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

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Richters L, Lange N, Renner R, Treiber N, Ghanem A, Tiemann K, Scharffetter-Kochanek K, Bloch W, Brixius K. Exercise-induced adaptations of cardiac redox homeostasis and remodeling in heterozygous SOD2-knockout mice. J Appl Physiol 111: 1431–1440, 2011. First published August 11, 2011; doi:10.1152/japplphysiol.01392.2010.—A reduced expression of the manganese-dependent superoxide dismutase (SOD2) is characterized by increased cardiac oxidative stress. Oxidative stress has also been described in situations of physical exercise. We investigated the influence of physical exercise (EX; treadmill 1 h/day at 15 m/min, 5 days/wk, at an angle of 5° for a duration of 8 wk) on cardiac function [heart frequency (HF), echocardiography, morphometry], oxidative stress [reactive oxygen species (ROS)], and antioxidative defence capacity (peroxiredoxin 1–6) in male SOD2-knockout (SOD2_EX) and wild-type mice (WT_EX) compared with untrained age-matched animals (WT_CON; SOD2_CON). In SOD2_CON, heart weight, cardiomyocyte diameter, and cardiac ROS generation were significantly larger and peroxiredoxin isoforms 4–6 lower than in WT_CON. The vessel-to-cardiomyocyte ratio, cardiac VEGF-concentration, and cardiac function were similar in SOD2_CON and WT_CON. Both groups tolerated the exercise protocol well. In WT, exercise significantly increased vessel-to-cardiomyocyte ratio and ROS-generation and downregulated peroxiredoxin isoforms 4–6 and VEGF generation. The vessel-to-cardiomyocyte ratio, cardiac VEGF concentration, and cardiac ROS were not altered in SOD2_EX compared with SOD2_CON, but a significant upregulation of cardiac peroxiredoxin 1 and 4 was observed. Similar to the result observed in WT_EX, peroxiredoxin 3 was upregulated in SOD2_EX. Chronic exercise shifted the (mal)adaptive hypertrophic into a compensated dilated cardiac phenotype in SOD2_EX. In conclusion, downregulation of SOD2 induces a maladaptive cardiac hypertrophy. In this situation, physical exercise results in a further deterioration of cardiac remodeling despite an upregulation of the antioxidative defense system.

SOD2; oxidative stress; peroxiredoxin; exercise; cardiac

CELLULAR OXIDATIVE STRESS is induced by an imbalance between the formation of reactive oxygen species (ROS) and antioxidative defence mechanisms (for review see Ref. 18). Increased ROS generation has been implicated in the process of left ventricular remodelling during the development of heart failure (2, 35).

The superoxide dismutase enzymes (SOD) represents the first-line defense mechanism against increased ROS formation (32). Three SOD isoforms have been described: 1) SOD1, which is dependent on the presence of copper and zinc, is located within the cell and is responsible for 70–80% of the cellular SOD-activity (22); 2) SOD3 is located in the extracellular space and has been shown to be active in various tissues including the heart (43); and 3) SOD2, which is dependent on the presence of manganese, is located at the inner membrane of the mitochondria and is responsible for the cleavage of ROS generated by the respiratory chain, i.e., 10–20% of the total cellular ROS generation (22, 47).

Transgenic mouse models have been described with either a homozygous or a heterozygous knockout for SOD2. The homozygous SOD2 mutants (SOD2-Cje) produced by Li were not viable (31). The enlarged hearts with a dilated left ventricular cavity and reduced left ventricular wall thickness, which were found in these animals, suggested cardiomyopathy (31).

A mouse model with a heterozygous deficiency of SOD2 characterized by an increased cardiac formation of ROS and reactive nitrogen radical species (RNS) at day 10 after birth has been proposed as suitable to investigate age-dependent alterations in heart failure (56). Only recently, the development of a progressive congestive heart failure has been shown in a heart/muscle-specific SOD2 knockout mouse model (39), whereas an overexpression of SOD2 protected against myocardial ischemia/reperfusion injury (8). Thus antioxidative defence by SOD2 in the mitochondria seems to critically influence cardiac remodeling and the development of heart failure. In this context, it is important to mention that the protein expression of the SOD2 is modulated by a variety of physiological [e.g., TNF-α (55)] and environmental factors [e.g., ionizing radiation (40)] resulting in large inter-individual variance of the SOD2 protein expression level (46).

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 defence 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 function, cardiac angiogenesis) following endurance training in mice with a heterozygous deficiency of SOD2. Sedentary, untrained WT and SOD2-knockout animals were measured for control.

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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 equipped with a linear array transducer operating at an emission frequency of 15 MHz (harmonic-mode) with frame rates up to 280 Hz.

Analysis of cardiomyocyte diameter and vessel density. Small heart muscle samples were fixed in 4% buffered PFA, rinsed in cacodylate buffer three times, and then treated with 1% uranyl acetate in 70% ethanol for 8 h to enhance the contrast. The heart samples were then treated with 1% uranyl acetate in 70% ethanol for 8 h.

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.

Materials. The following primary antibodies were used for immunohistochemistry: anti-AKT phosphor-specific pT308, polyclonal, derived from amino acids sequence 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 (pTGPy) (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-isoprostann) immunoglobulins (all from DakoCytomation, 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

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 equipped with 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.
(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 immuno-
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 SOD+/− 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 mitochondrium (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).
which has been described in newborn SOD2-pensated for under sedentary conditions. In addition, apoptosis, phic cardiomyopathy. However, cardiac function is still com-
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cardiac oxidative stress occurs in myocardium
as muscle 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-defi-
ciency 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 mitochon-
drial 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 tis-

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−/− 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
Straßburger and colleagues (56) has confirmed that heterozy-
gous SOD2-deficient mice represent a model of cardiac failure,
since the hearts of SOD2-deficient mice developed hypertro-

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 phys-
ical exercise in SOD2−/+ 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-isoprostan
and nitrotyrosin formation in the cardiomyocytes of MnSOD-
knockout mice.

Antioxidative capacity in heart failure. There is a controver-
sial 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 destruc-
tive potential (52). In addition to catalase and the seleno-

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−/+ 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$^{-/-}$ mice. *P < 0.05 vs. WT-Sed. #P < 0.05 vs. SOD$^{-/-}$-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: phospholamban 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 mitochondrial 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 \(H_2O_2\) is decreased in the SOD2 \(^{-/-}\) knockout mice, since no further transition from the superoxide to \(H_2O_2\) 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 littersmates. 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 \(H_2O_2\) 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-MEK-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-1\(\beta\) or TNF-\(\alpha\) (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 the 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 elektroparamagnetic 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 antioxidant treatment options before they become clinically relevant.

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**DISCLOSURES**

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

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