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Original Article

Sildenafil reverses the hypertrophy of mice right ventricle caused by hypoxia but does not reverse the changes in the myosin heavy chain isoforms

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Abstract: In this study, we investigated the effect of hypoxia and concomitant sildenafil treatment on MHC isoforms in hypoxia-induced hypertrophied right ventricles. Right ventricular hypertrophy was induced in mice by exposing them to hypoxic stimulus (11% ambient oxygen) in a normobaric chamber for 20 days. 45 mice were used in this study, distributed randomly into three groups: the first group served as a control (CO), the second group was exposed to hypoxia for 20 days without sildenafil treatment (HY), and the third group was given sildenafil orally at a dose of 30 $mg.kg^{-1}.day^{-1}$ plus exposure to hypoxia for 20 days (HS). Relative amounts of MHC isoforms were calculated using two ELISA kits containing antibodies against α and β MHC, and by SDS-PAGE. Compared with the CO group, the HY group showed a significant increase in right ventricle weight/left ventricle plus septum ratio (Fulton's ratio). The HS group showed a significant decrease in Fulton's ratio compared with the HY group, but not with the CO group. Expression of the MHC-β isoform was significantly increased in the HY group compared with the CO group. There was no significant difference in MHC-β between the HY group and the HS group. Plasma atrial natriuretic peptide level was significantly higher in HY group than HS group and did not return to normal after sildenafil treatment. Conclusion: sildenafil reversed the right ventricular hypertrophy induced by hypoxia but did not decrease the expression of MHC-β to normal levels.

Keywords: Sildenafil, myosin heavy chains, hypertrophied right ventricle, hypoxia

Introduction

Myosin is a mechanoenzymatic protein of the cardiac muscle. The structural and enzymatic properties of myosin determine most of the contractile features of cardiac muscle. Myosin is a large molecule with a molecular weight of ~500 kDa, composed of two heavy chains (MHCs) and four light chains. There are two distinct heavy chain isoforms in mammalian cardiac muscle: MHC-α (fast) and MHC-β (slow). Abundant evidence in the literature indicates that the contractile properties of the cardiac muscle and mechanical performance of the heart are strongly correlated with the type of MHC expressed in the cardiac muscle under different conditions. Myosin isoforms transition from the faster MHC-α to the slower MHC-β during the progression of human heart failure [1] and mechanical challenges imposed on the heart [2]. Hormonal factors like thyroid hormone cause increased MHC-α and decreased MHC-β expression [3], and a similar transition seen during normal developmental processes [4]. Adult mammals predominately express one of these cardiac MHC isoforms [1, 5]. From a functional point of view, α-MHC has twice the actin-activated ATPase activity of MHC-β, and roughly three times the actin sliding speed [5]. Also, the release rate of MgADP is roughly twofold faster for MHC-α [6]. The rate of tension redevelopment decreases as the expression of MHC-α decreases and that of MHC-β increases, and actomyosin cross-bridge cycling under high strain is threefold higher with MHC-α [7, 8].

Sildenafil is a selective, potent and orally active phosphodiesterase-5 enzyme inhibitor (PDE-5), which is the major PDE subtype pres-

ent in pulmonary blood vessels [8] and is highly expressed in lung vessels compared to other tissues. PDE-5 mainly inactivates the second messenger cyclic guanosine monophosphate (cGMP), by controlling its rate of degradation, thus inhibiting this enzyme with sildenafil leads to an increase in the concentration of cGMP. The increased concentration of cGMP causes vascular smooth muscle relaxation. Although sildenafil was first prescribed for erectile dysfunction treatment [9], it may be used with caution for the treatment of some cardiovascular diseases, since it is effective for treating pulmonary hypertension by increasing the cGMP level, and thus offers the possibility of selective pulmonary vasodilatation and antiproliferative effects [10]. The action of sildenafil in the lungs and the heart is enhanced as there is an upregulation in the expression of PDE5 in pulmonary hypertension [11]. Sildenafil therapy stimulates endothelial nitric oxide synthase (NOS) production and basal nitric oxide release [12]. The presser effect of hypoxia is almost abolished, and the plasma cGMP level increased significantly by sildenafil treatment, even when nitric oxide synthase is impaired, thus sildenafil offers a novel approach to reducing the effect of hypoxia-induced pulmonary hypertension in both humans and mice [13]. PDE5 is highly expressed in the hypertrophied heart compared with the normal heart [14]. PDE-5 inhibitors deactivate multiple elements of the hypertrophy signaling pathway triggered by pressure overload [15]. The efficacy of sildenafil has also demonstrated in reducing myocardial lesions and the remodeling of both cardiomyocytes and vascular smooth muscle in NOS inhibitor-treated rats. Also, it has been shown that the chronic administration of sildenafil restores the plasma cGMP level in L-NAME-induced cardiac hypertrophy, while normal hearts show no effect after sildenafil administration [16].

Chronic hypoxia can be induced by exposing animals to normal air at hypobaric pressures resembling those experienced by organisms living at high altitude, or by exposure to oxygen-poor air at normal pressure (e.g. 10% O_2). Hypoxia plays a significant role in the development of pulmonary hypertension by causing a sequence of morphological changes in the small pulmonary arteries and arterioles [17]. Animals exposed to chronic hypoxia have been used for decades to induce pulmonary vascular remodeling, compared with those changes develop in human, which induces right ventricular hypertrophy. Vascular remodeling reveals a muscularization of a subset of arterioles in the lung and proliferation of the longitudinal smooth muscle cells in the intima of small arteries, which is a classical feature of human pulmonary vascular remodeling [17]. From the above description of the effects of sildenafil and the changes produced by hypoxia, we tried in this work to explore the changing MHC isoforms in mouse hypertrophied right ventricles and whether sildenafil treatment can alleviate the changes caused by hypoxia.

Materials and methods

Animals, experimental grouping and hypoxic condition

The experiments were performed on 45 male Swiss Balb\c albino mice (10 weeks of age, 26.62 ± 3.2 g) obtained from the animal house of Jordan university of science and technology. They were housed in a controlled environment with a 12 h light/12 h dark cycle at room temperature throughout the whole experimental period. Laboratory chow and water were supplied *ad libitum*. The mice were randomly assigned to three groups: the control group (CO), containing 15 mice; the hypoxia group (HY), containing 15 mice exposed to normobaric hypoxia (11% ambient oxygen) for 20 days; and the hypoxia with sildenafil treatment group (HS), containing 15 mice exposed to normobaric hypoxia (11% ambient oxygen) for 20 days plus sildenafil administered orally at a concentration of 30 $mg.kg^{-1}.day^{-1}$. The concentration of the drug in water was adjusted for body weight and daily water intake. Each group was divided into two subgroups. The first group, consisting of 5 mice, was used for morphometric measurement, and second, consisting of 10 mice, was for biochemical measurements. Hypoxia was induced in a specially constructed plexiglass chamber that enabled the oxygen percentage to be maintained at 11% with 89% nitrogen for 20 days under normobaric conditions. All animal experimental protocols were reviewed and approved by Jordan University of Science and Technology animal care and use committee and all procedures were done according to the guidelines set by this committee.

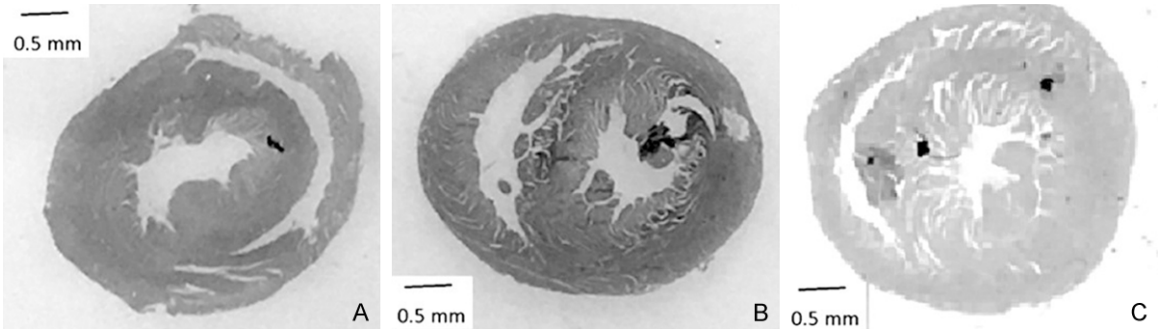

Figure 1. Cross section of the heart 2 mm below the upper edge of the ventricles. A. Normal heart. B. Heart from an animal exposed to hypoxia for 20 days. C. Heart from an animal exposed to 20 days of hypoxia plus sildenafil.

Tissue and blood collection

After euthanization of the mice with CO_2, the chest cavity was quickly opened, and the intact heart was removed and washed in chilled phosphate buffered saline to remove the blood. Both atria and great vessels were removed, and the heart weight was measured. Under a dissecting microscope, the right ventricle free wall was completely removed from the left ventricle wall and the intraventricular septum. To assess right ventricular hypertrophy, the right ventricle and left ventricle plus septum were blotted dry and their weights were calculated to obtain Fulton's ratio (right ventricle weight/left ventricle plus septum weight) and right ventricle/body weight ratio. The obtained tissues were stored at -80°C for further analysis. Blood samples were taken for assessment of hematological parameters, triiodothyronine (T_3) and atrial natriuretic peptide (ANP) plasma levels, in a heparinized capillary pipette from the descending aorta, before removal of the heart.

Quantitative determination of MHC-α and MHC-β by ELISA

The relative amounts of both MHCs in right ventricular muscle samples were quantified using commercially available colorimetric ELISA kits (Mybiosource). Two sets of ELISA kits were used containing wells coated with specific antibodies against mouse cardiac MHC-α (cat. no. MBS092927) and MHC-β (cat. no. S107247). The sensitivity of the antibodies is high, and they can detect MHC at a concentration of 2 pg MHC per 1 ml supernatant. The protein concentration in cardiac muscle homogenates was determined using an assay kit obtained from Bio-Rad Laboratories, Inc. (Hercules, CA, USA).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to separate the two cardiac MHCs. Tissue samples from the right ventricular wall, frozen in liquid nitrogen, were ground in a frozen ceramic bowl using a mortar. The muscle powder was mixed with Laemmli protein sample loading buffer (Bio-Rad Laboratories, Inc.) plus 2.5%$^{v}/_{v}$ 2-mercaptoethanol. SDS-PAGE was run on a long gel (SCIEPLAS). The optimum voltage for long gel electrophoresis was a continuous 100 V/slab. After separation, the gels were stained with 0.1% Coomassie Brilliant Blue R250 in 40% methanol and 10% acetic acid. Silver stain was also used to visualize myosin bands when the protein concentration in the sample was not enough to be visualized by Coomassie stain. After the staining and destaining procedures, the gels were scanned with a computerized scanner and densitometer (GS 800; Bio-Rad Laboratories, Inc.). Since the difference in molecular weight of the two heavy chains is small, making the separation of these proteins difficult for the purpose of scanning and quantification of each heavy chain, we used a very porous polyacrylamide gel (5%). **Figure 2** shows a typical separation of MHCs of the right ventricle muscle in the HY and HS groups compared with MHCs in control animals. The MHC-β was identified by running protein extract from mouse soleus muscle which expresses high level of MHC-β [18].

Assay of plasma atrial natriuretic peptide (ANP) and triiodothyronine (T_3) levels

To measure the plasma levels of ANP and T_3, 0.5 ml blood was collected in a 3.2% citrate tube. Plasma was then obtained by centri-

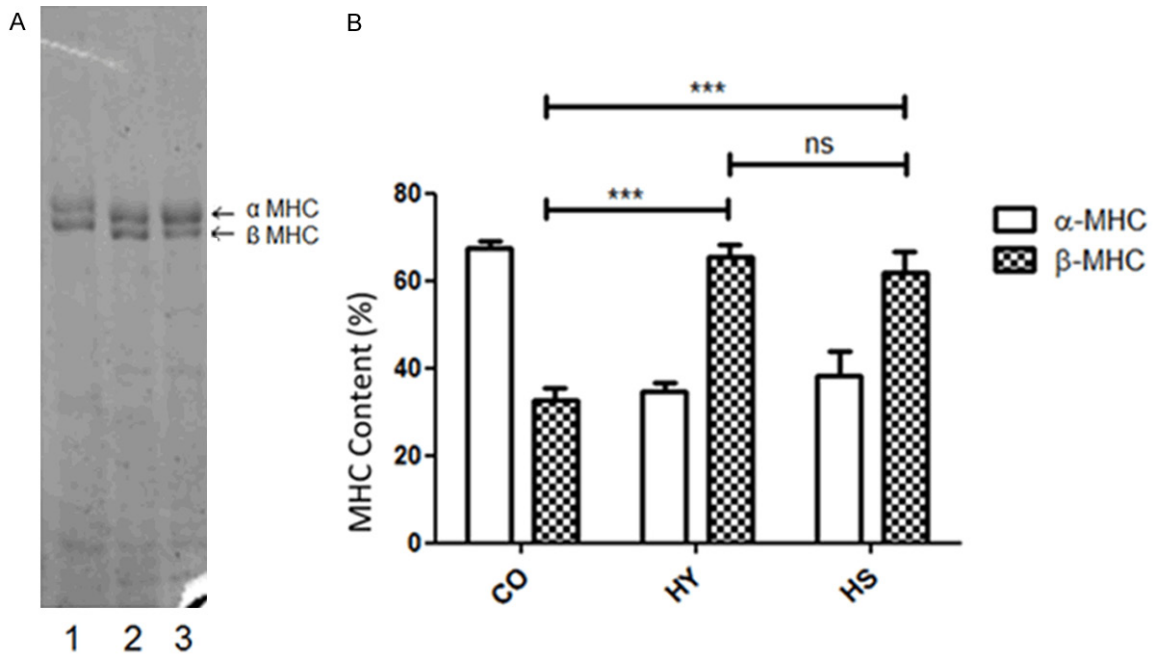

Figure 2. Effects of hypoxia and hypoxia with sildenafil on expression of myosin heavy chain isoforms. A. Representative SDS gel showing expression of α and β MHC. Lane 1: hypoxia group, lane 2: hypoxia with sildenafil group, lane 3: control group. B. Relative expression of MHC-β in the three experimental groups. Hypoxia caused expression of more MHC-β and sildenafil failed to reverse the changes caused by hypoxia. CO (control group); HY (hypoxia for 20 days without treatment); and HS (hypoxia for 20 days plus treatment with sildenafil). ***P<0.01 HY and HS vs. CO.

Table 1. Blood parameters in three experimental groups (15 animals in each group) expressed as mean ± SD

Parameter	control	hypoxia	Hypoxia + sildenafil
Red blood cells (× 10^6)	6.5 ± 0.3	9.3 ± 0.4*	9.1 ± 0.2*
Hb (g/dl)	13.9 ± 0.3	17.0 ± 0.1*	16.8 ± 0.2*
Hematocrit (%)	41.1 ± 1	63.3 ± 2.7*	65. 7 ± 3.1*
plasma T_3 (ng/dl)	53.1 ± 1.6	65.3 ± 2.1*	64.1 ± 1.2*
Plasma ANP (ng/dl)	286.3 ± 15.2	655 ± 89.3*	501 ± 60.5*,#

*P<0.05 versus control group. #P<0.05 versus hypoxia group.

fugation at 3,000 rpm for 10 min at 4°C and stored at -20°C until the assay. Plasma ANP levels were detected by ELISA using a kit supplied by Invitrogen; Thermo Fisher Scientific, Inc. (cat. no. EIAANP). T_3 plasma levels were measured using a mouse T_3 ELISA kit from Mybioscience (cat. no. MBS9914475).

Statistical analysis

MHC-α and MHC-β expression was calculated as a percentage of total myosin in the cardiac muscle. The results are expressed as the mean ± SE. Differences between the groups were calculated using Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Statistical differences between two means were determined by Student's t-test when two groups were compared, while comparisons between more than two groups were performed by one-way ANOVA analysis of variance followed by Tukey post hoc test. A value of $P<0.05$ was taken as evidence of a significant difference.

Results

Effects of hypoxia and hypoxia with sildenafil on blood parameters, T_3 and ANP

Mice exposed to hypoxia for 20 days (n=15) compared to control group (n=15) showed a significant increase (P<0.05) in red blood cell count, hemoglobin concentration, hematocrit values (**Table 1**). The red blood cell count was increased from 6.5 ± 0.3 to 9.3 ± 0.4 × 10^6/μL, the Hemoglobin was increased from 13.9 ± 0.3 to 17.0 ± 0.1 g/dl and hematocrit was increased from 41.1 ± 1 to 63.3 ± 2.7%. All these changes in blood parameters indicate that the hypoxic stimulus was strong enough to cause its known effects on the body.

Table 2. Animals data expressed as mean ± SD

Parameter	Control group n=10	hypoxia group n=10	Hypoxia and sildenafil group n=10
Body weight (g)	26.62 ± 1.20	26.58 ± 1.10	25.14 ± 0.83
Fulton's index RV/LV + septum (mg/mg)	0.25 ± 0.05	0.36 ± 0.06*	0.27 ± 0.05#
RV/body weight (mg/g)	0.89 ± 0.03	1.30 ± 0.07*	1.02 ± 0.04#

RV: right ventricle, LV: left ventricle. *P<0.05 versus CO group and #P<0.05 versus HY group.

The T_3 blood levels were increased significantly (P<0.05) in mice exposed to hypoxia for 20 days (65.3 ± 2.1 ng/dl) and those given sildenafil during hypoxia exposure (64.1 ± 1.2 ng/dl) relative to control group (53.3 ± 1.6 ng/dl). Concomitant treatment with sildenafil during hypoxia did not significantly affect the alteration in hematological parameters and T_3 levels shown in the hypoxic group. ANP plasma levels were significantly elevated (P<0.05) in hypoxia group (655 ± 89.3 ng/dl) and hypoxia plus sildenafil treatment (501 ± 60.5 ng/dl) as compared to control group (286.3 ± 15.2 ng/dl).

Right ventricular hypertrophy

Table 2 shows the changes in right ventricle weight in three experimental groups of animals. The body weight did not differ in hypoxia group (26.58 ± 1.1 gm) and sildenafil treated group (26.62 ± 1.2 gm) relative to control group (25.14 ± 0.83 gm). Exposure of mice to hypoxia induced right ventricular hypertrophy, as indicated by an increased right ventricular weight/body weight ratio. Fulton's index (right ventricular free wall weight/left ventricular plus septum weight) was used as a reliable indicator of right ventricular hypertrophy. Fulton's index was significantly increased (P< 0.05) in mice upon exposure to hypoxia for 20 days (0.36 ± 0.06) compared to control group (0.25 ± 0.05). Treatment with sildenafil significantly (P<0.05) reduced Fulton's ratio compared with the hypoxia group (0.27 ± 0.05). **Figure 1** shows cross sections of the heart (2 mm below the upper edge of the ventricles). The right ventricle thickness was greatest in the hypoxia group (0.54 ± 0.07 mm) compared to control group (0.3 ± 0.02 mm) and sildenafil treatment group (0.31 ± 0.03 mm).

Effects of hypoxia and hypoxia with sildenafil on MHC isoforms

Figure 2A shows the electrophoresis pattern of MHC-α and MHC-β in the three experimental groups of mice. The relative proportion of MHC-α and MHC-β is shown in **Figure 2B**, as determined by ELISA using specific antibodies against the two MHCs. Compared with the CO group, the expression of both MHCs was changed in the HY and HS groups. The relative expression of MHC-β was increased in the HY group compared with CO group (66 ± 3% versus 33 ± 3%, P<0.01). Sildenafil treatment failed to reverse the change in the relative expression of MHC (63 ± 6% in SH versus 66 ± 3% in HY). This trend in expression of the two MHC was also seen in scanning of MHC bands separated by SDS-PAGE.

Discussion

Cardiac muscle myosin has two heavy chain isoforms, MHC-α and MHC-β which differ in biochemical and biomechanical properties that correlate with the mechanical properties of muscle [19]. There are many pathophysiological factors that can modify the relative expression of the two MHC isoforms. One of these factors is cardiac hypertrophy. Cardiac muscle that expresses more MHC-β shows low ATPase activity and can generate force with a remarkably economical pattern [19]. In our study, we explored the effect of hypoxia on the expression of MHC isoforms and the ability of sildenafil treatment to reverse the changes in MHC expression. Mice tolerate normobaric hypoxia (11% O_2) very well, and all of the mice in this work survived after living in hypoxic conditions for 20 days, which is in accordance with the previous study [20] wherein mice were exposed to 10% O_2 for 4 weeks without lethal effect. We used 11% O_2 because the experiments were performed at an altitude of ~650 m above sea level where barometric pressure is ~700 mmHg. The partial pressure of inspired O_2 in the chamber was ~85 mmHg and this represents severe hypoxic condition in human [21]. Hypoxia is both a cause and a result of pulmonary hypertension, which subsequently leads to right ventricular hypertrophy.

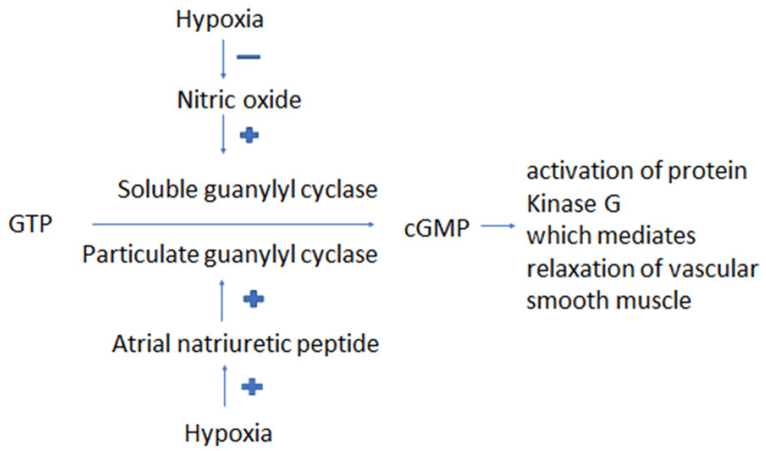

Figure 3. Schematic diagram showing the effect of hypoxia on cGMP production. Hypoxia affects the production of nitric oxide (NO) and atrial natriuretic peptide (ANP) in pulmonary vascular smooth muscle cells. Both NO and ANP raise intracellular cGMP levels. Hypoxia increases the production of ANP and decreases NO production. NO stimulates soluble guanylyl cyclase to convert guanosine triphosphate (GTP) to GMP, while ANP activates particulate guanylyl cyclase, found on the cell membrane, leading to the formation of cGMP, which mediates the relaxation of vascular smooth muscle.

Vascular smooth muscle relaxation is primarily mediated by cyclic cGMP, an "intracellular messenger", via its second messenger protein kinase G (PKG). PKG activation decreases the intracellular Ca2+ concentration and thus induces relaxation of the muscle cells. Production of cGMP is affected by hypoxia through two opposing mechanisms: an inhibitory one through inhibition of nitric oxide synthesis (NO), and a second excitatory one through production of more ANP (**Figure 3**).

The gene expression of the cardiac hormone ANP in the right ventricle was reportedly increased by almost three-fold after 3 weeks of hypoxia [26]. Our results showed that the ANP level was increased during hypoxia and this is due to partly to hypoxia effect per se and partly by increased load on right ventricle. It seems that its effect was blunted by a concomitant decreased in NO production; the action of ANP on vascular smooth muscle is through particulate guanylyl cyclase (GC-A) which is found at a high level in the mouse lung [27]. Hypoxic conditions markedly decrease the expression and activity of GC-A in pulmonary endothelial cells [27]. The blunted effect of decreased NO production, and decreased activity and expression of GC-A, explains the overall hypoxic effect of an increase in pulmonary vascular resistance and thus more load on the right ventricle, leading to its hypertrophy. Plasma ANP was significantly higher in HY group than HS group because of the greater overload in the HY group. However, the ANP level was not returned to normal after sildenafil treatment. It seems that both hypoxia [28] itself and the greater overload caused by vasoconstriction were the stimuli for ANP release from the hypoxic heart muscle. The pressure load imposed on the right ventricle was reversed by sildenafil, but hypoxia stimulus was there, and this may explain why ANP level did not return to control levels.

Fetal gene expression is increased in cardiac hypertrophy [22] and one of these genes is MHC-β. We reported here that hypoxia was associated with an increase in the expression of MHC-β in the right ventricles of mice, and this expression was not reversed by concomitant treatment with sildenafil. The shift in MHC expression towards more MHC-β by hypoxic stimulus was reported previously by many investigators [23, 24] but to the best of our knowledge, this is the first report which shows that sildenafil failed in reversing this shift in cardiac MHC type in spite of regression of the cardiac hypertrophy caused by hypoxia. The right ventricular mass was increased significantly in the HY group and this was accompanied by upregulation of MHC-β in the HY group compared with the CO group (**Figure 1**). This upregulation of MHC-β could be an adaptive mechanism to decrease ATPase activity and thus lead to more economical force generation from myocardial contraction, to preserve O_2 consumption and increase myocardial energy under hypoxic conditions. The increase in MHC-β expression in the human heart has also been reported in pathological hypertrophy [25]. Increased load pressure caused by pulmonary vasoconstriction is the main factor causing right ventricular hypertrophy.

cGMP is broken down to guanosine monophosphate (GMP) by PDE5 enzyme, so inhibiting PDE5 enzyme will increase the cGMP level. Since PDE5 enzyme is found in large amounts in the lungs, it is logical to use a specific inhibitor (sildenafil) of this enzyme to cause relaxation in the smooth muscle of the pulmonary vascular tree and thus decreased induction of right ventricular hypertrophy. Sildenafil inhibits the breakdown of cGMP in vascular smooth muscle. PDE5 is found in abundance throughout the muscularized pulmonary vascular tree, and its inhibition with sildenafil leads to a decrease in pulmonary resistance [29]. Our results showed that sildenafil reversed the right ventricular hypertrophy caused by hypoxia, and this was primarily due to decreased breakdown of cGMP. Recently, it has been reported that treatment with sildenafil, in both *in vivo* and *in vitro* preparations, upregulated endothelial NO synthase in the lungs and increased the plasma levels of nitrates and nitrites in rats exposed to hypoxia for 2 weeks [30, 31]. This upregulation of NO synthase could result in more cGMP production and thus less vasoconstriction during hypoxia in the presence of sildenafil. A morphological and hemodynamic study showed that sildenafil treatment ameliorates the rise in right ventricle pressure, right ventricular hypertrophy and decreases the wall thickness of lung vessels caused by hypoxia for two or four weeks [31]. The main finding of our study is the inability of sildenafil to decrease the increment in expression of MHC-B in mice exposed to hypoxia for three weeks in spite of pronounced effect of sildenafil on the regression of right ventricle hypertrophy (**Table 2**).

Pressure and volume overload are well-known conditions causing cardiac hypertrophy, which is associated with changes in the expression of cardiac MHC isoforms [23]. Hypoxia for 48 h increases MHC-β transcript levels *in vitro* and in *in vivo* rat models [32]. Cardiac muscle expressing more MHC-β shows a greater economy of contraction, and this increases the efficiency of hypertrophied muscle contraction [23]. In this work we demonstrated that hypoxia-induced hypertrophy was associated with more MHC-β expression in the right ventricles of the mice. The right ventricles expressed more MHC-β even when the hypertrophy was ameliorated by sildenafil treatment. This might indicate that the hypoxia increased the expression of MHC-β regardless of whether there was hypertrophy or not. This agrees with the findings of other investigators [24] in rats where hypoxia caused a fourfold increase in MHC-β expression in nonhypertrophied left ventricle muscle. This dissociation between cardiac hypertrophy and expression of increased MHC-β has also been reported [24, 33] in nonhypertrophied rat ventricles. In our study, we found that nonhypertrophied mouse right ventricles expressed more MHC-β in the presence of hypoxia. This suggests that hypoxic stress has a direct effect on the expression of MHC-β. It is well documented that hypoxia stimulates the expression of many fetal genes, one of which encodes MHC-β. Our findings suggest that hypoxia itself is involved in the expression of more MHC-β and this could be mediated through hypoxia-inducible factor-1α (HIF-1α). It seems that the effect of hypoxia on the expression of MHC-β gene was not affected by sildenafil which reverses right ventricular hypertrophy through its pulmonary vasodilation effect. Hypoxia is a condition common in fetal heart development and the failing adult heart; HIF-1α is a good candidate regulator that could be responsible for the postnatal switch and the return to the fetal gene program. However, we cannot attribute expression of more MHC-β caused by hypoxia to this factor alone, since cardiac hypertrophy is also associated with more expression of HIF-1α in the absence of cardiac hypoxia [34].

T_3 level as well as hemoglobin values were significantly increased in both HY and HS groups and this indicates that hypoxia stimulus was strong. We measured the T_3 level for two purposes; first to make sure the hypoxia stress is there and second to see whether increased T_3 help in reduction of MHC-β expression since increased T_3 associated with expression of more MHC-α expression. It has been reported that an increased T_3 concentration suppresses the expression of MHC-β mRNA [35]. Hypoxia was associated with an increased plasma T_3 concentration (**Table 1**). The presence of more thyroid hormone was associated with the expression of more MHC-α. Our results showed that the T_3 level was slightly increased in both the HY and HS groups. The effect of hypoxia on MHC expression

appeared to cancel out the effect of increased T_3 on the expression of MHC, or this slight increase in T_3 may not have affected, or only slightly affected, MHC expression.

In conclusion, the shift in MHC is likely due to effect of hypoxia on the cardiac muscle itself, or due to load imposed on the right side of heart due to pulmonary vasoconstriction caused by the stress of hypoxia. Sildenafil treatment reduces right ventricular hypertrophy caused by hypoxia, but it does not reverse the changes in MHC expression.

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Disclosure of conflict of interest

None.

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