

# Establishment of a Rabbit Model of Chronic Obstructive Sleep Apnea and Application in Cardiovascular Consequences

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## Abstract

**Background:** Although obstructive sleep apnea (OSA) has been recognized as a major risk factor for cardiovascular complications and its clinical features are well characterized, it is difficult to replicate the OSA hypoxic model in humans. We aimed to establish an experimental rabbit model for chronic OSA and to explore its application to measure blood pressure (BP), myocardial systolic function, and oxidative stress.

**Methods:** The rabbit model for OSA was established by repeatedly closing the airway and then reopening it. A tube specially designed with a bag that could be alternately inflated and deflated according to a predetermined time schedule, resulting in recurrent airway occlusions and chronic intermittent hypoxia (CIH) imitating OSA patterns in humans, was used. Twenty-four rabbits were randomly divided into obstruction, sham, and control groups, and their upper airways were alternately closed for 15 s and then reopened for 105 s in a 120-s-long cycle, for 8 h each day over 12 consecutive weeks. Before and after the experiment, the BP of each rabbit was monitored. Levels of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in the serum, superoxide dismutase (SOD) activity, malondialdehyde (MDA) and reactive oxygen species (ROS) contents, as well as Na<sup>+</sup>-K<sup>+</sup>-ATPase/Ca<sup>2+</sup>-ATPase activities in cardiac muscle were examined. In addition, cardiac functional parameters were measured using echocardiography.

**Results:** After 3 months, all rabbits in the obstruction group manifested sleepiness performance similar to that observed in OSA patients. Traces of airflow and SpO<sub>2</sub> showed that this model mimicked the respiratory events involved in OSA, including increased respiratory effort and decreased oxygen saturation. Gradually, the BP rose each month. CIH led to obvious oxidative stress and injured myocardial systolic performance. The serum levels of IL-6 and TNF- $\alpha$  increased significantly ( $64.75 \pm 9.05$  pg/ml vs.  $147.00 \pm 19.24$  pg/ml and  $59.38 \pm 8.21$  pg/ml vs.  $264.75 \pm 25.54$  pg/ml, respectively, both  $P < 0.001$ ). Compared with the sham and the control groups, myocardial activities of Na<sup>+</sup>-K<sup>+</sup>-ATPase/Ca<sup>2+</sup>-ATPase and SOD in the obstruction group decreased markedly, while ROS and MDA content increased.

**Conclusions:** These results show that the rabbit model for OSA simulates the pathophysiological characteristics of OSA in humans, which implies that this animal model is feasible and useful to study the mechanisms involved in the cardiovascular consequences of OSA.

**Key words:** Airway Obstruction; Animal Model; Obstructive Sleep Apnea; Rabbit

## INTRODUCTION

Obstructive sleep apnea syndrome (OSAS) is a common sleep-disordered breathing (SDB) disease characterized by recurrent episodes of partial or complete upper airway collapse.<sup>[1]</sup> As a prevalent chronic disease, OSAS has been recognized as a potential major risk factor for the morbidity and mortality of many cardiovascular complications,<sup>[2-4]</sup> which may depend on the severity of the nocturnal apnea periods causing exposure to repeated chronic intermittent hypoxia (CIH).

Although the clinical features of obstructive sleep apnea (OSA) have been well characterized, many aspects of the pathogenesis and pathophysiology of the disorder remain

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poorly understood. The pathophysiologic mechanisms proposed for OSA include sympathetic hyperactivation, impairment of vasomotor reactivity, vascular inflammation, oxidative stress, endothelial dysfunction, and metabolic disorders, which are closely related to the function of the left ventricle.<sup>[5]</sup> Furthermore, it is difficult to replicate this characterized OSA hypoxic model in humans. Accordingly, animal models provide useful tools to investigate the pathophysiological mechanisms determining the consequences of OSA and to mimic some manifestations of OSA in humans under well-controlled experimental conditions. Therefore, in the present study, we aimed to establish an experimental rabbit model for chronic OSA by applying a repetitive pattern of controlled airway obstructions mimicking the ones experienced by OSA patients. We tested the feasibility of this model and explored its effects by measuring blood pressure (BP), myocardial systolic function, and oxidative stress.

## METHODS

### Animals and experimental protocols

All experiments were performed on a total of 24 adult male and female rabbits, each weighing 2.5–3.0 kg, which were obtained from the Wuhan University Center for Animal Experiments. The animals were randomized into three groups ( $n = 8$  each): an obstruction group, a sham group, and a normoxic control group (no intervention). The animal protocols in this study were approved by the Committee on the Use of Animals for Teaching and Research of the Medical School of Wuhan University. These protocols also conformed to the China Committee of Experimental Animal Care, and the regulations were in accordance with National Institutes of Health guidelines.

### Obstructive sleep apnea model setup

#### Endotracheal obstruction device and implantation

An obstruction device was constructed from a silicone tube, which was 15 cm in length and 2 mm in outer diameter. The silicone tubing was closed at one end with a small foam rubber ball bag. The open end was connected to a controller [Figure 1], which consisted of a programmable

chip, batteries, and a digital time switch. The bag can be inflated and deflated at different times according to the design requirements, which is regulated by the control delivery system with a digital time switch and a pressure feedback system. Upon increasing the pressure within the silicone tubing, the stretched bag would inflate gently to 10 times its original diameter [Figure 1].

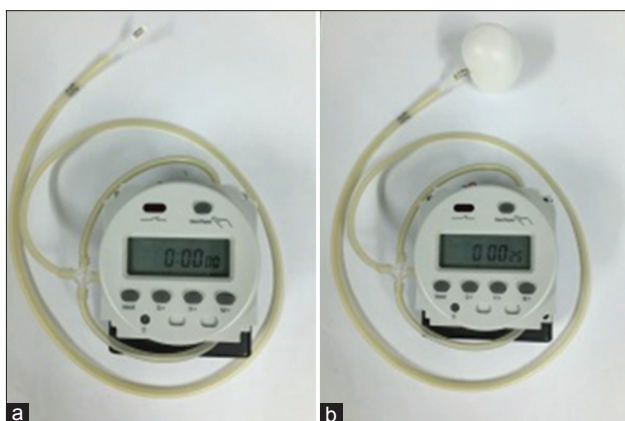
The surgical process for implanting the obstruction device was as follows: after anesthetizing each rabbit with 20% urethane (1.0–1.2 g/kg) through an intraperitoneal injection, which was supplemented hourly with a 15% initial dose to maintain adequate anesthesia, the rabbit was fixed on an animal operating table in the supine position. A longitudinal incision was then made in the midcervical region and a tracheostomy was carried out beneath the cricoid cartilage. First, a 1.5-cm midline incision was made just above the sternum. Second, the connective tissue and sternohyoid muscles were gently spread to visualize the trachea. Third, a small hole located two cartilage rings below the thyroid was made in the trachea using a hypodermic needle. The top of the obstruction device (the bag) was inserted into the hole and was fixed effectively by a piece of silk suture attached to the cartilage to avoid the risk of the tube being pulled into the trachea lumen or out of the hole. Because the bag just entered the airway lumen, it did not affect the airflow when it was not inflated. However, upon inflation, the obstruction device gradually blocked the airway, partially to completely, as the bag became larger and larger. These events therefore simulated the recurrent episodes of upper airway obstruction that occur in humans. The open end of the silicone tube was then tunneled subcutaneously and fixed to the neck on the back side, which was connected to the control device to ensure that it remained outside the animal's body. The control device and battery were put into a jacket worn by the rabbit. The sham rabbits were subjected to the same surgical procedure with a tracheostomy and a tube implanted, but no airway obstructions were applied. The normoxic control rabbits were subjected to no intervention. After recovery from surgery, all animals had *ad libitum* access to food and water throughout all experiments.

### Hypoxia exposures

Experiments were performed in the rabbit model for OSA, which was established by closing the airway and reopening it for 8 h/day (from 8:00 a.m. to 4:00 p.m.) for 12 consecutive weeks. In this study, the upper airway was alternately given 15 s of closing (75% SaO<sub>2</sub> at the nadir) and 105 s of reopening (summit SaO<sub>2</sub> 99%) in a 120-s-long cycle, which was the equivalent of 30 hypoxic events per hour. The inflation and deflation of the bag were regulated by the control delivery system with a digital time switch. The animals in the sham group had the same surgical procedure as the obstruction group; however, the controller was not triggered.

### Evaluation of the obstructive sleep apnea model

In this study, rabbit behavioral observations, such as somnolence, objective airflow, and oxygen saturation measurement, were used to assess whether the animal model



**Figure 1:** Schematic diagrams of the airway occlusion system. The deflated (a) and inflated (b) bag.

was successful. The ear oximeters for oxygen saturation measurements (pulse oxygen saturation meter analyzer, 8008J) were provided by the Nonin Medical Inc., USA. The airflow was digitally recorded and analyzed using a multichannel sleep diagnostic system (SOMNOscreen Plus Tele PSG, SOMNOmedics GmbH, Germany).

### Measurement of blood pressure

The BP of rabbits in a conscious and unrestrained condition was measured every month using tail-cuff plethysmography (RBP-1 noninvasive blood pressure analyzer, Chengdu Instrument Factory, China), and three measurements for each time point were averaged.

### Left ventricular systolic performance by echocardiography

Before the obstructive apnea model began and 3 months later, the left ventricular (LV) functions of each animal were measured using transthoracic echocardiography with a phased-array system (Sonos7500, Philips Ultrasound, Bothell, WA, USA) and a 2.3 MHz probe. The parameters measured included left ventricle ejection fraction (LVEF), left ventricle end-diastolic volume (LVEDV), left ventricle end-systolic volume (LVESV), LV percent fractional shortening (FS), stroke volume (SV), and E/A ratio (combining early filling velocity [E] with late filling velocity [A]).

### Collection and storage of blood and tissues

Blood samples were drawn from the ear vein of each rabbit before the OSA model began. After the study, rabbits were sacrificed by taking their blood through intracardiac puncture. Blood samples were collected for centrifugation, and sera were stored at  $-80^{\circ}\text{C}$  for later analyses of interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Heart tissues were harvested and fixed in 4% phosphate-buffered formaldehyde for reactive oxygen species (ROS) evaluation. The remaining parts of the cardiac muscle were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for later studies of superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, as well as  $\text{Na}^+\text{-K}^+\text{-ATPase}$  and  $\text{Ca}^{2+}\text{-ATPase}$  activities.

### Measurement of blood interleukin-6 and tumor necrosis factor- $\alpha$ levels

The serum levels of IL-6 and TNF- $\alpha$  were detected by solid-phase sandwich enzyme-linked immunosorbent assays, and absorbances were measured at 450 nm using a microplate reader (BioTek ELx800, USA). The ELISA kits specific for IL-6 and TNF- $\alpha$  were from Boster Biological Technology Ltd. (Wuhan, China).

### Measurements of oxidative stress biomarkers in cardiac tissues

Frozen cardiac specimens from the rabbits were homogenized in tissue lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). After lysis for 15 min in ice, the homogenates were centrifuged at 3000 r/min for 15 min. SOD activity, MDA and ROS contents in the supernatant were measured using commercially available kits (Jiancheng Bioengineering, Nanjing, China). All assays were conducted according to the instructions of the manufacturer. The assay kit for SOD in

cardiac tissue uses thiazole salt to detect superoxide anions and produces a colored product, and whose absorbance was measured at a wavelength of 450 nm. ROS levels were assessed using the DCF-HDA (Molecular Probes) fluorescence assay. MDA contents were determined spectrophotometrically by measuring the presence of thiobarbituric acid-reactive substances. The activities of cardiac sarcolemmal  $\text{Na}^+\text{-K}^+\text{-ATPase}$  and  $\text{Ca}^{2+}\text{-ATPase}$  were assessed in homogenates by measuring inorganic phosphorus released by the action of  $\text{Na}^+\text{-K}^+$ - and  $\text{Ca}^{2+}\text{-Mg}^{2+}$ -dependent hydrolysis of ATP.

### Statistical analyses

All data are expressed as mean  $\pm$  standard error (SE). Statistical analysis was performed by analysis of variance (ANOVA) using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). Two-way ANOVA tests were used to ascertain whether BP, parameters of LV systolic performance (LVEF, LVEDV, LVESV, FS, and SV), and blood inflammatory markers (IL-6, TNF- $\alpha$ ) varied with time and/or group (obstruction, sham, and normal control). One-way ANOVA test was employed to assess differences between groups in cardiac oxidative stress biomarkers (SOD, ROS, MDA,  $\text{Na}^+\text{-K}^+\text{-ATPase}$ , and  $\text{Ca}^{2+}\text{-ATPase}$ ). When the differences were significant, Newman-Keuls posttest was performed. A value of  $P < 0.05$  was considered a significant difference. The figures were made using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

## RESULTS

### Rabbit behaviors

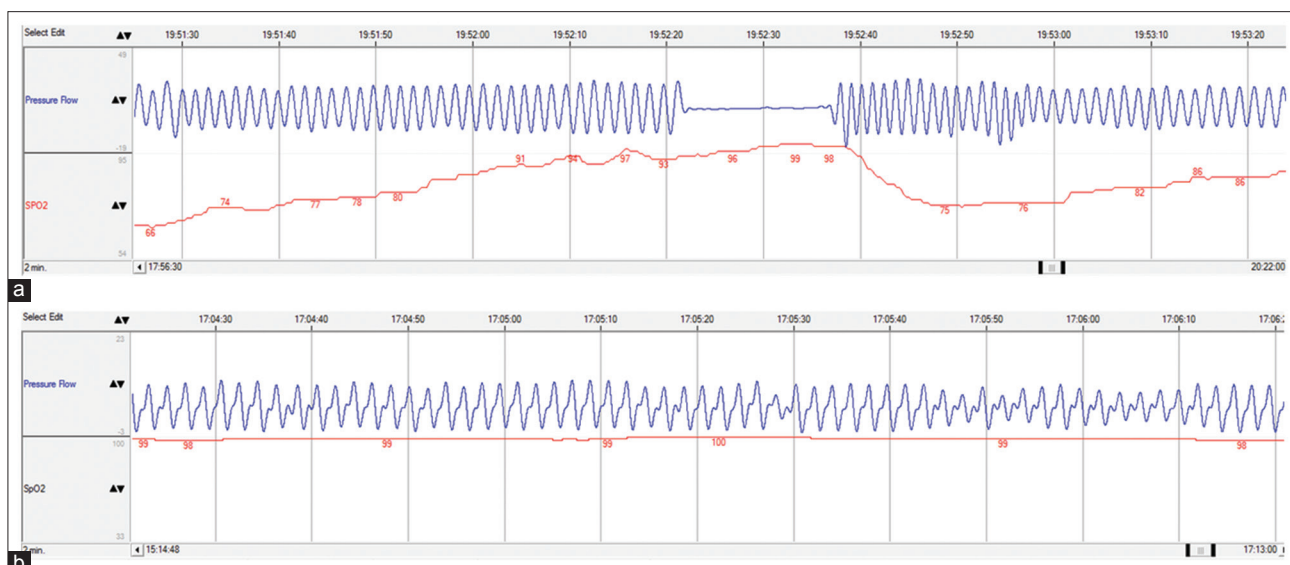
During the first 3–5 days of the experimental period, the rabbits struggled with restless or suffocation when the obstruction device was initiated and their breathing efforts against the closed airway increased markedly. After adaption to the restricted airway during the 1<sup>st</sup> week, the animals progressively became somnolent and fatigued. During the course of the study, the rabbits became increasingly sleepy with increases in the frequency and degree of obstruction. The animals in the sham group and in the control group did not have these manifestations.

### Variation of pulse oxygen saturation ( $\text{SpO}_2$ )

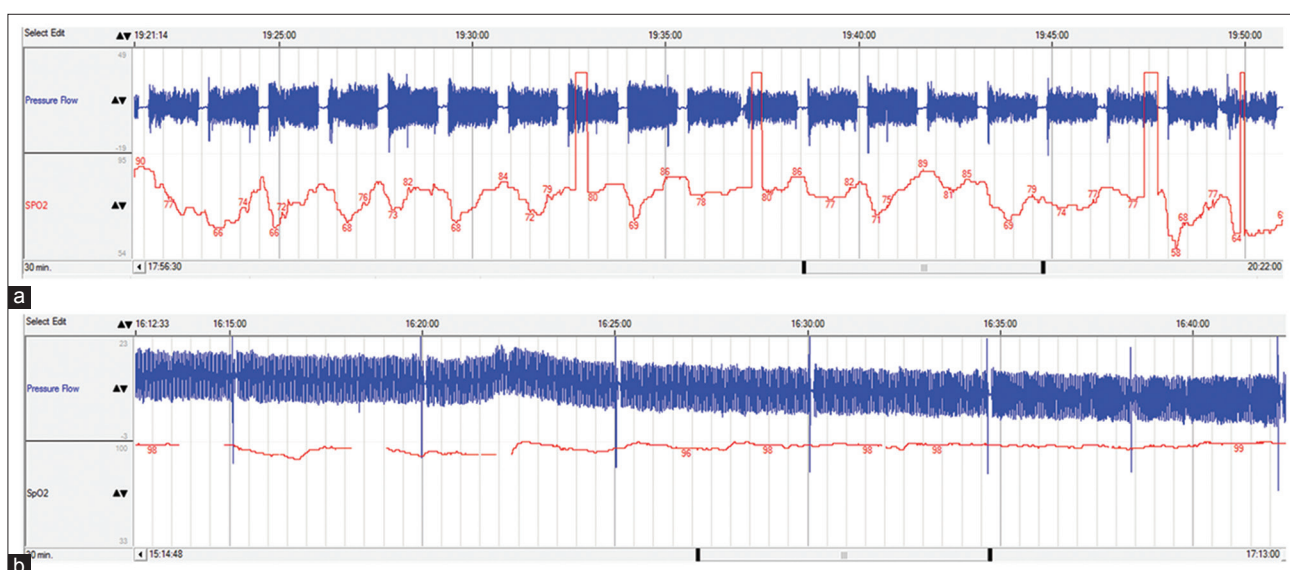
Figures 2 and 3 show examples of the airflow and the oxygen saturation traces recorded during one of the airway obstructions applied to the rabbits in the obstruction group. No airflow and decreased oxygen saturation accompanied every airway closure. When the airway reopened, the breathing efforts of the rabbit increased.  $\text{SpO}_2$  consistently decreased as a result of the occlusion. The  $\text{SpO}_2$  fell from the highest value ( $99 \pm 1$ ) % to the lowest value ( $75 \pm 2$ ) %. As expected, after some delay from the start of the occlusion,  $\text{SpO}_2$  exhibited a transient dip. However, no obvious changes of air flow or  $\text{SpO}_2$  occurred in the sham animals, in which the average  $\text{SpO}_2$  was ( $99 \pm 1$ ) %.

### Blood pressure measurements

When compared with sham group and the control group, rabbits in the obstruction group showed significant BP



**Figure 2:** Representative tracings of 120-s signal recordings. (a) During the 15 s airway obstruction, airflow was nil owing to airway closure caused by the bag inflation (upper trace), and the rabbits' arterial oxygen saturation ( $SpO_2$ ) exhibited a transient decrease (lower trace). The  $SpO_2$  fell from 99% to 75%. (b) No obvious changes of flow and  $SpO_2$  occurred in the sham animals. The average  $SpO_2$  was  $(99 \pm 1) \%$ .



**Figure 3:** Representative tracings of 30-min signal recordings. (a) During each 15 s airway obstruction for every 120 s as a cycle in a rabbit, no airflow (upper trace) and decreased oxygen saturation (lower trace) accompanied every airway closure. (b) In the sham animals, the airflow and  $SpO_2$  were stable in a 30-min recording.

elevations in response to CIH [Figure 4]. The BP started to rise from the end of the first month and gradually increased with the duration of the experiment. Animals in the sham and in the control groups did not develop hypertension.

### Changes of left ventricular systolic function

Before the CIH experiments, there were no significant differences in the baseline parameters of cardiac function among the rabbits in the obstruction, sham, or control groups. However, 3 months of repeated airway obstruction and CIH resulted in sustained decreases in LV systolic performance of the rabbits. Compared with 3 months earlier, the related parameters of cardiac function, such as LVEF, FS, and SV, decreased obviously while both LVESV and LVEDV

increased significantly. The E/A ratio in the obstruction group was  $>1$  in baseline and 3 months later it was  $<1$ . However, echocardiography showed no significant changes in these parameters in either the sham or control rabbits before or after the experiments. These data suggested that CIH caused by repeated airway obstruction injured the LV physiology of the animals [Table 1].

### Myocardial ATPase, superoxide dismutase, malondialdehyde, and reactive oxygen species

Compared with the sham and normal groups, in the obstruction group, the myocardial tissue activities of SOD,  $Na^+K^+$ -ATPase, and  $Ca^{2+}$ -ATPase decreased significantly; but in contrast, the contents of MDA and ROS increased

considerably [Table 2]. These results implied that CIH could irritate obvious oxidative stress.

### Serum values of interleukin-6 and tumor necrosis factor- $\alpha$

After the 3 months of the study, the rabbits in the obstruction group showed significantly higher IL-6 values than rabbits in the sham and the control groups [Table 3]. There was no significant difference between the sham and the control rabbits. In addition, the TNF- $\alpha$  level was significantly higher in the obstruction group than in the sham and the control groups, and again, the difference between the sham and the control groups was not significant.

## DISCUSSION

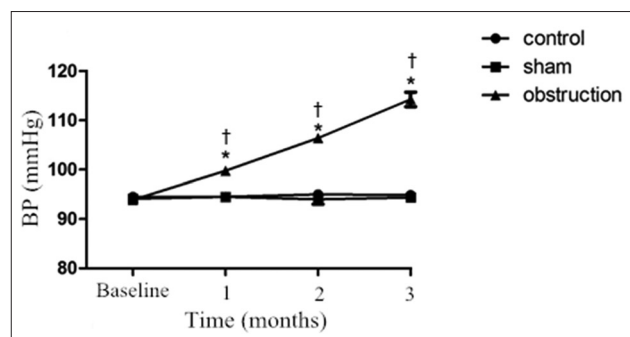
We have developed a new experimental rabbit model for repeated airway obstruction, which imitates the

physiological events that occur in human OSA. Successful operation of the model was confirmed by behavioral observation of sleepiness, increased breathing efforts, and postapnea arterial oxygen desaturations. The results show that after 3 months, the CIH rabbit model caused an elevation of BP and myocardial oxidative stress of the animals and injured their LV systolic function. We conclude that this experimental model provides a potentially selective tool for investigating the mechanisms involved in the cardiovascular consequences of OSA.

### Models for obstructive sleep apnea

An animal model is an experimental design that permits the judgment of pathophysiologies and treatment strategies of human diseases in a reproducible and standardized manner.<sup>[6]</sup> Based on this general definition, the OSA model validation should meet at least one criterion of homologous, predictive, or isomorphic.<sup>[6]</sup> However, most OSA models are partially isomorphic, focusing on one aspect of the human SDB. Animal models used to mimic and study SDB are either spontaneous or induced.<sup>[6]</sup> Spontaneous models for OSA have been documented in the English bulldogs<sup>[7,8]</sup> and in a kind of female pig in Guangxi, China,<sup>[9]</sup> which were reported to have an abnormal narrowing upper airway anatomy. Those models may reproduce all the clinical features of human SDB;<sup>[10]</sup> however, their relatively low availability has enhanced interest in induced models, which create models that mimic at least one important aspect of the human disease.

Induced models can be invasive or noninvasive. Invasive models reproduce OSA in tracheostomized animals installed with an intermittently blocked endotracheal tube.<sup>[11-14]</sup> Noninvasive models, such as the CIH model, are easier to



**Figure 4:** Blood pressure (BP) in rabbits exposed to chronic intermittent hypoxia vs. the sham group or the control group. \* $P < 0.0001$  versus control; † $P < 0.0001$  vs. sham.

**Table 1: Left ventricular systolic function indexes at baseline and 3 months in obstruction, sham, and control groups ( $n = 8$  in each group)**

Items	Baseline					3 months				
	Control	Sham	Obstruction	F	P	Control	Sham	Obstruction	F	P
LVEF (%)	73.75 ± 1.96	74.02 ± 1.70	72.91 ± 2.14	0.71	0.50	73.86 ± 3.63	73.03 ± 1.34	64.79 ± 2.60*‡¶	27.84	0.000
LVEDV (ml)	2.58 ± 0.21	2.76 ± 0.41	2.91 ± 0.44	0.17	0.84	2.60 ± 0.26	2.86 ± 0.53	5.59 ± 1.49*‡¶	25.56	0.000
LVESV (ml)	0.81 ± 0.08	0.82 ± 0.09	0.87 ± 0.11	1.53	0.24	0.80 ± 0.09	0.84 ± 0.14	1.58 ± 0.83*‡¶	6.47	0.006
FS (%)	40.23 ± 2.05	40.45 ± 1.91	39.87 ± 1.86	0.85	0.44	40.38 ± 1.78	40.48 ± 1.77	31.31 ± 2.10*‡¶	63.16	0.000
SV (ml/s)	1.92 ± 0.21	1.91 ± 0.31	1.92 ± 0.17	0.01	0.99	1.97 ± 0.22	1.99 ± 0.27	1.45 ± 0.15*‡¶	15.83	0.000

Values were shown as mean ± SE. \* $P < 0.001$  vs. control; † $P < 0.005$  vs. control; ‡ $P < 0.001$  vs. sham; § $P < 0.01$  vs. sham; ¶ $P < 0.001$  vs. baseline; ¶ $P < 0.05$  vs. baseline. SE: Standard error; LVEF: Left ventricle ejection fraction; LVEDV: Left ventricle end-diastolic volume; LVESV: Left ventricle end-systolic volume; FS: Fractional shortening; SV: Stroke volume.

**Table 2: Oxidative stress biomarkers of myocardial tissues in three groups ( $n = 8$  in each group)**

Parameters	Control group	Sham group	Obstruction group	F	P
Na <sup>+</sup> -K <sup>+</sup> -ATPase ( $\mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$ )	9.94 ± 0.37	9.98 ± 0.54	6.35 ± 0.37*†	183.29	0.000
Ca <sup>2+</sup> -ATPase ( $\mu\text{mol}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$ )	5.53 ± 0.26	5.53 ± 0.29	3.06 ± 0.27*†	216.47	0.000
SOD (NU/ml)	437.50 ± 43.25	418.25 ± 38.58	291.75 ± 36.88*†	31.88	0.000
MDA (nmol/ml)	3.24 ± 0.25	3.18 ± 0.23	5.05 ± 0.48 *†	79.60	0.000
ROS (fluorescence intensity/mg)	4100.38 ± 246.66	4143.25 ± 249.10	7365.63 ± 570.16*†	187.93	0.000

Values were shown as mean ± SE. \* $P < 0.001$  vs. control; † $P < 0.001$  vs. sham. SE: Standard error; ROS: Reactive oxygen species; SOD: Superoxide dismutase; MDA: Malondialdehyde.

**Table 3: Serum values of IL-6 and TNF- $\alpha$  at baseline and 3 months in three groups (pg/ml,  $n = 8$  in each group)**

Parameters	Control group	Sham group	Obstruction group	F	P
IL-6					
Baseline	62.50 $\pm$ 10.33	64.12 $\pm$ 7.45	64.75 $\pm$ 9.05	0.13	0.880
3 months	63.75 $\pm$ 5.82	67.25 $\pm$ 10.87	147.00 $\pm$ 19.24* <sup>†‡</sup>	101.85	0.000
TNF- $\alpha$					
Baseline	61.88 $\pm$ 10.60	65.88 $\pm$ 9.91	59.38 $\pm$ 8.21	0.93	0.410
3 months	60.50 $\pm$ 8.54	65.00 $\pm$ 10.64	264.75 $\pm$ 25.54* <sup>†‡</sup>	389.58	0.000

Values were shown as mean  $\pm$  SE. \* $P < 0.001$  vs. control; <sup>†</sup> $P < 0.001$  vs. sham; <sup>‡</sup> $P < 0.001$  vs. baseline. SE: Standard error; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-6: Interleukin-6.

use because they reproduce a single aspect of the human disease – the repetitive hypoxia-reoxygenation process. Since the first description in the early 1990s,<sup>[15]</sup> these models have been widely used to evaluate various consequences of OSA. Animals either breathe with a mask or are put in specific chambers or cages, where they intermittently breathe nitrogen-enriched air to produce hypoxia, alternating with oxygen or air for the reoxygenation.<sup>[15-19]</sup> Compared to other models, the CIH models allow the animals to be exposed to hypoxic conditions for very long periods that can last several months,<sup>[20]</sup> allowing the investigation of chronic consequences that develop in human disease. However, the lack of an upper airway occlusion is an important limitation of this CIH model. In this study, we developed an OSA model in which a repeated upper airway obstruction was induced in healthy adult rabbits. As a kind of airway obstruction, the model was designed primarily as a tool to research the cardiovascular consequences of OSA.

### Obstructive sleep apnea and left ventricular diastolic dysfunction

Some clinical studies support the view that chronic OSA has important effects on LV function. LV diastolic dysfunction and LV hypertrophy occur in patients with OSA even before the development of hypertension and other cardiovascular diseases.<sup>[21]</sup> Furthermore, in a rat CIH model, there was a significant elevation both in LVESD and in LVESV along with the decreases both in LVEF and in LVFS.<sup>[22]</sup> Interestingly, significant changes of LV function were found in this study, which were in agreement with previous results.<sup>[22,23]</sup> As shown in Table 1, there were significant differences in LVEF, LVEDV, LVESV, FS, and SV among the obstruction, sham, and control groups.

However, the underlying molecular mechanisms of cardiac function damage caused by CIH remain poorly understood. Sympathetic nerve hyperinnervation may participate in the structural alterations,<sup>[23]</sup> and cardiomyocyte apoptosis<sup>[22,24-26]</sup> has been recognized to be involved in cardiovascular diseases. In the present study, we detected obvious cardiac inflammation and oxidative stress in the myocardial tissues of rabbits with chronic airway obstruction, with upregulated cardiac inflammatory and oxidative stress factors. Considering there were no significant differences between the control and the sham groups, these results suggest that the cardiac inflammation

and oxidative stress observed were specifically triggered by the CIH stimulus of recurrent airway obstructions, and may be an involvement that mediates myocardial injury. Even though oral antioxidants, such as N-acetylcysteine<sup>[27]</sup> and carbocysteine,<sup>[28]</sup> may slightly improve sleep disorders by attenuating oxidative stress in patients with OSA syndrome, little information has been reported on the effects of antioxidant treatment on the OSA-induced cardiovascular events.

### Obstructive sleep apnea and hypertension

Several epidemiological studies have identified OSA as a risk factor for systemic hypertension, but a direct etiologic relationship has not been definitively established. A number of animal models have been employed to investigate the effects of OSA on BP;<sup>[29,30]</sup> however, the most widely used model for this consists of subjecting rodents to a hypoxia/normoxia pattern by breathing air with variable oxygen concentrations.<sup>[31]</sup> Fletcher *et al.*<sup>[15]</sup> first reported this repetitive episodic hypoxia in rats, which induced a diurnal elevation of BP. Since then, many investigations have duplicated those experimental results using different IH paradigms and different animals.<sup>[11,32-36]</sup> Nevertheless, models based on changing the air concentration do not allow us to study the potential consequences of labored breathing efforts against an obstructed airway.

One of the purposes of this study was to systematically examine the effects of OSA on BP. The present study represents the mid-term application of an induced model of repetitive upper airway occlusion. We found that weeks of an upper airway obstruction result in sustained daytime hypertension. Exposure to CIH is thought to be responsible for the marked increase in sympathetic nerve activity and consequential hypertension.<sup>[37-40]</sup> In another study,<sup>[41]</sup> we reported that BP, after 21 days of CIH, was significantly higher than that of before CIH, the renal sympathetic activity was significantly enhanced, and the serum norepinephrine level was higher. These results indicate that CIH causes hypertension and overactivity of the sympathetic nervous system.

### Study limitations

For technical reasons, we did not perform EEG and could not analyze the animals' sleep architecture. Furthermore, intrathoracic pressure changes were not monitored. Because behavior, airflow and oxygen saturation were observed in

this study, we think these limitations did not influence the assessment of outcomes.

In conclusion, according to our findings, the OSA animal model developed using a tracheal airway occlusion was made on practical grounds. Application of this model may result in the development of cardiovascular abnormalities including hypertension, myocardial dysfunction induced by CIH, and oxidative stress. Therefore, this model provides a useful tool to investigate the mechanisms involved in the consequences of OSA.

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### Conflicts of interest

There are no conflicts of interest.

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