Adaptive Medicine 12(2): 42-49, 2020
DOI: 10.4247/AM.2020.ACA247

Original Article

Amelioration of Chronic Intermittent Hypobaric Hypoxia on the Damaged Carotid Sinus Baroreflex in Fructose-Induced Metabolic Syndrome Rats

Yun-Hui Xie[1, #], Hui Guo[2, #], Yan Wei[3], Xin-Qi Guo[1], Hong Hua[1], Hui-Jie Ma[1, *], and Yi Zhang[1, *]

[1] Department of physiology, Hebei Medical University, Shijiazhuang 050017, Hebei;
[2] Department of Gynaecology and Obstetrics, Fourth Hospital of Hebei Medical University, Shijiazhuang 050000, Hebei, and
[3] Institute of Cardiovascular, Southwest Medical University, Luzhou 646000, Sichuan, People's Republic of China.

High blood pressure is a cardiovascular risk factor and an important element in metabolic syndrome. It has been proved that chronic intermittent hypobaric hypoxia (CIHH) has anti-hypertensive effect through facilitating carotid sinus baroreflex (CSB) in renal vascular hypertension (RVH) rats. However, it is unclear whether CIHH has the same effect on CSB in fructose-induced metabolic syndrome (Mets) rats. The current study was to investigate the effect of CIHH on CSB and the potential cellular and molecular mechanisms using isolated carotid sinus perfusion technique. Male Sprague-Dawley rats were randomly divided into fructose-fed (Fruc-fed), CIHH, CIHH plus fructose-fed (CIHH+F) and control groups. Fruc-fed rats were fed with 10% fructose in drinking water. CIHH rats were exposed to simulated high-altitude hypoxia in a hypobaric chamber mimicking 5000 m altitude for 42 days, 6 h per day. CIHH+F rats received both 10% fructose drinking and CIHH treatment. The arterial blood pressure and body weight of animals were measured once a week. Carotid sinus perfusion technique was used to observe the CSB. Both body weight and SAP were increased in Fruc-fed rats compared to control, CIHH and CIHH+F rats. The CSB was inhibited in Fruc-fed rats with the decreased reflex gain and was enhanced in CIHH rats with the increased reflex gain compared to control rats. The inhibition of CSB in CIHH+F rats was improved compared to Fruc-fed rats. The facilitation of CSB in CIHH+F rats was partially abolished by glibenclamide, an ATP sensitive potassium channel (K_{ATP}) blocker. CIHH ameliorates the damaged CSB through activation of K_{ATP} channel in fructose-induced Mets rats.

Key words: metabolic syndrome, chronic intermittent hypobaric hypoxia (CIHH), carotid sinus, baroreflex, K_{ATP} channel

Introduction

The metabolic syndrome (Mets) is a complex metabolic disorders and displays as obesity, glycolipid metabolic disturbance, insulin resistance, and hypertension. Hypertension is the important element of Mets and the biggest threat to the cardiovascular system (3, 13, 18, 25).

A large number of studies have shown that chronic intermittent hypobaric hypoxia (CIHH) has cardiac protection, such as enhancement of myocardial antioxidation and promotion of recovery of cardiac function from ischemia/reperfusion (I/R) (19, 32, 38). Our previous study showed that CIHH has protective effect on heart against I/R injury and anti-arrhythmia (30, 31). Multiple mechanisms and signaling had been proposed for CIHH cardiac protection, such as promotion of myocardial anti-oxidation (1, 31), improvement of coronary circulation (34), inhibition of mitochondria DNA deletion (35), induction of heat shock proteins (33), promotion of NO production (5), activation of PKC and ATP-sensitive potassium channels (6, 37). In addition, CIHH has an anti-hypertension effect in renal vascular hypertension (RVH) and fructose-induced Mets rats (17, 36).

It is well known that the homeostasis of cardiovascular activity depends on the nervous and humoral regulations. The cardiovascular reflex is the basic manner of nervous regulation. The baroreflex, known as the buffer or depressor reflex, plays a key role in the maintenance of normal blood pressure (27). It was reported that impaired baroreceptor reflex occurred in various hypertension (15). Our previous

Corresponding authors: [1] Yi Zhang, Ph.D., Department of Physiology, Hebei Medical University, 361 Zhong Shan Dong Rd., Shijiazhuang 050017, Hebei, PRC. E-mail: zhyhenryphy@163.com; and [2] Hui-Jie Ma, Ph.D., Department of Physiology, Hebei Medical University, 361 Zhong Shan Dong Rd., Shijiazhuang 050017, Hebei, PRC. E-mail: lily564300@163.com
[#] These authors contribute equally to this work.
Received : June 23, 2020; Revised : June 27, 2020; Accepted : June 29, 2020.
 ISSN : 2076-944X. http://www.sast.org.tw

study suggested that CIHH could not alter arterial blood pressure under normal condition, but significantly reduce the decrease in arterial blood pressure in acute hypoxia condition (29). Recently, we found that CIHH has obvious anti-hypertensive effect in RVH rats (17), which might be related to the facilitation of CIHH on the carotid sinus baroreflex (12, 17).

Accordingly, we have reason to propose a hypothesis that CIHH antagonizes the hypertension through ameliorating the damaged carotid sinus baroreflex in fructose-induced metabolic syndrome rats. The current study was to investigate the effect of CIHH on CSB and the potential cellular and molecular mechanisms using isolated carotid sinus perfusion technique in fructose-induced Mets rats.

Materials and Methods

Animal and CIHH Treatment

Male adult Sprague-Dawley rats (170-190 g) in this study were obtained from the Experimental Animal Center of Hebei Province, PRC. The animal experimental protocols were approved by the Committee on the Use of Animals for Teaching and Research in Hebei Medical University and all experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996).

The rats were randomly divided into four groups: fructose-fed group (Fruc-fed), CIHH group, CIHH plus fructose-fed group (CIHH+F) and control group. The rats in Fruc-fed group were fed with 10% fructose in drinking water. The CIHH rats were exposed to hypobaric hypoxia mimicking 5000-m altitude (PO_2 = 108.8 mmHg, O_2: 14%) for 42 days, 6 h per day in a hypobaric chamber. The rats in CIHH+F group received both 10% fructose drinking and CIHH treatment. The rats in control group were given tap water and kept in a normoxic environment. All animals were housed in a temperature-controlled room (22 ± 1°C) with a 12 h light: 12 h dark photocycle. The body weight of animals was recorded weekly.

Measurement of Arterial Blood Pressure

The systolic arterial blood pressure (SAP) in conscious rats was measured by a tail-cuff pressure meter (LE5001, Panlab, Spain) once a week. SAP was measured three times in each rat and the mean value was calculated.

Perfusion of Left Isolated Carotid Sinus

The isolated carotid sinus perfusion was carried out with a modified method in our laboratory (8). The animals were anesthetized with urethane (1.0 g/kg, i.p.) and the trachea was cannulated for artificial ventilation. Carotid sinus areas were fully exposed by turning the trachea and esophagus in the rostral direction. The superior laryngeal nerve and sternohyoideus muscle were cut off. All bilateral aortic nerves, cervical sympathetic nerves, recurrent laryngeal nerves and right carotid sinus nerve were cut off. The common, external and internal carotid arteries and smaller arteries originating from these vessels were exposed and ligated, while carefully leaving the left carotid sinus nerve undisturbed. Ligation of the occipital artery at its origin from the external carotid artery excluded chemoreceptor involvement during change in carotid sinus pressure. A plastic tube was inserted into the distal end of left common carotid artery to serve as an inlet tube and a tube was inserted into the external carotid artery to serve as an outlet tube. The carotid sinus was then perfused with warm (37°C) oxygenated modified Krebs-Henseleit (K-H) solution (mM: NaCl 118.0, KCl 14.7, $CaCl_2$ 2.5, $MgSO_4$ 1.6, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.6, pH 7.35~7.45) bubbled with 95% O_2 and 5% CO_2. The intrasinus pressure (ISP) was monitored by a transducer (XH YP200) connected with the inlet tube. Sinus pressure and blood pressure (BP) were recorded simultaneously by the RM-6240 multi-channel physiological recording system (Chengdu Instrument Factory, Sichuan, PRC). The body temperature was maintained at 37.0~37.5°C throughout the experiment. At the end of the experiment, the animals were sacrificed by an over-dose of urethane (3.0 g/kg, i.v.).

Recording of Baroreflex

After perfusion of the left carotid sinus with the K-H solution, ISP was kept at 100 mmHg for 20 min and was then rapidly lowered to 0 mmHg from which ISP was elevated to 250 mmHg *via* a pulsatile ramp by regulating the speed of peristaltic pump, which was automatically controlled by a program designed by our laboratory (8). It took 0.5 min for ISP to be increased from 0 to 250 mmHg. The process was repeated at an interval of 5 min to check the stability of the baroreflex.

By perfusing the left carotid sinus with K-H solution and elevating the ISP, a functional curve and functional parameters were obtained. Data for the ISP-MAP relationships were collected and fitted to a sigmoidal logistic function curve (the baroreceptor function curve). The baroreflex gain was calculated as the ratio of change in MAP to the change in ISP (ΔMAP/ΔISP, expressed as mmHg/

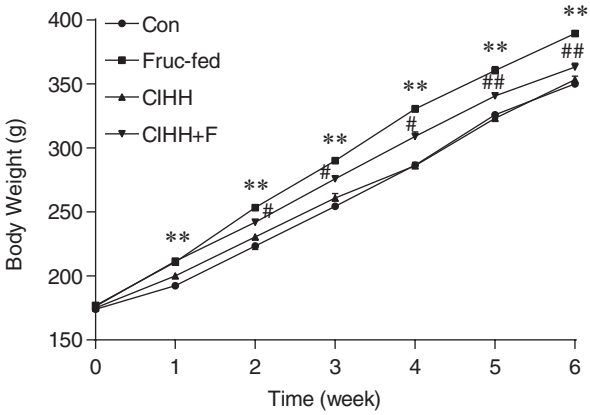

Fig. 1. Effect of chronic intermittent hypobaric hypoxia (CIHH) on body weight in fructose-induced metabolic syndrome rats. Con: control group, Fruc-fed: Fruc-fed group, CIHH:CIHH group, CIHH+F: CIHH plus Fruc-fed group. Data were expressed as mean ± SD. $n = 6$ for each group. $^{**}P < 0.01$ *vs.* Con, $^{\#}P < 0.05$ $^{\#\#}P < 0.01$ *vs.* Fruc-fed.

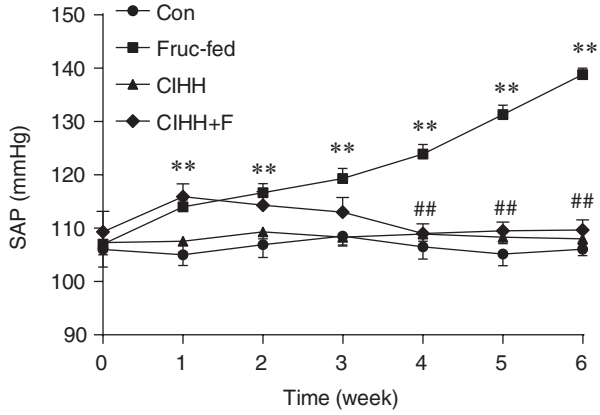

Fig. 2. Effect of chronic intermittent hypobaric hypoxia (CIHH) on systolic arterial blood pressure (SAP) in fructose-induced metabolic syndrome rats. Con: control group, Fruc-fed: Fruc-fed group; CIHH:CIHH group, CIHH+F: CIHH plus Fruc-fed group. Data were expressed as mean ± SD. $n = 6$ for each group. $^{**}P < 0.01$ *vs.* Con, $^{\#\#}P < 0.01$ *vs.* Fruc-fed.

mmHg), which was considered to be the marker of the baroreceptor reflex sensitivity. The functional parameters of baroreflex include threshold pressure (TP), equilibrium pressure (EP), saturation pressure (SP), operating range (OR), peak slope (PS) and reflex decrease of MAP (RD). TP was the ISP at which MAP decreased 5 mmHg in response to the increase of ISP, SP was the ISP at which MAP just showed no further reflex decreasing with an increase in ISP, EP was the ISP that equaled to systemic mean arterial pressure (MAP), and OR was calculated through SP minus TP.

Data Analysis

All data are expressed as mean ± SD. Statistical analysis was conducted using One-way analysis of variance (ANOVA) followed by a Student-Newman-Keuls's post hoc test for comparison among multiple groups. Paired *t*-test was used to compare the effect before and after drug administration. $P < 0.05$ was considered statistically significant.

Results

Effect of CIHH on Body Weight

Compared with control, CIHH and CIHH+F rats, the body weight of Fruc-fed rats was increased significantly ($P < 0.01$). The body weight was lower in CIHH+F rats than Fruc-fed rats ($P < 0.05$-0.01), and there was no difference of body weight between Control and CIHH rats ($P > 0.05$, Fig. 1). The result indicates that CIHH has no action on the normal body weight, but alleviates the increase of body weight and obesity in fructose-induced Mets rats.

Effect of CIHH on SAP

The baseline SAP was not different among the groups ($P > 0.05$). SAP in fructose-fed rats began to increase at the end of first week during fructose feeding and was significant higher than control and CIHH rats ($P < 0.01$). CIHH treatment normalized the increase of SAP in fructose-fed rats after the fourth week of CIHH treatment ($P < 0.01$, Fig. 2). The result indicates that CIHH has no effect on the normal SAP, but prevents the hypertension in fructose-induced Mets rats.

Effect of CIHH on Carotid Sinus Baroreflex

Compared with control rats the CSB functional curve was shifted rightward in Fruc-fed rat (Figure 3B). The peak slope (PS, 0.27 ± 0.03 *vs.* 0.36 ± 0.02), reflex decrease (RD, 30.67 ± 3.36 *vs.* 38.83 ± 2.35 mmHg) were decreaed significantly ($P < 0.01$), and the threshold pressure (TP, 76.28 ± 2.90 *vs.* 64.22 ± 1.30), equilibrium pressure (EP, 103.08 ± 3.83 *vs.* 94.37 ± 2.72) and saturation pressure (SP, 179.30 ± 3.00 *vs.* 171.93 ± 2.81 mmHg) were increased significantly ($P < 0.01$, Table 1). Also the gain curve of baroreceptor reflex was shifted downward in Fruc-fed rats (Figure 3C). It suggests that the baroreflex was inhibited in fructose-induced Mets rats.

Compared with the control rats the CSB func-

Table 1. Effects of CIHH on the functional parameters of CSB in fructose-induced metabolic syndrome rats

	TP/mmHg	EP/mmHg	SP/mmHg	OR/mmHg	PS	RD/mmHg
Con	64.22 ± 1.30	94.37 ± 2.72	171.93 ± 2.81	107.71 ± 1.78	0.36 ± 0.03	38.83 ± 2.354
Fruc-fed	76.28 ± 2.90**	103.08 ± 3.83**	179.30 ± 3.00**	103.01 ± 0.96**	0.27 ± 0.03**	30.67 ± 3.36**
CIHH	52.83 ± 1.98**	87.84 ± 4.37*	166.73 ± 3.53*	113.90 ± 1.98**	0.52 ± 0.03**	52.58 ± 3.16**
CIHH-F	59.20 ± 2.32**$^{\#\#}$	94.20 ± 3.35$^{\#\#}$	175.28 ± 2.07*$^{\#\#}$	116.08 ± 3.97**$^{\#\#}$	0.43 ± 0.04**$^{\#\#}$	46.62 ± 2.73**$^{\#\#}$

CSB: carotid sinus baroreflex, Con: control group, Fruc-fed: Fruc-fed group, CIHH: CIHH group, CIHH-F: CIHH-F group, TP: threshold pressure, EP: equilibrium pressure, SP: saturation pressure, OR: operating range, PS: peak slope, RD: reflex decrease in MAP. The date was expressed as Mean ± SD, $n = 6$ for each group. *$P < 0.05$, **$P < 0.01$ *vs*. Con., $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs*. Fruc-fed.

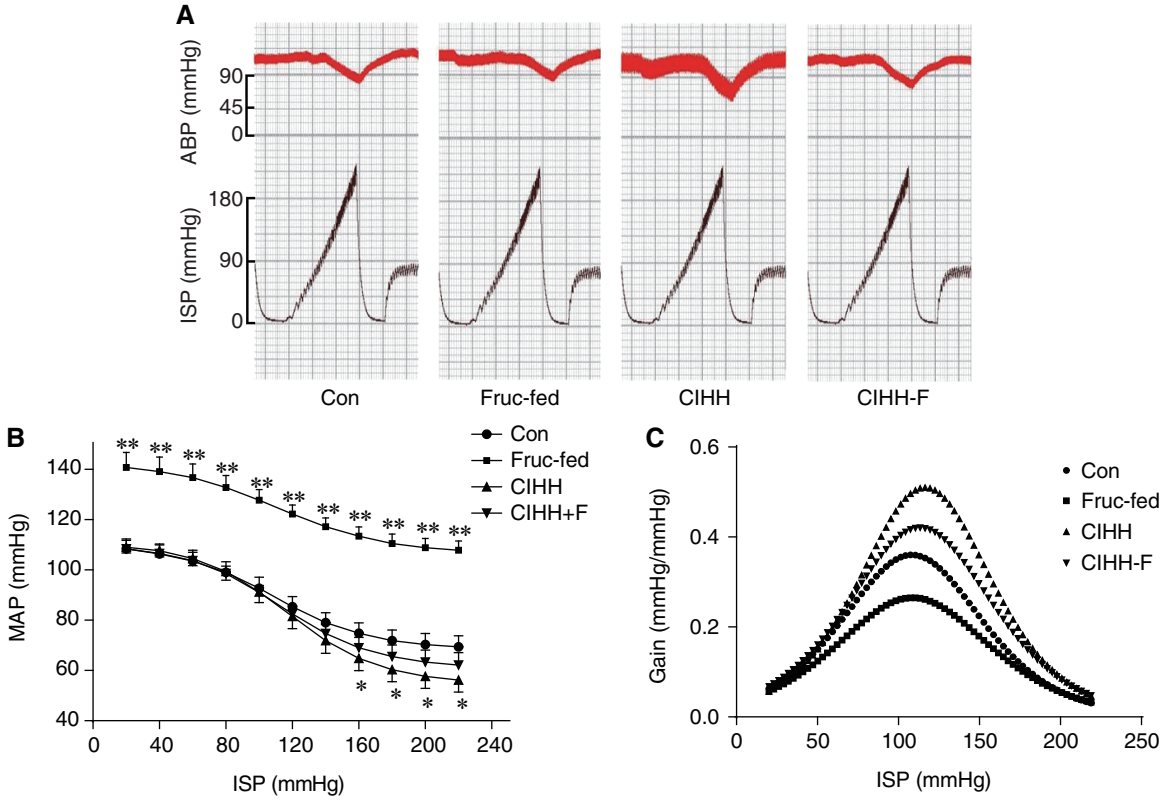

Fig. 3. Effect of chronic intermittent hypobaric hypoxia (CIHH) on carotid sinus baroreceptor reflex in fructose-induced metabolic syndrome rats. A. Oringinal recording of reflex. B. Functional curve of carotid sinus baroreceptor reflex. C. Gain curve of baroreceptor reflex. Con: Control group, Fruc-fed: Fruc-fed group, CIHH: CIHH group, CIHH+F: CIHH plus Fruc-fed group, ISP: intrasinus pressure, ABP: arterial blood pressure, MAP: mean arterial pressure, Data were expressed as Mean ± SD. $n = 6$ for each group,*$P < 0.05$, **$P < 0.01$ *vs.* Con

tion curve was shifted leftward in CIHH rats (Figure 3B). The PS (0.52±0.03 *vs.* 0.36 ± 0.02) and RD (52.58 ± 3.16 *vs.* 38.83 ± 2.35 mmHg) was increased significantly ($P < 0.01$), and the TP (52.83 ± 1.98 *vs.* 64.22 ± 1.30), EP (87.84 ± 4.37 *vs.* 103.08 ± 3.83), and SP (166.73 ± 3.53 *vs.* 179.30 ± 3.00 mmHg) were decreased significantly ($P < 0.05$-0.01, Table 1). Also the gain curve of CSB was shifted upward in CIHH rats (Figure 3C). It suggests that the baroreflex was enhanced in CIHH rats.

Compared with Fruc-fed rats the CSB functional curve was shifted leftward in CIHH+F rats (Figure 3B). The PS (0.43 ± 0.03 *vs.* 0.27 ± 0.03) and, RD (46.62 ± 2.73 *vs.* 30.67 ± 3.36 mmHg) were increased significantly ($P < 0.01$), and the TP (58.53 ± 2.82 *vs.* 76.28 ± 2.90), EP (94.20 ± 3.35 *vs.* 103.08

Table 2 Effect of Gli on function parameters of CSB in fructose-induced metabolic syndrome rats.

	TP/mmHg	EP/mmHg	SP/mmHg	OR/mmHg	PS	RD/mmHg
Con	64.22 ± 1.30	94.37 ± 2.72	171.93 ± 2.81	107.71 ± 1.78	0.36 ± 0.03	38.83 ± 2.35
Con + Gli	63.05 ± 1.35	94.04 ± 2.15	172.93 ± 1.35	109.88 ± 0.47	0.35 ± 0.03	38.72 ± 1.31
Fruc-fed	76.28 ± 2.90**	103.08 ± 3.83**	179.30 ± 3.00 **	103.01 ± 0.96**	0.27 ± 0.03**	30.67 ± 3.36**
Fruc-fed + Gli	72.76 ± 5.02$^{\#\#}$	103.08 ± 4.98$^{\#\#}$	177.62 ± 2.56$^{\#}$	104.67 ± 2.74$^{\#\#}$	0.27 ± 0.05$^{\#\#}$	31.23 ± 4.78$^{\#\#}$
CIHH	52.83 ± 1.98**	87.84 ± 4.37**	166.73 ± 3.53*	113.90 ± 1.98**	0.52 ± 0.03**	52.58 ± 3.16**
CIHH + Gli	61.70 ± 2.72$^{\#\#}$	95.71 ± 1.38$^{\#\#}$	172.59 ± 1.30	110.88 ± 1.60$^{\#\#}$	0.43 ± 0.05$^{\#\#}$	46.38 ± 2.40$^{\#\#}$
CIHH-F	59.20 ± 2.32**	94.20 ± 3.35	175.28 ± 2.07*	116.08 ± 3.97**	0.43 ± 0.04**	46.62 ± 2.73**
CIHH-F + Gli	63.72 ± 4.09^{+}	101.91 ± 6.24^{+}	175.61 ± 2.81	111.89 ± 1.61^{+}	0.36 ± 0.04^{++}	41.12 ± 2.99^{++}

Gli: glibenclamide, CSB: carotid sinus baroreflex, Con: Control group, Fruc-fed: Fruc-fed group, CIHH: CIHH group, CIHH-F: CIHH-F group, TP, threshold pressure; EP, equilibrium pressure; SP, saturation pressure; OR, operating range; PS, peak slope; RD, reflex decrease in MAP. The date was expressed as Mean ± SD $n = 6$ for each group. *$P < 0.05$, **$P < 0.01$ *vs.* Con., $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs.* Fruc-fed or CIHH, $^{+}P < 0.05$, $^{++}P < 0.01$ *vs.* CIHH-F

Table 3 Effect of L-NAME on function parameters of CSB in fructose-induced metabolic syndrome rats.

	TP/mmHg	EP/mmHg	SP/mmHg	OR/mmHg	PS	RD/mmHg
Con	64.22 ± 1.30	94.37 ± 2.72	171.93 ± 2.81	107.71 ± 1.78	0.36 ± 0.03	38.83 ± 2.35
Con + *L*-NAME	64.22 ± 2.25	95.04 ± 1.50	173.77 ± 2.66	109.55 ± 1.54	0.36 ± 0.02	38.57 ± 2.96
Fruc-fed	76.28 ± 2.90**	103.08 ± 3.83**	179.30 ± 3.00**	103.01 ± 0.90**	0.27 ± 0.03**	30.67 ± 3.36**
Fruc-fed + *L*-NAME	74.10 ± 3.20	103.08 ± 3.16	177.11 ± 1.80	103.69 ± 2.20	0.29 ± 0.04	31.05 ± 3.35
CIHH	52.83 ± 1.98**	87.84 ± 4.37**	166.73 ± 3.53*	113.90 ± 1.98**	0.52 ± 0.03**	52.58 ± 3.16**
CIHH + *L*-NAME	52.50 ± 2.37	89.36 ± 3.82	168.91 ± 4.28	116.42 ± 2.81	0.49 ± 0.02	52.69 ± 3.37
CIHH-F	59.20 ± 2.32**	94.20 ± 3.35	175.28 ± 2.07*	116.08 ± 3.97**	0.43 ± 0.04**	46.62 ± 2.73**
CIHH-F + *L*-NAME	57.69 ± 2.89	93.53 ± 5.24	176.62 ± 1.87	116.58 ± 2.09	0.43 ± 0.05	46.56 ± 4.39

CSB: carotid sinus baroreflex, Con: Control group, Fruc-fed: Fruc-fed group, CIHH: CIHH group, CIHH-F: CIHH-F group, TP: threshold pressure, EP: equilibrium pressure, SP: saturation pressure, OR: operating range, PS: peak slope, RD: reflex decrease in MAP. The date was expressed as Mean ± SD $n = 6$ for each group. *$P < 0.05$, **$P < 0.01$ *vs*. Con.

± 3.83) and SP (175.28 ± 2.07 *vs.* 179.30 ± 3.00 mmHg) were decreased significantly ($P < 0.05$ - 0.01, Table 1). Also the gain curve of CSB was shifted upward in CIHH+F rats (Figure 3C). It suggests that CIHH improves the baroreflex injury in Fruc-fed rats.

Effects of Glibenclamide on the Improvement of CSB by CIHH

Perfusion with K-H solution containing glibenclamide (Gli,10 umol/l), an ATP-sensitive potassium channel, had no effect on functional parameters of baroreflex in control and CIHH rats, but the change of TP, OR, PS and RD in CIHH+F rats was relieved by Gli ($P < 0.05$-0.01, Table 2). The result indicates that improvement of CSB by CIHH in Mets rats can be blocked partially by Gli.

Effects of L-NAME on the Improvement of CSB by CIHH

Perfusion with K-H solution containing L -NAME (100 μM) has no effect on CSB function parameters in all rats including CIHH+F rats ($P > 0.05$, Table 3), which indicates NO is not involved in improvement of CSB by CIHH.

Discussion

High fructose-fed rats can display the similar symptom in human Mets, such as hypertension, weight increase, hyperglycemia, hyperlipemia, and insulin

resistance after long-time feeding with high fructose, which is widely used for the Mets study (9). In this study, we explored the effect of CIHH on baroreflex by using perfusion technique in isolated carotid sinus area in the fructose-induced Mets rats. The result showed that the baroreflex curve was shifted rightward with the decrease of PS and RD, and increase of TP and EP in Mets rats, which suggests that CSB function is damaged. On the contrary, the baroreflex curve was shifted leftward with the increase of PS and RD, and the decrease of TP and EP, which suggests that CIHH treatment could improve the damaged CSB in Mets rats.

The elevated blood pressure is a cardiovascular risk factor. High blood pressure and cardiovascular damage are often existed in metabolic syndrome patients. Malik *et al.* reported that the incidence of hypertension was 84.2% for male and 76.7% for female in patients with Mets. Along with the increase of Mets constituents, blood pressure is also increased (2, 11). So anti-hypertension is a key strategy for prevention of cardiovascular damage in Mets. It was reported that CIHH has decompression effect in essential hypertension patients and spontaneously hypertensive rats (20, 24). Our previous study showed that CIHH decrease the elevated blood pressure in renal vascular hypertension rats, which might be related with the facilitation of baroreflex and the enhancement of relaxation function in resistant vessel (12, 17). In this study, the depression effect of CIHH was confirmed in Mets rats firstly, which suggests that depression effect of CIHH is universal for all kinds of hypertension.

The development and progress of hypertension in Mets involves several mechanisms. Firstly, hyperinsulinism in Mets can increase the excitability of sympathetic nerve system (26). Increased sympathetic tone activates adrenal gland and sympathetic system directly, activates the signaling pathway for promotion of cellular proliferation, and enhances proliferation and migration of arterial smooth muscle cell, leading to blood vessel remodeling. Also, plasma catecholamine is increased, resulting in blood pressure elevation (10). In addition, hyperinsulinism increases sensibility of vessel wall and kidney to salt, decrease Na^+ reabsorption in proximal kidney tubules and glomerular filtration, leading to water-sodium retention and blood pressure increase (7, 16). Secondly, the increased plasma free fatty acids (FFA) in lipid metabolism disturbance activated a non-oxidative pathway to increase ROS production; at same time redundant triglyceride deposited in cells (lipotoxicity) promotes tissue fibration and apoptosis, which is related with capillary vessel disfunction and hypertension (4). So the over-activity of sympathetic system, lipid metabolism disturbance and lipotoxicity are involved in the creation of hypertension in Mets. Kacimi et al reported that CIHH adaptation can decrease the sympathetic excitability and reactivity of myocardium to catecholamine (14). Our previous study showed that CIHH improved carbohydrate and lipid metabolic disturbance in Mets rats (36), which might be another mechanism for anti-hypertension effect of CIHH.

CSB, one of the most important regulation mechanisms for cardiovascular activity, plays key role in homeostasis of arterial blood pressure. When blood pressure elevated, the stretch of vessel wall stimulates the baroreceptor to firing. The afferent nerves impulse produces the increase of cardiac vagus nerve outflow and the decrease of cardiac sympathetic nerve outflow. As a result, the cardiac output is decreased due to the attenuated myocardial contraction and peripheral resistance is reduced due to arterial vessel relaxation, which results in the decrease of blood pressure. Conversely, the decreased blood pressure leads to the elevation of blood pressure through opposite process by the inhibition of reflex (28). Consistent with our previous study that CIHH decreased blood pressure through facilitating baroreflex in RVH rats (17), present study confirmed the facilitation effect of CIHH on baroreflex and demonstrated the anti-hypertension effect of CIHH through improvement the damaged baroreceptor reflex in fructose-feed-induced Mets rats.

NOMA *et al*. found the ATP-sensitive potassium channels (K_{ATP}) in isolated ventricular myocytes from guinea pigs in 1983 firstly (23). A great number of researches demonstrated that K_{ATP} were also existed in vascular smooth muscle cell (30). K_{ATP} channel is closed under normal physiological condition and open in ischemia/hypoxia. Activation of the K_{ATP} channels in vascular system can hyperpolarize the membrane of vascular smooth muscle (VSM) cell, relax VSM and enhance activity of the carotid sinus baroreceptor through stretching the wall of VSM (22). In the present study, we found that Gli, a non-selective blocker of K_{ATP} channels, could eliminate the facilitation of CIHH on the CSB partially, which suggests that opening of K_{ATP} channels is involved in the facilitation of CIHH on the CSB in fructose-feed-induced Mets rats.

Nitric oxide (NO), a key signal molecule, is distributed widely in the various tissues like vessel system, central nerve system, and immune system and plays an important role in the regulation of body function (21). NO is generated from L-arginine by NO synthase (NOS) in multiple cells (include neuron). Vascular endothelial cell can release NO into blood under stimulation of pressure-stretch or chemicals. It was reported that NOS is existed in sensory neurons abundantly, suggesting the regulation of NO

on baroreceptors activity. In this study, however, the perfusion with K-H solution containing NOS inhibitor L-NAME (100 μmol/l) had no effect on the facilitation of CIHH on the CSB, which suggests that NO does not contribute to the improvement of CIHH on baroreflex in fructose-fed-induced Mets rats.

In conclusion, this study demonstrated for the first time that CIHH decreases the high blood pressure through facilitation of carotid sinus baroreceptor reflex in fructose-fed-induced Mets rats. The facilitation effect of CIHH on CSB is might related with the activation of K_{ATP} channels.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Aguilar, M., Gonzalez-Candia, A., Rodriguez, J., Carrasco-Pozo, C., Canas, D., Garcia-Herrera, C., Herrera, E.A. and Castillo, R.L. Mechanisms of cardiovascular protection associated with intermittent hypobaric hypoxia exposure in a rat model: Role of oxidative stress. *Int. J. Mol. Sci.* 19: 366, 2018.
2. Aijaz, B., Ammar, K.A., Lopez-Jimenez, F., Redfield, M.M., Jacobsen, S.J. and Rodeheffer, R.J. Abnormal cardiac structure and function in the metabolic syndrome: a population-based study. *Mayo. Clin. Proc.* 83: 1350-1357, 2008.
3. Araujo, P.A.O., Silva, M.G., Borba, E.F. and Shinjo, S.K. High prevalence of metabolic syndrome in antisynthetase syndrome. *Clin. Exp. Rheu.* 36: 241-247, 2018.
4. de Jongh, R.T., Serne, E.H., Ijzerman, R.G., de Vries, G. and Stehouwer, C.D. Free fatty acid levels modulate microvascular function: relevance for obesity-associated insulin resistance, hypertension, and microangiopathy. *Diabetes* 53: 2873-2882, 2004.
5. Ding, H.L., Zhu, H.F., Dong, J.W., Zhu, W.Z., Yang, W.W., Yang, H.T. and Zhou, Z.N. Inducible nitric oxide synthase contributes to intermittent hypoxia against ischemia/reperfusion injury. *Acta. Pharmacol. Sin.* 26: 315-322, 2005.
6. Ding, H.L., Zhu, H.F., Dong, J.W., Zhu, W.Z. and Zhou, Z.N. Intermittent hypoxia protects the rat heart against ischemia/reperfusion injury by activating protein kinase C. *Life Sci.* 75: 2587-2603, 2004.
7. Fujita, T. Insulin resistance and salt-sensitive hypertension in metabolic syndrome. *Nephrol. Dia. Transplant* 22: 3102-3107, 2007.
8. Gao, L., Guan, Y., Cui, F., Liu, Y.X., Zhou, Z.N. and Zhang, Y. Facilitation of chronic intermittent hypobaric hypoxia on carotid sinus baroreflex in anesthetized rats. *Chinese. J. Physiol.* 55: 62-70, 2012.
9. Giani, J.F., Mayer, M.A., Munoz, M.C., Silberman, E.A., Hocht, C., Taira, C.A., Gironacci, M.M., Turyn, D. and Dominici, F.P. Chronic infusion of angiotensin-(1-7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. *Am. J. Physiol.* 296: E262-E271, 2009.
10. Grassi, G. Sympathetic overdrive and cardiovascular risk in the metabolic syndrome. *Hypertensions Res.* 29: 839-847, 2006.
11. Grundy, S.M. Pre-diabetes, metabolic syndrome, and cardiovascular risk. *J. Am. Coll. Cardiol.* 59: 635-643, 2012.
12. Guan, Y., Gao, L., Ma, H.J., Li, Q., Zhang, H., Yuan, F., Zhou, Z.N. and Zhang, Y. Chronic intermittent hypobaric hypoxia decreases beta-adrenoceptor activity in right ventricular papillary muscle. *Am. J. Physiol.* 298: H1267-1272, 2010.
13. Kachur, S., Morera, R., De Schutter, A. and Lavie, C.J. Cardiovascular Risk in Patients with Prehypertension and the Metabolic Syndrome. *Curr. Hypertens. Rep.* 20, 15, 2018.
14. Kacimi, R., Richalet, J.P. and Crozatier, B. Hypoxia-induced differential modulation of adenosinergic and muscarinic receptors in rat heart. *J. Appl.Physiol.* 75: 1123-1128, 1993.
15. Kishi, T., Hirooka, Y., Kimura, Y., Sakai, K., Ito, K., Shimokawa, H. and Takeshita, A. Overexpression of eNOS in RVLM improves impaired baroreflex control of heart rate in SHRSP. Rostral ventrolateral medulla. Stroke-prone spontaneously hypertensive rats. *Hypertension* 41, 255-260, 2003.
16. Krikken, J.A., Lely, A.T., Bakker, S.J. and Navis, G. The effect of a shift in sodium intake on renal hemodynamics is determined by body mass index in healthy young men. *Kidney Int.* 71: 260-265, 2007.
17. Li, N., Guan, Y., Zhang, L., Tian, Y., Zhang, Y. and Wang, S. Depressive effects of chronic intermittent hypobaric hypoxia on renal vascular hypertension through enhancing baroreflex. *Chinese. J. Physiol.* 59: 210-217, 2016.
18. Li, W., Song, F., Wang, X., Wang, L., Wang, D., Yin, X., Cao, S., Gong, Y., Yue, W., Yan, F., Zhang, H., Sheng, Z., Wang, Z. and Lu, Z. Prevalence of metabolic syndrome among middle-aged and elderly adults in China: current status and temporal trends. *Ann. Med.* 50: 345-353, 2018.
19. Mallet, R.T., Manukhina, E.B., Ruelas, S.S., Caffrey, J.L. and Downey, H.F. Cardioprotection by Intermittent Hypoxia Conditioning: Evidence, Mechanisms and Therapeutic Potential. *Am. J. Physiol.* 315: H216-H232, 2018.
20. Meerson, F.Z., Barbarash, N.A., Dvurechenskaia, G., Prokina, N.S. and Saltykova, V.A. [Natriuretic and antihypertensive effects of acute hypoxia in animals with spontaneous hereditary hypertension]. *Biull. Eksp. Biol.* 90: 142-144, 1980.
21. Moncada, S., Palmer, R.M. and Higgs, E.A. Nitric oxide: physiology, pathophysiology, and pharmacology . *Pharmacol. Rev.* 43: 109-142, 1991.
22. Morris, C.E. Mechanosensitive ion channels. *J. Membr. Biol.* 113: 93-107, 1990.
23. Noma, A. ATP-regulated K+ channels in cardiac muscle. *Nature* 305: 147-148, 1983.
24. Serebrovskaya, T.V., Manukhina, E.B., Smith, M.L., Downey, H.F. and Mallet, R.T. Intermittent hypoxia: cause of or therapy for systemic hypertension? *Exp. Biol. Med.* 233: 627-650, 2008.
25. Shimamoto, K. and Ura, N. Mechanisms of insulin resistance in hypertensive rats. *Clin. Exp. Hypertens.* 28: 543-552, 2006.
26. Stepniakowski, K.T., Goodfriend, T.L. and Egan, B.M. Fatty acids enhance vascular alpha-adrenergic sensitivity. *Hypertension* 25: 774-778, 1995.
27. Vitela,, M., Herrera-Rosales, M., Haywood, J.R. and Mifflin, S.W. Baroreflex regulation of renal sympathetic nerve activity and heart rate in renal wrap hypertensive rats. *Am .J. Physiol.* 288: R856-862, 2005.
28. Wallbach, M. and Koziolek, M.J. Baroreceptors in the carotid and hypertension-systematic review and meta-analysis of the effects of baroreflex activation therapy on blood pressure. *Nephrol. Dial. Transplant.* 33: 1485-1493, 2017.
29. Zhang, Y., Zhong, N., Gia, J. and Zhou, Z. Effects of chronic intermittent hypoxia on the hemodynamics of systemic circulation in rats. *Jan. J. Physiol.* 54: 171-174, 2004.
30. Zhang, Y., Zhong, N. and Zhou, Z.N. Effects of intermittent hypoxia on action potential and contraction in non-ischemic and ischemic rat papillary muscle. *Life Sci.* 67: 2465-2471, 2000.
31. Zhang, Y., Zhong, N., Zhu, H.F. and Zhou. Z,N. [Antiarrhythmic and antioxidative effects of intermittent hypoxia exposure on rat myocardium]. *Sheng Li Xue Bao* 52: 89-92, 2000.
32. Zhang, Y. and Zhou, Z.N. Beneficial effects of intermittent hypobaric hypoxia on the body. *Zhongguo yingyong shenglixue zazhi* 28: 504-509, 2012.
33. Zhong, N., Zhang, Y., Fang, Q.Z. and Zhou, Z.N. Intermittent

hypoxia exposure-induced heat-shock protein 70 expression increases resistance of rat heart to ischemic injury. *Acta. Pharmacol. Sin.* 21: 467-472, 2000.

34. Zhong, N., Zhang, Y., Zhu, H.F., Wang, J.C., Fang, Q.Z. and Zhou, Z.N. Myocardial capillary angiogenesis and coronary flow in ischemia tolerance rat by adaptation to intermittent high altitude hypoxia. *Acta. Pharmacol. Sin.* 23: 305-310, 2002.
35. Zhong, N., Zhang, Y., Zhu, H.F. and Zhou, Z.N. Intermittent hypoxia exposure prevents mtDNA deletion and mitochondrial structure damage produced by ischemia/reperfusion injury. *Sheng Li Xue Bao* 52: 375-380, 2000.
36. Zhou, J.J., Wei ,Y., Zhang, L., Zhang, J., Guo, L.Y., Gao, C., Li, D.P. and Zhang, Y. Chronic intermittent hypobaric hypoxia prevents cardiac dysfunction through enhancing antioxidation in fructose-fed rats. *Can. J. Physiol. Pharmacol.* 91: 332-337, 2013.
37. Zhu, H.F., Dong, J.W., Zhu, W.Z., Ding, H.L. and Zhou, Z.N. ATP-dependent potassium channels involved in the cardiac protection induced by intermittent hypoxia against ischemia/reperfusion injury. *Life sci.* 73: 1275-1287, 2003.
38. Zhuang, J. and Zhou, Z. Protective effects of intermittent hypoxic adaptation on myocardium and its mechanisms. *Biol. Signals Receptor* 8: 316-322, 1999.