Hypoxia-Inducible Factor-1α in Pulmonary Artery Smooth Muscle Cells Lowers Vascular Tone by Decreasing Myosin Light Chain Phosphorylation

Short Communication

Yu-Mee Kim,* Elizabeth A. Barnes,* Cristina M. Alvira, Lihua Ying, Sushma Reddy, David N. Cornfield

Rationale: Hypoxia-inducible factor-1α (HIF-1α), an oxygen (O₂)-sensitive transcription factor, mediates transcriptional responses to low-O₂ tension states. Although acute hypoxia causes pulmonary vasoconstriction and chronic hypoxia can cause vascular remodeling and pulmonary hypertension, conflicting data exist on the role of HIF-1α in modulating pulmonary vascular tone.

Objective: To investigate the role of smooth muscle cell (SMC)–specific HIF-1α in regulating pulmonary vascular tone.

Methods and Results: Mice with an SMC-specific deletion of HIF-1α (SM22α-HIF-1α−/−) were created to test the hypothesis that pulmonary artery SMC (PASMC) HIF-1α modulates pulmonary vascular tone and the response to hypoxia. SM22α-HIF-1α−/− mice exhibited significantly higher right ventricular systolic pressure compared with wild-type littermates under normoxia and with exposure to either acute or chronic hypoxia in the absence of histological evidence of accentuated vascular remodeling. Moreover, myosin light chain phosphorylation, a determinant of SMC tone, was higher in PASMCs isolated from SM22α-HIF-1α−/− mice compared with wild-type PASMCs, during both normoxia and after acute hypoxia. Further, overexpression of HIF-1α decreased myosin light chain phosphorylation in HIF-1α−/−null SMCs.

Conclusions: In both normoxia and hypoxia, PASMC HIF-1α maintains low pulmonary vascular tone by decreasing myosin light chain phosphorylation. Compromised PASMC HIF-1α expression may contribute to the heightened vasoconstriction that characterizes pulmonary hypertension. (Circ Res. 2013;112:1230-1233.)

Key Words: hypoxia ■ hypoxia-inducible factor-1α ■ oxygen sensing ■ pulmonary hypertension ■ vascular tone

Organismal survival requires cells to sense and respond to changes in oxygen tension. Hypoxia-inducible factor-1α (HIF-1α), an oxygen (O₂)-sensitive transcription factor, facilitates cellular responses to hypoxia by regulating genes involved in angiogenesis, oxygen transport, and energy metabolism. The pulmonary vasculature responds to hypoxia with vasoconstriction and remodeling; however, the role of HIF-1α in the pulmonary circulation remains unclear.

Sustained hypoxic exposure results in pulmonary hypertension, a disease characterized by pulmonary artery (PA) vasoconstriction and neomuscularization. In a murine model, haploinsufficiency of HIF-1α attenuates hypoxia-induced increases in PA pressure, right ventricular hypertrophy, and pulmonary vascular remodeling, suggesting that HIF-1α might play a role in increasing pulmonary vascular tone. However, recent data in PA smooth muscle cells (PASMCs) indicate that HIF-1α regulates the expression of an ion channel subunit that mitigates vasoconstriction. Insight into the role that HIF-1α plays in modulating pulmonary vascular tone and in the pathogenesis of pulmonary hypertension is limited, in part, by neonatal lethality in mice lacking HIF-1α. Thus, to test the hypothesis that PASMC HIF-1α modulates both basal PA tone and the response to hypoxia, we created mice with an SMC-specific deletion of HIF-1α.

Methods

Transgenic mice with selective deletion of HIF-1α in SMCs were created by crossbreeding SM22α-promoter–driven Cre mice expressing the Cre reporter ROSA26 (R26R; kindly provided by Marlene Rabinovitch, MD, Stanford University) with HIF-1αtm3Rsjo/J; Jackson Laboratories). HIF-1α homozygous floxed mice contain the HIF-1α exon 2 flanked by LoxP sites. The Institutional Animal Care and Use Committee at Stanford University approved all the procedures and protocols governing the care and use of laboratory animals. Right ventricular...
systolic pressure (RVSP) was measured after exposure to either acute or chronic hypoxia. Histological analysis was performed on lung tissue. Western blotting was performed to determine myosin light chain (MLC) phosphorylation (pMLC) in PASMCs. Full details can be found in the online Data Supplement.

Results

SM22α-HIF-1α−/− Mice

The smooth muscle–specific protein SM22 is ubiquitously expressed in SMCs. SM22 promoter activity was found in the arterial SMCs, including the aorta and PA.3 LacZ reporter staining demonstrated the absence of β-galactosidase activity in tissues derived from wild-type (WT) SM22α-HIF-1α−/− mice (Figure 1A and 1C). In contrast, prominent X-gal staining was found in SMCs of the PA and aorta from SM22α-HIF-1α−/− mice (Figure 1B and 1D, respectively). Both HIF-1α mRNA and protein were undetectable in aortic SMCs and PASMCs isolated from SM22α-HIF-1α−/− mice, confirming deletion of HIF-1α in vascular SMCs (Figure 1E and 1F, respectively). In addition, PASMC HIF-2α protein expression was not different between WT and HIF-1α−/− mice (Online Figure I). Although the myocardiump of SM22α-HIF-1α−/− mice demonstrated patchy X-gal staining, HIF-1α protein expression in the heart did not differ between WT and HIF-1α−/− mice under either normoxic or hypoxic (21 days) conditions (data not shown). In addition, left ventricular function, as measured by echocardiography, did not differ between the 2 groups (Online Table I).

SMC-Specific Loss of HIF-1α Increases Pulmonary Vascular Tone

At baseline, RVSP was higher in SM22α-HIF-1α−/− mice compared with WT (Figure 2A), despite only normoxic exposure. After chronic hypoxia, RVSP increased in both groups but was significantly higher in SM22α-HIF-1α−/− mice compared with controls. Heart rate, cardiac output, left ventricular function, hematocrit, and body weight did not differ in the 2 genotypes, either at baseline or after chronic hypoxia (Online Table I). Moreover, the increased RVSP in SM22α-HIF-1α−/− mice was observed in the absence of differences in the number of muscularized arterioles between the 2 groups (Figure 2B), suggesting that the relatively higher RVSP in SM22α-HIF-1α−/− mice is not attributable to differential vascular remodeling.

To address the possibility that hypoxic pulmonary vasoconstriction differs between SM22α-HIF-1α−/− and WT mice, RVSP was measured during 15 minutes of acute hypoxia (10% O2) and then during exposure to 40% O2 (Figure 2C). Acute hypoxia increased RVSP in both groups, but RVSP remained higher in SM22α-HIF-1α−/− mice compared with WT mice. With exposure to 40% oxygen, RVSP decreased in both groups but remained higher in the SM22α-HIF-1α−/− mice.

Loss of HIF-1α in PASMC Increases MLC Phosphorylation

pMLC augments the contractile state of vascular SMCs by facilitating myosin and actin filament interaction.7 To investigate the molecular mechanism leading to increased pulmonary vascular tone in SM22α-HIF-1α−/− mice, we measured pMLC in PASMCs isolated from the 2 groups of mice. pMLC was >2-fold higher in PASMCs isolated from SM22α-HIF-1α−/− mice compared with WT mice under baseline normoxia (Figure 3A). Although acute hypoxia increased pMLC in both groups, pMLC remained significantly higher in HIF-1α−/− PASMCs compared with WT PASMCs.

To ensure that HIF-1α modulation of pMLC in human and murine PASMCs, human PASMCs were transfected with HIF-1α–targeted small interfering RNA (siHIF-1α). Depletion of endogenous HIF-1α increased pMLC expression (Figure 3B). Under hypoxic conditions, pMLC expression increased in both groups. However, pMLC expression increased significantly more in HIF-1α–depleted human PASMCs compared with cells transfected with nontargeting control small interfering RNA.

To investigate whether overexpression of HIF-1α would rescue the enhanced pMLC observed in the mouse PASMCs null for HIF-1α, HIF-1α−/− PASMCs were transfected with empty vector, pcDNA3, or a constitutively active form of

Nonstandard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>hypoxia-inducible factor-1α</td>
</tr>
<tr>
<td>MLC</td>
<td>myosin light chain</td>
</tr>
<tr>
<td>PA</td>
<td>pulmonary artery</td>
</tr>
<tr>
<td>PASMC</td>
<td>pulmonary artery smooth muscle cell</td>
</tr>
<tr>
<td>pMLC</td>
<td>phosphorylated myosin light chain</td>
</tr>
<tr>
<td>RVSP</td>
<td>right ventricular systolic pressure</td>
</tr>
<tr>
<td>SMC</td>
<td>smooth muscle cell</td>
</tr>
<tr>
<td>WT</td>
<td>wild type</td>
</tr>
</tbody>
</table>
HIF-1α (Figure 3C). Transfection with the constitutively active construct effectively restored HIF-1α expression in the null cells. Overexpression of HIF-1α decreased pMLC under both normoxic and hypoxic conditions.

To address the phosphorylation of MLC in vivo, lung tissues from SM22α-HIF-1α−/− and WT mice exposed to normoxia or chronic hypoxia were examined by Western immunoblot (Figure 4). Overall, pMLC was increased in SM22α-HIF-1α−/− mice compared with WT. After chronic hypoxia, pMLC expression increased in both groups but was significantly higher in SM22α-HIF-1α−/− mice compared with WT. Taken together, these results demonstrate that in PASMCs, HIF-1α decreases pMLC, providing evidence that HIF-1α modulates pulmonary vascular tone through effects on pMLC.

**Discussion**

This is the first report to demonstrate that selective deletion of HIF-1α in SMCs increases pulmonary vascular tone under both normoxic and hypoxic conditions and increases PASMC pMLC. These novel results suggest that SMC HIF-1α plays a previously undescribed role in maintaining the normally low pressure of the pulmonary vasculature at baseline and in mitigating hypoxic pulmonary hypertension. Taken together, the observations that in SM22α-HIF-1α−/− mice pMLC is increased and RVSP is elevated in the absence of accentuated muscularization indicate a primary role for SMC HIF-1α in modulating vascular tone specifically, not vascular remodeling.

HIF-1α mediates, in part, SMC cell proliferation in response to hypoxia.10 In a previous study, compared with controls, mice haploinsufficient for HIF-1α had decreased RVSP, fewer muscularized vessels, and reduced PA wall thickness after chronic hypoxia, suggesting a blunted vasoproliferative response. In contrast, in this report, complete loss of HIF-1α in SMCs raised RVSP in the absence of an increase in the number of muscularized arteries. Thus, a global decrease in HIF-1α may mitigate against the development of pulmonary hypertension, although an absence of HIF-1α in SMCs alone potentiates the response to hypoxia. HIF-1α may play distinct cell-specific roles in the pulmonary vasculature. Because HIF-1α was deleted only in SMCs in this report, HIF-1α activation in other cell types such as endothelial cells may increase arterial muscularization under hypoxia via HIF-1α-mediated induction of growth.
Thus, cell-specific alterations of HIF-1α expression in the pulmonary vasculature, including decreased expression in SMCs, may play a pathogenic role in pulmonary hypertension. Consequently, cell-specific modulation of HIF-1α might represent a viable therapeutic strategy to address pulmonary hypertension, a disease without either cure or definitive treatment. Loss of HIF-1α in PASMCs increased pulmonary vascular tone and pMLC under both normoxic and hypoxic conditions, although the physiological significance of the mild RVSP elevation under normoxic conditions is uncertain. Overexpression of HIF-1α decreased pMLC, providing further evidence for the importance of HIF-1α activity in regulating pMLC and vascular tone. Although it seems that HIF-1α plays a role in maintaining the normally low pulmonary vascular tone by decreasing pMLC in SMCs, the mechanistic link between HIF-1α and pMLC remains unknown. HIF-1α may directly or indirectly induce factors that reduce pMLC (eg, MLC phosphatase) or repress the activity of proteins that increase pMLC (eg, Rho kinase and MLC kinase).

We conclude that SMC-specific HIF-1α plays a previously undescribed role in maintaining low tone in the normoxic pulmonary circulation and in regulating the response to hypoxia by decreasing pMLC. We speculate that compromised PASMC HIF-1α expression or activity contributes to the vasoconstriction that characterizes pulmonary hypertension and thus represents a novel therapeutic target.

Acknowledgments

We thank M. Rabinovich for the SM22α-promoter–driven Cre/ROSA26 reporter mice and A.J. Giaccia for the HIF-1α (constitutively active) construct.

Sources of Funding

This work was supported by funding from the National Institutes of Health HL060784 and HL0706280 (D.N. Cornfield).

Disclosures

None.

References


Novelty and Significance

What Is Known?

- Hypoxia-inducible factor-1α (HIF-1α) is an oxygen (O2)-sensitive transcription factor that facilitates the cellular adaptation to hypoxia.
- Sustained hypoxia exposure results in pulmonary hypertension, a disease characterized by pulmonary artery vasoconstriction and neomuscularization.
- In murine models, haploinsufficiency of HIF-1α protects against hypoxia-induced pulmonary hypertension, but the specific function of HIF-1α in pulmonary artery smooth muscle cells (SMCs) is not known.

What New Information Does This Article Contribute?

- Tissue-specific deletion of HIF-1α in pulmonary artery SMCs increases pulmonary vascular tone, both at baseline and in response to hypoxia.
- HIF-1α decreases the phosphorylation of myosin light chain, a key determinant of SMC tone.

Pulmonary hypertension causes substantial morbidity and has a high mortality rate. Although haploinsufficiency of HIF-1α protects against hypoxia-induced pulmonary hypertension, cell-specific functions of HIF-1α are unknown. This study demonstrates that SMC-specific loss of HIF-1α increases pulmonary vascular tone without increased pulmonary artery muscularization. Moreover, we report that SMC-specific loss of HIF-1α increases pulmonary vascular tone by augmenting myosin light chain phosphorylation. Thus, SMC-specific HIF-1α plays a previously undescribed role in maintaining the low tone of the normoxic pulmonary circulation and in mitigating hypoxia-induced increases in tone. Compromised pulmonary artery SMC HIF-1α activity may contribute to the vasoconstriction that characterizes pulmonary hypertension.
Hypoxia-Inducible Factor-1α in Pulmonary Artery Smooth Muscle Cells Lowers Vascular Tone by Decreasing Myosin Light Chain Phosphorylation
Yu-Mee Kim, Elizabeth A. Barnes, Cristina M. Alvira, Lihua Ying, Sushma Reddy and David N. Cornfield

Circ Res. 2013;112:1230-1233; originally published online March 19, 2013;
doi: 10.1161/CIRCRESAHA.112.300646
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/112/9/1230

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2013/03/19/CIRCRESAHA.112.300646.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/
SUPPLEMENTAL MATERIAL

Methods

Genotyping
To identify the Cre gene, primers (forward: 5'-CCGGTTATTCAACTTGCAACC-3'; reverse: 5'-CTGCATTACCGGTGATGCAACC-3') were used to generate a 149bp PCR product.\(^1\) To identify the Cre reporter gene, ROSA26, primers (Jackson Laboratories protocol: Gt(ROSA)26Sortm1sor, forward: 5'AAAGTCGCTCTGAGTTTATAT; mutant reverse: 5'-GCGAAGAGTTTGTCCTCAACC-3'; WT reverse: 5'GGAGCGGGAGAAATGGATATG-3') were used to amplify a 340bp fragment from mutant (ROSA26-R) and a 550bp fragment from WT mice. To identify the floxed HIF-1\(\alpha\) gene, primers (Jackson Laboratory protocol: Hif1\(\alpha\)tm3Rsjo, forward 5'TGCTCATCAGTTGCCACTT; reverse 5'GTTGGGGCAGTACTGGAAAG-3') were used to amplify two DNA fragments at 700bp and 650bp, representing mutant (HIF-1\(\alpha\)flox/flox) and WT mice, respectively.

X-Gal Staining of the Aorta and Pulmonary Artery
To identify SM22\(\alpha\)-promoter-driven Cre recombinase activity, aorta and pulmonary artery (PA) tissues were fixed with 2% paraformaldehyde (PFA) and 0.2% glutaraldehyde (GTA) for 1h at 4°C and stored in OCT at -80°C. Frozen tissues were sectioned, and then stained with 1mg/ml X-Gal overnight at 37°C using the \(\beta\)-Galactosidase Reporter Gene Staining Kit (Sigma-Aldrich).

Hemodynamic Assessments
Adult SM22\(\alpha\)-HIF-1\(\alpha\)/- and WT littermate mice matched for age and gender were used in each group. To measure vasoreactivity, mice were anesthetized with 1.5-2.0% isofluorane and right ventricular systolic pressure (RVSP) measurements were obtained using a 1.4F Millar catheter (Millar Instruments) at baseline (40% O\(_2\), 10min.), during acute hypoxia (10% O\(_2\), 15 min.), and with return back to baseline (40% O\(_2\), 10 min.), or chronic hypoxia (10% O\(_2\), 3 wks) as previously described.\(^2\) Left ventricular (LV) fractional shortening, ejection fraction, cardiac output, and heart rate were evaluated by echocardiography using the GE Vivid 7 ultrasound machine with a 13MHz probe (GE Healthcare).

Arterial Muscularization
Left lungs were prepared for morphometric analysis by barium gelatin injection via the PA to label peripheral pulmonary arteries as previously described.\(^3\) The lung was inflated and fixed with 10% formalin at 20cm H\(_2\)O pressure. Each lung section, containing the mid-portion of the barium-injected lung parallel to the hilum, was embedded in paraffin. Movat's pentachrome staining of formalin-fixed tissues was used to evaluate PA muscularization. Arterial muscularization was assessed in 15 random fields per mouse (x400 magnification) by calculating the proportion of muscularized peripheral (alveolar duct and wall) pulmonary arteries to total peripheral pulmonary arteries.

Primary Cell Culture
Primary aorta (AoSMC) and pulmonary artery smooth muscle cells (PASMC) were isolated from SM22\(\alpha\)-HIF-1\(\alpha\)/- and littermate control mice using a modified elastase/collagenase digestion protocol.\(^4\) Aorta and PA tissues were digested in dispersion medium containing 40\(\mu\)mol/L CaCl\(_2\), 0.5mg/mL elastase (Worthington Biochemical), 0.5mg/mL collagenase (Worthington Biochemical), 0.2mg/mL soybean trypsin inhibitor (Worthington Biochemical), and 2mg/mL albumin (Sigma-Aldrich) for 20min. at 37°C. After filtration with
100µm cell strainers, cells were incubated with Dynabeads® (Invitrogen) coated with anti-CD31 and anti-CD102 antibodies (BD Biosciences) for 15min., in order to deplete endothelial cells expressing CD31 and CD102. Remaining SMC were collected through centrifugation at 225g for 6min. and cultured in DMEM containing 10% FBS with antibiotics (Invitrogen/Gibco).

**Real-Time Quantitative RT-PCR**

Total RNA was isolated from cultured cells using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol. Total RNA was reverse-transcribed using Superscript II Reverse Transcriptase (Invitrogen) per the manufacturer’s instructions. HIF-1α mRNA was quantified using preverified TaqMan® Assays-on-Demand Gene Expression Products (Invitrogen) targeting HIF-1α exons 2 and 3 and normalized to 18S ribosomal RNA using Applied Biosystems Comparative C_T Method.

**siRNA Transfection**

Human pulmonary artery smooth muscle cells (hPASMC) derived from a 51 yr-old were purchased from Lonza and grown according to the manufacturer’s protocol. At 50-70% confluency, cells were transfected using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer’s protocol. In brief, siRNA specific for human HIF-1α, siHIF-1α (Thermo Scientific Dharmacon), or scrambled siRNA, siNC (Thermo Scientific Dharmacon), at a final concentration of 50nM was transfected per 60mm plate. 24h post-transfection, cells were refed with fresh media. After an additional 24h, cells were incubated in either normoxic (20% O_2) or hypoxic (1% O_2) conditions for 1h. Cells were lysed in mild extraction buffer (20mM Tris-HCl pH 7.5, 1mM Na_3VO_4, 1mM PMSF, 10µg/mL aprotinin, 10µg/mL leupeptin) to obtain cytosolic fractions. Pelleted nuclei were lysed in nuclear buffer (250mM sucrose, 150mM NaCl, 20mM Tris-HCl pH 7.5, 10mM KCl, 1mM EDTA, 0.1% SDS, 1mM DTT, 1mM Na_3VO_4, 1mM PMSF, 10µg/mL aprotinin, 10µg/mL leupeptin) to obtain nuclear fractions.

**HIF-1α-Add-Back**

The HIF-1α expression plasmid, HIF-1α (CA), containing the double mutant P402A/P564A was a kind gift from Dr. A.J. Giaccia, Stanford University. Empty vector, pcDNA3, served as a transfection control. Mouse pulmonary artery smooth muscle cells (mPASMC) lacking HIF-1α expression (HIF-1α−) were transfected by Lipofectamine LTX/PLUS method (Invitrogen) per the manufacturer’s instructions. In brief, cells at 50-80% confluency were transfected with 5µg of DNA per 60mm plate. 24h post-transfection, cells were refed with fresh media. After an additional 24h, cells were incubated in either normoxic or hypoxic (1% O_2) conditions for 1h.

**Western Immunoblotting**

mPASMC were incubated in normoxic or hypoxic conditions (1% O_2, 1h) and then lysed with RIPA extraction buffer containing protease and phosphatase inhibitors (Thermo Scientific). Protein content was determined by the Lowry method (Bio-Rad). HIF-1α-add-back mPASMC (HIF-1α−) were lysed with 0.5% NP-40 Buffer (50mM Tris-HCl pH 7.5, 150mM NaCl, 2.5mM EDTA, 0.5% NP-40, 1mM Na_3VO_4, 1mM PMSF, 10µg/mL aprotinin, 10µg/mL leupeptin). Lung tissue samples from SM22α-HIF-1α−/− and WT mice exposed to normoxia or chronic hypoxia were homogenized with RIPA buffer (150mM NaCl, 50mM Tris-HCl pH 7.5, 0.1% SDS, 1% Triton X-100, 0.25% sodium deoxycholate, 1mM EDTA, 1mM Na_3VO_4, 1mM PMSF, 10µg/mL aprotinin, 10µg/mL leupeptin). Protein content was quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific). 10µg of protein/sample were subjected to SDS-PAGE analysis. Immobilon-P (Millipore) membranes were incubated with primary antibodies to detect HIF-1α.
(Cayman Chemical), HIF-2α (Cayman Chemical), MLC (Sigma), pMLC (Abcam), TATA binding protein (Abcam), α-tubulin (Sigma), or β-actin (Sigma), re-incubated with horseradish peroxidase-conjugated secondary antibodies, followed by detection with ECL reagents (GE Healthcare).

**Statistical Analysis**

Results are expressed as means ± SEM. Statistical significance was assessed with Student’s t-test (paired). A p value of < 0.05 was taken as the threshold level for statistical significance. All experiments were repeated a minimum of three times.

**Online Figure I. Loss of HIF-1α in mPASMC does not affect HIF-2α expression.** Expression of HIF-1α and HIF-2α in PASMC isolated from SM22α-HIF-1α−/− and WT mice by Western immunoblot under normoxia and after exposure to acute hypoxia.

**Online Table I. Hemodynamic Assessments of SM22α-HIF-1α−/− and WT Mice**

<table>
<thead>
<tr>
<th></th>
<th>Normoxia</th>
<th></th>
<th>Chronic Hypoxia</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>SM22α-HIF1α−/−</td>
<td>WT</td>
<td>SM22α-HIF1α−/−</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.9 ± 1.1 (11)</td>
<td>44.0 ± 0.83 (14)</td>
<td>54.8 ± 1.4 (14)</td>
<td>56.7 ± 1.3 (14)</td>
</tr>
<tr>
<td>Cardiac Output (mL/min)</td>
<td>19.7 ± 1.9 (9)</td>
<td>20.4 ± 1.7 (9)</td>
<td>16.5 ± 1.5 (10)</td>
<td>16.4 ± 1.0 (10)</td>
</tr>
<tr>
<td>LV Fractional Shortening (%)</td>
<td>35.6 ± 1.4 (10)</td>
<td>36.4 ± 1.4 (12)</td>
<td>37.9 ± 2.0 (10)</td>
<td>36.7 ± 1.5 (10)</td>
</tr>
<tr>
<td>LV Ejection Fraction (%)</td>
<td>71.7 ± 1.8 (10)</td>
<td>72.6 ± 1.7 (12)</td>
<td>74.1 ± 2.1 (10)</td>
<td>72.9 ± 1.7 (10)</td>
</tr>
<tr>
<td>Heart Rate (bpm)</td>
<td>426.4 ± 14.6 (15)</td>
<td>460.5 ± 11.0 (17)</td>
<td>466.6 ± 22.5 (10)</td>
<td>467.3 ± 11.2 (10)</td>
</tr>
<tr>
<td>Body Weight (g)</td>
<td>24.84 ± 0.65 (5)</td>
<td>24.56 ± 0.48 (5)</td>
<td>20.42 ± 0.18 (5)</td>
<td>19.80 ± 0.64 (4)</td>
</tr>
</tbody>
</table>

Values of parameters are expressed as means ± SEM. ( ): number of mice per group.
References


