Structural and functional prevention of hypoxia-induced pulmonary hypertension by individualized exercise training in mice

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Am J Physiol Lung Cell Mol Physiol 306: L986–L995, 2014. First published April 11, 2014; doi:10.1152/ajplung.00275.2013.—Pulmonary hypertension (PH) is a disease with a poor prognosis characterized by a vascular remodeling and vessel wall cell proliferation, followed by an increase in pulmonary vascular resistance (27). It is characterized by abnormal changes of small vessels may include the production of proliferative and vasoconstrictive factors, such as endothelin-1, as well as the curbing of the production of vasodilatory and antiproliferative factors, such as nitric oxide (NO) and prostacyclin, which have been shown to contribute to PH development (6). NO is produced by nitric oxide synthase (NOS). It activates the soluble guanylate cyclase (sGC), thereby increasing the amount of cGMP in smooth muscle cells, which activates protein kinase G with several downstream targets, causing vessel dilatation (37). In this way, NO contributes to the local adaptation of pulmonary blood perfusion to ventilation. Phosphodiesterases (PDEs) represent a superfamily of enzymes that inactivate cAMP and cGMP. So far, 11 isoenzymes have been described, with different substrate specificities and different tissue distribution patterns. PDE inhibitors stabilize cAMP and cGMP and thus differentially regulate their levels, depending on the respective selectivity profile (21).

The current treatment options for PH include PDE5 inhibitors, endothelin receptor-antagonists, prostanoids, or a combination thereof, as well as oxygen supply, diuretics, and anticoagulants (20, 27). Although curative treatment of PH has been achieved in animal models (45), it is not transferable to human disease yet.

The initial symptoms of PH, like dyspnea, fatigue, and syncope, together with a compromised exercise capacity, lead to a vicious circle with reduced quality of life, loss of mobility and independence, and finally enhanced mortality. Until just a few years ago, exercise training has been thought to be dangerous for PH patients, bearing a risk of potentially fatal cardiovascular compromise (13). However, the study of Meerleers and colleagues indicated that physical training improved 6-min walking distance and the quality of life in patients with severe PH (36). Since then, a variety of studies in humans appeared that showed beneficial effects of exercise training at least for the 6-min walking distance, quality of life scores, peak oxygen consumption, heart rate, systolic pulmonary arterial pressure, work capacity, or pulmonary blood flow in different forms of PH (4, 7, 24, 34, 40). In idiopathic pulmonary arterial hypertension de Man et al. found no increase in the 6-min walking distance but an increased muscle capillarization (11). Long-term safety of various exercise intervention programs has been demonstrated although exercise training was not completely harmless (22, 23). Nevertheless, in spite of these
studies, the effects of exercise training on the structural changes of the pulmonary circulation, i.e., vascular remodeling, are still unclear. Such investigations can be preferentially performed in animal studies, since histological investigations of the lung are needed to address this issue. For the animal model of monocrotaline (MCT)-induced PH, conflicting results of exercise effects on the vascular remodeling process were presented recently (17, 18). Therefore, we aimed to set up a mouse model of chronic hypoxia-induced PH to address the role of regular exercise training on pulmonary vascular structural remodeling. Moreover, we took an approach to uncover possible signaling mechanisms underlying the effects of exercise training. In addition, we compared the effects of exercise training with those of a therapy with the PDE5 inhibitor Sildenafil, applied in a low dosage, to uncover potential synergistic or adverse effects of exercise and pharmacological treatment.

MATERIALS AND METHODS

Experimental design. Adult male C57BL/6J mice were obtained from Charles River Laboratories (Sulzfeld, Germany). During the time of the study, mice were housed in standard cages at 22–24°C and had access to food and water ad libitum. Mice were randomly distributed into the following groups: placebo-treated mice in normoxia (Nox Plac), placebo-treated mice in hypoxia (Hox Plac), Sildenafil-treated mice in hypoxia (Hox Sild), Sildenafil-treated mice in hypoxia that performed a daily exercise training (Hox Sild + Train), and placebo-treated mice that performed a daily exercise training (Hox Plac + Train).

It has been shown that mice that have access to a running wheel voluntarily run an average of >4 h/24 h (1). For that reason and because of our observations in previous studies we have conducted, we acted on the assumption that our C57BL/6J mice that we have also used in previous studies are in general inherent to running. For practical reasons, training and exercise testing were performed under normoxic conditions. Sildenafil was applied one time daily at a dose of 100 mg·kg⁻¹·day⁻¹, diluted in 2% methylcellulose using a feeding needle. Placebo-treated mice received 2% methylcellulose. Physical performance of mice was assessed in the beginning of the study, before the mice were randomly distributed into groups. Further performance tests were undertaken after 7 days of hypoxia, after 14 days of hypoxia, and after 21 days of hypoxia. The transmitter for the measurement of right ventricular systolic pressure (RVSP) was implanted after 19 days of hypoxia, 2 days before the final assessment of physical performance. After the third performance test, mice were prepared for surgery as described below, the implanted radiotransmitter was removed, and the lungs were taken for molecular analysis, morphometry, and immunohistochemistry.

Assessment of physical performance of mice. Physical performance of mice was assessed using rodent treadmill spiroergometry in a custom-made setup as described before (32). The treadmill was part of a spiroergometer closed-chamber system connected to a computer with Metabolic Performance Testing System analytical software. Air was pumped into the chamber at a rate of 0.5 l/min. On the other side of the chamber, a flexible tube was connected with gas analyzers. The change of oxygen concentration was recorded continuously. Before every physical performance test session, the spiroergometer was calibrated with a standardized gas mixture. Before the measurements, mice were allowed to run slowly on a treadmill for a few minutes to limber up, get used to the setup before testing their maximum exercise capacity training, and to assure that all mice are willing to run on the treadmill. This warm-up is common practice in rodent exercise training (32). Accordingly, mice can be randomly assigned into the groups (described in Experimental design) after the habituation, without any further selection process. After acclimatization, the mice performed a continuous progressive exercise test until exhaustion, defined by the refusal of the animal to continue running. The test started at a speed of 0.15 m/s at 25° inclination (30). Treadmill speed was increased stepwise by 0.05 m/s every 3 min. Oxygen and carbon dioxide concentrations were measured continuously by gas analyzers. At the end of the test, the maximum walking speed, walking distance, and maximum oxygen consumption (VO₂max) were documented. Physical performance of mice was assessed under normoxic conditions.

Exercise training of mice. Maximal running speed, VO₂max, and maximal running distance of all mice were assessed initially as described above. Next, the animals were randomly divided into five groups as described in Experimental design. Mice in the training groups were trained in normoxia at 60% of their initial physical capacity (maximal running speed) 5 days/week for 30 min each. One time per week, physical performance was reassessed as described above, to individually adapt the intensity of the exercise training for the following week (5). Sildenafil-treated mice and placebo-treated control mice that did not perform exercise were exposed daily to hypoxia for the same time period as exercising mice.

Mouse model of chronic hypoxia-induced PH. All investigations involving animals were approved by the local authorities (Regierungspräsidium, Giessen, Germany). Adult male C57BL/6J mice, purchased from Charles River, were exposed to normobaric hypoxia (10% O₂) in a ventilated chamber for 21 days as described previously to induce hypoxic pulmonary vascular remodeling (45). Normoxic animals were kept under the same conditions at 21% O₂. For technical reasons, daily exercise training was performed in normoxia. These short time periods of normoxic exposure had a slight impact on the development of experimental hypoxia-induced PH, since those mice showed differences in RVSP, right heart hypertrophy, and small vessel muscularization compared with mice that stay continuously in hypoxia, a common experimental setup in our laboratory (35, 44). Conversely, animals that are exposed to intermittent hypoxic episodes develop PH (57). Sedentary control mice were kept in normoxia for the same time period as exercising mice.

Telemetric measurement of right ventricular pressure. RVSP in mice was measured with an implanted radiotelemetry system, consisting of a fluid-filled catheter, connected to a transmitter (TA11PA-N101, Dataquest A.R.T. 2.1; Data Sciences), exactly as described previously (45). The device was implanted 2 days before the final assessment of the physical performance of the mice, after 19 days of hypoxia. Before implantation, the catheter was filled with an antithrombotic gel and sanitized overnight in 2% glutaraldehyde. Mice were anesthetized with an intraperitoneal injection of a mixture of ketamine and xylazine. The catheter was inserted in the right ventricle via the jugular vein under ultrasound control. The correct position of the catheter was monitored by displaying the pressure amplitude of the right ventricle on the computer monitor. The catheter was fixated on the vein and the surrounding tissue with a surgical knot. After surgery, animals were housed individually and were allowed to recover for 2 days. The RVSP was measured during the final performance test on the treadmill.

Heart and lung explantation. After the final performance test, the catheter was removed carefully. Mice were killed by an overdose of ketamine and xylazine after the right heart catheter had been removed under anesthesia. The lung was flushed with sterile saline (0.9% NaCl) to wash out all blood cells. The right lung was immediately snap-frozen in liquid N₂ for molecular investigations. The left lung was fixed in formalin for immunohistochemical investigations.

Right ventricular hypertrophy. Right ventricular hypertrophy was quantified by calculating the ratio of the dried right ventricular and left ventricular plus septum mass. Hearts were removed directly after fixation under a magnifying lens (M55; Leica Microsystems, Nussloch, Germany). The left ventricle, including the septum, was dissected from the right ventricle. Both parts of the heart were dried for

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3 wk at room temperature. Heart ratio was calculated by dividing the weight of the right ventricle by the weight of the left ventricle plus septum (19).

**Vascular morphology.** The degree of muscularization was determined from mouse lung sections as described previously (15, 46). Paraffin-embedded lung sections of 3 μm were double-stained with α-smooth muscle muscle actin antibody to detect smooth muscle tissue and von Willebrand factor antibody to detect vessels. Morphological assessment of small vessel muscularization (outer diameter: 20–70 μm) was performed via computer-assisted analysis (Leica Q Win Standard analyzing software) at ×400 magnification under a microscope. The analytical software detected vessels that appeared in a brown color because of staining with anti-von Willebrand factor antibody. Muscle tissue appeared in a violet color. Thus, with the help of the software, it was possible to distinguish between nonmuscularized vessels (no smooth muscle cells detectable with anti-α-smooth muscle muscle actin staining), partially muscularized (at least one smooth muscle cell ~75% circumference with anti-α-smooth muscle muscle actin staining), and fully muscularized (>75% of circumference with anti-α-smooth muscle muscle actin staining).

**Quantitative real-time RT-PCR.** To assess mRNA expression levels in whole lung homogenates of mice, real-time RT-PCR was performed as described previously (47). Total mRNA was isolated from homogenized murine lung tissue using RNeasy Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Isolated RNA of each sample (1 μg) was converted to cDNA by reverse transcription using the iScript cDNA Synthesis Kit (Bio-Rad, Munich, Germany) under the following conditions: one cycle at 25°C for 5 min; one cycle at 42°C for 30 min; one cycle at 85°C for 5 min.

Relative quantification of the mRNA expression of the enzymes (Table 1) was performed by real-time PCR using the iQ SYBR Green Supermix according to the manufacturer’s instructions (Bio-Rad). Per reaction, a 25-μl mixture was used containing 12.5 μl iQ SYBR Green Supermix, 0.5 μl forward and reverse primer, 9.5 μl sterile water, and 2 μl of the 1:5 diluted cDNA template. A negative control (nontemplate control) was carried out in each run. The real-time PCR experiments were executed with a Mx3000P real-time PCR system (Stratagene, Heidelberg, Germany) under the following conditions: one cycle at 95°C for 10 min, then 40 cycles at 95°C for 10 s, 59°C for 10 s, 72°C for 10 s, followed by a dissociation curve. The intron-spanning primers were designed using the sequence information from the NCBI database. Expression levels are represented as ΔCT values, normalized to the ubiquitous β2-microglobulin b2m reference gene. The primers employed in this study are enlisted in Table 1.

**Statistics.** For statistical analysis, data were tested for normality of distribution by Kolmogorov-Smirnov test. All data except RVSP (Fig. 2A) were normally distributed. Therefore, for testing normally distributed data, significance of differences among groups was tested by one-way ANOVA. Bonferroni was used for post hoc testing between hypoxia and treatment groups. For analyzing mRNA expression data (Fig. 4), an unpaired two-sided Student’s t-test was employed unless otherwise stated. All data are given as means ± SE. For not normally distributed data (Fig. 2A) Kruskal Wallis test with Dunn’s post hoc test were employed. Statistical significance was defined as P < 0.05.

**RESULTS**

Exercise training improved physical performance capacity of mice that lived in chronic hypoxia. Mice that were kept in hypoxia for 21 days (Hox Plac) showed a significantly improved physical performance capacity than mice that were kept in normoxia (Nox Plac), as indicated by a significant decrease of VO2max (Fig. 1A). In contrast, mice that performed a daily exercise training and received a placebo (Hox Plac + Train) or were treated with Sildenafil alone (Hox Sild) or in combination with daily exercise training (Hox Sild + Train) showed a VO2max similar to normoxic healthy mice (Fig. 1A). These results were partly supported by changes of maximum walking distance until exhaustion (Fig. 1B); treatment with Sildenafil in combination with daily exercise training (Hox Sild + Train) led to a significant improvement of the maximum walking distance compared with mice that were kept in hypoxia alone (Hox Plac) (Fig. 1B).

A hypoxia-induced increase in RVSP is prevented by regular exercise training. Mice subjected to chronic hypoxia (Hox Plac) developed moderate PH. Two days before the final assessment of exercise performance of the mice a telemetric sensor was implanted into the animals, as described above and before (45). Telemetric measurements of RVSP showed that chronic hypoxia-induced increase in RVSP was prevented in mice that lived under hypoxic conditions and performed regular exercise training (Hox Plac + Train); RVSP was reduced to a similar level as that of normoxic mice (Nox Plac). Treatment with Sildenafil alone (Hox Sild), however, showed no significant effect (Fig. 2A), demonstrating a suboptimal dosing of Sildenafil that was chosen to allow the determination of synergistic effects of Sildenafil application plus physical exercise.

Right ventricular hypertrophy is not changed in response to regular exercise training in hypoxia-exposed mice. Hypoxia-exposed mice developed right ventricular hypertrophy (Hox Plac vs. Nox Plac). Surprisingly, even though RVSP was not increased in hypoxic exercising mice, treatment with Sildenafil, daily exercise training, or both in combination did not affect right heart hypertrophy (Fig. 2B).

### Table 1. Primers employed in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>pde1a</td>
<td>5'-GCATCGGTGCTACTGAACTG-3'</td>
<td>5'-AGACTACGAGCTGGTTTTGA-3'</td>
</tr>
<tr>
<td>pde1b</td>
<td>5'-GGCTGATCGAGGAGCTGTTA-3'</td>
<td>5'-CGAAGTTTCCGAGTTATTC-3'</td>
</tr>
<tr>
<td>pde1c</td>
<td>5'-ACACGTCAAGGACATGCTA-3'</td>
<td>5'-GCTGTGGAAGGTTCTGCTT-3'</td>
</tr>
<tr>
<td>pde4a</td>
<td>5'-GCATCGGTGCTACTGAACTG-3'</td>
<td>5'-AGACTACGAGCTGGTTTTGA-3'</td>
</tr>
<tr>
<td>pde4b</td>
<td>5'-GCATCGGTGCTACTGAACTG-3'</td>
<td>5'-AGACTACGAGCTGGTTTTGA-3'</td>
</tr>
<tr>
<td>scg3</td>
<td>5'-CCACCGGCGAGAACCCTATCA-3'</td>
<td>5'-GCTGTGGAAGGTTCTGCTT-3'</td>
</tr>
<tr>
<td>scgb1</td>
<td>5'-TGGCGAAGGATCGGCCGTTGA-3'</td>
<td>5'-AGATGACGCTTTGGCTCTT-3'</td>
</tr>
<tr>
<td>enox</td>
<td>5'-ACAGCATCTTCTGCCCACACAG-3'</td>
<td>5'-TGCAAGCTGAGGAAAGAAG-3'</td>
</tr>
<tr>
<td>inos</td>
<td>5'-AGACAGGCGAGGCTACATCA-3'</td>
<td>5'-TGCAAGCTGAGGAAAGAAG-3'</td>
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pde, Phosphodiesterase; scg, soluble guanylate cyclase; enos, endothelial nitric oxide synthase; inos, inducible nitric oxide synthase.
Small vessel muscularization in hypoxia-exposed mice. The hallmark of hypoxia-induced PH is an increased muscularization of small pulmonary vessels. To determine the degree of PH and the effect of regular exercise training in hypoxia-treated mice, vessels were classified as nonmuscular (no smooth muscle cells detectable with anti-α-smooth muscle actin staining), partially muscularized (at least one smooth muscle cell ~75% circumference with anti-α-smooth muscle actin staining), and fully muscularized (>75% of circumference with anti-α-smooth muscle actin staining). In normoxic controls (Nox Plac) 30.6 ± 4.7% of small pulmonary vessels were nonmuscularized, and 13.2 ± 2.2% were fully muscularized (Fig. 3). In contrast, in hypoxia-treated mice (Hox Plac), 6.4 ± 1.2% of small pulmonary vessels were nonmuscularized, whereas 31.4 ± 2.4% of small vessels were fully muscularized (Fig. 3). Regular exercise training (Hox Plac + Train) and treatment with Sildenafil (Hox Sild) both significantly reduced small vessel muscularization [20.8 ± 4.7% nonmuscular vessels and 17.3 ± 4.6% fully muscular vessels (Hox Plac + Train), and 19 ± 3% nonmuscular vessels and 21 ± 4% fully muscular vessels (Hox Sild)], indicating that regular exercise training exerts an inhibiting effect on smooth muscle cell proliferation to a similar extent as pharmacological treatment. When training was performed and Sildenafil was applied (Hox Sild + Train), 20.8 ± 2.8% of small pulmonary vessels were nonmuscularized and 15.8 ± 3.1% were fully muscularized, indicating that there was no additional effect of a combination of both treatments (Fig. 3).

Signaling pathways underlying exercise-induced effects in hypoxic mice. To delineate possible signaling pathways underlying the effects of regular exercise training and a synergism between training and Sildenafil application, we next focused on the NO-sGC-PDE axis. This analysis revealed that 1) the mRNA expression of PDE1b, PDE4a, PDE4b, sGCa3, and inducible nitric oxide synthase was significantly upregulated upon chronic hypoxia, but regular exercise training did not affect this regulation (Fig. 4, A–C). Relative mRNA expression of both endothelial nitric oxide synthase (eNOS) and PDE5 was significantly increased in lung homogenate of hypoxia- and placebo-treated mice compared with normoxic control animals (Fig. 4, D and E); and 2) the eNOS and PDE5 mRNA expression showed lower levels in lung homogenates from hypoxic mice who performed regular exercise training compared with hypoxic mice without training. Nevertheless, for eNOS, the expression was still higher in the trained group compared with normoxic mice. The mRNA expression of PDE5 in mice that performed regular exercise training, in contrast, was not different from PDE5 expression in normoxic mice. In contrast, no mRNA regulation of PDE1a and sGCh1 was found in response to hypoxia or training (Fig. 4, F and G). The mRNA expression of PDE1c was unchanged in hypoxia-treated mice compared with normoxic controls. However, mice that performed regular exercise training and received placebo drug treatment showed a significant upregulation in PDE1c expression compared with normoxic and hypoxia-treated control mice who received a placebo drug treatment.

DISCUSSION

The data presented here show that regular exercise training 1) improved aerobic exercise performance capacity, prevented from 2) the decrease in the maximal walking distance, and 3) the increase of RVSP of mice that have lived under chronic hypoxic conditions for 3 wk. Chronic exposure to hypoxia is a common animal model that has been used extensively to study PH (2, 16, 35, 44, 53), since it is highly reproducible within one animal strain (49). However, chronic hypoxia in mice (fraction of inspired O₂ <0.1) only induces a mild form of the disease in mice, for example, relatively small changes in small vessel muscularization. In the future, besides our study, it will be interesting to investigate the impact of exercise training also in animal models of PH that represent a more severe manifestation of the disease and also represent other forms of PH, like the vascular endothelial growth factor receptor blocker Sugen 5416 in combination with chronic hypoxia in rats, or MCT in rats. All data obtained from animal models always have to be interpreted carefully.
The findings in our study are nevertheless in line with most studies in humans with established PH (4, 7, 24, 34, 40). In addition to the human studies and the rare animal studies in MCT-induced PH (10, 26), our data revealed that the increase in small vessel muscularization that occurs in hypoxia-induced PH was completely prevented by physical exercise. Such exercise-associated effects on structural alterations of vessels have not yet been described for hypoxia-induced PH so far.

The maximum walking distance, employed in our study as a measure of the physical fitness, is, to a certain degree, comparable with the 6-min walking distance used in clinical studies with patients. The 6-min walking distance is a parameter that is commonly used to estimate functional capacity in patients with PH. It is well reproducible and, more importantly, it correlates with \( \dot{V}O_{2\text{max}} \) (41) and peak oxygen consumption determined by maximal cardiopulmonary exercise testing (38). However, this might merely be true for patients with PH: due to their severely impaired exercise capacity, there might be a particular close association between submaximal exercise (6-min walking distance) and maximal exercise (38), and, thus, both types of exercise can only be compared with a limited degree.

The \( \dot{V}O_{2\text{max}} \) represents the maximum capacity of an organism to transport and use oxygen. It reflects the physical fitness of the individual and represents a suitable predictor of mortality in many chronic cardiopulmonary diseases (3). Earlier studies on endurance training in mice (43, 50), as well as in rats (33), revealed an increase of \( \dot{V}O_{2\text{max}} \) in response to regular exercise training. However, in those studies, exercise training was usually performed as voluntary wheel running, and, therefore, neither exercise intensity nor the individual fitness levels of the animals were suitably controlled. In contrast, the model employed by us allowed an individual adjustment of the training load of each animal. This adjustment of training intensity has been reported to evoke physiological adaptation to exercise similar as in humans. Endurance training at an intensity corresponding to 60% of \( \dot{V}O_{2\text{max}} \) can be compared with the recommendations for patients with severe disease, since it corresponds to moderate exercise intensity. In addition, the setup allowed for adequate controlling and quantification of the outcome of the study, since the treadmill for mice functions as a spiroergometer (30, 58). When \( \dot{V}O_{2\text{max}} \) was tested at a range of inclinations, Kemi et al. discovered that inclinations...
between 15° and 35° resulted in the highest peak of maximal oxygen consumption (30). Therefore, mice in the present study trained at 25° inclination. We demonstrated here that chronic hypoxic mice were able to reach the same maximum oxygen uptake as healthy animals in a 3-wk program of running exercise. Several factors of oxygen supply and demand determine VO2max. One of the factors that determines the oxygen supply is the transport of oxygen in the muscle. It comprises lung diffusion, stroke volume, blood volume, and capillary density of the skeletal muscle (51). Oxygen delivery, not skeletal muscle oxygen extraction, has been considered to be the primary limiting factor for VO2max in exercising humans (3). The hypoxic effect on VO2max in our study is consistent with other reports that have demonstrated a reduction of VO2max under hypoxic conditions, yet not always in chronic hypoxia (8, 25, 42). It can be assumed that VO2max may be constrained by the transfer of blood to the tissue and not by the transport of oxygen to the blood (8). Considering the chronic hypoxia-induced pulmonary vascular remodeling, it seems likely that the decrease of VO2max in response to hypoxia is a consequence of the increased perfusion resistance in the lung. Such an explanation was, however, excluded in our study, since vascular remodeling was prevented by exercise training in chronically hypoxic mice. A decrease in VO2max has also been shown in patients with PH (39, 52); however, in both of these cases PH occurred secondary to either scleroderma pigmentosum or chronic obstructive pulmonary disease.

Regular exercise training and Sildenafil treatment increased VO2max in our study. It was previously shown in rodents that exercise training induces cardiac and skeletal muscle hypertrophy (14, 30) and an increase in capillary density and mitochondria-to-myofibril volume in the heart (1). Also, an increase in skeletal muscle capillarization was found after exercise training in human idiopathic pulmonary hypertension (11). In healthy adults, the mitochondrial ATP production rate in isolated skeletal muscle mitochondria increases by up to 92%, depending on the substrate mixture, largely because of an increase in the activity of cytochrome c oxidase and an increase in mitochondria mass (56). It might be possible that the exercise-induced increase in VO2max did not target the hypoxia-induced physiological changes but that an increase in muscle and mitochondria mass compensates for the reduced oxygen delivery to the blood. By reason of the multitude of physiological adaptation processes in response to exercise, we cannot exclude various peripheral effects that lead to an increase in oxygen consumption in our mice.

Chronic alveolar hypoxia leads to morphological and functional changes of pulmonary resistance vessels (55). In our model of hypoxia-induced PH, chronic hypoxic mice showed a significant increase in small vessel muscularization that is supported by previous data (17, 18, 44, 54, 60). In both training groups, and in the Sildenafil-treated group, small vessel muscularization in response to hypoxia was largely prevented. Exercise-induced prevention of hypoxia-induced small vessel remodeling might be mediated by increasing vasodilatory mechanisms. However, at least on the mRNA level we did not find respective changes in the NOS-sGC-cGMP-PDE axis. This is in contrast to findings in the heart, where several studies showed an exercise-induced upregulation of eNOS protein expression in the aorta and coronary vessels (12, 59). In the lung, Chen and Li showed an improvement of endothelium-mediated vasorelaxation of the pulmonary artery in trained rabbits (9). Johnson et al. could not detect any pulmonary vasorelaxation in response to short-term exercise in pigs (28), whereas an increase of acetylcholine-induced relaxation and epithelial nitric oxide synthase (eNOS) protein expression in response to short-term exercise was found (29). However, these studies were not performed in chronic hypoxic animals.

With regard to the right heart, even though pathological small vessel muscularization and increase in RVSP were prevented by regular exercise training, no effect of training or of Sildenafil treatment on hypoxia-induced right heart hypertrophy was detectable. In contrast, in earlier studies, Zhao et al. had shown a small but significant reduction in right ventricle hypertrophy in response to Sildenafil treatment in mice (61), even though this group used a 75% lower dose of the drug. Although the differences to our study are not known, the fact that the chosen dosage of our study exerted no effect on right heart hypertrophy should have allowed the detection of synergistic effects of training and pharmacological treatment. Al-
though right ventricular hypertrophy was not affected by exercise training compared with chronic hypoxia alone, for the maximum walking distance, a combination of exercise with Sildenafil treatment seemed to be superior compared with training or pharmacological treatment alone. Investigations of the NO-sGC-PDE axis revealed major effects on eNOS and PDE5 expression that showed lower expression levels in the trained hypoxic group compared with hypoxia alone. Whereas an upregulation of PDE5 can be suggested to contribute to PH development by a higher degradation of cGMP, the downregulation of eNOS most likely is a secondary effect that could be interpreted to result from decreased PH and thus a decreased need of counterbalancing PH by an NOS increase. Interestingly, PDE1c, also being a cGMP-degrading enzyme, was

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Fig. 4. mRNA regulation in normoxic, chronically hypoxic, and trained hypoxic mouse lungs for the nitric oxide (NO)-soluble guanylate cyclase (sGC)-phosphodiesterase (PDE) pathway. Expression levels of mRNA as ΔCt for Pde1a (A), Pde1c (B), sGCh1 (C), PDE1b (D), PDE4a (E), PDE4b (F), sGCa3 (G), inducible nitric oxide synthase (iNOS, H), endothelial nitric oxide synthase (eNOS, I), and PDE5 (J). Data are given as means ± SE from n = 4–5 lungs/group. *P < 0.05, **P < 0.01, ***P < 0.001, and #P < 0.05 as per unpaired, one-sided t-test. ns, Not significant.
upregulated by exercise compared with hypoxia alone, which may limit the effects of physical training. The fact that right ventricular hypertrophy was prevented neither by exercise nor by Sildenafil treatment may be indicative for a direct effect of hypoxia on the right ventricle independent from the pulmonary arterial pressure although this conclusion has to be drawn cautiously, since we did not quantify cardiac output.

In summary, we have shown that not only the development of PH could be prevented by individualized exercise training but also that the vascular remodeling process occurring in chronic hypoxia was prevented. Therefore, exercise training might be an effective treatment option for PH patients, which directly affects the pathological cascade of PH. Furthermore, in future studies, in addition to testing the effect of exercise training in models that represent a more severe type of PH, it will also be interesting to investigate the effect of different types of training, like, for example, isometric strength training, which has recently been shown to exhibit marked differences in physiological adaptations compared with endurance training (31).

The results of our study indicate that exercise training does not “simply” prevent a pathological increase in RVSP by triggering vasodilatory mechanisms. This conclusion is supported by the fact that, in our study, training did not induce a vasodilatory shift of the NOS-sGC-PDE enzyme expression. However, because whole lung homogenate was used for expression studies, we cannot rule out cell type-specific changes that might only be detectable in isolated cells. Most interestingly, no preventive effect on right heart hypertrophy by exercise training was noted, which can either be caused by 1) a direct effect of hypoxia on the right heart leading to right ventricular hypertrophy or 2) an increased cardiac output upon training that results in similar pulmonary vascular resistance values under hypoxia alone and hypoxia plus training.

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DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

EVALUATION OF GROWTH AND FUNCTIONAL OUTCOMES IN PROGRESSIVE LUNG DISEASE

EXERCISE PREVENTS LUNG VESSEL REMODELING IN MICE


