The pentapotassium salt of indo 1 was micro-injected into fibers; this procedure avoids problems with the loading of organelles. The fluorescence of indo 1 was measured with a system consisting of a xenon lamp, a monochromator, and two photomultiplier tubes (PTI, Photo Med GmbH). Fluorescence signals were translated to [Ca²⁺]_i by using an intracellular calibration curve established in intact mouse muscle fibers.¹⁴ After the injection of indo 1, the fibers were allowed to rest for at least 60 minutes. Fibers that produced a force markedly lower than that before the injections were not used. The experimental protocol was started by first producing a single twitch and then, at 1-minute intervals, 350-ms tetani at 15 to 100 Hz. Fatigue was produced by applying 350-ms 70-Hz tetani at 1-second intervals until force was reduced to 40% of the control.

Experiments on Whole Soleus Muscles

Isolated soleus muscles were mounted at optimum length. After 30 minutes of rest, the muscle was electrically stimulated at 1-minute intervals with a single pulse or 2-second tetani at 10 to 70 Hz. Fatigue was produced by 700-ms 50-Hz tetani at 2-second intervals delivered until force was down to 40% of the control.

Immunoblot Analyses

Membrane proteins from FDB and soleus muscles (and also, for comparison, EDL muscles and pieces from the left ventricle of the heart) were isolated as previously described.¹⁵ A semiquantitative determination of different proteins was achieved by Western or slot-blot analysis with various amounts of protein to ensure that staining intensity was within the linear range of analysis.¹⁶ The primary antibodies and concentrations used were anti-SERCA1 antibody (MA3-912, Affinity BioReagents; 1:2500), anti-SERCA2 antibody (MA3-919, Affinity BioReagents; 1:1000), anti-Na⁺,K⁺-ATPase α_1 -subunit antibody (MA3-929, Affinity BioReagents; 1:250), anti-Na⁺,K⁺-ATPase α_2 -subunit antibody (No. 06-168, Upstate Biotechnology; 1:1000), anti-Na⁺,K⁺-ATPase β_1 -subunit antibody (No. 06-170, Upstate Biotechnology; 1:1000), and anti-ryanodine receptor antibody (MA3-925, Affinity BioReagents; 1:5000). Values are expressed in arbitrary units and normalized to the mean of sham muscles (100%).¹⁷

TABLE 1. Gross Characteristics and Heart Performance in Sham and CHF Rats

	Sham	CHF
Body weight, g	408 \pm 21 (5)	389 \pm 6 (11)
Body temperature, $^\circ\text{C}$	37.1 \pm 0.3 (9)	37.0 \pm 0.3 (11)
Heart rate, bpm	372 \pm 4 (9)	359 \pm 3* (11)
Locomotor activity, counts/h	1917 \pm 419 (7)	1643 \pm 371 (10)
SAP, mm Hg	131 \pm 6 (4)	106 \pm 10* (11)
LVEDP, mm Hg	5.3 \pm 0.5 (4)	27 \pm 3.8* (11)

SAP indicates systolic aortic pressure. Values are mean \pm SE (number of animals). Body temperature, heart rate, and locomotor activity were measured as the mean over 96 or 120 hours.

* $P < 0.05$ vs sham group.

Statistical Analysis

Values are presented as mean \pm SE. Unpaired *t* tests were used to establish statistical differences between CHF and sham groups, and the significance level was set at $P < 0.05$.

Results

Body weight, body temperature, and gross locomotor activity were not significantly different between sham and CHF rats, whereas the heart rate was slightly lower in CHF rats (Table 1).¹⁸ Hemodynamic measurements of heart function were severely pathological in CHF rats: systolic aortic pressure was significantly lower and LVEDP was significantly higher in CHF rats than in sham rats. A recent study using echocardiography shows that selecting rats with LVEDP > 15 mm Hg, as in the present study, ensures that the animals have a significant uncompensated heart failure.¹⁹ CHF rats also showed several clinical signs of severe heart failure, including pulmonary congestion, tachypnea, and pleural effusion (data not shown). Moreover, there was an extensive thin-walled scar in the left ventricle of all CHF rats. Finally, ECG records showed markedly pathological QRS complexes (broadened large Q waves) in four of five CHF rats, whereas ECG was normal in all three sham rats studied.

Experiments on Single FDB Muscle Fibers

One major difference was noted during the dissection of single muscle fibers: isolated fibers from CHF rats often did not respond to electrical stimulation, despite having a normal appearance in the dissection microscope, whereas fibers from sham rats were robust in this respect. To deal with this problem in CHF muscles, we adopted a procedure in which we dissected bundles of ≈ 3 intact fibers, and this bundle was transferred to the stimulation chamber. One fiber was then injected with indo 1, and when it had recovered from injection, as judged from the [Ca²⁺]_i transient and force response, the remaining fibers were cut open by a broken microelectrode; an all-or-none response to small increments of the stimulation strength verified activation of the injected fiber only. With this procedure, we had a success rate (number of fibers that could be used in experiments divided by the number of dissected fibers) of $\approx 20\%$ in CHF muscles compared with $\approx 80\%$ in sham muscles.

Mean data from twitch and tetanic contractions produced in unfatigued single muscle fibers did not show any signifi-

TABLE 2. Contractile Properties of Unfatigued Sham and CHF Muscle

	Sham	CHF
Single fibers of FDB muscles		
Twitch		
Force, mN	0.07±0.01 (11)	0.12±0.02* (6)
Contraction time, ms	38.6±2.1 (11)	37.5±3.1 (6)
Half-relaxation time, ms	40.9±2.8 (11)	45.8±5.9 (6)
Tetanus (70 Hz)		
Force, mN	0.62±0.05 (11)	0.74±0.10 (7)
Half-relaxation time, ms	92.4±5.3 (11)	90.3±6.6 (7)
[Ca ²⁺] _i , μmol/L	1.04±0.14 (9)	1.41±0.38 (5)
Force-[Ca ²⁺] _i relationship		
F _{max} , mN	0.64±0.07 (9)	0.82±0.15 (5)
Ca ₅₀ , μmol/L	0.42±0.03 (9)	0.41±0.06 (5)
Constant N	3.73±0.24 (9)	3.47±0.54 (5)
Frequency for 50% F _{max} , Hz	23.3±1.7 (11)	21.5±4.0 (5)
Soleus muscles		
Muscle wet weight, mg	153±5 (10)	164±4 (10)
Twitch		
Force, N/g wet muscle	0.79±0.08 (10)	1.01±0.06* (10)
Contraction time, ms	88.8±5.4 (10)	106.4±4.6* (10)
Half-relaxation time, ms	143.2±20.1 (10)	145.8±8.1 (10)
Tetanus (50 Hz)		
Force, N/g wet muscle	7.14±0.32 (10)	7.84±0.24 (10)
Half-relaxation time, ms	172.5±11.1 (10)	213.8±10.0* (10)
Frequency for 50% force, Hz	10.4±0.8 (10)	8.1±0.4* (10)

Values are mean±SE (number of experiments) for single fibers and isolated soleus muscles of sham and CHF rats. Twitch contraction time was measured as the time from the onset of contraction until peak force was attained.

**P*<0.05 vs sham group.

cant differences between sham and CHF fibers, except for a higher twitch force in CHF fibers (Table 2). However, during 70-Hz (and also 100-Hz) tetanic stimulation, there was a trend for both [Ca²⁺]_i and force to be higher in CHF fibers than in sham fibers (Figure 1), but the difference was not significant (*P*>0.2). Resting [Ca²⁺]_i was not significantly different between the two groups: 43.6±10.4 nmol/L in sham fibers (*n*=9) and 45.7±10.8 nmol/L in CHF fibers (*n*=5).

Force-[Ca²⁺]_i curves of unfatigued fibers were constructed by measuring mean force (*F*) and [Ca²⁺]_i during the final 100

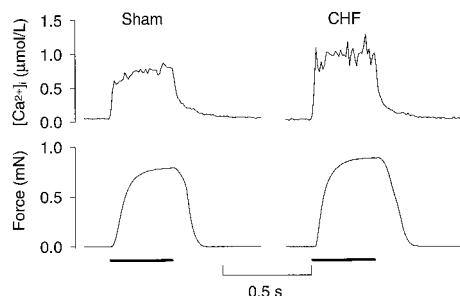


Figure 1. Original records of [Ca²⁺]_i and force from 70-Hz tetani produced in unfatigued single FDB fibers of sham and CHF muscle. Period of stimulation is indicated below force records.

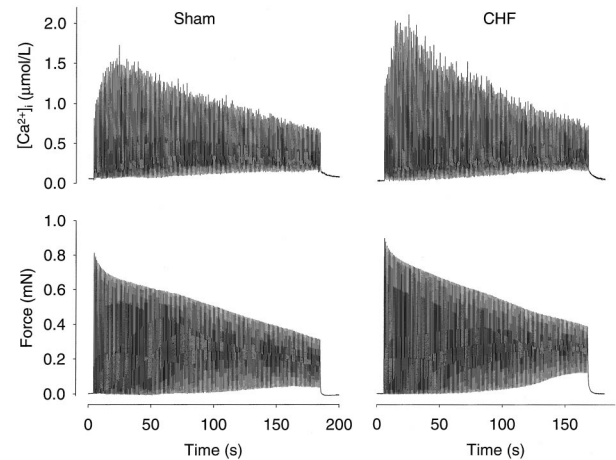


Figure 2. Original records of [Ca²⁺]_i and force from representative fatigue runs produced in single FDB fibers of sham and CHF muscle.

ms of stimulation in tetani of different frequencies and fitting data points to the following equation: $F = F_{\max} [Ca^{2+}]_i^N / (Ca_{50}^N + [Ca^{2+}]_i^N)$, where F_{\max} is the force at saturating [Ca²⁺]_i, Ca_{50} is the [Ca²⁺]_i giving 50% of F_{\max} , and N is a constant describing the steepness of the function. Neither of these parameters nor the frequency giving half-maximum force (obtained by linear interpolation) was significantly different between sham and CHF fibers (Table 2).

Original representative records of [Ca²⁺]_i and force obtained during fatiguing stimulation are shown in Figure 2, and mean data from all fibers studied are presented in Figure 3. The rate of force decline during fatigue was similar in CHF and sham fibers (Figure 3A). Although the mean tetanic [Ca²⁺]_i was higher in CHF fibers than in sham fibers throughout the fatigue runs (Figure 3B), this difference was not statistically significant at any time point. The fibers depicted in Figure 2 show a clear increase of baseline force in fatigue, and this increase was larger in the CHF fibers. This was a general finding, and at the end of fatiguing stimulation, baseline force (measured immediately before the onset of the last tetanic contractions) tended to be larger in CHF fibers (6.7±3.1% of the control tetanic force, *n*=7) than in sham fibers (2.4±0.7%, *n*=9), but the difference was not significant (*P*=0.16). The increase in baseline force in fatigue suggests a marked slowing of relaxation, and this was confirmed by measurements of the half-relaxation time in fatigue, which was significantly increased both in CHF and sham fibers. The half-relaxation time in fatigue was 260±71 ms in CHF fibers and 186±25 ms in sham fibers, which represents an increase by 175±63% and 99±31%, respectively. Thus, again the slowing of relaxation tended to be larger in CHF fibers, but the difference was not significant (*P*=0.25).

In Figure 3C, we show average [Ca²⁺]_i records obtained immediately after tetanic stimulation in control conditions and in fatigue. In control conditions, these “tails” of [Ca²⁺]_i were similar in CHF and sham fibers. However, in fatigue, the [Ca²⁺]_i tails were markedly slower in CHF fibers, and baseline [Ca²⁺]_i (measured as the mean over 100 ms obtained

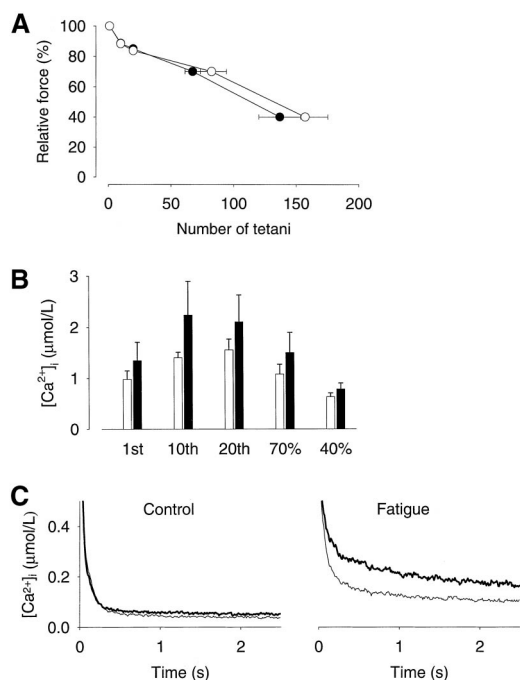


Figure 3. A, Mean \pm SEM data of tetanic force during development of fatigue in single FDB muscle fibers of sham (\circ , $n=9$) and CHF (\bullet , $n=7$) muscle. Data shown are the 1st, 10th, and 20th fatiguing tetanus and the tetani by which 70% and 40% force was produced. B, Mean \pm SEM values of tetanic $[Ca^{2+}]_i$ during fatigue measured at the same time points as in panel A. Open bars indicate sham ($n=7$); solid bars, CHF ($n=5$). C, Average $[Ca^{2+}]_i$ tails obtained after 70-Hz tetani produced under control conditions (left) and at the end of fatiguing stimulation (right). Thin line indicates sham; thick line, CHF. Time axes start at the end of tetanic stimulation.

1 second after the end of stimulation) was significantly ($P<0.05$) higher in CHF fibers (206 ± 37 nmol/L, $n=5$) than in sham fibers (120 ± 10 nmol/L, $n=7$). One minute after the end of fatiguing stimulation, baseline $[Ca^{2+}]_i$ had recovered substantially in both groups, and there was no longer any significant difference between CHF fibers (67 ± 16 nmol/L) and sham fibers (44 ± 11 nmol/L).

Recovery of tetanic force and $[Ca^{2+}]_i$ after fatigue was studied by producing tetani at regular intervals for 30 minutes. There was no significant difference between CHF and sham fibers at any time point, and at 30 minutes of recovery, tetanic force was $81\pm7\%$ and $76\pm8\%$ in CHF fibers ($n=7$) and sham fibers ($n=8$), respectively; corresponding values for tetanic $[Ca^{2+}]_i$ were $76\pm7\%$ ($n=5$) and $92\pm6\%$ ($n=7$).

Experiments on Soleus Muscles

Twitch contraction and tetanic relaxation in unfatigued soleus muscles were slower in CHF animals than in sham animals (Table 2). In addition, the twitch force was higher and the frequency required to produce 50% tetanic force was lower in CHF muscles than in sham muscles. Original force records from typical fatigue runs in sham and CHF muscles are shown in Figure 4, and mean data are presented in Figure 5. During fatiguing stimulation, peak tetanic force fell more slowly in CHF muscles than in sham muscles (Figure 5A), and the number of tetani required to bring peak force down to

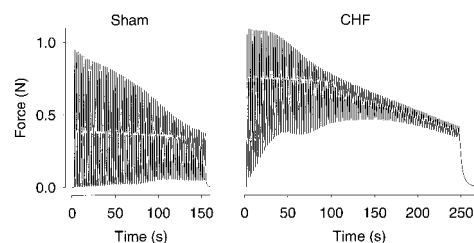


Figure 4. Original force records from typical fatigue runs in soleus muscles from sham and CHF rats.

40% of the original was significantly ($P<0.05$) higher in CHF muscles (103 ± 4 tetani) than in sham muscles (76 ± 7 tetani). A further difference between the two groups was that baseline force increased rapidly during fatigue in CHF muscles. Thus, at the end of fatiguing stimulation, it amounted to $24.1\pm2.2\%$ of the original peak tetanic force, which was significantly ($P<0.01$) higher than the value in sham muscles ($5.9\pm3.0\%$). In accordance, the half-relaxation time of the last fatiguing tetanus was markedly longer in CHF muscles (1896 ± 163 ms) than in sham muscles (538 ± 57 ms, $P<0.001$). During the 30-minute recovery period after fatiguing stimulation, peak tetanic force was generally lower in CHF muscles than in sham muscles (Figure 5B).

Immunoblot Analyses

The contractile results and $[Ca^{2+}]_i$ measurements described above would indicate some changes in SERCA in CHF muscles. Therefore, immunoblot analyses of the expression of fast-type and slow-type SR Ca^{2+} -ATPase (SERCA1 and SERCA2, respectively) were performed (Figure 6). As expected, SERCA1 was the predominant form in fast-twitch EDL and FDB muscles, whereas only traces of SERCA1 expression were seen in the soleus muscle, and none were seen in the heart. On the other hand, SERCA2 was expressed in slow-twitch soleus muscles and the heart (not shown) but not in the two fast-twitch muscles (only EDL shown). Furthermore, there was not a significant difference between SERCA1 expression in CHF and sham FDB muscles (Figure 6A) or between SERCA2 expression in CHF and sham soleus muscles (Figure 6B). Thus, the slowed force and $[Ca^{2+}]_i$ handling observed in CHF muscle cannot be explained by a reduced expression of SERCA. In FDB muscles, we also performed immunoblot analyses of several other proteins. Again, no significant difference between CHF and sham muscles was found regarding SR Ca^{2+} -release channels (ie, ryanodine receptors; $98\pm9.0\%$ versus $100\pm8.7\%$, respec-

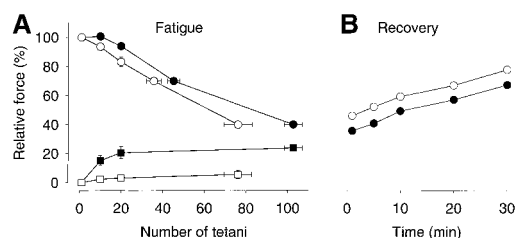


Figure 5. Mean data of baseline and tetanic force during fatigue (A) and recovery (B) in sham (\square and \circ , $n=10$) and CHF (\blacksquare and \bullet , $n=10$) soleus muscles.

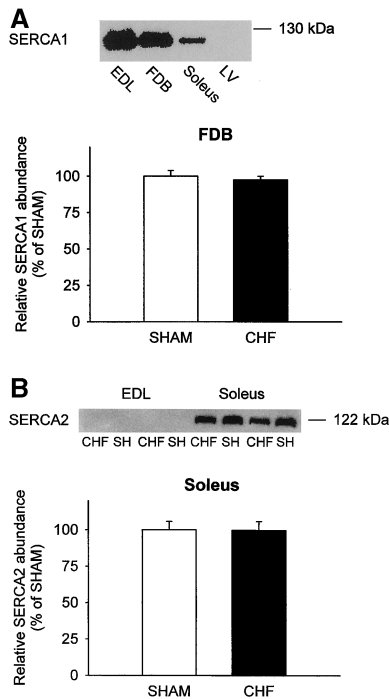


Figure 6. A, SERCA1 is expressed in fast-twitch (EDL and FDB) muscles, whereas the expression is small in slow-twitch (soleus) muscles and undetectable in the left ventricle of the heart (LV) (immunoblots). Bar graph shows mean data of SERCA1 expression in FDB from sham ($n=11$) and CHF ($n=13$) rats; note that there was no difference between the two groups. B, SERCA2 is expressed in slow-twitch soleus muscles but not in fast-twitch EDL muscles (immunoblots). Mean data showed no difference in SERCA2 expression between soleus muscles from sham (SH, $n=11$) and CHF ($n=13$) rats (bar graph).

tively) or different isoforms and subunits of the sarcolemma Na^+, K^+ -ATPase (α_1 , $94 \pm 13.2\%$ versus $100 \pm 11.6\%$, respectively; α_2 , $103 \pm 8.2\%$ versus $100 \pm 7.3\%$, respectively; and β_1 , $99 \pm 3.2\%$ versus $100 \pm 3.7\%$, respectively).

Discussion

The major results of the present study are as follows: (1) single fibers from the hindlimb FDB muscle of CHF rats were more susceptible to damage during dissection; (2) in the unfatigued state, there were no differences or only moderate differences in contractile function and $[\text{Ca}^{2+}]_i$ handling between CHF and sham muscle; and (3) in the fatigued state, there was a marked reduction of the rate of $[\text{Ca}^{2+}]_i$ removal from the myoplasm in single fibers and a marked slowing of force relaxation of soleus muscles from CHF rats.

The present functional differences between CHF and sham muscles cannot be explained by inactivity in CHF rats, inasmuch as there was no difference in the locomotor activity pattern of the two groups, a finding that agrees with previous results obtained in rats with CHF.^{4,7,18} Rather, it appears likely that some systemic factors are involved in the development of skeletal muscle dysfunction in CHF, as recently reviewed by Lunde et al.²⁰ For instance, a major increase of tumor necrosis factor- α has been observed in rats with CHF,⁵ and tumor necrosis factor- α may (possibly together with other cytokines) induce myopathy and apoptosis.^{21,22}

The single fibers of FDB muscles had twitch contraction and half-relaxation times of ≈ 40 ms, and the stimulation frequency required to give half-maximal tetanic force was ≈ 20 Hz, compared with 90 to 150 ms and 10 Hz, respectively, in soleus muscles (Table 2). Thus, the single FDB muscle fibers would be fast-twitch fibers, whereas the soleus muscles would consist mainly of slow-twitch fibers, which fits with the expression pattern of SERCA isoforms.²³

The present study is the first in which force and $[\text{Ca}^{2+}]_i$ were measured in intact, single, skeletal muscle fibers of the rat. Single fibers were dissected from the hindlimb FDB muscle because the fibers of this muscle are short, which is advantageous for microdissection and dye injection. One disadvantage with the use of this muscle is that it contains almost exclusively fast-twitch fibers. In the present study, we observed larger differences between intact soleus muscles from CHF and sham rats than between the single fast-twitch FDB fibers of the two groups. Thus, it would have been interesting to measure force and $[\text{Ca}^{2+}]_i$ in single slow-twitch fibers from soleus muscles. However, soleus fibers are long, and their diameters are small, which make them less suitable for single-fiber experiments.

Increased Risk of Damage During Dissection of CHF Muscle Fibers

During the dissection of fibers, we observed a markedly increased fragility in muscles from CHF rats, and fibers that had a normal appearance in the dissection microscope often did not respond to electrical stimulation. The reason for this difference between CHF and sham muscles is not clear. One possibility is that the extracellular matrix is affected in skeletal muscles of CHF animals, and it may be hypothesized that this change precedes intrinsic changes in the muscle cells, as has been shown in failing hearts.²⁴ In line with this, it has recently been shown that in skeletal muscles from rats with CHF, apoptosis was first seen in interstitial cells, and it has been speculated that damage to endothelial cells diminishes the delivery of nutrients to the muscle cells, leading to secondary muscle cell damage.⁵ Interestingly, we observed larger changes in contractile function in the highly vascularized and oxygen-dependent soleus muscle, which, therefore, would be expected to be more vulnerable.

An increased susceptibility to mechanical damage of CHF skeletal muscle suggests that these muscles are more easily damaged during eccentric contractions. To the best of our knowledge, this intriguing possibility has not been specifically investigated.

Minor Changes of Muscle Function in Unfatigued CHF Muscle

In muscles in the unfatigued state, we found only moderate differences in contractile function and Ca^{2+} handling between CHF and sham rats. In accordance, immunoblot analyses showed no difference between the two groups in the expression of SERCA isoforms, ryanodine receptors, or different subunits of sarcolemma Na^+, K^+ -ATPase.

The small effects on contraction and $[\text{Ca}^{2+}]_i$ of unfatigued single fast-twitch fibers observed in the present study are markedly different from the results of a previous study

(Perreault et al⁹) on fiber bundles from fast-twitch muscle of rats with CHF, in which major abnormalities were observed. CHF was induced by ligation of the left coronary artery in both studies; the degree of heart failure and infarct size are comparable; and in both cases, experiments were performed 6 weeks after inducing CHF. Thus, it is likely that the conflicting results are due to the different approaches used to assess muscle function. In the previous study, the Ca^{2+} -sensitive photoprotein aequorin was loaded into bundles of muscle fibers by means of macroinjection into the interstitial space.⁹ This procedure inevitably must put a large stress on the muscle cells because a large amount of the protein is forced into the myoplasm. If the increased fragility during microdissection that we observed is taken into account, it is possible that recovery after the macroinjection of aequorin was markedly impaired preferentially in muscles from CHF rats. This would also explain the apparent conflict between the large effects on contractile function observed in the study of Perreault et al and the modest changes in contractile function and protein expression observed with similar models of CHF in the present and other^{7,10} studies. An alternative explanation might be that the isolated single fibers used in the present study are not representative of the whole muscle; eg, it could be that the fibers that contracted after dissection were the only fibers with almost unaltered properties. Although we cannot fully exclude this possibility, we consider it unlikely, because muscles from CHF rats contracted normally in early stages of dissection, and it was not until reaching the state of isolating a single cell from a bundle of ≈ 3 fibers that an increased fragility was observed.

Changes of Function in Fatigued CHF Muscle

In the single-fiber experiments, changes of tetanic force and $[\text{Ca}^{2+}]_i$ were not different between CHF and sham fibers at any time point during fatigue. The fatigue pattern observed in both groups of fibers, with an initial increase of tetanic $[\text{Ca}^{2+}]_i$ accompanied by a 10% to 20% force reduction followed by decreases of both tetanic $[\text{Ca}^{2+}]_i$ and force, is very similar to that previously observed in single mouse FDB muscle fibers, albeit the present rat fibers appear more fatigue resistant.²⁵ Nevertheless, CHF fibers tended to have higher tetanic $[\text{Ca}^{2+}]_i$ throughout fatiguing stimulation, and in the fatigued state, baseline $[\text{Ca}^{2+}]_i$ was significantly higher in CHF fibers than in sham fibers. This finding is consistent with a slower SR Ca^{2+} uptake in CHF fibers, which became manifest during fatigue.^{26,27}

In fatigued soleus muscles, force relaxation was markedly slower in CHF muscles than in sham muscles. In principle, a slowing of relaxation is a consequence of slowed crossbridge kinetics and/or slowed Ca^{2+} removal from the myoplasm, which mainly occurs by Ca^{2+} being taken up by the SR.²⁸ A major slowing of crossbridge kinetics would also be manifested as a marked reduction of the rate of force development at the start of tetanic contraction. In the present study, the rate of force development during fatigue was not markedly different in muscles from CHF and sham rats (data not shown). Thus, the pronounced slowing of relaxation in fatigued CHF soleus muscles was most likely due to an impaired Ca^{2+} uptake into the SR, which is also found in

cardiomyocytes from rats with heart failure, in diaphragms from rabbits with heart failure, and probably in the present CHF single fibers.^{11,29}

Based on the above results, the following model can be proposed. Because there is only a modest or no difference between unfatigued CHF and sham muscles regarding speed of relaxation and Ca^{2+} removal from the myoplasm, no major difference in the number of SR Ca^{2+} pumps between the two groups is expected, which fits with the present immunoblot results and published data.⁷ However, during fatigue, a slowing of the rate of SR Ca^{2+} uptake becomes manifest, indicating that the Ca^{2+} pumps in CHF muscles are more sensitive to fatigue-induced changes. The situation would then be similar to that in aged muscle, in which the rate of SR Ca^{2+} pumping may be reduced and in which an increased sensitivity to posttranslation modifications of SR Ca^{2+} pumps has been observed.³⁰ Interestingly, it has been shown that the impairment of SR Ca^{2+} pump function in aged muscle predominantly occurs in slow-twitch soleus muscles, whereas fast-twitch gastrocnemius muscles are not affected.³¹ This agrees with the present results, in which the largest effects of CHF were seen in soleus muscles.

During the present type of isometric contractions, peak tetanic force fell more slowly in CHF muscles than in sham muscles. This might be taken as an index of an increased fatigue resistance in CHF muscles, which would be opposite the reduced endurance observed in patients with CHF. However, the marked slowing of relaxation in fatigue would cause a decreased exercise tolerance during normal types of locomotion, which involve alternating movements, because slowed relaxation of antagonist muscles will impede the intended movements.³²

Conclusions

Functional impairments can be observed in skeletal muscle isolated from rats with CHF. In fast-twitch muscle, the impairment appears to mainly involve structures surrounding the muscle cells, but an impaired SR Ca^{2+} pumping also seems to become manifest during fatigue. In slow-twitch muscle, SR Ca^{2+} pumping appears to be impaired, and this becomes especially clear during fatigue.

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