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# Hypoxia training attenuates left ventricular remodeling in rabbit with myocardial infarction

Chun-Xiao WAN[1,2], Yun-Feng LAN[3], Hui JIANG[1], Jie HUANG [1], Rui-Sheng LI [4], Sheng BI[1], Jian-An LI[5]

[1]*Rehabilitation Medicine Center, Chinese PLA General Hospital, Beijing 100853, China*

[2]*Rehabilitation Medicine Department, Wuxi No.2 Hospital Affiliated with Nanjing Medical University, Nanjing 214002, Jiangsu Province, China*

[3]*Department of Healthcare, Hainan Branch of Chinese PLA General Hospital, Sanya 572000, Hainan Province, China*

[4]*Research and Technology Service Center, 302 Hospital of PLA, Beijing 100039, China*

[5]*Rehabilitation Medicine Center, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China*

**Abstract**

**Objective** Previous studies showed that hypoxia preconditioning could protect cardiac function against subsequent myocardial infarction injury. However, the effect of hypoxia on left ventricular after myocardial infarction is still unclear. This study therefore aims to investigate the effects of hypoxia training on left ventricular remodeling in rabbits post myocardial infarction. **Methods** Adult male rabbits were randomly divided into three groups: group SO (sham operated), group MI (myocardial infarction only) and group MI-HT (myocardial infarction plus hypoxia training). Myocardial infarction was induced by left ventricular branch ligation. Hypoxia training was performed in a hypobaric chamber (having equivalent condition at an altitude of 4000 m, $F_iO_2$14.9%) for 1 h/day, 5 days/week for four weeks. At the endpoints, vascular endothelial growth factor (VEGF) in the plasma was measured. Infarct size and capillary density were detected by histology. Left ventricular remodeling and function were assessed by echocardiography. **Results** After the 4-week experiment, compared with the group SO, plasma VEGF levels in groups MI (130.27 ± 18.58 pg/mL, $P < 0.01$) and MI-HT (181.93 ± 20.29 pg/mL, $P < 0.01$) were significantly increased. Infarct size in Group MI-HT (29.67% ± 7.73%) was deceased remarkably, while its capillary density (816.0 ± 122.2/mm$^2$) was significantly increased. For both groups MI and MI-HT, left ventricular end-diastolic and end-systolic dimensions were increased whereas left ventricular ejection fraction was decreased. However, compared with group MI, group MI-HT diminished left ventricular end-diastolic (15.86 ± 1.09 mm, $P < 0.05$) and end-systolic dimensions (12.10 ± 1.20 mm, $P < 0.01$) significantly and improved left ventricular ejection fraction (54.39 ± 12.74 mm, $P < 0.05$). **Conclusion** Hypoxia training may improve left ventricular function and reduce remodeling via angiogenesis in rabbits with MI.

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**Keywords:** Hypobaric hypoxia; Myocardial infarction; Left ventricular remodelling; Infarct size; Vascular endothelial growth factor

## 1 Introduction

Acute myocardial infarction (MI) is one of the leading causes of death in the world. Survival has improved greatly due to the significant improvement in the prevention, diagnosis and treatment in the initial phases of acute MI.[1] However, cardiac mortality still remains high in the recent years.[2,3] It was reported that cardiac remodeling post MI is an adaptive response to MI. It will result in the left ventricular (LV) dilatation and loss of global contractile function.[4] Clinical trials and animal experiments had illustrated that LV remodeling played an important role in the development of heart failure and the increase of mortality, mainly resulted in sudden cardiac death.[5,6] How to develop an appropriate strategy in the early stage of post-MI to limit the adverse remodeling has been proposed in many researches.[4,7]

Hypoxia exposure has been recognized as an efficient protective intervention to increase cardiac ischemic tolerance. It was reported as early as in the late 1950s that the incidence of MI was lower in people who lived at high altitude.[8] An epidemiological survey from New

**Correspondence to:** BI Sheng, MD, Rehabilitation Medicine Center, Chinese PLA General Hospital, Beijing 100853, China. E-mail: bisheng301@gmail.com

**Telephone:** +86-10-55499057 **Fax:** +86-10-55499057

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Mexico revealed that even living at moderate elevations (2100 m) could lead to protection against death from ischemic heart disease.[9] Recently, hypoxia was performed as a preconditioning approach to provide cardioprotection in the subsequent ischemia/reperfusion (I/R) injury. It was demonstrated that rats exposed to hypobaric hypoxia (7000 m, 8 h/day, and 35 exposures) improved cardiac tolerance against I/R injury.[10,11] However, the optimal altitude and duration of hypoxia exposure and their effects on patients after MI are still unclear. This study therefore aims to examine the effects of moderate hypoxia training (4000 m, 1 h/day) for four weeks on heart function in rabbits after MI.

## 2 Methods

Twenty-four adult male New Zealand white rabbits (2.0−2.5 kg) were randomly divided in three groups: group SO (sham-operated), group MI (MI only) and group MI-HT (MI plus hypoxia training). MI was induced by left ventricular branch (LVB) ligation. After recovery for 7 days, rabbits in group MI-HT were put in a hypobaric hypoxia chamber at a target altitude of 4000 m for one hour per day, five days a week, for four weeks. At the same time, rabbits in groups SO and MI were kept in an ordinary room which has usual air condition.

Animals were free to access water and a standard laboratory diet and maintained in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). All procedures were approved by the ethics committee of Chinese PLA general hospital.

### 2.1 Model of MI

After induction of anesthesia with 3% (v/v) sodium pentobarbital (1 mL/kg, *iv*), a left lateral thoracotomy was performed through the fourth intercostal space. The pericardium was opened and the proximal left ventricular branch was ligated by 6-0 silk suture, approximately 2 cm inferior to the left anterior coronary artery. The infarction was confirmed by the ST segment elevation over 0.2 mV on electrocardiography (ECG) leads Ⅱ and V1 (as shown in Figure 1) and colour changes. Sham-operated rabbits underwent the same cardiac exposure but without ligation of the coronary artery.

The rabbits were kept in cage for recovery for seven days after surgery. Antibiotics were consecutively administered for five days ($4 \times 10^5$ U penicillin/day, *im*).

### 2.2 Hypoxia training

Rabbits in group MI-HT were subjected to hypoxia training in a hypobaric chamber at the target stimulating altitude of 4000 m, for 1 h/day, 5 days a week for four weeks. Barometric pressure in the chamber was lowered at 3 m/s from the normal barometric pressure. It reached and maintained at 64.64 kPa (equivalent to 4000 m, $F_iO_2$ 14.9%) for one hour. Then the pressure in the chamber increased back to normal barometric pressure at the same velocity. The rabbits were kept in cages in ordinary indoor air environment after hypoxia exposure. The hypoxia chamber was maintained at a temperature of 22 ± 0.5°C with a relative humidity of 60% ± 5%. The other two groups of animals were always kept in ordinary indoor air environment for the same period of time.

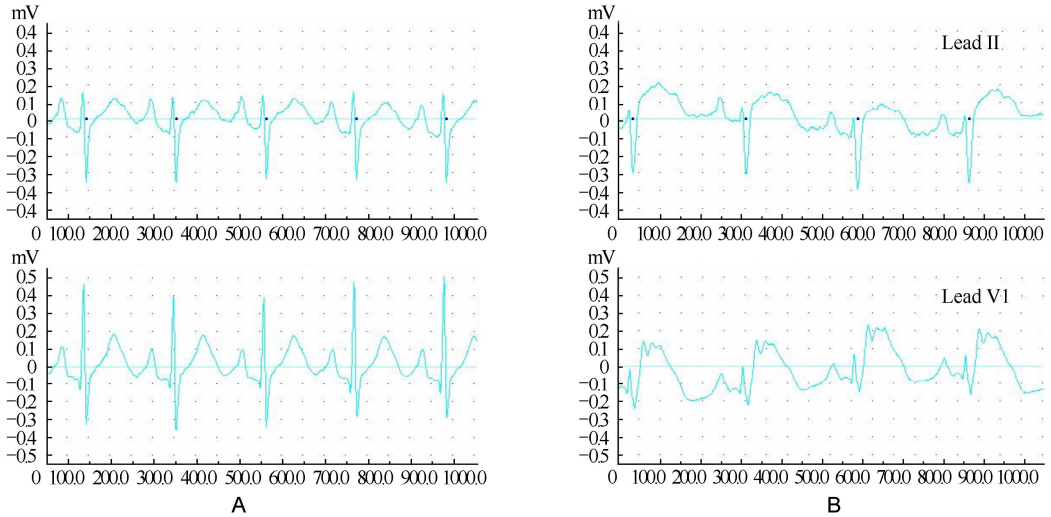


**Figure 1. Electrocardiography analysis of MI.** (A): Before left ventricular branch was ligated, ST segment was normal; (B): Five minutes after left ventricular branch was ligated, ST segment in ECG leads II and V1 elevated over 0.2 mV indicated successful inducing of MI. MI: myocardial infarction.

### 2.3 Detection of VEGF levels

At the end of experiment, 2 mL blood samples were drawn from the central artery of the rabbit's ear and centrifuged at 3000 r/min at 4°C for 15 min. The plasma was frozen immediately in liquid nitrogen and was stored at −80°C. The concentration of vascular endothelial growth factor (VEGF) was determined using VEGF enzyme-linked immunosorbent assay kit (BPB Biomedicals, Franklin, USA) following the manufacturer's protocol.

### 2.4 Examination of echocardiography

At the end of the 4-week training, all rabbits were performed echocardiography tests respectively. Rabbits were anesthetized and placed in the supine position. Transthoracic echocardiography was performed with 10-MHz ultrasound sector probe using a GE Vingmed Vivid 7 system (GE Vingmed Ultrasound AS, Horten, Norway). The left ventricular end-systolic diameter (LVEDS) and left ventricular end-diastolic diameter (LVEDD) were measured from M-mode tracings according to the American Society for Echocardiology leading-edge method.[12] The left ventricular ejecting fraction (EF) and the fractional shortening (FS) were calculated using the following formulas: EF (%) = $[(LVEDD^3–LVEDS^3)/LVEDD^3] \times 100$, FS (%) = $[(LVEDD–LVEDS)/LVEDD] \times 100$. Data from three consecutive cardiac cycles were averaged. All measurements were performed by an animal cardiologist blind to the study.

### 2.5 Measurement of infarct size

Rabbits were sacrificed by intravenously injection of potassium chloride after four weeks' intervention. Hearts were harvested and immediately washed with repeated flushing of the coronary artery with normal saline. Three cross sections (1 cm in thickness) of the heart were obtained at basal, middle, and apical levels for measuring myocardial infarct size. Each cross section of heart tissue was then stained with 2% triphenyl tetrazolium chloride (TTC) for infarct area analysis. Briefly, all heart sections were placed on a tray with a scaled vertical bar to which a digital camera was attached. The infarct size was expressed as a percentage of total LV area. The sections were photographed from directly above at a fixed height. The images obtained were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Silver Spring, USA).

### 2.6 Measurement of capillary density

Hearts were embedded in optimal cutting temperature compound (OCT; Miles, Elkhart, USA), then cut transversely into slices approximately 5-μm as described previously.[13] Capillary densities in the peri-infarcted areas were evaluated by immunohistochemical staining to detect CD31 (1: 100, Santa Cruz Biotechnology, Santa Cruz, USA) expression by endothelial cells. The capillary was defined as a round structure with a diameter of less than 10-μm. Five fields from each sample were randomly selected for counting by three independent blinded pathologists. The number of positive cells in each high-power field was converted to cells per square mm.

### 2.7 Statistics

All data were analyzed using SPSS 14.0 software (SPSS, Chicago, USA) and were expressed as mean ± SD. ANOVAs were performed with the Student-Newman-Keuls test to detect significance in multiple groups. Survival was analyzed by the method of Kaplan and Meier. Differences were considered significant at the level of $P < 0.05$.

## 3 Results

A total of 24 adult male rabbits were recruited in the experiment. Six animals were excluded from analysis for two reasons: (1) perioperative death, within the first seven days after surgery (four rabbits); (2) the elevation of ST segment in ECG leads Ⅱ and V1 < 0.1 mV after left ventricular branch occluded (two rabbits). The rest of 18 rabbits were included in the study with their mortality was followed up for four weeks after surgery. During day 1 to day 28 in the training period, two rabbits of group MI died due to cardiac death. None of animals in the sham-operated and hypoxia training died during the 4-week period of the experiment.

The experiment showed that 28-day survival in group MI-HT (100%) was much higher than the one in group MI (66.67%, $P < 0.05$), but was the same as that in group SO (100%).

### 3.1 VEGF levels

At the end of experiments, VEGF levels were increased significantly in groups MI (130.27 ± 18.58 pg/mL, $P < 0.01$) and MI-HT (181.93 ± 20.29 pg/mL, $P < 0.001$), compared to those in group SO (86.00 ± 12.43 pg/mL). VEGF levels in group MI-HT showed higher values than those in group MI as shown in Table1($P < 0.01$).

### 3.2 Infarct size

Heart sections were obtained after the experiment and infarct size in three groups were detected by TTC staining. After four weeks' hypoxia training, the animals in group MI-HT demonstrated a significantly lower infarct size (29.67% ± 7.73%) compared to the infarct size in MI animals without hypoxia training (41.25% ± 7.59%, $P < 0.01$), (Figure 2A).

### 3.3 Capillary density

The capillary densities in the peri-infarcted areas were increased significantly in groups MI (533 ± 101/mm$^2$, $P < 0.01$) and MI-HT (816 ± 122/mm$^2$, $P < 0.01$) when compared with the group SO (Figure 3). It was found such effect was much more prominent in group MI-HT than in group MI ($P < 0.01$).

### 3.4 Left ventricular remodeling

Echocardiographic data are presented in Table 1 and Figure 2B. After 4-week intervention, groups MI and MI-HT experienced LV dilatation with significant increase of LVEDD and LVESD and decrease of EF. However, hypoxia training reversed the dilatation of LV by reducing the LVESD (12.10 ± 1.20 mm) and LVEDD (15.86 ± 1.09 mm), respectively. EF was significantly higher in the hypoxia training group (54.39% ± 12.74%) than in group MI (38.13% ± 5.59%, $P < 0.05$).

## 4 Discussion

In the present study, we confirmed that adaptation

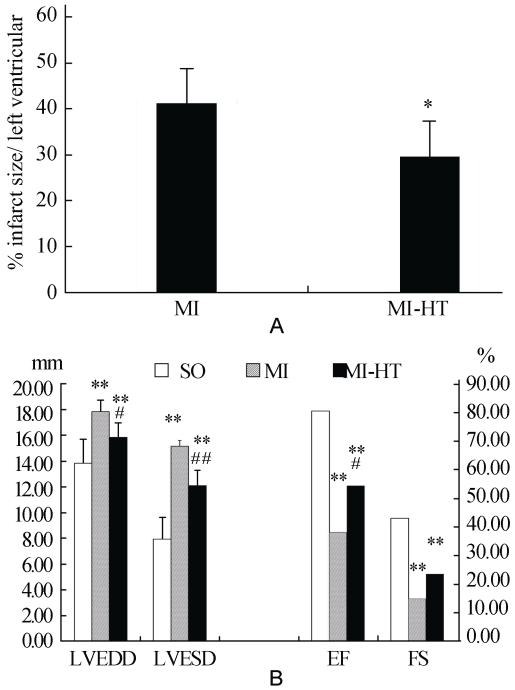


**Figure 2. Infarct size and LV remodeling in the experiment.** (A): Infarct size of heart, expressed as percentage of infarct area in the whole heart post 4-week experiments; (B): LVEDD, LVESD, EF and FS parameters were detected by echocardiography in groups SO, MI and MI-HT at the end of experiments. Compared with group SO, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with group MI, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. EF: ejection fraction, FS: fractional shortening; LV: left ventricular; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; MI: myocardial infarction; MI-HT: myocardial infarction plus hypoxia training; SO: sham operated.

**Table 1. After four weeks' invention, VEGF levels and LV geometry parameters.**

| Group | *n* | VEGF (pg/mL) | LVEDD (mm) | LVESD (mm) | EF% | FS% |
|---|---|---|---|---|---|---|
| SO | 6 | 86.00 ± 12.43 | 13.84 ± 1.87 | 7.94 ± 1.70 | 80.58 ± 7.38 | 42.99 ± 7.70 |
| MI | 4 | 130.27 ± 18.58$^{**}$ | 17.83 ± 0.89$^{**}$ | 15.17 ± 0.45$^{**}$ | 38.13 ± 5.59$^{**}$ | 14.85 ± 2.59$^{**}$ |
| MI-HT | 6 | 181.93 ± 20.29$^{**\#}$ | 15.86 ± 1.09$^{**\#}$ | 12.10 ± 1.20$^{**\#\#}$ | 54.39 ± 12.74$^{**\#}$ | 23.59 ± 7.21$^{**}$ |

Results are expressed as mean ± SD. Compared with group SO, $^{*}P < 0.05$, $^{**}P < 0.01$; compared with group MI, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$. EF: ejection fraction, FS: fractional shortening; LV: left ventricular; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; MI: myocardial infarction; MI-HT: myocardial infarction plus hypoxia training; SO: sham operated.

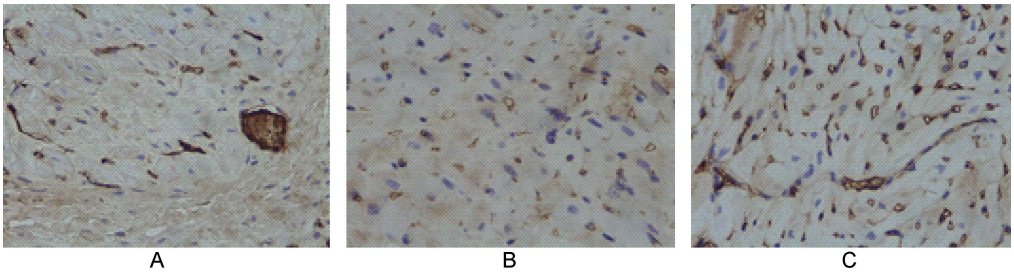


**Figure 3. Images of capillary density in the peri-infarction area.** (A): In group SO; (B): In group MI; (C): In group MI-HT (× 200). SO: sham operated, MI: myocardial infarction, MI-HT: myocardial infarction plus hypoxia training.

of MI rabbits to high altitude hypoxia training at 4000 m for four weeks could enhance the cardioprotection by diminishing infarct size, increasing capillary density, preventing from LV hypertrophy and improving cardiac function.

Acute mortality from MI has decreased in the last decades with the advantages of prevention, diagnosis and treatments.[1] However, the incidence of long-term mortality in patients with MI is still high. Several reports showed that the 3-month, 1-year, and 2-year death rate were 7.9%, 12.7%, and 18.6% in patients after initial acute MI, respectively.[14] Adverse LV remodeling after MI is recognized as a major cause of cardiac dysfunction,[15] which is often associated with increased morbidity and mortality.[16] Recently, a clinical trial investigated 53 patients with acute MI underwent complete reperfusion and received anti-remodeling medical treatment and found that six-month later, 55% patients displayed LV remodeling and left ventricular ejection fraction (LVEF) deterioration.[17] Similar results were obtained from other studies. For example, it was reported that 48 of 148 patients with acute MI exhibited an increase in LV end-diastolic volume and a decrease in LVEF at six months follow-up.[18] Many evidences demonstrated that low LVEF were an important independence predictor of mortality.[19,20] Mortality in patients with LVEF $\leq$ 20, 21−35 and $\geq$ 36 were 45%, 22% and 13%, respectively ($P$ = 0.002).[19] Therefore how to limit the cardiac remodeling, preserve the LV function and improve a long-term survival post-infarct has emerged as the growing challenges in the health system.[16] In our study, we aim to improve LV function by hypoxia training and found that a 4-week hypoxia training could decrease LVEDD and LVESD, enhanced LVEF for animals with MI and consequently diminish the mortality. These data indicated that such 4-week hypoxia training may be an effective approach to provide cardioprotection in patients with MI.

In this study, hypoxia training was applied to reserve LV remodeling and improve cardiac function. We had demonstrated that compared with the group SO, groups MI and MI-HT both showed the dilatation of LV and decline of LVEF. However, the 4-week hypoxia training (group MI-HT) exhibited lower LVEDD, LVESD and higher LVEF than those in group MI, which indicated that hypoxia training could reserve heart remodeling after MI. Hypoxia, as a physiological stimulation, has long been recognized to increase the resistance of the heart to ischemia–hypoxia injury.[8] The low incidence of MI and mortality from coronary heart disease in populations living in high altitude areas was noticed.[21] Several studies have unveiled that hypobaric hypoxia preconditioning can protect the isolated heart against subsequently ischemia/reperfusion injury, improving major manifestations such as infarct size,[10] cardiac contractility,[22] and ventricular arrhythmia.[23] Wang, *et al*.[24] studied that rats were exposed either in hypobaric hypoxia condition (at an altitude of 5000 m, 4 h/day) or in air room for 4 weeks, then hearts were isolated and performed 30-min globe ischemia and 45-min reperfusion, the difference of infarct size (20.5% ± 5.3% in hypoxia *vs*. 42.1% ± 3.8% in control, $P < 0.01$) and LV contractility between groups were observed. Guo, *et al*.[25] found that guinea pigs were subjected to hypoxia exposure significantly increased cardiac tolerance to ischemia/reperfusion injury, which was confirmed by an improved recovery of contractile function and an increased coronary flow. Naghshin, *et al*.[26] exposed C57BL/6J mice to chronic hypoxia for four weeks and then assessed cardiac function by echocardiography and pressure-volume loop analyses. They found that LVEF were increased in chronic hypoxia-exposed animals compared to controls. In our study, rabbits with MI were subjected to hypoxia training at 4000 m altitude for 1-h per day. Four weeks later, we revealed that the improvements of LVEDD (15.86 ± 1.09 mm), LVESD (12.10 ± 1.20 mm) and LVEF (54.39 ± 12.74 mm) were much more significant in group MI-HT than in group MI. Our study confirmed that the moderate intensity of hypoxia training also elicited cardioprotection by limiting LV remodeling after MI.

At the end of the experiments, we detected the elevation of VEGF levels and capillary density. VEGF plays an essential role in angiogenesis process.[27,28] It was demonstrated that hypoxia stress could stimulate VEGF increase as a major cellular response, which involved in the development of new microvasculature.[29,30] In some researches, neo-angiogenesis was demonstrated by the remarkable increase of average capillary count in left ventricles in the hypoxia-exposure mice with respect to control, companied by the elevated levels of VEGF and VEGF-R2.[31] Sasaki, *et al*.[32] confirmed the effects of hypoxic preconditioning on stimulating myocardial angiogenesis after MI. In their research, rats were firstly put to systemic hypoxic exposure (10% ± 0.4% $O_2$) for 4-h and then underwent left anterior descending coronary artery (LAD) occlusion. Data showed that hypoxia preconditioning significantly increased both capillary and

arteriolar densities and elevated VEGF levels after two (33%) and three (63.3%) weeks of LAD occlusion. Similarly, a study of mice was raised in the hypobaric hypoxia chamber ($F_iO_2$ 11.1%) for 28 days and 42 days revealed that capillary density increased in LV without myocardium hypertrophy.[33] It is well-known that angiogenesis following MI could improve LV function. Many researches had demonstrated that capillary density increased after MI resulted in LVEF improvement.[34,35] In this study, we performed hypoxia training on rabbits post myocardial infarction and compared the changes of VEGF and capillary density. The data illustrated that hypoxia training could augment capillary density and VEGF levels significantly.

In our experiment, we performed hypoxia training at the intensity of $F_iO_2$, 14.9% (equivalent to altitude 4000 m) for 1-h per day, totally for four weeks. It is popular that experimental protocols of hypoxia exposure vary greatly in cycle length, severity, frequency and total numbers of hypoxic episodes. These factors are critical in determining whether hypoxia is beneficial or harmful.[36] In some papers, some negative changes was proposed in some hypoxia experiments, such as hypertension, arrhythmias, coronary heart disease, heart failure, insulin resistance, fatty liver, pulmonary edema and cerebral edema.[37] A study of hypoxia exposure ($F_iO_2$5% or 10%) recorded that infarct size was increased with $F_iO_2$5% hypoxia significantly, whereas decreased with $F_iO_2$10% hypoxia, indicating that the adequate oxygen content is a key factor in cardioprotection.[36] A study on rats received cycles of hypoxia exposure (15 s 100%$N_2$ and 15 s 21% $O_2$, repeated 8 h/day) for 5 weeks, the rats underwent hypoxia with these parameters resulted in the increases of hematocrit and right heart mass.[38] Diverse parameters of hypoxia will contribute to various effects. How to avoid these risks and enhance the benefits and safety of hypoxia training is an important strategy in clinical practice. In our study, the protocol was hypoxia training at altitude of 4000 m, 1 h per day for 4 weeks and was recorded the improvement of LV remodeling and LV function. It may provide a feasible, acceptable and beneficial protocol for clinical practice.

In the present study, we concentrated on the benefits of cardiac remodeling of hypoxia training on MI and determined cardiac function at the endpoints of the experiments. We should measure the LVEDD, LVESD and EF before, immediately after ligation and hypoxia training respectively to check the conditions of hearts accurately. However, in the experiment, we strictly followed a uniform standard method to complete the production model of myocardial infarction. Moreover, molecular mechanism involved in the process would be explored in the further study.

In summary, our study had observed the effects of hypoxia training on rabbits post myocardial infarction and confirmed that moderate hypoxia could increase capillary density, decrease infarct size and restore LV remodeling post myocardial infarction, which contribute to the improvement of LV function and mortality. This study may provide a feasible and practical approach for the recovery of MI.

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