Exercise-induced adaptations of cardiac redox homeostasis and remodeling in heterozygous SOD2-knockout mice

L. Richters,1,5 N. Lange,1,5 R. Renner,1 N. Treiber, A. Ghanem, K. Tiemann, K. Scharffetter-Kochanek, W. Bloch,1 and K. Brixius1

1Department of Molecular and Celluar Sport Medicine, Institute of Cardiology and Cardiology Medicine, German Sport University Cologne, Cologne; 2Department of Dermatology and Allergology, University of Ulm, Ulm; 3Clinic and Policlinic II, University of Bonn, Bonn; 4University Hospital of Muenster, Department of Cardiology and Angiology, Muenster; and 5Department of Obstetrics and Gynecology, University Hospital of Cologne, Cologne, Germany

Submitted 1 December 2010; accepted in final form 3 August 2011

Physical exercise has been shown to go along with an increased formation of oxygen-derived free-radical species due to the increased activity of the respiratory chain (11). The release of ROS from the mitochondria into the cytosol may contribute to a contractile improvement (49), induce tissue adaptation (14, 57), and in the long term result in an adaptive increase in the antioxidative defense system (42). Whereas there is clear evidence that physical activity may improve cardiovascular function and prevent cardiovascular disease (for review, see Ref. 63), it remains unclear whether an exercise-induced temporary increase in mitochondrial ROS formation may improve or worsen the cardiac situation if the activity of a central mitochondrial antioxidative defence mechanism like SOD2 is hampered. Therefore, this study investigates cardiac adaptation (cardiac growth, left ventricular wall thickness, cardiac angiogenesis) following endurance training in mice with a heterozygous deficiency of SOD2. Sedentary, untrained WT and SOD2-knockout animals were measured for control.
EXERCISE-INDUCED ADAPTATIONS OF CARDIAC REDOX HOMEOSTASIS AND REMODELING

METHODS AND MATERIALS

Animals and endurance training. Heterozygous SOD2-knockout mice (SOD2+/−) were generated as described previously (56). Experiments were performed in 14 male SOD2+/− and 14 wild-type (WT) mice at the age of 8 wk. Half the animals of each group remained sedentary, i.e., lived in their cages without additional interventions, whereas the others underwent a specific treadmill endurance training program. Moderate endurance exercise is a usual setting to stabilize cardiovascular function in human beings (26). To transfer these conditions into the mouse model, we choose a 5 days/1-h treadmill intervention. The inclination of 5° was chosen to enforce the running process. Preliminary experiments have provided evidence that 15 m/min is a moderate velocity for the mice. The training was performed for 8 wk.

The study was performed according to the US National Institutes of Health guidelines and was approved by the local ethics committee. At the end of this period, the animals were killed, and their hearts were isolated for analysis. The experiments were approved by the local ethics committee. Immediately after the excision, the heart was divided into two parts. One part was shock frozen for biochemical analysis. The other part was incubated in 4% paraformaldehyde (PFA) for 6 h for tissue fixation.

Echocardiography. High-resolution mouse echocardiography was performed using commercially an available ultrasound system equipped with a linear array transducer operating at an emission frequency of 15 MHz (harmonic-mode) with frame rates up to 280 Hz (HDI-5000, Philips Medical Systems, Bothell, WA). Data sets were analyzed on a Zeiss KS300 microscope using the software for morphometric analysis Science Imaging (Surface and microanalysis Science Imaging, Germany) and stained with methylene blue. Cardiomyocyte diameter was measured for 40 different cells with a maximum of 5 cells/face field. Vessel density was determined by counting the capillaries around a cross-sectioned cardiomyocyte. The vessel density was determined for 40 different cells with a maximum of 5 cells/face field.

Immunohistochemical analysis. Immunohistochemical preparation and analysis was performed as described before (4). For intensity analysis of immunostaining in cardiomyocytes, we measured the gray values of 40 cardiomyocytes from five randomly selected areas of each slice. The intensity of immunostaining (Nikon Eclipse microscope coupled to a 3-chip CCD camera) was reported as the mean of measured cardiomyocyte gray value minus the mean background gray value (Optimas 6.01 image analysis program). The intensity of immunostaining was evaluated in a blinded fashion. The areas were not quantified, but we chose the area of single muscle cells. The measurement was performed by two observers.

Specificity of the antibody was controlled by an immunohistochemical staining of a slice from the same muscle tissue on the same microscope slide in the same experimental setting without using the primary antibody during the staining procedure. Whether a specific staining was achieved was decided by microscopic comparison of the staining intensity of the two slices.

Materials. The following primary antibodies were used for immunohistochemistry: anti-AKT phosphor-specific pT308, polyclonal, derived from amino acids sequences surrounding Thr308 in humans (Biomol, Hamburg, Germany); anti-diphosphorylated ERK1 and 2 (Sigma Deisenhofen, Germany); anti-active caspase 3 polyclonal antibody (BD Bioscience, Heidelberg, Germany); anti-8-epi PGF2α (8-isoprostan, NatuTec, Frankfurt, Germany); anti-active p38 pAb, polyclonal rabbit (pTGPγ) (Promega, Madison, WI); anti-nitrotyrosine (Upstate, Lake Placid, NY); anti-peroxiredoxin 1–6 sampler kit (Acris, Hiddenhausen, Germany).

As secondary antibodies, biotinylated polyclonal goat anti-rabbit, goat anti-mouse (ERK1/2), and rabbit anti-goat (8-isoprostano) immunoglobulins (all from DakoCytonation, Glostrup, Denmark) were used.

VEGF-ELISA. To detect the concentration of VEGF in the cardiomyocytes, an ELISA was performed using the mouse VEGF immunoassay kit (Biosource). The shock-frozen cardiac tissue was pulverised and homogenized. The analysis was performed by an ELISA reader (Molecular Devices, kinetic microplate reader, MWG Biotech, Ebersberg, Germany/High Point, NC) at 450 nm. Data were collected by SoftMax Pro (Molecular Devices, Sunnyvale, CA). Final analysis was carried out with regard to main protein concentration and dilution.

Statistical analysis. All data are presented as means ± SE. Data was analyzed using Student’s t-test for unpaired data. Significance was considered at P value of <0.05.

RESULTS

Morphometric analysis. Heart-to-body weight ratio and cardiomyocyte diameter were significantly higher in SOD2+/− mice compared with WT. No alterations were observed in the untrained groups regarding end-diastolic volume and ejection fraction. In WT animals, physical exercise significantly increased heart-to-body weight ratio (Fig. 1A) and cardiomyocyte diameter (Fig. 2). End-diastolic volume (Fig. 1B) and cardiac ejection fraction (Fig. 1C) did not change.

The exercise-induced cardiac adaptation was missing in trained compared with untrained SOD2+/− mice, since heart-to-body weight ratio (Fig. 1A) was similar in both groups. In addition, a maladaptive cardiac remodeling seems to take place in exercising SOD2+/− mice. Cardiomyocyte diameter was unchanged (Fig. 2), but a significant increase in end-diastolic volume was observed in trained SOD2+/− mice compared with their untrained counterparts (Fig. 1B). Echocardiographic measurements provided evidence that cardiac function represented by the ejection fraction (Fig. 1C) was preserved in trained SOD2+/− mice.

Hypertrophic signaling. Since mechanical stretch has been shown to result in an activation of the MAP kinase signaling (53), we investigated long-term alterations in the phosphorylation of ERK1/2 and p38. Hearts were investigated after a 2-mo training intervention rather than immediately after an exercise session.

Akt/protein kinase B phosphorylation was similar under sedentary [WT vs. SOD2+/−: 6.61 ± 1.63 vs. 8.51 ± 2.83 arbitrary grey values (ArGV)] and trained conditions (WT vs. SOD2+/−: 7.97 ± 1.53 vs. 6.89 ± 2.08 ArGV) (Fig. 3A). The same holds true for the phosphorylation of p38 (sedentary, WT vs. SOD2+/−: 6.90 ± 1.44 vs. 6.67 ± 2.23 ArGV; trained, WT vs. SOD2+/−: 6.00 ± 1.78 vs. 6.79 ± 1.74 ArGV) (Fig. 3B). ERK1/2 phosphorylation was similar in WT and SOD2+/− under sedentary conditions (5.75 ± 1.09 vs. 6.15 ± 0.91 ArGV). Long-term physical endurance exercise, however, significantly decreased phosphorylated ERK1/2 in SOD2+/− mice.
(5.24 ± 0.79 ArGV) but not in WT mice (4.87 ± 0.94 ArGV) (Fig. 3C).

**ROS-formation.** The intensity of 8-isoprostane immunostaining indicating the oxidative stress situation was significantly increased in adult untrained SOD2+/− mice compared with WT mice (Fig. 3). In SOD2+/− mice, physical exercise did not lead to a further increase of 8-isoprostane formation compared with sedentary SOD2+/− mice, whereas a significant increase in 8-isoprostane staining was observed in the trained WT mice (Fig. 4).

Enhanced formation of ROS in conjunction with nitric oxide may result in the increased formation of nitrotyrosine, which has been described in the cardiac tissue of 10-day-old SOD2+/− mice (56). However, in the cardiac tissue of adult SOD2+/− mice (16.2 ± 2.1 ArGV), a similar intensity of nitrotyrosine staining was observed as in WT mice (15.6 ± 2.2 ArGV) irrespective of the performance of physical exercise (WT: 16.5 ± 2.7 ArGV vs. SOD2+/−: 15.7 ± 2.0).

**Apoptosis.** There are reports suggesting a relationship between SOD2 and apoptosis (15). Since caspase 3 activation, which is a key mediator for DNA fragmentation in the final cascade of apoptosis (for review, see Ref. 64), has been shown to be significantly increased at day 6 after birth in SOD2+/− mice, we measured caspase 3 activation in immunohistochemical stainings of cardiac left ventricular tissue of adult sedentary and trained SOD2+/− and in WT mice to further characterize the cardiac situation. Cardiac caspase 3 activation was similar in all four groups investigated (sedentary WT 8.4 ± 2.1 ArGV, sedentary SOD2+/−: 6.8 ± 1.1 ArGV, exercising WT: 6.9 ± 1.2 ArGV, exercising SOD2+/−: 7.7 ± 1.4 ArGV).

**Cardiac vessel density and plasma VEGF-concentration.** Exercise has been shown to induce angiogenesis. Therefore, we investigated the vessels per cardiomyocyte ratio as well as the cardiac VEGF-content in hearts of untrained and trained WT and SOD2+/− mice. Capillarization was similar in untrained WT and SOD2+/−-deficient animals (Fig. 5A). Exercise was associated with an increase in cardiac capillarization only in WT mice. Cardiac VEGF concentration was similar in untrained WT and SOD2+/−-deficient mice and in untrained and trained SOD2+/−-deficient mice. However, cardiac VEGF-concentration was decreased in trained but not untrained WT mice (Fig. 5B).

**Expression of peroxiredoxins.** To investigate the adaptation of the antioxidative defence system, we performed immunohistochemical stainings of cardiac left ventricular tissue of adult sedentary and trained SOD2+/− mice and wild-type (WT) mice. *P < 0.05.
histochemical stainings of peroxiredoxin 1–6 in ventricular tissue of sedentary and trained WT and SOD2+/− mice.

Peroxiredoxin 1, which is localized in the cytosol (41), was equally distributed in sedentary WT and SOD2+/− mice (Fig. 6A). Exercise significantly increased peroxiredoxin 1 expression in the cardiomyocytes of SOD2+/− mice but not in WT mice.

The protein expression of peroxiredoxin 2, another cytosolic peroxiredoxin (41), was significantly increased in SOD2+/− mice under basal conditions (Fig. 6B). Exercise did not alter peroxiredoxin 2 expression in WT but decreased peroxiredoxin 2 expression in SOD2+/−-deficient mice to a level similar to that observed in sedentary WT mice (Fig. 6B).

The protein expression of peroxiredoxin 3, which is only localized at the mitochondrion (41), was similar in WT and SOD2+/− mice under sedentary conditions (Fig. 6C). We observed an upregulation of peroxiredoxin 3 in trained WT mice compared with the sedentary WT group. An upregulation of peroxiredoxin 3 was also observed in the trained SOD2+/− mice (Fig. 6C).

The cardiac expression of peroxiredoxin 4 [localized at the endoplasmic reticulum and the extracellular space (51)] is depressed in sedentary SOD2+/− mice compared with WT mice (Fig. 7A). Whereas the protein expression of peroxiredoxin 4 was downregulated in WT mice following endurance training, the same exercise regime induced an upregulation of peroxiredoxin 4 in SOD2+/− mice.

Density of peroxiredoxin 5 (Fig. 7B) and peroxiredoxin 6 (Fig. 7C) was significantly decreased in SOD2+/−-deficient mice compared with WT mice. Exercise significantly downregulated both isoforms in cardiac tissue of WT mice, whereas their expression was not altered by exercise in SOD2+/− mice (Fig. 7, B and C).

Fig. 3. Alterations of AKT (A), p38 (B), and ERK1/2 (C) phosphorylation in Sed and Train WT and SOD2+/− mice. *P < 0.05 vs. Sed.

Fig. 4. Alterations of 8-isoprostane immunostaining in Sed and Train WT and SOD2+/− mice. *P < 0.05 vs. wild-type Sed.
which has been described in newborn SOD2-deficient mice. In addition, apoptosis, being the mechanism responsible for the breakdown of the mitochondrial compartments of a cell. There is evidence that the protein expression of peroxiredoxin 1 depends on mechanical stimuli or the presence of nitric oxide (13). Both mechanisms may be of importance for the development of cardiac failure. In our study, an upregulation of the peroxiredoxin 1 expression was observed in the hearts of newborn mice with a partial deficiency of SOD2 (56). Therefore, it has been proposed that this mouse model may represent a model of compensated maladaptive cardiac hypertrophy. A dilated cardiomyopathic phenotype, i.e., end-stage heart failure or cardiac decompensation, only develops in response to physical exercise in SOD2-deficient mice. Although there is evidence from the literature that physical exercise increases cellular oxidative stress (as reviewed by Ref. 3), a chronic increase of the oxidative cellular stress situation as a pathophysiological inducer of the cardiac remodeling may be excluded from our study since we did not observe alterations in the 8-isoprostanate and nitrotyrosin formation in the cardiomyocytes of MnSOD-knockout mice.

Antioxidative capacity in heart failure. There is a controversy discussion whether the antioxidant defence mechanisms are preserved in heart failure (1, 5, 24). Aside from cleavage of the superoxide anion, the control of the elimination of H2O2, which results from dismutation of the superoxide anion, is of special importance since this molecule is readily converted to the hydroxyl radical, a molecule with a high cellular destructive potential (52). In addition to catalase and the selenium-glutathione peroxidases, peroxiredoxins comprise a group of enzymes, which are most likely of major importance under stress conditions (reviewed by Ref. 50). Since the molar efficiencies of peroxiredoxins are by orders of magnitude smaller than those of catalase and the selenium-containing glutathione peroxidases, it has been suggested that they are predominantly required under stress conditions (50).

Peroxiredoxin 1 is present in both cytosol and nuclear compartments of a cell. There is evidence that the protein expression of peroxiredoxin 1 depends on mechanical stimuli or the presence of nitric oxide (13). Both mechanisms may be of importance for the development of cardiac failure. In our study, an upregulation of the peroxiredoxin 1 expression was only observed in the trained SOD2-deficient mice. It has been shown that cytoplasmic peroxiredoxin 1 inhibited the activation and nuclear translocation of the redox-sensitive transcription factor NF-kappaB (23). Whether this reaction may protect the mice from a further progression into cardiac failure or whether this mechanism may induce the maladaptive transition from the hypertrophic into the dilative cardiomyopathy is not known.

DISCUSSION

This study investigated cardiac function in an experimental mouse model with a heterozygous knockout of the manganese-dependent superoxide dismutase (SOD2). A special focus of this study was to investigate cellular adaptation in response to physical exercise in a situation with an already increased cardiac oxidative radical stress. We showed that SOD2-deficiency increased cellular oxidative stress and induced the development of a compensatory cardiac hypertrophy. The antioxidative defense capacity was upregulated in adult mice with SOD2 deficiency. Thus a further increase of mitochondrial ROS induced by physical activity was well compensated for, at least in the long term. This situation, however, may hamper exercise-induced adaptation of the cardiovascular tissue.

Oxidative stress and heart failure. There are several studies indicating that cardiac oxidative stress occurs in myocardium during the progression of cardiac remodeling and failure (2, 35). Superoxide dismutases represent “first-line” antioxidative enzymes, because they are responsible for the transformation of superoxide anions into hydrogen peroxides, which then can be cleaved to H2O by diverse hydrogen peroxidase systems. The manganese-dependent superoxide dismutase isoform is mainly responsible for the breakdown of the mitochondrial superoxide anions (29). In line with this, oxidative stress is increased in hearts of newborn mice with a partial deficiency of SOD2 (56). Therefore, it has been proposed that this mouse model may represent an experimental model of heart failure (56). The study provides evidence that the cardiac oxidative stress is still elevated in the SOD2-deficient mice at the age of 4 mo, indicating that a partial deficiency of the SOD2 system cannot be completely compensated for by other antioxidative defence systems even at older age. In addition, the hypothesis of Straubburger and colleagues (56) has confirmed that heterozygous SOD2-deficient mice represent a model of cardiac failure, since the hearts of SOD2-deficient mice developed hypertrophic cardiomyopathy. However, cardiac function is still compensated for under sedentary conditions. In addition, apoptosis, which has been described in newborn SOD2-deficient mice, vanished in the adult animals. Thus the sedentary heterozygous SOD2-deficient mouse model used in this study resembles a model of compensated maladaptive cardiac hypertrophy. A dilated cardiomyopathic phenotype, i.e., end-stage heart failure or cardiac decompensation, only develops in response to physical exercise in SOD2-deficient mice. Although there is evidence from the literature that physical exercise increases cellular oxidative stress (as reviewed by Ref. 3), a chronic increase of the oxidative cellular stress situation as a pathophysiological inducer of the cardiac remodeling may be excluded from our study since we did not observe alterations in the 8-isoprostanate and nitrotyrosin formation in the cardiomyocytes of MnSOD-knockout mice.

Antioxidative capacity in heart failure. There is a controversial discussion whether the antioxidant defence mechanisms are preserved in heart failure (1, 5, 24). Aside from cleavage of the superoxide anion, the control of the elimination of H2O2, which results from dismutation of the superoxide anion, is of special importance since this molecule is readily converted to the hydroxyl radical, a molecule with a high cellular destructive potential (52). In addition to catalase and the selenium-glutathione peroxidases, peroxiredoxins comprise a group of enzymes, which are most likely of major importance under stress conditions (reviewed by Ref. 50). Since the molar efficiencies of peroxiredoxins are by orders of magnitude smaller than those of catalase and the selenium-containing glutathione peroxidases, it has been suggested that they are predominantly required under stress conditions (50).

Peroxiredoxin 1 is present in both cytosol and nuclear compartments of a cell. There is evidence that the protein expression of peroxiredoxin 1 depends on mechanical stimuli or the presence of nitric oxide (13). Both mechanisms may be of importance for the development of cardiac failure. In our study, an upregulation of the peroxiredoxin 1 expression was only observed in the trained SOD2-deficient mice. It has been shown that cytoplasmic peroxiredoxin 1 inhibited the activation and nuclear translocation of the redox-sensitive transcription factor NF-kappaB (23). Whether this reaction may protect the mice from a further progression into cardiac failure or whether this mechanism may induce the maladaptive transition from the hypertrophic into the dilative cardiomyopathy is not known.
Fig. 6. Immunostaining of peroxiredoxin 1 (A), peroxiredoxin 2 (B), and peroxiredoxin 3 (C) in hearts of Sed and Train WT and SOD^{−/−} mice. *P < 0.05 vs. WT-Sed. #P < 0.05 vs. SOD^{−/−}-Sed (p SOD^{−/−} Sed vs. SOD^{−/−}-Train, prx 2 = 0.056).
Fig. 7. Immunostaining of peroxiredoxin 4 (A), peroxiredoxin 5 (B), and peroxiredoxin 6 (C) in hearts of Sed and Train WT and SOD\textsuperscript{+/−} mice. *P < 0.05 vs. WT-Sed. #P < 0.05 vs. SOD\textsuperscript{−/−}-Sed.
In contrast to peroxiredoxin 1, peroxiredoxin 2 was only upregulated in sedentary SOD−/− mice but decreased in a situation of dilated cardiomyopathy to a similar level to that described for WT mice. These results are in contrast to previous findings in rats (10), in which the protein expression of peroxiredoxin 2 was found to correlate with the severity of heart failure. Whether this is a species-dependent finding is not known yet. In a previous publication from our group (5), we also did not observe significant alterations of peroxiredoxin 2 expression in end-stage human heart failure. However, peroxiredoxin 2 expression was increased in hyperdynamic hearts of two genetically altered mouse models: phosphorylamban knockout (PLN KO) and protein phosphatase 1 inhibitor 1 overexpression (I-1 OE) (9, 44).

Peroxiredoxin 3 is the only peroxiredoxin isoform that is only located in the mitochondria and not in other cellular compartments (60). Peroxiredoxin 3 protects cells against mitochondrially derived oxidative stress (7, 12, 30, 36). Despite a deficiency of SOD2, the protein expression of peroxiredoxin 3 was similar in WT and SOD−/− mice under sedentary conditions. We observed an upregulation of peroxiredoxin 3 in both exercising groups compared with the sedentary WT and SOD−/− mice. This indicates that the upregulation of peroxiredoxin 3 is a typical adaptation of the cardiac muscle toward physical exercise. There is an indication that the protein expression of peroxiredoxin 3 is regulated by c-myc (19, 62) and that exercise-induced activation of STAT3 signaling increases c-myc (58).

Peroxiredoxin isoforms 4–6 are downregulated in MnSOD knockout mice under sedentary conditions, which is in line with previous findings in human heart failure (5). This downregulation may be due to the fact that the intracellular concentration of H₂O₂ is decreased in the SOD2−/− knockout mice, since no further transition from the superoxide to H₂O₂ takes place due to the SOD2 deficiency and a decreased activity of the Cu/Zn SOD (56). A downregulation of peroxiredoxin 4–6 was also observed in the hearts of trained WT mice compared with their untrained littermates. This situation is different to the one described above, because exercise in healthy individuals goes along with an upregulation of MnSOD and glutathione peroxidase (21), resulting in a better cleavage of ROS. The following decrease in H₂O₂ may lead to a reactive downregulation of those isoforms. Less is known about the mechanisms underlying peroxiredoxin 4–6 downregulation. It has been described that NF-kappaB may suppress peroxiredoxin 6 expression (16). Whereas it has been shown that serum samples of heart failure patients induce a specific profile of pro- and anti-inflammatory mediators leading to an increase in NF-kappaB activation in human pulmonary artery endothelial cells (25), the NF-kappaB mechanism may not explain the exercise-induced peroxiredoxin 6 downregulation in WT mice, since NF-kappaB activation has been shown to be either unaltered (37) or downregulated (6) in physical exercise.

**Tissue adaptation in MnSOD-knockout mice.** Physical activity increased cardiac angiogenesis in WT mice, which was paralleled by a decrease of the cardiac tissue VEGF concentration. This was an unexpected finding, assuming neoangiogenesis is due to VEGF content (27). Lloyd and coworkers showed that the VEGF concentration increased a few days after starting the endurance training but declined very shortly after this increase (33). Seeing that the training started 2 mo before our observations can be interpreted as a reactive downregulation after successfully completed angiogenesis.

ERK1/2 are activated at the end of a mitogenic Raf-ERK-cascade (45), which is induced by growth factors such as EGF, PDGF, or various cytokines. The MAP kinase p38 is activated for example by interleukin-1β or TNF-α (48). Whereas ERK1/2 is important for cell proliferation (respectively, cardiac hypertrophy) and cell proliferation (59, 61), activation of p38 seems to be responsible for apoptosis and inflammatory processes (20). In the present study, we did not observe alterations of ERK1/2 or p38 activation in MnSOD knockout mice. In addition, p38 phosphorylation was not affected by physical exercise. Very interestingly, we observed a slight decrease of ERK1/2 phosphorylation in WT mice and a significant decrease of ERK1/2 phosphorylation in MnSOD knockout mice. It has been described that a decrease in ERK1/2 phosphorylation may go along with an increase in cellular apoptosis (59). However, we did not observe increased apoptosis after physical exercise in the mice of the present study.

**Antioxidative treatment: perspectives for heart failure therapy.** It has been suggested that the MnSOD-knockout mice may provide a model to study heart failure due to the increased cardiac oxidative stress (56). Only recently, it has been shown by electroparamagnetic resonance that formation of superoxide anion was increased more than twofold in failing compared with nonfailing human myocardium. This was paralleled by a decrease in the protein expression of MnSOD (54). Thus MnSOD knockout mice really provide a model to study heart failure interventions. In very recent animal studies, it has been shown that exercise specifically upregulates MnSOD, thereby reducing mitochondrial ROS-production even in old animals (21). Although we observed an adaptation of the antioxidative peroxiredoxin system, ROS generation was not altered. However, due to the genetic modification, an upregulation of the MnSOD-protein may not be possible.

Several recently published studies showed that there might be a future advantage of antioxidative therapy in cardiac MnSOD-deficient systems (28, 34). Assuming the presented mice are a good model to study heart failure and heart insufficiency, they also might be a safe method to study the effect of antioxidative treatment options before they become clinically relevant.

**ACKNOWLEDGMENTS**

The authors thank Mojgan Gilhav, Bianca Collins, and Annika Voß for excellent technical help. This work contains data from the doctoral thesis of L. Richter and R. Renner.

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**REFERENCES**


