Sarcalumenin is essential for maintaining cardiac function during endurance exercise training

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Submitted 22 August 2008; accepted in final form 2 June 2009

Sarcalumenin (SAR), a Ca$^{2+}$-binding protein located in the longitudinal sarcoplasmic reticulum (SR), regulates Ca$^{2+}$ reuptake into the SR by interacting with cardiac sarco(endoplasmic reticulum Ca$^{2+}$-ATPase 2a (SERCA2a). We have previously demonstrated that SAR deficiency induced progressive heart failure in response to pressure overload; despite mild cardiac dysfunction in sham-operated SAR knockout (SARKO) mice (26). Since responses to physiological stresses often differ from those to pathological stresses, we examined the effects of endurance exercise on cardiac function in SARKO mice. Wild-type (WT) and SARKO mice were subjected to endurance treadmill exercise training (~65% of maximal exercise ability for 60 min/day) for 12 wk. After exercise training, maximal exercise ability was significantly increased by 5% in WT mice ($n=6$), whereas it was significantly decreased by 37% in SARKO mice ($n=5$). Cardiac function assessed by echocardiographic examination was significantly decreased in accordance with upregulation of biomarkers of cardiac stress in SARKO mice after training. After training, expression levels of SERCA2a protein were significantly downregulated by 30% in SARKO hearts, whereas they were significantly upregulated by 59% in WT hearts. Consequently, SERCA2 activity was significantly decreased in SARKO hearts after training. Furthermore, the expression levels of other Ca$^{2+}$-handling proteins, including phospholamban, ryanodine receptor 2, calsequastatin 2, and sodium/calcium exchanger 1, were significantly decreased in SARKO hearts after training. These results indicate that SAR plays a critical role in maintaining cardiac function under physiological stresses, such as endurance exercise, by regulating Ca$^{2+}$ transport activity into the SR. SAR may be a primary target for exercise-related adaptation of the Ca$^{2+}$ storage system in the SR to preserve cardiac function.

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impact of SAR deficiency on the expression and activity of SERCA2a in the heart and on cardiac function after endurance exercise training.

MATERIALS AND METHODS

Animal preparation. Generation of SARKO mice has been described previously (38). SARKO and C57BL/6J WT mice (8–10 wk of age) were bred at Yokohama City University. All mice used in the present study came from the same genetic background. All animal care and study protocols were approved by the Animal Ethics Committees of Yokohama City University School of Medicine and Waseda University, and the investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (National Institutes of Health Publication No. 85–23, revised 1996).

Maximal exercise ability and treadmill endurance exercise training. Mice were randomized into four groups: sedentary WT (SED-WT) and sedentary SARKO (SED-SARKO) mice, and WT (ET-WT) and SARKO (ET-SARKO) mice subjected to endurance exercise training.

Animals ran on a rodent motor-driven treadmill (MANUAL, LE 8700 series, Panlab, Barcelona, Spain) with adjustable belt speed (0–150 cm/s). The treadmill apparatus was equipped with adjustable-amperage (0–2 mA) shock bars at the rear of the belt, through which mild electrical stimulation (grid shock <1 mA) was applied to encourage the mice to run. A detector located above the shock grid measured the number of shock stimuli received by each mouse.

First, mice were acclimated to the treadmill via three 15-min running sessions with mild shock stimulation and a belt speed of 30 cm/s. After acclimation, all mice underwent a treadmill exercise test to determine their exercise ability before the endurance exercise training described below. A similar assessment was made during and after training for comparison purposes. The belt speed of the treadmill was set to 30 cm/s at the beginning of each test. It was then increased linearly by 2 cm/s every 30 s until the mice could not continue to run regularly on the treadmill, or until they had rested on the shock grid more than three times. The final belt speed achieved by each mouse was considered to be that mouse's maximal exercise ability. Maximal exercise ability was determined by averaging the maximal belt speeds of at least three measurements for each mouse; there was an inter-mission of at least 1 h between each measurement. Workloads of endurance exercise training were then adjusted for each mouse in accordance with its maximal exercise ability.

Before the start of each exercise training session, each mouse performed a 5-min warm-up at 40% of its maximal speed. ET-WT and ET-SARKO mice then ran on the treadmill (at 0° inclination) at 65% of their maximal speeds for 60 min/day, 5 days/wk, for 12 wk. Each mouse’s maximal exercise ability was reevaluated every 4 wk, and each mouse’s workload was adjusted again based on its current maximal speed (Supplemental Fig. 1). (The online version of this article contains supplemental data.) For sedentary mice, running skill was maintained by treadmill running for 15 min at 0° inclination at a belt speed of 30 cm/s, 3 days/wk.

Citrate synthase activity. As a marker for endurance training, the myocardial citrate synthase (CS) activity was measured at 37°C in the presence of 0.2% Triton X-100 with 20 μg protein sample, as previously described (27, 32). CS activity was also measured in soleus muscle homogenates to assess the efficacy of endurance exercise training.

Cardiac function assessed by echocardiography. Mice were anesthetized with an intraperitoneal injection of Avertin (250 μg/g) and subjected to echocardiography, as described in our laboratory’s previous publications (28, 38). Since we have observed that the heart rates of mice decrease after intraperitoneal injection of Avertin, reaching stable minimal levels around 15–20 min after injection (Supplemental Fig. 2), we obtained the echocardiographic data around 15–20 min after injection of Avertin. After the final assessment of cardiac function after endurance training, heart and skeletal (soleus) muscles were immediately placed in chilled phosphate-buffered saline to remove all residual blood. Hearts were then weighed, and left ventricles were immediately frozen in liquid nitrogen and stored at −80°C.

Quantitative RT-PCR analysis. Total RNA was isolated from various tissues using TRIZol reagent (Invitrogen, Carlsbad, CA), as recommended by the manufacturer. Generation of cDNA and RT-PCR analysis was performed as described previously (36, 37). The primers for PCR amplification were designed based on the mouse nucleotide sequences of atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP). The mRNA levels of interest were normalized to mouse glyceraldehyde-3-phosphate dehydrogenase.

Immunoblot analysis. We prepared protein samples from the left ventricular tissues of the sedentary and trained mice, which had been immediately frozen and stored at −80°C after death of the animals. Immunoblot analyses were performed as described previously (26, 36). Briefly, tissues were defrosted to 0°C and homogenized in a chilled homogenization buffer [in mM: 50 Tris (pH 8.0), 1 EDTA, 1 EGTA, 1 dithiothreitol, and 200 sucrose] with protease inhibitors (Complete Mini, Roche, Basel, Switzerland). Protein content was determined using the Coomassie Plus protein assay (Pierce Chemical, Rockford, IL), and BSA (0.1–1 mg/ml) was used as a standard. The protein samples (20 μg) were separated in the same gel by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). When the molecular size of target proteins was different, polyvinylidene difluoride membranes were cut in accordance with their size. When the molecular size of target proteins was similar, we reused the same membrane for a different antibody after washing the membrane with a stripping buffer [in mM: 62.5 Tris (pH 8.0), 100 2-mercaptoethanol, and 2% SDS]. Antibodies used in the present study are shown in Supplemental Table 1. After application of a secondary antibody, quantification of the target signals was performed using the LAS-3000 imaging system (FUJIFILM, Tokyo, Japan). The protein levels of interest were normalized to rat β-actin. For reuse, a membrane was washed with a stripping buffer at 55°C for 10 min and was washed three times with 0.1% Tris buffered saline-Tween 20 buffer.

SR Ca2+-ATPase activity was measured in triplicate spectrophotometrically at 37°C, as described previously with some modifications (18). Briefly, using 5 μg of SR protein from mice heart tissues, the reaction was carried out at 37°C in a reaction medium [in mM: 30 TES, 100 KCl, 5 NaN3, 5 MgCl2, 0.5 EGTA, and 2% SDS]. Antibodies used in the present study are shown in Supplemental Table 1. After application of a secondary antibody, quantification of the target signals was performed using the LAS-3000 imaging system (FUJIFILM, Tokyo, Japan). The protein levels of interest were normalized to rat β-actin. For reuse, a membrane was washed with a stripping buffer at 55°C for 10 min and was washed three times with 0.1% Tris buffered saline-Tween 20 buffer.

SR Ca2+-ATPase activity was measured in triplicate spectrophotometrically at 37°C, as described previously with some modifications (18). Briefly, using 5 μg of SR protein from mice heart tissues, the reaction was carried out at 37°C in a reaction medium [in mM: 30 TES, 100 KCl, 5 NaN3, 5 MgCl2, 0.5 EGTA, and 4 ATP, with or without 0.5 CaCl2]. The reaction was preincubated at 37°C for 5 min. The reaction was started at 37°C by adding SR protein to the medium. After 5 min, the reaction was stopped by adding 0.5 ml of ice-cold 10% trichloroacetic acid solution, and the mixture was placed on ice. Inorganic phosphate was measured by using U2001 (Hitachi), as described previously (8). Ca2+-ATPase activity was calculated by subtracting the ATPase activity in the presence of 0.5 mM EGTA (no added Ca2+) from the activity in the presence of 0.5 mM CaCl2.

Statistical analysis. All values are expressed as means ± SE. Comparisons of data from multiple groups were performed by unpaired ANOVA followed by the Student Newman-Keuls post hoc test. Statistical significance was defined as P < 0.05.

RESULTS

Effects of endurance exercise training on exercise ability in SARKO mice. Before the start of endurance exercise training, exercise ability was examined in WT and SARKO mice by a treadmill-based exercise stress test, described above. Maximal exercise ability, as evaluated by maximal belt speed, was lower in SARKO mice (n = 16, 65.0 ± 3.6 cm/s) than in WT mice.
n/H1100516, 74.1 cm/s), although it did not reach a statistical significance (P = 0.059). As expected, maximal exercise ability in sedentary animals (SED-WT and SED-SARKO mice) did not significantly change during the 12-wk training period (data not shown). In ET-WT mice, maximal exercise ability gradually increased during endurance exercise training, whereas, in ET-SARKO mice, it gradually decreased (Fig. 1). Whenever a change in a mouse’s maximal exercise ability was detected by a regular treadmill test, that mouse’s training workload was adjusted based on its current maximal speed (Supplemental Fig. 1). Maximal exercise ability after endurance exercise training significantly increased by 5% in ET-WT mice, whereas it actually decreased by 37% in ET-SARKO mice compared with their ability measured before the training regime began (Fig. 1).

**Exercise training did not improve CS activity in SARKO mice.** We observed no difference between WT and SARKO mice in terms of CS activity of skeletal or cardiac muscle at a basal condition. After the endurance exercise training, ET-WT mice exhibited increased CS activity of soleus muscle (Fig. 2A), indicating an appropriate effect of the training program on working muscles. In accordance, they also exhibited increased CS activity of cardiac muscle (Fig. 2B), which is consistent with several previous studies (1, 20), although most of previous studies have demonstrated that CS activity is not increased or little increased by endurance exercise in rodent hearts (4, 19). In ET-SARKO mice, on the other hand, CS activity was not increased in either soleus or cardiac muscle (Fig. 2).

**Endurance exercise training resulted in cardiac dysfunction in SARKO mice.** To examine the effect of endurance exercise training on cardiac function, we investigated it using transthoracic echocardiography. Before endurance exercise training, all parameters listed in Table 1 were similar between WT and SARKO mice, including body weight, heart rate, left ventricular fractional shortening, thickness of myocardial walls, and ejection time. After endurance exercise training, left ventricular fractional shortening was significantly decreased in ET-SARKO mice, whereas it was not changed in ET-WT mice (Table 1). As we expected, the diameter of the end-diastolic left ventricular chamber was significantly increased in ET-SARKO mice. Furthermore, ejection time was significantly prolonged in ET-SARKO mice, and their heart rate corrected velocity of circumferential fiber shortening was significantly lower (Table 1).

**Biomarkers of cardiac stress were increased in ET-SARKO hearts.** To examine the effect of endurance exercise training on the myocardium itself, we measured molecular markers of

![Fig. 2. Citrate synthase (CS) activity after ET. After ET, CS activity of soleus muscle (A) and cardiac muscle (B) was increased in ET-WT mice, but not in ET-SARKO mice. Values are means ± SE; n = 5 for each group. SED, sedentary; NS, not significant.](http://ajpheart.physiology.org/)

**Fig. 1.** The effect of endurance exercise training (ET) on maximal exercise ability in sarcalumenin (SAR) knockout (SARKO) mice. Maximal exercise ability, as evaluated by maximal belt speed, is already lower in SARKO mice than in wild-type (WT) mice before ET. During ET, maximal exercise ability gradually increased in WT mice, whereas it actually decreased in SARKO mice in a time-dependent manner. Maximal exercise ability after ET was significantly increased in WT mice, whereas it was actually decreased in SARKO mice compared with their maximal exercise ability before the training. Values are means ± SE; n = 6 and 5 for WT and knockout (KO), respectively.
cardiac stress, such as ANF and BNP mRNAs. These were significantly upregulated in ET-SARKO mice (Fig. 3). Endurance exercise training did not affect the expression of ANF and BNP mRNAs in ET-WT mice.

Significant reductions in the expression of Ca2+ handling proteins in ET-SARKO mice. Since the expression levels of SERCA2a and other Ca2+ handling proteins are critical for the regulation of cardiac function, we examined them by Western blot analyses (Fig. 4, Table 2). Consistent with our laboratory’s previous report (26, 38), the expression levels of SERCA2a and other Ca2+ handling proteins that we examined were downregulated in SED-SARKO mice compared with those in SED-WT mice. After endurance exercise training, the expression level of SERCA2a protein was significantly increased by 59% in ET-WT mice, whereas it was reduced by 30% in ET-SARKO mice compared with sedentary mice of each group’s respective genotype. Endurance exercise training also resulted in a further significant downregulation of both total and phosphorylated PLN proteins in ET-WT mice, but not in ET-SARKO mice. The SERCA2a-to-PLN protein ratio was significantly decreased in ventricular muscles of ET-SARKO mice (Table 2). The ratio of phosphorylated threonine 17 PLN to total PLN protein was significantly lower in ET-SARKO than in ET-WT, but that of serine 16 to total PLN protein was not (Table 2). It should be noted that intraperitoneal injection of Avertin did not affect the phosphorylation status of serine 16 and threonine 17 in PLN (Supplemental Fig. 2).

The expression levels of calsequestrin 2 (CSQ2) and ryanodine receptor type 2 (RyR2) proteins in SED-SARKO mice were comparable to those in SED-WT mice, while those of sodium/calcium exchanger 1 (NCX1) protein were even higher in ET-SARKO mice after endurance exercise training. As measured in myocardial homogenates, maximal Ca2+-ATPase activity was lower in SED-SARKO mice than in ET-WT mice. The expression levels of ANF (A) and brain natriuretic peptide (BNP) mRNAs were significantly upregulated in ET-SARKO mice. Quantitative RT-PCR analyses revealed that the expression levels of ANF (A) and BNP (B) mRNAs were significantly upregulated in the ventricles of ET-SARKO mice. The expression levels observed in SED-WT mice were set as 100% as a control. mRNA expression was normalized by GAPDH. Values are means ± SE; n = 5 for each group.

### Table 1. Cardiac function after endurance exercise training

<table>
<thead>
<tr>
<th></th>
<th>SED-WT</th>
<th>ET-WT</th>
<th>SED-SARKO</th>
<th>ET-SARKO</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW, g</td>
<td>24.2±1.3</td>
<td>32.6±1.9</td>
<td>26.3±1.7</td>
<td>31.1±1.3</td>
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<td>HR, beats/min</td>
<td>464±15</td>
<td>474±15</td>
<td>438±18</td>
<td>436±18</td>
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<tr>
<td>LV weight, mg</td>
<td>115±9</td>
<td>111±7</td>
<td>111±7</td>
<td>93±6</td>
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<tr>
<td>LV-to-BW weight ratio, mg/g</td>
<td>3.55±0.25</td>
<td>3.58±0.15</td>
<td>3.38±0.12</td>
<td>3.26±0.15</td>
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<tr>
<td>LV FS, %</td>
<td>35.6±1.1</td>
<td>35.5±1.1</td>
<td>34.7±1.2</td>
<td>34.9±2.6</td>
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<tr>
<td>LVIDd, mm</td>
<td>4.1±0.08</td>
<td>4.12±0.11</td>
<td>4.03±0.06</td>
<td>4.25±0.12</td>
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<td>IVSTd, mm</td>
<td>0.77±0.03</td>
<td>0.79±0.05</td>
<td>0.77±0.02</td>
<td>0.71±0.05</td>
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<tr>
<td>LVPWd, mm</td>
<td>0.76±0.03</td>
<td>0.75±0.04</td>
<td>0.76±0.04</td>
<td>0.76±0.06</td>
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<tr>
<td>Ejection time, ms</td>
<td>60±1</td>
<td>60±1</td>
<td>64±2</td>
<td>66±3</td>
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<tr>
<td>Vcf, circumferences/s</td>
<td>2.14±0.06</td>
<td>2.13±0.07</td>
<td>2.12±0.10</td>
<td>2.14±0.17</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of mice. SED, sedentary; WT, wild-type mice; ET, endurance exercise training; SARKO, sarcalumenin-knockout mice; Pre, before ET; Post, after ET; BW, body weight; HR, heart rate; LV, left ventricle; FS, fractional shortening; LVIDd, LV internal dimensions at end diastole; IVSTd, interventricular septum thickness at end diastole; LVPWd, LV posterior wall thickness at end diastole; Vcf, corrected velocity of circumferential fiber shortening. Significant difference vs. Pre: *P < 0.05 and **P < 0.01; vs. WT: ^P < 0.05; and vs. SED: âP < 0.05.
the basis of that mouse' maximal exercise ability. Accordingly, intensity of each mouse's exercise regime was determined on undertaken by WT mice (Supplemental Fig. 1), because the taken by SARKO mice was significantly lower than that tation to not only pathological, but also physiological, stresses. an important role in preserving cardiac function during adap-

physiological stresses, the present data indicate that SAR plays
dysfunction (38). Since exercise is one of the most common
loaded stress induced by transverse aortic constriction (26),
SAR gene. Along the same lines, we have recently demon-
cardiac dysfunction in mice that harbor systemic ablation of the

Table 2. The expression of calcium handling proteins after endurance exercise training

<table>
<thead>
<tr>
<th></th>
<th>SED-WT</th>
<th>ET-WT</th>
<th>SED-SARKO</th>
<th>ET-SARKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAR</td>
<td>100±5</td>
<td>108±9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SERCA2</td>
<td>100±10</td>
<td>159±13</td>
<td>74±4*</td>
<td>52±6‡</td>
</tr>
<tr>
<td>PLN</td>
<td>100±6</td>
<td>123±8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-PLN Ser 16</td>
<td>100±3</td>
<td>120±11</td>
<td>95±5</td>
<td>82±3†‡</td>
</tr>
<tr>
<td>p-PLN Thr 17</td>
<td>100±4</td>
<td>112±7</td>
<td>92±6</td>
<td>78±4‡</td>
</tr>
<tr>
<td>SERCA2/PLN</td>
<td>100±5</td>
<td>132±12</td>
<td>94±4</td>
<td>75±10‡</td>
</tr>
<tr>
<td>p-PLN Ser 16/PLN</td>
<td>100±4</td>
<td>98±8</td>
<td>113±4</td>
<td>116±8</td>
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<tr>
<td>p-PLN Thr 17/PLN</td>
<td>100±1</td>
<td>98±4</td>
<td>93±2</td>
<td>88±2*‡</td>
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<tr>
<td>RyR2</td>
<td>100±5</td>
<td>100±10</td>
<td>97±6</td>
<td>68±8‡‡</td>
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<tr>
<td>CSQ2</td>
<td>100±7</td>
<td>101±4</td>
<td></td>
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</tr>
<tr>
<td>NCX1</td>
<td>100±10</td>
<td>139±11</td>
<td>124±3*</td>
<td>92±11‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 mice for each group. The expression level in SED-WT mice was referred to as a control. Protein expression was normalized by β-actin. SAR, sarcalumenin; SERCA2, sarco(endo)plasmic reticulum Ca2+-ATPase 2; PLN, phospholamban; p-PLN: phosphorylated phospholamban; RyR2, ryanodine receptor 2; CSQ2, calsequestrin 2; NCX1, sodium/calcium exchanger 1. Significant difference vs. WT: *P < 0.05 and †P < 0.01; vs. SED: ‡P < 0.05 and §P < 0.01.

DISCUSSION

The most striking finding in the present study is that long-
term (12 wk) endurance exercise training induced a significant cardiac dysfunction in mice that harbor systemic ablation of the SAR gene. Along the same lines, we have recently demonstrated that SARKO mice failed to adapt to pressure-over-

It should be noted that the absolute training intensity under-
taken by SARKO mice was significantly lower than that undertaken by WT mice (Supplemental Fig. 1), because the intensity of each mouse’s exercise regime was determined on the basis of that mouse’s maximal exercise ability. Accordingly,

CS activity in soleus muscle after endurance exercise training was significantly lower in ET-SARKO mice than in ET-WT mice (Fig. 2). Since skeletal muscle CS activity is a marker for mitochondrial content (a hallmark of endurance exercise) and muscle oxidative capacity, this result indicates that our exercise training program is sufficient to enhance the exercise ability of WT mice, but insufficient to enhance that of SARKO mice. Although this may explain a number of the negative effects on SARKO mice that were caused by exercise training in the present study, it is, nevertheless, very difficult to explain why ET-SARKO mice exhibited progressive cardiac dysfunction. We assume that inadequate adaptation to endurance exercise in ET-SARKO mice caused impaired cardiac function, the primary insult, which, secondarily, resulted in a number of negative effects on SARKO mice caused by training.

The mechanism by which endurance exercise induced pro-
gressive cardiac dysfunction in SARKO mice is a critical question. One observation that may be relevant to this question is the significant decrease in the expression and activity of SERCA2a in ET-SARKO mice. A number of previous studies have reported that endurance exercise training increased the expression and/or activity of SERCA2a in healthy (9, 10, 20, 22, 30, 35) or diseased rodents (6, 21, 24, 34, 39); similarly, we found that the expression and activity of SERCA2a increased after endurance exercise training in control mice. Yet other studies have demonstrated that endurance exercise training does not change the expression and/or activity of SERCA2a (3, 4) or Ca2+-transients (12) in rodents. It is worth noting that these conflicting results may have their origins in such factors as differences in species, exercise protocols, and/or condition of the subjects; few studies, however, have shown that endurance exercise decreases the expression and/or activity of SERCA2a. Therefore, our results found in ET-SARKO mice were so remarkable that it is very important to investigate why SAR deficiency caused the significant reduction in the expression and activity of SERCA2a under endurance exercise training.

Our laboratory’s recent study has demonstrated that SAR interacts with SERCA2 to enhance the protein stability of SERCA2a (26). Since exercise training usually increases pro-
tein synthesis and degradation in muscle (11, 23), we assume that endurance exercise training also increased the turnover rate of SERCA2a protein. Then we postulate that SAR deficiency induced a progressive degradation of SERCA2a protein due to impaired protein stabilization under endurance exercise training and resulted in the significant decrease in the expression of SERCA2a in ET-SARKO mice. Importantly, the present study demonstrated that endurance exercise training slightly increased the expression levels of SERA protein in WT hearts, in accordance with a significant increase in the expression of SERCA2a protein. To our knowledge, this is the first report to show the effect of endurance exercise training on the expression of SERA protein. These data suggest that SERA is a key regulatory protein to maintain the expression level of SERCA2a protein under pathophysiological stresses. In addition, the ratios of SERCA2a to PLN protein and phosphorylated threonine 17 PLN to total PLN protein were significantly decreased in the ventricular muscles of ET-SARKO mice, indicating that SERCA2a activity was inhibited by PLN more in ET-SARKO mice than in other groups. Taken together, this evidence shows that SAR deficiency induced a significant reduction in SERCA2a activity and deterioration of the Ca²⁺ storage system in the SR under endurance exercise stress, which is very likely to play a primary role in the exercise-induced cardiac dysfunction exhibited by ET-SARKO mice.

Interestingly, in addition to the decreases in the SERCA2a and PLN proteins that interact with SAR in the longitudinal SR, other Ca²⁺ handling proteins, such as RyR2, CSQ2, and NCX1, were also significantly downregulated in ET-SARKO mice, which has not been investigated in pressure-overloaded SARKO hearts (26). These abnormalities probably contribute to the further impairment of cardiac function during endurance exercise training. We assume that the downregulation of RyR2, CSQ2, and NCX1 could be a secondary phenomenon that occurs under physiological stress conditions, as SAR does not directly interact with these proteins. The mechanism of these discrepant responses to different stresses in SARKO mice is currently not clear; it is an important question that should be addressed in future studies.

In one way, the results of the present study somewhat contradict those of a recent report by Zhao et al. (40), which showed that skeletal muscles from SARKO mice are highly resistant to fatigue compared with those from WT mice. The same authors have also demonstrated that SOCE was promoted in SARKO skeletal muscle by the upregulation of MG29 (40). They proposed that the promotion of SOCE played a role in making skeletal muscle more fatigue resistant (40). In the present study, however, we did not detect any expression of MG29 protein in either WT or SARKO hearts, before or after exercise training, although we used the same membranes for our Western blot analyses (data not shown). This observation is consistent with a previous study (29). Currently, we cannot explain the exact reason for the disagreement between the results of Zhao et al. (40) and our own. A possible explanation is the difference in the exercise programs our two groups used to evaluate the exercise performance of SARKO mice. Further investigation is needed to clarify whether a defect of MG29 may cause the negative responses to exercise in SARKO cardiac muscle cells.

In conclusion, we found that cardiac function and maximal exercise ability were significantly impaired in SARKO mice after endurance treadmill exercise training. These impairments were due, at least in part, to a significant downregulation of SERCA2a and other Ca²⁺ handling proteins and to a deterioration of the Ca²⁺ storage system in the SARKO heart under endurance exercise. Thus present study indicates that SAR plays a critical role in maintaining cardiac function under physiological stresses, such as endurance exercise, by regulating Ca²⁺ transport activity into the SR. SAR may be a primary target for exercise-related adaptation of the Ca²⁺ storage system in the SR to preserve cardiac function.

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