Temperature-dependent skeletal muscle dysfunction in rats with congestive heart failure

H.-M. Schiøtz Thorud, E. Verburg, P. K. Lunde, T. A. Strømme, I. Sjaastad, and O. M. Sejersted. Temperature-dependent skeletal muscle dysfunction in rats with congestive heart failure. J Appl Physiol 99: 1500–1507, 2005. First published June 2, 2005; doi:10.1152/japplphysiol.00807.2004.—Abnormalities in the excitation-contraction coupling of slow-twitch muscle seem to explain the slowing and increased fatigue observed in congestive heart failure (CHF). However, it is not known which elements of the excitation-contraction coupling might be affected. We hypothesize that the temperature sensitivity of contractile properties of the soleus muscle might be altered in CHF possibly because of alterations of the temperature sensitivity of intracellular Ca\textsuperscript{2+} handling. We electrically stimulated the in situ soleus muscle of anesthetised rats that had 6-wk postinfarction CHF using 1 and 50 Hz and using a fatigue protocol (5-Hz stimulation for 30 min) at 35, 37, and 40°C. Ca\textsuperscript{2+} uptake and release were measured in sarcoplasmic reticulum vesicles at various temperatures. Contraction and relaxation rates of the soleus muscle were slower in CHF than in sham at 35°C, but the difference was almost absent at 40°C. The fatigue protocol revealed that force development was more temperature sensitive in CHF, whereas contraction and relaxation rates were less temperature sensitive in CHF than in sham. The Ca\textsuperscript{2+} uptake and release rates did not correlate to the difference between CHF and sham regarding contractile properties or temperature sensitivity. In conclusion, the discrepant results regarding altered temperature sensitivity of contraction and relaxation rates in the soleus muscle of CHF rats compared with Ca\textsuperscript{2+} release and uptake rates in vesicles indicate that the molecular cause of slow-twitch muscle dysfunction in CHF is not linked to the intracellular Ca\textsuperscript{2+} cycling.

Alterations in the excitation-contraction (EC) coupling have been proposed to explain the contractile dysfunction in skeletal muscle seen during CHF (25, 40). Previous studies have suggested that changes in Ca\textsuperscript{2+} handling in the sarcoplasmic reticulum (SR) contribute to increased fatigue in CHF, but the results are not conclusive. Williams and Ward (44) and Spanenberg et al. (32) observed accelerated Ca\textsuperscript{2+} uptake and release rates in SR vesicles from mixed and fast-twitch muscles in rats with moderate heart failure. However, Perreault et al. (22) showed reduced Ca\textsuperscript{2+} release and Lunde et al. (16) observed reduced rate of Ca\textsuperscript{2+} removal from the cytoplasm in fiber bundles and intact fibers from fast-twitch muscle in rats with CHF. Recently, Reiken et al. (25) and Ward et al. (40) demonstrated ryanodine receptor hyperphosphorylation and impaired Ca\textsuperscript{2+} release in fast- and slow-twitch muscles from postinfarction rats.

Because the role of altered intracellular Ca\textsuperscript{2+} cycling in skeletal muscle dysfunction in CHF is unclear, we searched for a method by which Ca\textsuperscript{2+} release and reuptake could be modified so as to reveal the importance of these processes to the overall EC coupling. Temperature might affect Ca\textsuperscript{2+} handling and the contractile apparatus differently and, in addition, affect rates of metabolism (29). Especially, both metabolism and the intracellular cycling of Ca\textsuperscript{2+} have temperature sensitivities that are probably different from that of the contractile machinery. We have previously shown that high-energy phosphates and lactate accumulation are not significantly affected in working soleus muscles of CHF rats (18, 35). Therefore, we hypothesized that, if Ca\textsuperscript{2+} release and reuptake are affected in the CHF condition, this could be reflected in parallel alterations of temperature sensitivity of Ca\textsuperscript{2+} release and uptake and of contractile parameters. We tested this hypothesis by correlating contractile parameters with Ca\textsuperscript{2+} release and uptake in SR vesicles.

We examined contractile properties, metabolism, and SR function at temperatures within the physiological range in the soleus muscle from anesthetized rats with 6-wk postinfarction CHF and from sham-operated (Sham) rats. We found altered temperature sensitivity of soleus muscles from CHF rats compared with Sham, and contrary to our hypothesis the data indicate that the reason for this difference was not changes in SR Ca\textsuperscript{2+} handling proteins or metabolism. A possible explanation for the temperature-sensitive muscle dysfunction during CHF could be alterations within the contractile proteins.

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METHODS

The experimental procedures conformed to the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Norwegian Animal Research Authority. The rats were housed in standard cages with a 12:12-h day-night light cycle. Temperature (22 ± 2°C) and humidity (55 ± 5%) were continuously monitored, and the animals had access to standard laboratory rat chow (Standard Rodent Diets, SCANBUR BK, Nittedal, Norway) and water ad libitum.

Animal preparation. Under Isofluran (Abbott Scandinavia, Solna, Sweden) anesthesia (2–2.5% in 30/70% O2/N2O), male Wistar rats (315 ± 2 g) (Møllegaard and Bomholtgård, Lille Skensved, Denmark) were subjected to ligation of the left coronary artery [myocardial infarction (MI) group] as described by Tønnessen et al. (37). Sham rats were used as controls. Postoperatively, the rats were given 0.2 mg/kg buprenorphine (Temgesic, Schering-Plough Europe, Brussels, Belgium) subcutaneously.

The survival rate after 6 wk was 41 and 94% in the MI and Sham groups, respectively. The remaining MI and Sham rats were anesthetized as described above, and a blood pressure catheter (Mikro-Tip Transducer SPR-407, Millar Instruments) was inserted into the right common carotid artery to measure systolic arterial pressure. The catheter was advanced into the left ventricle to measure left ventricular end-diastolic pressure (LVEDP). In the MI group, 73% of the rats had a LVEDP of ≥15 mmHg and were included in the study (CHF group). The CHF and Sham rats (n = 71) were assigned either to studies of contractility (n = 47) or SR Ca2+ handling (n = 24). Both the contractility and the SR Ca2+ measurements were designed for the soleus muscle. The distribution of rats in those experiments concerning contractile measurements was as follows: 35°C: CHF, n = 14; Sham, n = 6; 37°C: CHF, n = 6; Sham, n = 9; 40°C: CHF, n = 6; Sham, n = 6. During the contractility experiments, the Isofluran concentration was adjusted (2–2.5%) to maintain a stable systolic arterial pressure. To maintain a body temperature of 38°C (4), a heating pad connected to a thermostat (PRODAB LB750 Controlunit, Uppsala, Sweden) was placed under the rat. In rats assigned to contractility experiments, the skin was removed from the right leg, and the soleus muscle was isolated in situ with intact blood supply as previously described (19, 38). The Achilles tendon was cut adjacent to the calcaneus, and the distal gastrocnemius and plantaris tendons were separated from the distal soleus tendon and cut. To ensure that movements of the gastrocnemius and plantaris did not interfere with the soleus muscle, the thin fascia between the soleus and gastrocnemius-plantaris was split halfway up the soleus, distal to the blood vessels supplying the soleus muscle. The sciatic nerve was cut distal to the hip. The leg was then fixed on a platform, and the distal soleus tendon was attached to a force transducer (force-displacement transducer FT03, GRASS Medical Instruments). Two steel hooks positioned at the proximal and distal end of the muscle were used as stimulation electrodes, and the muscle was kept moist at a constant temperature (35, 36, 37, or 40°C). Eighty microliters of just-thawed, ryanodine receptor, and the release was followed until it leveled.

Ca2+ uptake and release in SR vesicles. SR Ca2+ uptake and release were measured in crude soleus muscle homogenate based on the methods of O’Brien (21), as modified by Li et al. (13). Immediately after the rats were killed, the soleus muscles were dissected out. While keeping the muscles cooled on ice, blood and tendons were removed and the muscles were weighed. The muscles were homogenized in ice-cold buffer (1:10 wet wt/vol, pH 7.9) containing sucrose (300 mM), sodium azide (5 mM), EDTA (1 mM), l-histidine (40 mM), and Tris·HCl (40 mM) at 25,000 rpm for 3 × 20 s with a 20-s break between each burst (Polytron 1200, Kinematica AG, Luzern, Switzerland). The homogenates were then immediately frozen and stored for up to 2 mo in liquid nitrogen until analysis of SR function.

The SR Ca2+ uptake and release were measured in an assay buffer (pH 7.0) containing KCl (165 mM), HEPES (22 mM), oxalate (7.5 mM), sodium azide (11 mM), N,N,N’,N’-tetrais(2-pyridylmethyl) ethylenediamine (5.5 μM), MgCl2 (4.5 mM), and Tris·HCl (9 mM). The rates of Ca2+ uptake into and release from SR vesicles were monitored using the Ca2+-binding dye Indo-1 (pentapotassium salt, Molecular Probes, Eugene, OR). Assay buffer (2.2 ml) was added to a plastic cuvette with stirring, and the buffer was heated to appropriate temperature (35, 36, 37, or 40°C). Eighty microliters of just thawed, vortexed homogenate (~8 mg of muscle tissue) were added to the cuvette before adding Indo-1 (1.3 μM). At an extravesicular free Ca2+ concentration ([Ca2+][free]) of 1.0 μM, MgATP (1.1 mM) was added to initiate Ca2+ uptake by the Ca2+-ATPase. When uptake had leveled off, pump activity was blocked with thapsigargin (1.5 μM). 4-Chloromercresol (5.5 mM) was then added to initiate Ca2+ release through the ryanodine receptor, and the release was followed until it leveled. Finally, to obtain minimal and maximal binding of Ca2+ to Indo-1, EGTA (3.3 mM) and Calgus (4.8 mM) were added, respectively.

The fluorescence of Indo-1 was monitored using a luminescence spectrometer (LS50B, Perkin Elmer, Beaconsfield, Buckinghamshire, UK). The sample was excited by a xenon lamp at 349 nm with a slit of 2.5 nm, and emission was measured at 405 and 495 nm with 8 nm slits. The 405-495 nm fluorescence ratio was sampled at 25 Hz.

The dissociation constant for Indo-1 was calculated using the following equation (10):

\[ [\text{Ca}^{2+}]_{\text{free}} = K_d (R - R_{\text{max}})/(R_{\text{max}} - R) (S_2/S_1) \]

where \( R_{\text{max}} \) is the ratio when Indo-1 is in the \([\text{Ca}^{2+}]_{\text{free}} \) form, \( R_{\text{max}} \) is the ratio when Indo-1 is saturated with Ca2+, \( K_d \) is the dissociation constant, and the factor \( S_2/S_1 \) is the ratio of the measured fluorescence intensity at 495 nm when Indo-1 is Ca2+ free or saturated, respectively.

Ca2+ uptake rate was calculated at \([\text{Ca}^{2+}]_{\text{free}} = 0.6 \mu M \), and maximal Ca2+ release rate was calculated from the steepest part of the...
curve after addition of 4-chloro-m-cresol. All calculations were performed in MATLAB 5.3 (MathWorks). Both ratios were corrected for protein content (μmol Ca\(^{2+}\)-min\(^{-1}\)g protein\(^{-1}\)), which was determined by micro BCA protein assay reagent kit (Pierce).

**Ca\(^{2+}\)-ATPase content.** The content of Ca\(^{2+}\)-ATPase in the soleus muscles was determined in crude muscle homogenate by steady-state incorporation of \(^{32}\)P from ATP as described by Everts et al. (8) and corrected for muscle protein content (nmol/g protein). The Ca\(^{2+}\)-ATPase content was measured in the same muscles as those analyzed for Ca\(^{2+}\) uptake and release rates.

**Statistics.** All data were reported as means ± SE. Differences between CHF and Sham and parameters within the same rat were tested by Student’s unpaired and paired t-test, respectively. P values for unpaired t-tests were Bonferroni corrected according to the number of hypotheses in the same data set. Differences between CHF and Sham during repeated measures were tested by comparing means of area under curve with a Student’s unpaired t-test, respectively. P values were corrected for unpaired t-test, respectively. P values were corrected for unpaired t-test, respectively. P values were corrected for unpaired t-test, respectively.

### RESULTS

**Animal characteristics.** Six weeks after ligation of the left coronary artery in the CHF rats, the MI comprised most of the free wall of the left ventricle. The left atrium was visually dilated as a sign of pulmonary congestion. Accordingly, lung weight was significantly increased 2.6-fold in CHF compared with Sham (Table 1). LVEDP was significantly elevated sevenfold in CHF compared with Sham (Table 1), indicating increased left ventricular filling pressure. We have previously demonstrated by echocardiography that rats with an LVEDP of ≥15 mmHg have a safe diagnosis of CHF (31). Systolic arterial pressure was significantly lower in CHF compared with Sham (Table 1), which suggests that the CHF rats have systolic dysfunction. Body weight was not significantly different in the CHF group compared with Sham (Table 1), and recently our laboratory demonstrated that the CHF rats had no skeletal muscle atrophy (16, 18, 35).

**Contractile properties and temperature dependence during 1- and 50-Hz stimulation (unfatigued muscles).** Independent of stimulation frequency and temperature, the overall contractile properties in the soleus muscle during stimulation at 1 and 50 Hz were significantly different in CHF and Sham. Maximal force in the soleus muscle was lower and maximal contraction and relaxation rates were slower in CHF compared with Sham (Fig. 1). The results were not statistically different for contraction rates relative to force (data not shown).

Comparing CHF and Sham treatment at the different temperatures revealed that at 35°C maximal force was 8% lower in CHF during stimulation at 50 Hz. Contraction rate was 18% slower in CHF at both stimulation frequencies, and relaxation rate was 31% slower in CHF at both frequencies at 35°C (P < 0.05). Contraction rate at 37°C was 24% slower in CHF at both stimulation frequencies (P < 0.05). There were no significant differences at 40°C in contractile properties between CHF and Sham at 1 or 50 Hz. Taken together, these results show that the difference between CHF and Sham was more prominent at 35°C compared with either 37 or 40°C (Fig. 1).

Maximal force was not temperature dependent except at 1 Hz in the Sham group where developed force significantly increased with increasing temperature. Maximal contraction rate increased with elevated temperature in both CHF and Sham. Maximal relaxation rate at 1 Hz increased significantly with increasing temperature only in the CHF group, and at 50 Hz the relaxation rate at 37°C was faster compared with both 35 and 40°C in both CHF and Sham (Fig. 1).

**Contractile properties and temperature dependence during 5-Hz stimulation (fatigue protocol).** There was no overall difference in maximal force in the soleus muscle between CHF and Sham at any of the tested temperatures during the 5-Hz stimulation protocol (Fig. 2A). However, maximal force fell significantly more rapidly during the fatigue protocol with increasing temperature in CHF. In Sham, there were no significant differences in force development between the different temperatures during stimulation (Fig. 2A).

At the end of the 30-min stimulation protocol, contraction rate at all temperatures was significantly decreased in both CHF and Sham compared with at start of stimulation (Fig. 2B). Overall maximal contraction rate was significantly slower in CHF compared with Sham at both 35 and 37°C but not at 40°C. In both CHF and Sham, overall contraction rate became significantly faster with increasing temperature, but contraction rate was significantly less temperature dependent in CHF compared with Sham (Fig. 2B).

Relaxation rate at all temperatures became significantly slower during the 5-Hz stimulation protocol in both CHF and Sham (Fig. 2C). Overall maximal relaxation rate was slower in CHF compared with Sham during the fatigue protocol at 35°C but not at 37°C. A tendency toward an overall slower relaxation in CHF was also observed at 40°C (P = 0.065). Overall maximal relaxation rate was significantly slower with increasing temperature in both CHF and Sham, but the relaxation rate was significantly less temperature sensitive in CHF compared with Sham (Fig. 2C).

In summary, the 5-Hz fatigue protocol reflected a slowed soleus muscle function in CHF compared with Sham, but the difference in muscle function between the two groups became less prominent with increasing temperature and was almost absent at 40°C. The fatigue protocol also reflected that force development was more temperature sensitive in CHF, whereas contraction and relaxation rates were less temperature sensitive in CHF compared with Sham during fatigue development (Fig. 2).

**ATP, CrP, and lactate.** ATP, CrP, and lactate content in stimulated and unstimulated soleus muscles were determined at the end of the 5-Hz stimulation protocol at 40°C. There were no significant differences in tissue content of high-energy phosphates and lactate between CHF and Sham. However, the 5-Hz stimulation induced a significant breakdown of ATP.
(47%) and CrP (45%) and an accumulation of lactate (141%) in the stimulated muscle in both CHF and Sham (Table 2).

**SR function.** In the crude homogenate from unstimulated soleus muscles, we measured Ca\(^{2+}\) uptake and release in SR vesicles at 35, 36, 37, and 40°C. There was a tendency, although not significant, toward overall higher Ca\(^{2+}\) uptake and release in the soleus muscle homogenates in CHF compared with Sham. However, at 36°C, uptake and release rates were significantly increased by 106 and 46%, respectively, in CHF compared with Sham (Fig. 3).

In CHF, the Ca\(^{2+}\) uptake and release rates were not significantly altered with temperature. In Sham, there were dips in uptake and release rates at 36°C, and Ca\(^{2+}\) uptake rate in Sham was significantly increased from 36 to 40°C (Fig. 3).

Ca\(^{2+}\) -ATPase content was quantified in crude homogenate from unstimulated soleus muscles, and there was no significant difference between CHF (46 ± 4 nmol/g protein) and Sham (39 ± 3 nmol/g protein). At 37°C, the uptake rates were 113 ± 27 and 86 ± 20 mol Ca\(^{2+}\)·mol Ca\(^{2+}\)-ATPase\(^{-1}\)·min\(^{-1}\) in CHF and Sham rats, respectively.

**DISCUSSION**

In this study, we demonstrated that the temperature sensitivity of the contraction and relaxation rates as well as the fatigue resistance in the soleus muscle were different in CHF and Sham rats, and the difference in muscle contractile function between the two groups was less pronounced at increasing temperatures within the physiological range. The slowing and altered temperature sensitivity of the soleus muscle during CHF probably cannot be explained by alterations in SR Ca\(^{2+}\) uptake pumps or release channels, because we did not find correlating differences in Ca\(^{2+}\) uptake and release rates in the soleus muscles between CHF and Sham.

**Contractile properties.** Although a very narrow temperature range was examined, contractile performance of the soleus muscle varied as predicted with temperature. Maximal developed force was only slightly temperature dependent over the 35–40°C range during stimulation at 1 and 50 Hz in the present study. This is in accordance with studies on normal rats that show that isometric tension in slow-twitch muscle increases with temperature but reaches a plateau at ~37°C (23, 24, 39). Previous studies on temperature effects on fatigue have been performed on healthy animals using experimental setups and stimulation protocols different from those used in the present study. Petrofsky and Lind (23) and Segal et al. (29) observed more pronounced fatigue at increasing temperature in slow-twitch muscle from normal rats (in vitro) and cats (in situ). Although we used a different protocol, it seems from our observations that this response is aggravated in soleus muscles from CHF rats. Maximal contraction rate increased with temperature at all stimulation frequencies examined, which is in agreement with previous studies (1, 23, 29). However, the temperature dependency of maximal relaxation rate was more variable in the present study (Fig. 1), and during the 5-Hz fatigue protocol overall relaxation rate decreased with temper-
Fig. 2. Stimulation (5 Hz) of the in situ prepared rat soleus muscle at 35, 37, and 40°C. A: maximal force. B: maximal contraction rate. C: maximal relaxation rate. At 35°C, there was an overall slowing of maximal contraction rate and maximal relaxation rate in CHF rats compared with sham rats ($P < 0.05$), and at 37°C there was an overall slowing of contraction rate in CHF compared with sham ($P < 0.05$). Force development was more temperature sensitive in CHF, whereas contraction and relaxation rates were less temperature sensitive in CHF compared with sham during fatigue development ($P < 0.05$). Data are means (35°C: CHF, $n = 14$; sham, $n = 6$; 37°C: CHF, $n = 6$; sham, $n = 9$; 40°C: CHF, $n = 6$; sham, $n = 6$).
skeletal muscle during CHF. On the other hand, SR Ca\(^{2+}\) cycling reflects an attempt by the muscle to compensate for slowed kinetics of the contractile machinery in skeletal muscle during CHF. On the other hand, SR Ca\(^{2+}\) uptake and release were measured in vitro, and thus signals that regulate SR Ca\(^{2+}\) handling could in theory be lost during preparation of Table 2. High-energy phosphates and lactate in the stimulated and unstimulated contralateral soleus muscle after 5-Hz stimulation at 40°C

<table>
<thead>
<tr>
<th>Muscle State</th>
<th>Sham (n = 6)</th>
<th>CHF (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unstimulated</td>
<td>Stimulated</td>
</tr>
<tr>
<td>ATP</td>
<td>5.1 ±0.4</td>
<td>2.9 ±0.3</td>
</tr>
<tr>
<td>CrP</td>
<td>5.5 ±1.0</td>
<td>3.1 ±0.6*</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.9 ±0.3</td>
<td>6.8 ±1.0*</td>
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</table>

Values are means ± SE (in mmol/kg wet wt). CrP, creatine phosphate. *P < 0.05 stimulated vs. unstimulated.

ature (Fig. 2). This variation is not easy to explain, and we can merely speculate that the rate of relaxation does not depend on a single temperature-sensitive step but that the process is more complex.

Contractile properties are affected by myosin isoform composition. There are reports of a fiber-type switch in muscles of CHF rats (17). However, Lunde et al. (18) did not find any change in the fiber-type composition of the soleus muscle in CHF rats. Lunde et al. (16) reported that the soleus muscle was more fragile in CHF rats compared with controls. The extracellular matrix and the viscoelastic properties of skeletal muscle might have changed in the CHF condition. We recently demonstrated that the activity of matrix metalloproteinases is higher in the soleus muscle in these animals, but it is not known whether this affects the composition of the extracellular matrix and the passive mechanical properties (36). Because matrix metalloproteinases activity was also higher in the EDL muscle, which does not show alterations of contractile parameters similar to those seen in the soleus muscle (18), it is not likely that viscoelastic properties in the soleus muscle are changed in a way that affects contractile parameters.

Altogether, soleus muscle function was slower in CHF compared with Sham, which is in agreement with previous findings (16, 18, 25, 35). Stimulation of the soleus muscle also revealed that the difference in muscle function between CHF and Sham was almost absent at 40°C compared with the markedly slower muscle function at 35°C in CHF. In normal rats, the temperature of active muscles during moderate exercise is 40°C compared with 36°C at rest (4, 7). One might therefore speculate that patients with CHF will experience increased effect of warming up compared with healthy individuals.

Although fatigue resistance was more temperature dependent in CHF than in Sham, we found that contraction and relaxation rates were less temperature sensitive during the fatigue protocol in CHF compared with Sham. Taken together, these results suggest a different temperature sensitivity of intracellular proteins involved in EC coupling in the soleus muscle of CHF compared with Sham rats.

Ca\(^{2+}\) handling in the SR. We observed that force development during fatigue was more temperature sensitive in CHF than Sham. Because one important component in skeletal muscle fatigue is a reduced SR Ca\(^{2+}\) release (12, 42, 43), SR Ca\(^{2+}\) release channels might be affected in soleus in CHF as proposed by Ward et al. (40) and Reiken et al. (25). The slowing of relaxation rate and reduced temperature sensitivity of relaxation rate during fatigue in CHF compared with Sham might also be related to SR Ca\(^{2+}\) handling, as Fryer and Neering (9) showed that, at temperatures higher than 20–25°C, the rate of relaxation in both rat slow- and fast-twitch muscles seems primarily to be determined by the rate of Ca\(^{2+}\) reuptake into the SR.

We measured Ca\(^{2+}\) uptake and release rates in homogenates of soleus muscles at the same temperatures as we measured contractile properties. Interestingly, and in contrast to the slowed muscle contraction and relaxation rates, there was a tendency toward increased SR Ca\(^{2+}\) release and uptake in CHF compared with Sham. This is in agreement with previous studies showing accelerated SR Ca\(^{2+}\) uptake and release at 37°C in fast-twitch and mixed muscle from rats with moderate heart failure (32, 44). One could speculate that the accelerated SR Ca\(^{2+}\) cycling reflects an attempt by the muscle to compensate for slowed kinetics of the contractile machinery in skeletal muscle during CHF. On the other hand, SR Ca\(^{2+}\) uptake and release were measured in vitro, and thus signals that regulate SR Ca\(^{2+}\) handling could in theory be lost during preparation of

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the muscle homogenate. One should also keep in mind that we measured SR Ca$^{2+}$ handling in unstimulated soleus muscles, and this might not reflect what happens in the stimulated soleus. However, we did find muscle dysfunction in the unfatigued soleus muscle when stimulating at 1 Hz. If this dysfunction were related to alterations in SR Ca$^{2+}$ handling in CHF, we should have been able to detect it also in homogenates obtained from resting muscle.

We did not find a significant change in the tissue content of Ca$^{2+}$-ATPase in the soleus muscle by steady-state $^{32}$P incorporation, and observations in our laboratory using the same rat model showed no difference in mRNA and protein expression of Ca$^{2+}$-ATPase (SERCA2) (P. K. Lunde, unpublished results). In contrast to our results, Simonini et al. (30) showed reduced protein and mRNA expression of SERCA2 in the soleus muscle of female Sprague-Dawley rats with CHF. Nevertheless, unchanged or reduced Ca$^{2+}$-ATPase protein content cannot explain the increased Ca$^{2+}$ uptake we observed in soleus in CHF compared with Sham. Proteins regulating SERCA2 in soleus in rats have to our knowledge not yet been identified (6).

We observed that both Ca$^{2+}$ uptake and release were temperature insensitive in CHF, but in Sham Ca$^{2+}$ uptake increased from 36 to 40°C. Previous studies examining Ca$^{2+}$ uptake in healthy mice and rats have found increased SR Ca$^{2+}$ uptake with increasing temperature from 15 to 37°C and then a leveling off of Ca$^{2+}$ uptake from 37 to 41°C in homogenate from fast-twitch and mixed muscle (27, 41). The strange reduction in both Ca$^{2+}$ uptake and release from 35 to 36°C in the Sham group was reproduced in control experiments (not shown) and was markedly different from the CHF group. We have no explanation for this phenomenon, which, if reproduced, calls for further investigation. The increase in Ca$^{2+}$ uptake rate with temperature from 36 to 40°C in Sham but not in CHF could be related to the less temperature-dependent relaxation rate during the fatigue protocol in soleus in CHF compared with Sham.

Metabolism. The temperature-dependent muscle dysfunction seen in CHF could be the result of abnormalities in muscle metabolism (17). The development of fatigue is associated with a reduction in cytoplasmic ATP and CrP concentration, and increasing muscle temperature accelerates metabolic rate and glycolysis (2–4, 26, 28). However, muscle content of high-energy phosphates and lactate in the soleus muscle was not different between CHF and Sham at the end of the 5-Hz stimulation protocol at 40°C. In addition, previous studies in our laboratory using the same rat model showed no differences in high-energy phosphates and lactate in soleus between CHF and Sham after the 5-Hz stimulation protocol at 35–37°C (18, 35). Consequently, metabolism does not seem to be more rate limiting in the soleus muscle in CHF than in Sham.

Possible explanations for skeletal muscle dysfunction during CHF. The present and our laboratory’s previous studies suggest that atrophy, reduced blood supply, altered metabolism, and abnormalities in SR Ca$^{2+}$ pumps and release channels cannot explain the contractile dysfunction observed in the soleus muscle in CHF (16, 18, 35).

The muscle dysfunction in CHF may reside in the contractile machinery, as indicated by our preliminary results from measurements of Ca$^{2+}$ transients in isolated soleus muscle fibers (15). This could involve changes in Ca$^{2+}$ sensitivity of the myofilaments or in cross-bridge interactions. It is not known whether the binding of Ca$^{2+}$ to troponin C is temperature dependent in slow-twitch muscle. Stephenson and Williams (33) observed that the Ca$^{2+}$ sensitivity of the contractile apparatus in slow-twitch skinned fibers was temperature independent between 3 and 35°C, but the investigators would not totally exclude a temperature effect because of a large variation in the data set. To our knowledge, no study has investigated the temperature dependence of Ca$^{2+}$ sensitivity in skeletal muscle in the temperature range of our contractile measurements (35–40°C). Although Ca$^{2+}$ binding to troponin C is the primary site for the Ca$^{2+}$ sensitivity of the contractile apparatus, the myosin regulatory light chains, which are parts of the myosin heads, could play an important modulatory role. During muscle contraction, the increase in myoplasmic Ca$^{2+}$ activates the Ca$^{2+}$/calmodulin-dependent myosin light kinase and leads to phosphorylation of the regulatory light chains. This increases the Ca$^{2+}$ sensitivity of the myofilaments (34). Investigators have shown that the phosphorylation status of slow-twitch skeletal muscle might be altered during CHF (25), and we speculate that this could affect the Ca$^{2+}$ sensitivity of the contractile apparatus in CHF.

In conclusion, in rats with CHF, soleus muscle function is slowed, and the temperature sensitivity of the contractile properties is different in CHF and Sham. The muscle dysfunction during CHF is not associated with alterations of the SR Ca$^{2+}$ pumps or release channels or with changes in metabolism. Our results rather indicate that CHF causes other intrinsic abnormalities in the EC coupling, possibly within the contractile machinery.

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