High-intensity exercise training in mice with cardiomyocyte-specific disruption of Serca2

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1Department of Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim; 2Institute for Experimental Medical Research, Oslo University Hospital Ulleval, Oslo; 3Center for Heart Failure Research, University of Oslo, Oslo; 4Department of Medical Imaging, St. Olavs Hospital, Trondheim; 5Department of Laboratory Medicine, Children’s Health and Women’s Health, Norwegian University of Science and Technology, Trondheim; 6Department of Pathology and Medical Genetics, St. Olavs Hospital, Trondheim; 7Department of Cardiology, Oslo University Hospital Ulleval, Oslo; and 8Department of Cardiology, St. Olavs Hospital, Trondheim, Norway

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Ericsson M, Andersson KB, Amundsen BH, Torp SH, Sjaastad I, Christensen G, Sejersted OM, Ellingsen Ø. High-intensity exercise training in mice with cardiomyocyte-specific disruption of Serca2. J Appl Physiol 108: 1311–1320, 2010. First published February 18, 2010; doi:10.1152/japplphysiol.01133.2009.—Several lines of evidence suggest that reduced Serca2 (SERCA2) function is essential for sustaining cardiac pump function and exercise capacity. Hence, a reduction in SERCA2 abundance is expected to reduce work performance and maximal oxygen uptake (V̇O2max) and to limit the response to exercise training. To test this hypothesis, we compared V̇O2max and exercise capacity in mice with cardiac disruption of Serca2 (SERCA2 KO) with control mice (SERCA2 FF). We also determined whether the effects on V̇O2max and exercise capacity could be modified by high-intensity aerobic exercise training. Treadmill running at 85–90% of V̇O2max started 2 wk after serca2 gene disruption and continued for 4 wk. V̇O2max and maximal running speed were measured weekly in a metabolic chamber. Cardiac function was assessed by echocardiography during light anesthesia. In sedentary SERCA2 KO mice, the aerobic capacity was reduced by 50% and running speed by 28%, whereas trained SERCA2 KO mice were able to maintain maximal running speed despite a 36% decrease in V̇O2max. In SERCA2 FF mice, both V̇O2max and maximal running speed increased by training, while no changes occurred in the sedentary group. Left ventricle dimensions remained unchanged by training in both genotypes. In contrast, training induced right ventricle hypertrophy in SERCA2 KO mice. In conclusion, the SERCA2 protein is essential for sustaining cardiac pump function and exercise capacity. Nevertheless, SERCA2 KO mice were able to maintain maximal running speed in response to exercise training despite a large decrease in V̇O2max.

calcium transport; maximal oxygen uptake; cardiomyopathy; conditional knockout; heart failure; sarco(endo)plasmic reticulum ATPase type 2

Efficient Ca2+ handling by the cardiac sarco(endo)plasmic calcium ATPase (SERCA2) is a major determinant in sustaining myocardial function (5, 15, 21). The SERCA2 protein sequesters Ca2+ into the sarcoplasmic reticulum during diastole by removing cytosolic Ca2+2+, and its function correlates with cardiac contractility and relaxation. During exercise training, increased aerobic capacity is closely associated with cardiomyocyte contractility and increased SERCA2 function (9, 12, 33–35). These observations are consistent with the notion that regulation of cardiomyocyte SERCA2 function is essential for enhanced cardiac pump capacity and thus for sustaining an increase in maximal oxygen uptake (V̇O2max) in response to exercise. V̇O2max is a measure of aerobic capacity and a strong predictor of cardiovascular morbidity and mortality (24). Several studies (9, 34) have shown that high-intensity exercise training may improve both cardiac function and SERCA2 function. Changes in V̇O2max may result from increased oxygen consumption due to exercise training or from decreased oxygen consumption in skeletal muscles due to inactivity or impaired cardiac function (36). Hence, exercise testing is a useful way of assessing the capacity of the cardiovascular system.

Several lines of evidence suggest that reduced SERCA2 function would lead to impaired cardiac contractility and changes in V̇O2max (33). In the present study, we examined the relationship between V̇O2max and exercise capacity in adult mice with inducible cardiomyocyte-specific disruption of Serca2 (SERCA2 KO) (2), and we investigated whether the effects on V̇O2max and exercise capacity could be modified by high-intensity aerobic exercise training. In SERCA2 KO mice, effective compensatory mechanisms, such as enhanced L-type Ca2++ and Na+/Ca2++ exchanger (NCX1) activity, delay the development of severe heart failure by several weeks (2). These mechanisms are sufficient for cardiac function at rest within the time window of the study but may be inadequate for increased cardiac demand during exercise.

Materials and Methods

Mice. Serca2flox/flox (SERCA2 FF) and Serca2flox/floxTg(MHC-MerCreMer) (SERCA2 KO) mice, fully backcrossed onto the B6/J background, are described elsewhere (2, 3). The resulting adult SERCA2 KO mice are unrecoverable until rapid disruption of the Serca2 gene in the presence of tamoxifen, whereas control SERCA2 FF mice remain unaffected (2). All experiments were approved and performed in accordance with the Norwegian National Committee for Animal Welfare Act, which closely conforms to the NIH guidelines (NIH Publication No. 85–23, revised 1996). Mice were housed in an approved animal facility on a 12:12-h light:dark cycle with free access to RM1 rodent pellets (Scanbur, Oslo, Norway) and water. To avoid physiological changes due to diurnal rhythm, all experiments were performed during the dark hours. All groups of mice were fed with tamoxifen base (TS646, Sigma-Aldrich, Oslo, Norway) added to nonpelleted feed (100 mg/200 g; RM1 FG SQC, 811004; Scanbur) for 7 days to induce disruption of the Serca2 gene in the SERCA2 KO

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mice (4). Thereafter, the mice received standard RM1 pellets. SERCA2 KO and SERCA2 FF mice were randomly assigned to training or sedentary groups. The start of the experiment was defined as day 1 of tamoxifen feeding; the end of the experiment was defined as 45 days later, when the mice were killed. In a recent study, myocardial Serca2 mRNA was reduced to <5% after 7 days and SERCA2 protein abundance was reduced to 13% of SERCA2 FF controls 2 wk after the first day of tamoxifen, using a four-injection protocol (2). Another recent study (2) showed that the feed protocol used in this study was as efficient as intraperitoneal injections, producing animals with Serca2 mRNA reduced to <5% of FF controls 4 days after the start of tamoxifen feed administration. In the present study, myocardial Serca2 mRNA and SERCA2 protein reduced similarly 6 wk after start of tamoxifen administration (see Fig. 2 in Results).

**Exercise training, aerobic capacity, and running performance.** The duration of the training protocol was determined in a pilot study (n = 20). On 49 ± 5 days after tamoxifen treatment, both training and sedentary SERCA2 KO mice showed marked reduced physical activity and refused to run on the treadmill. In the full study, SERCA2 FF (n = 16) and SERCA2 KO (n = 25) were killed on day 45 after tamoxifen treatment. High-intensity exercise training starting 2 wk after Serca2 gene disruption was performed as interval running on a treadmill (25° inclination), 8-min bouts at 85–90% of VO2max interspersed with a 2-min recovery at 40–50%, in 1-h sessions 5 days per wk for 4 wk. The details of test and training protocols have previously been described (13). Sedentary mice were tested weekly for VO2max and respiratory exchange ratios (VCO2/VO2). Work economy was estimated by submaximal tests at a set running speed (4a). The maximal running speed was defined as the maximal speed the mice managed to run on the upper half of the treadmill for one whole minute during the VO2max test (13, 32). All VO2max data were normalized to body weight (ml kg−0.75 min−1; Ref. 30). An O2 analyzer (Servomex Pni1155; Servomex) and CO2 analyzer (LAIR 12; M&C Instruments; range of measurements 0–1%) assessed the mean O2 and CO2 content in the metabolic chamber.

**Echocardiography.** Cardiac function was evaluated in 19 mice (SERCA2 FF: n = 7 for training and n = 7 for sedentary; SERCA2 KO: n = 13 for training and n = 11 for sedentary), 2 and 6 wk after tamoxifen treatment, using trans-thoracic high resolution echocardiography (Vevo770; Visual Sonics, Toronto, ON, Canada). Echocardiography was performed during light isoflurane anaesthesia (1.0–2.25%; Abbott, Solna, Sweden) in 100% O2 (800 ml/min). Anesthesia was adjusted to keep the respiration rate at 70–90 bpm. The RMV712 scanhead (Visual Sonics), center frequency of 35 MHz and axial resolution of 50 μm, was used for image acquisition. Left ventricle end-diastolic and stroke volumes were measured by tracing systolic and diastolic endocardium in the parasternal long-axis view. Left atrial diameter was measured in the same view. Right ventricular dimensions were not evaluated due to a difficult position behind the sternum. Echocardiographic data were stored digitally and analyzed offline, using the enclosed software (version v.2.3.0).

**Tissue harvesting.** At the 45-day endpoint, organs were harvested from all mice (n = 41) after cervical dislocation in isoflurane anaesthesia. Hearts were separated into atria (auricles included), left ventricle (septum included), and right ventricular free wall, and weighed separately before immersion in liquid N2. Lungs and liver were weighed, fixed in 4% buffered formaldehyde, and paraffin embedded. Soleus muscles and part of right lung were weighed separately before immersion in liquid N2. The right tibia was collected for gravimetric analyses. The wet and dry weight of the right lung was determined before and after the lung was dried in a vacuum centrifuge for 75 min.

**SDS-PAGE and Western blot analysis.** Total protein lysates were prepared from left ventricles as described previously (2). Protein content was measured by a Micro BCA assay (Pierce 23235; Pierce Biotechnology, Rockford, IL) using BSA as the standard. Samples were electrophoresed in SDS-PAGE gels (7.5%, 15%, or gradient 4–20%; Bio-Rad and Pierce) and transferred to 0.45-μm PVDF membranes (GE Healthcare Amersham Biosciences, Oslo, Norway). Membranes were blocked with 5% BSA in TBS-Tween (anti-P-Thr286 CamKII) or in 5% skimmed milk in TBS-Tween (other antibodies). Antibodies for protein detection were as follows: polyclonal SERCA2a (a kind gift from Frank Wuytack, Katholieke Universiteit, Leuven), CamKII P-Thr286 (PA1–22993; Affinity BioReagents), and CamKIIβ (a kind gift from Donald Bers, University of California, Davis, CA) (NCX1) (30a). Blots were incubated with horseradish-conjugated sheep anti-mouse IgG, donkey anti-rabbit IgG (GE Healthcare Biosciences), or donkey anti-goat IgG (HAF109; R&D Systems) secondary antibodies and developed with ECL or ECLplus reagents (GE Healthcare Biosciences). Images were acquired in a LAS-4000 CCD detection system (Fuji Photo Film Europe, Düsseldorf, Germany). After immunoblottting, membranes were stained with Coomassie Brilliant Blue R250 (Sigma-Aldrich) and imaged for verification of equal protein loading and sample transfer (1).

**Gene expression quantification by RT-quantitative PCR analysis.** Total RNA was isolated from right ventricles (10 mg) and left ventricles (30 mg) using an Rneasy kit including ProteinaseK and DNAse steps as described in the protocol (Qiagen, Oslo, Norway). RNA concentrations were measured in a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples were quality checked using RNA 6000 Nano LabChips in a 2100 Bioanalyzer instrument (Agilent Technologies, Palo Alto, CA). Samples with RNA integrity number (RIN) values >7.5 were accepted. Total RNA (25 ng/μl) was reverse transcribed using iScript (Bio-Rad Laboratories, Oslo, Norway). PCR reactions were run in triplicate with an equivalent of 1.25 ng transcribed RNA in a final volume of 25 μl on a HT7900 sequence detection instruments (Applied Biosystems, Foster City, CA) with predesigned Taqman assays for the following mRNA transcripts: Serca2 (Atp2a2, Mm01201431_m1), atrial

**Table 1. Weights and gravimetric data of trained and sedentary male SERCA2 FF and SERCA2 KO mice**

<table>
<thead>
<tr>
<th>Organ Weights</th>
<th>SERCA2 FF</th>
<th>SERCA2 KO</th>
<th>SERCA2 FF</th>
<th>SERCA2 KO</th>
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<tbody>
<tr>
<td></td>
<td>Sedentary</td>
<td>Exercised</td>
<td>Sedentary</td>
<td>Exercised</td>
</tr>
<tr>
<td>n</td>
<td>7</td>
<td>11</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Body weight (2 wk, g)</td>
<td>29.0 ± 3.1</td>
<td>29.9 ± 1.4</td>
<td>29.6 ± 1.6</td>
<td>29.3 ± 2.3</td>
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<tr>
<td>Body weight (6 wk, g)</td>
<td>29.2 ± 1.4</td>
<td>28.2 ± 2.3</td>
<td>29.6 ± 1.6</td>
<td>31.7 ± 1.8</td>
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<tr>
<td>Lung weight (mg)</td>
<td>169 ± 9</td>
<td>239 ± 42*</td>
<td>176 ± 29</td>
<td>238 ± 45*</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.35 ± 0.1</td>
<td>1.26 ± 0.2</td>
<td>1.43 ± 0.1</td>
<td>1.69 ± 0.26*</td>
</tr>
<tr>
<td>Heart weight/tibia length, mg/cm</td>
<td>80 ± 9</td>
<td>94 ± 19</td>
<td>82 ± 14</td>
<td>98 ± 14</td>
</tr>
<tr>
<td>Lung weight/tibia length, mg/cm</td>
<td>90 ± 5</td>
<td>132 ± 25§</td>
<td>95 ± 16</td>
<td>128 ± 23§</td>
</tr>
<tr>
<td>Liver weight/tibia length, g/cm</td>
<td>0.78 ± 0.02</td>
<td>0.70 ± 0.03</td>
<td>0.74 ± 0.02</td>
<td>0.92 ± 0.03*</td>
</tr>
<tr>
<td>Soleus muscle weight, mg</td>
<td>7.6 ± 1.7</td>
<td>7.5 ± 1.2</td>
<td>9.0 ± 1.5</td>
<td>8.5 ± 0.9</td>
</tr>
</tbody>
</table>

Data are means ± SD. SERCA2 FF, sarco(endo)plasmic reticulum ATPase type 2 control mice; SERCA2 KO, mice with cardiac disruption of Serca2. *P < 0.05, difference between sedentary groups. †P < 0.05, ‡P < 0.01, difference between trained groups. § P < 0.01, difference within genotype.

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natriuretic peptide (Nppa, Mm01255747_g1), brain natriuretic peptide (Nppb, Mm00435304_g1), endothelin-1 (Edn1, Mm00438656_m1), PGC-1α (Ppargc1a, Mm00447180_ml), and ribosomal protein L32 (Rpl32, Mm02528467_g1). Relative gene expression was determined using relative standard curves and PCR efficiency corrections for each assay. Results were normalized to Rpl32. Similar results were obtained with normalization to Gapdh (mRNA analysis, SERCA2 FF: n = 10 for trained and n = 10 for sedentary; SERCA2 KO: n = 9 for trained and n = 8 for sedentary). For citrate synthase activity, the left ventricular myocardium (10–12 mg) was homogenized in 50 mM Tris·HCl, pH 8.0, and determined as previously described (28).

Histochemistry. Paraffin-embedded tissues (heart, lung, liver, and soleus muscle) were cut into 4-μm sections and stained with hematoxylin-erythrosin-saffron and PERL staining for Fe2⁺ deposits. Liver sections were stained with periodic acid Schiff stain (Sigma-Aldrich 395B-1KT) for estimation of glycogen content. Sections were scored in a blinded setup by two independent examiners (M. Ericsson and S. H. Torp) using the following scale: 0, histologic baseline with no glycogen; 1, slightly increased glycogen; and 2, marked increase in glycogen. Capillary density in soleus muscle was estimated by immunohistochemistry of vascular endothelium for von Willebrandt factor (1:3,000; Dako A082; Dako, Glostrup, Denmark), 30 min at room temperature. Sections

Fig. 1. Treadmill performance in sarcoplasmic reticulum ATPase type 2 (SERCA2) control (SERCA FF) mice (A, C, and E) and in mice with cardiac disruption of Serca2 (SERCA2 KO: B, D, and F). Time course is shown for maximum oxygen uptake (VO2max; A and B), maximum running speed (C and D), and work economy (E and F). Respiratory exchange ratio (RER) is shown at VO2max at 2 and 6 wk (G). Two-way ANOVA of the change from week 2 to week 6 within individual mice detected the following effects: main effect of genotype: P < 0.001 for VO2max and P < 0.001 for maximum running speed; main effect of training: P < 0.001 for VO2max and P < 0.001 for maximum running speed; and genotype/training group interaction: P = 0.72 for VO2max and P < 0.05 for maximum running speed. For two-way ANOVA analysis, #P < 0.05 between genotype; *P < 0.05 between training group for one-way ANOVA analysis at the 6-wk endpoint. In E and F, comparison is made by Students’ t-test between genotype (#P < 0.05), since submaximal running speeds were achieved at different speed levels for training and sedentary mice, respectively. FFSED and FFTR denote sedentary and trained SERCA2 FF mice, respectively; KOSED and KOTR denote sedentary and trained SERCA2 KO mice, respectively. Data are means ± SD; n = 7 SERCA2 FF and n = 11 SERCA2 KO for sedentary mice; n = 7 SERCA2 FF and n = 11 SERCA2 KO for training mice.
were pretreated with pressure-boiling for 10 min in Target Retrieval Buffer (Dako S1699), and incubated in 3% hydrogen peroxide to block endogenous peroxidase activity. Positive staining was visualized with Envision+ (Dako K5007). Antibodies were diluted in 1% BSA in PBS. Nuclei were counterstained with hematoxylin for 2 min.

Perfusion fixation of hearts. For morphometric assessment of the right ventricular free wall, nine hearts from trained and sedentary SERCA2 FF and SERCA2 KO mice were retrogradely perfused with oxygenated Tyrode’s solution (in mM: 140 NaCl, 5.4 KCl, 1.8 CaCl2, 0.4 NaH2PO4, 0.5 MgCl2, 5 HEPES, and 5.5 glucose, pH 7.4), with a pressure of 70 mmHg. Hearts were stopped in diastole by switching to Tyrode’s solution with high KCl (10.8 mM), followed by perfusion with 4% phosphate-buffered paraformaldehyde and paraffin embedding (8, 22).

Fig. 2. Serca2 mRNA and SERCA2a protein abundance in SERCA2 FF and KO myocardium at 6-wk endpoint. A: Serca2 mRNA in the left (LV) and right (RV) ventricles. LV: n = 10 SERCA2 FF and n = 8 SERCA2 KO for sedentary mice; n = 10 SERCA2 FF and n = 9 SERCA2 KO for training mice. RV: n = 10 SERCA2 FF and n = 16 SERCA2 KO for sedentary mice; n = 13 SERCA2 FF and n = 13 SERCA2 KO for training mice. B: estimate of residual SERCA2a protein in KO sedentary LV. C: SERCA2a abundance in LV in C (n = 6 for each group). Within genotype data are normalized to sedentary values. Data are means ± SD.

Statistics. Data are expressed as means ± SD. Statistical calculations were made by ANOVA with Scheffé’s post hoc test, Mann-Whitney test, and Student’s t-test. P < 0.05 was considered as significant. Where Leven’s test for homogeneity of variances were <0.05, a Kruskal-Wallis nonparametric test was used. Area under curve analysis was made for progressive time course evaluation. Repeated measurement ANOVA was made for longitudinal comparisons, and two-way ANOVA was used for main effect analysis.
2 wk after Serca2 gene disruption, when <13% SERCA2 protein remained in SERCA2 KO mice (2). There were no differences in body weight VO2max, running speed, or respiratory exchange ratio among the four groups of mice at this time point (Table 1; Fig. 1). Heart dimensions were similar, except for dilation of the left atrium in SERCA2 KO mice (Table 2), consistent with previous findings (2). At 6 wk, both Serca2 mRNA and SERCA2 protein were strongly reduced (<5%) in SERCA2 KO (Fig. 2) in agreement with previous findings (2).

Aerobic capacity and running speed in sedentary mice. In SERCA2 KO mice, there was a linear decline in VO2max from week 3. At the 6-wk endpoint, VO2max in SERCA2 KO was 50% of control SERCA2 FF values (Fig. 1, A and B). In contrast, VO2max remained unchanged throughout the time course in SERCA2 FF mice. Thus at the endpoint, there was a strong correlation between reduced VO2max and reduced SERCA2 protein abundance in the myocardium. Despite the large decline in VO2max, SERCA2 KO mice were able to maintain maximal running speed up to 5 wk before a marked 26% decline occurred between week 5 and week 6 (Fig. 1D). In sedentary SERCA2 FF mice, the maximal running speed was maintained throughout the whole period. At the 6-wk endpoint, the maximal running speed in sedentary SERCA2 KO was 43% of sedentary SERCA2 FF. Cardiac function measured by echocardiography showed a significant decrease in stroke volume and cardiac output in sedentary and trained SERCA2 KO compared with SERCA2 FF counterparts (Fig. 3, A and B). However, left ventricular fractional shortening and ejection fraction were not significantly different between SERCA2 KO and SERCA2 FF mice at 6 wk.

Effect of training on aerobic capacity and running speed. We next investigated whether the decline in VO2max and response to training in SERCA2 KO mice could be modified by high-intensity aerobic exercise training. In training SERCA2 KO mice, there was a progressive decline in VO2max from 3 to 6 wk in parallel to sedentary SERCA2 KO mice (Fig. 1B). At the 6-wk endpoint, VO2max in SERCA2 KO was reduced to 67% of the 2-wk baseline value. In contrast, SERCA2 FF controls mounted a normal response to exercise training with increased VO2max throughout the training period (Fig. 1A). In trained mice at 6 wk, VO2max in SERCA2 KO was reduced to 54% of SERCA2 FF values. At this time point, the decline in VO2max in SERCA2 KO was significantly less in trained than in sedentary mice (Fig. 1B). Exercise training also induced a transient increase in running speed (Fig. 1, C and D) and significantly improved work economy (Fig. 1, E and F) in SERCA2 KO mice compared with sedentary SERCA2 KO.

Myocardial response to exercise training. Within both genotypes, stroke volume was higher in trained compared with sedentary counterparts after the intervention, consistent with a positive response to exercise training (Fig. 3A), whereas cardiac output at rest remained unchanged (Fig. 3B). Left ventricular fractional shortening and ejection fraction and left atrial diameter and lung weight were also similar in trained and sedentary SERCA2 KO (Tables 1–2). The values were similar to previous findings at 4 and 7 wk (2, 7). These findings indicate that training did not have a detrimental effect on cardiac function in SERCA2 KO mice.

Neither SERCA2 KO nor SERCA2 FF mice responded to exercise training by left ventricular hypertrophy or chamber dilation (Table 2). In trained SERCA2 KO, the right ventricular weight increased to 178% of sedentary SERCA2 KO (Fig. 4A), corresponding to increased right ventricular free wall thickness (0.49 ± 0.01 vs. 0.31 ± 0.01 mm; P < 0.05). Sparse interstitial fibrosis was detected in sections from the right ventricle myocardium in trained SERCA2 KO but not in sedentary SERCA2 KO (data not shown). No fibrosis was seen in SERCA2 FF mice, independent of training. In the left ventricles of trained SERCA2 KO, the abundance of Anp and endothelin-1 (Edn1), but not Bnp, mRNA was increased compared with sedentary counterparts (Fig. 4, B, D, and F). In the right ventricle, however, the abundance of Anp, Bnp, and Edn1 transcripts all tended to increase in trained SERCA2 KO (Fig. 4, C, E, and G).

Previous studies have shown that the activation state of CamKIIβ, as well as the phosphorylation status of the SERCA2 regulatory peptide phospholamban, may be modified by exercise training. In 6-wk SERCA2 KO left ventricles, we did not find any effect of exercise on the abundance of CamKIIβ or any increase in the phosphorylation status of threonine 286-CamKII (Fig. 5, A–C) or in the abundance or phosphorylation status of phospholamban (data not shown) compared with sedentary SERCA2 KO. Also, the protein abundance of NCX1 was slightly increased in trained SERCA2 KO mice compared with sedentary counterparts (Fig. 5D).

Several studies have shown that the metabolic status of the heart is altered in heart failure. We found that left ventricle citrate synthase activity was slightly but not significantly increased by exercise training in the left ventricles of trained SERCA2 KO and SERCA2 FF mice compared with their respective sedentary counterparts (Fig. 5D). There was also no statistically significant change in PGC-1α mRNA expression in
Peripheral adaptations in SERCA2 KO mice. In healthy mice, exercise training introduces adaptive changes, such as increased peripheral blood supply and improved alveolar ventilation, to meet the increased metabolic demand during exercise. In SERCA2 KO mice, training did not further increase lung weight compared with sedentary SERCA2 KO controls. Pulmonary tissue morphology appeared normal with no increase in water content or fibrosis. Small amounts of iron deposits indicating pulmonary congestion were found in 18 out of 24 SERCA2 KO lungs, independent of training.

The liver weights of trained SERCA2 KO mice increased to 118% of sedentary SERCA2 KO controls and to 133% in exercised SERCA2 FF controls (P < 0.05; Table 1). Hema-toxylin-erythrosin-saffron staining of liver sections revealed
irregular morphology, such as pleomorphic hepatocytes in SERCA2 KO mice (Fig. 6A), indicating “metabolic strained liver.” There was no sign of centrolobular ischemia in the liver sections from either sedentary or trained SERCA2 KO mice, suggesting that circulation in the liver was sufficient. Two-way ANOVA detected higher capillary density in soleus muscle in SERCA2 KO than in SERCA2 FF mice (Fig. 6D).

DISCUSSION

The present study shows that despite cardiac disruption of the Serca2 gene and very low expression levels of the SERCA2 protein in mice, exercise performance and even \( V_{O2max} \) were surprisingly well maintained for several weeks. Maximum running speed was better maintained than aerobic capacity as assessed by treadmill running. Moreover, the SERCA2 KO mice tolerated intensive training for several weeks. However, the training effect was small compared with SERCA2 FF control mice. Eventually, aerobic capacity and maximum running speed declined but not in parallel. The training effect in SERCA2 KO mice was probably due to peripheral factors, since running speed could be maintained in the face of a declining \( V_{O2max} \). Given the central role of SERCA2 in sarcoplasmic calcium handling, it was surprising that SERCA2 KO mice were able to maintain contractility without severe cardiac dysfunction at 4 wk, when myocardial SERCA2 protein was <5% of SERCA2 FF values (2). However, it is consistent with the observation that cardiomyocyte shortening and isoproterenol stimulation in isolated cells is remarkably well preserved at a 6-Hz stimulation frequency at this time point (2).

Aerobic capacity and physical performance after Serca2 disruption. Andersson et al. (2, 4) have previously shown that SERCA2 protein is rapidly reduced in the myocardium after disruption of the Serca2 gene. At 2 wk, cardiac SERCA2 protein is reduced to 13% and at 4 wk to <5% of control levels (2).

Surprisingly, we found no differences between SERCA2 FF and SERCA2 KO mice in \( V_{O2max} \), respiratory exchange ratio, and maximum running speed 2 wk after the start of tamoxifen. It may be that the remnant cardiac SERCA2, in the range of 13% of control values, is sufficient for normal cardiac function, including treadmill running.

After 7 wk, cardiomyocyte function is severely deteriorated (2). In the present study, the changes in \( V_{O2max} \) followed the previously reported time course of cardiomyocyte contractility, supporting the notion that \( V_{O2max} \) is closely linked to cardiomyocyte contractility and myocardial function.

Running performance is closely correlated to \( V_{O2max} \) (13, 31, 32), and we hypothesized that changes in \( V_{O2max} \) would affect the maximal running speed. In contrast, we found that changes in maximal running speed followed a time course that differed significantly from that of \( V_{O2max} \). Sedentary SERCA2
KO mice maintained maximal running speed until cardiac function and $\text{VO}_2\text{max}$ were markedly reduced and signs of heart failure were evident, 6–7 wk after Serca2 disruption. Our results indicate that peripheral adaptations preserved running speed to a greater extent in trained than in sedentary SERCA2 KO. This observation is consistent with improved work economy secondary to exercise training.

**Aerobic capacity in exercise training mice after Serca2 disruption.** We found that $\text{VO}_2\text{max}$ decreased slightly less in trained than in sedentary SERCA2 KO mice but followed a parallel time course. These observations indicate that adaptive mechanisms during exercise training may counteract the decrease in $\text{VO}_2\text{max}$ in SERCA2 KO mice.

According to previous findings, the abundance of NCX1 in sedentary SERCA2 KO mice was slightly upregulated (2). In the present study, NCX1 protein abundance was increased further by exercise training in SERCA2 KO but not in SERCA2 FF. This suggests that sarcolemmal Ca$^{2+}$ transport may be even further increased in SERCA2 KO mice. However, this observation cannot explain the full extent of the compensatory mechanisms occurring in Serca2-deficient hearts.

In line with the study by Andersson et al. (2), we also found a significantly lower heart rate in sedentary SERCA2 KO compared with sedentary SERCA2 FF. This was suggested to be a result of Serca2 gene disruption also in sinoatrial nodal cells, which are found to be dependent on proper cycling of Ca$^{2+}$ over the sarcoplasmic reticulum (2, 14, 19). Exercise training improved heart rate in SERCA2 KO mice and may therefore partly explain the maintained cardiac output in training SERCA2 KO mice. Together with the trend for a smaller decrease in stroke volume, we suggest that cardiac function is improved in trained compared with sedentary SERCA2 KO. This difference could also result from peripheral changes and/or delayed deterioration due a combination of transient compensatory mechanisms and a blunted adaptive improvement in response to exercise. However, these possibilities need further experimental exploration.

**Running speed in exercise training mice after Serca2 disruption.** In response to exercise training, physical performance, assessed as maximal running speed, was markedly reduced in SERCA2 KO mice, compared with SERCA2 FF controls. This is consistent with the notion that normal function of SERCA2 is essential for cardiomyocyte function. However, the time course of changes in maximal running speed differed from that of $\text{VO}_2\text{max}$. An initial increase in maximal running speed from weeks 2–4, despite a small reduction in $\text{VO}_2\text{max}$, indicated peripheral adaptation mechanisms, which is also supported by enhanced work economy (4a, 25, 27). From week 4 to week 6, maximal running speed returned to baseline values while $\text{VO}_2\text{max}$ was markedly reduced, paralleling further decline in cardiomyocyte function and at the same time indicating peripheral compensation. The present study started 1 wk after withdrawal of tamoxifen feeding. Therefore, we would not expect effects of tamoxifen on skeletal muscle function that would affect the $\text{VO}_2\text{max}$ measurements.

**Measures of cardiac dysfunction after Serca2 disruption.** Our data did not support the hypothesis that $\text{VO}_2\text{max}$ might be a better indicator of reduced cardiomyocyte function than echocardiography or in vitro single cell cardiomyocyte contractility. Dilatation of the left atrium, as measured by echocardiography or in vitro single cell cardiomyocyte contractility, has been shown to discriminate left ventricular dysfunction in vivo (7). We found that the left atrial diameter was significantly increased in both sedentary and in trained SERCA2 KO mice 2 wk after Serca2 disruption, suggesting increased left atrial filling pressure (2). In contrast, we found
no difference in left ventricle dimensions or function, $V_{O2\text{max}}$, or maximal running speed at this time point. This is consistent with the findings of Andersson et al. (2) and Louch et al. (18) showed that left ventricular systolic function was preserved in SERCA2 KO compared with SERCA2 FF. Those results suggested that primarily diastolic function was compromised. It is, however, important to notice that resting echocardiographic data and $V_{O2\text{max}}$ under training reflect different modes of cardiac function.

Exercise training induced right ventricle hypertrophy in SERCA2 KO. Pathophysiological stimuli such as hypoxia can induce pulmonary hypertension (10, 20), and increased levels of endothelin-1 in trained SERCA2 KO support this mechanism (16, 17, 29). Pulmonary hypertension, secondary to left ventricle failure, may induce a hypertrophic response in the right ventricle, which seems to be accentuated by exercise training.

Normobaric hypoxia, but not exercise training, is a powerful stimulus for ANP release, pulmonary hypertension, and ventricle hypertrophy (6, 10, 20, 26). In SERCA2 KO mice, peripheral adaptations due to reduced blood oxygen content are supported by the changes seen in liver morphology (26) and by increased capillary density in skeletal muscles from sedentary SERCA2 KO mice (4a). The diminished training response and lack of induction of cardiac regulatory proteins and hypertrophic markers in SERCA2 FF and SERCA2 KO myocardium may be due to the 4-wk training period in this study compared with the 6-wk training period and more robust responses reported in other studies (2, 9, 11, 13, 23, 33, 34).

In conclusion, we have shown that under conditions of strongly reduced SERCA2 protein abundance in the hearts of SERCA2 KO mice, there is a striking divergence between the aerobic capacity and running speed during high-intensity exercise. The aerobic capacity is closely linked to SERCA2 abundance and cardiac function, whereas the running speed is well preserved, most probably due to peripheral adaptations. Furthermore, SERCA2 KO mice are able to respond to high-intensity exercise training with improved cardiac function and peripheral adaptation.

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DISCLOSURES


REFERENCES


