Influence of cardiac function on intermittent hypoxia in rats fed with high-fat diet

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ABSTRACT

A high-fat diet (HFD) accumulates fat in the cardiovascular system, alters the metabolism, and affects cardiac function. Dyslipidemia is associated with the development of sleep apnea syndrome (SAS), which is associated with intermittent hypoxia (IH); however, it is unclear whether SAS affects cardiac function in patients with dyslipidemia. The purpose of this study was to evaluate how IH affects cardiac function in rats fed with a HFD. Male 5-week-old Sprague-Dawley rats of two groups (normal diet (SD) and HFD) were divided into IH-exposed and unexposed groups. Zinc protoporphyrin-9 (ZnPPIX) was administered as a heme oxygenase-1 (HO-1) inhibitor to the SD and IH + HFD groups, and cardiac function and blood viscosity were examined. In the IH + HFD group, echocardiography showed an increased fractional shortening (FS), which peaked on day 4. Western blot analysis revealed an increase in HO-1 after 2 weeks. This peak continued even after the HO-1 inhibitor and ZnPPIX were administered. One cause of increased FS is the stagnation of blood flow due to an increased blood viscosity. To be able to send highly viscous blood to every corner of the body, the heart must contract strongly. Therefore, HO-1 is released by the body as a biological defense reaction. HO-1 has a vasodilatory effect and suppresses hyper constriction. Thus, IH exposure to HFD causes and drives transient hyper constriction, releasing HO-1 as a biological response. This led to dilated blood vessels, after which the FS returned to normal.

1. Introduction

Hypertension, cardiac remodeling, and other complications of SAS have been studied using rodent models of intermittent hypoxia (IH) induced by short cycles of hypoxia-normoxia [1–3]. Suppression of the protein kinase B (PKB), also known as Akt, mammalian target of rapamycin (mTOR) pathway (Akt-mTOR pathway) using a proprietary IH exposure device induces autophagy in rat myocardium, and blocking this induction with an inhibitor has been reported to lead to heart failure [4]. This suggests that autophagy suppresses the heart failure (HF) development in the early stages of SAS. No other confounding factors were found in the early stages of SAS. Obesity, hyperlipidemia, diabetes, and non-alcoholic fatty liver disease (NAFLD) are thought to increase the risk of cardiovascular diseases in patients with SAS. However, the association between SAS and dyslipidemia remains controversial.

SAS correlates with diabetes, hypertriglyceridemia, increased interleukin-6 (IL-6), insulin resistance, fatty liver, liver fibrosis, and inflammation [5], high density lipoprotein-cholesterol (HDL-C), but not with cholesterol, triglycerides (TG), or low-density lipoprotein-C (LDL-C) [6]. It is associated with elevated C-reactive protein (CRP) levels, insulin resistance, hepatitis, ballooning, and fibrosis, but not fatty liver [7]. We previously reported that lipid accumulation in the liver after HFD loading improved after IH exposure [8].

HFD has been thought to promote the heart failure due to pressure loading and ischemia-reperfusion, but recently, the effect of HFD on the heart failure has been attracting attention [9]. After HFD loading, peroxisome proliferator-activated receptor-alpha/beta/gamma (PPAR-α/β/γ) transcriptional activation induces the fat-metabolizing enzyme CD36 and suppresses the onset of the heart failure in a pathological model [10–12]. Conversely, in diabetes/obesity models (STZ, Kir6.2, ob/ob), fatty acid synthesis decreased, L-type Ca2+ channel activity increased, and Ca2+/Calmodulin-Dependent Protein Kinase II (CaMKII) activated immediately after pressure loading and sudden death due to arrhythmia occurred [13].

Fatty acid metabolism is enhanced by acute pressure loading and beta-adrenergic receptors (β-AR) stimulation, but fatty acid metabolism

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disorders are less in the early stages of the heart failure and become more prominent as heart failure progresses, and inhibitors of fatty acid-metabolizing enzymes, such as CPT1, are useful for treating the heart failure [14].

Obesity has been implicated in increased risk of heart disease, including arrhythmias and ischemic heart disease [15–19]. Obesity caused by HFD leads to the accumulation of fat in the cardiovascular system, changes metabolism, and affects the heart function. Obesity and lipid abnormalities are associated with the onset of SAS, which is associated with intermittent hypoxia.

SAS and dyslipidemia were recapitulated by IH and a HFD, respectively, in rats. It is known that IH causes hemoconcentration, and dyslipidemia increases blood viscosity, both of which are risk factors for thrombus formation.

It remains unclear whether SAS affects cardiac function in patients with dyslipidemia, and whether dyslipidemia promotes thrombosis. The purpose of this study was to investigate the effects of IH exposure on cardiac function after HFD loading.

2. Materials and methods

2.1. Animal model

All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85–23, revised 1996). All experiments performed in this study were approved by the Experimental Animal Committee of Tokyo Medical University (approval numbers: H310009, R2-0127, and R3-0042).

Male 5-week-old Sprague–Dawley rats were divided into two groups according to the diet they consumed: the standard diet (SD) and HFD groups. After 2 weeks, they were further divided into two groups based on IH exposure: the IH-exposed and non-exposed groups. After 2 weeks, they were further divided into two groups based on IH exposure: the IH-exposed and non-exposed groups. After 2 weeks, they were further divided into two groups based on the treatment: the SD and IH groups.

2.2. Echocardiographic studies

Echocardiography was performed under inhalational anesthesia with 2.5 L/min isoflurane (Mylan Inc., Tokyo, Japan) using Noblus (Hitachi Ltd., Tokyo, Japan). A 2D parasternal short-axis view of the left ventricle (LV) was obtained at the level of the papillary muscles. When suitable images were obtained on the axis, 2D targeted M-mode tracings were recorded. Two consecutive heartbeats in each frame were analyzed to measure the heart rate (HR), LV anterior wall thickness in diastole (LAVWd) and systole (LAVWs), LV posterior wall thickness in diastole (LPWd) and systole (LPWs), LV internal dimensions at end diastole (LVDd), and LV internal dimensions at end systole (LVDs). LV fractional shortening (FS) was calculated as LVDd minus LVDs normalized for LVDd as an index of LV systolic performance. We confirmed very small intra-and inter-observer variability, along with high reproducibility, in the same animals as those used in our echocardiographic measurements.

2.3. Western blot analysis

The homogenized proteins from the frozen hearts were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 15% gel) and subjected to Western blot analysis. The blots were probed with one of the following primary antibodies: HO-1 (ab13248) and GAPDH (ab8245) (all from Abcam, Richmond, Canada). For chemiluminescence detection, peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG (Promega, Madison, WI, USA) was used as the secondary antibody. Band densities were measured using an ImageQuant™ LAS 4000 mini (GE Healthcare UK Ltd., Buckinghamshire, England).

2.4. Hematology and blood biochemistry

The blood collected was quickly divided into two evacuated blood collection tubes. One used Venetject™ vacuum blood collection tubes.
Influence of IH 4 days on hematology, body weight, and the heart weight.

These hematological analyses measured red blood cell (RBC) count, hemoglobin (Hb) concentration, and hematocrit (Ht) level. Blood biochemical analyses measured Total cholesterol (T-cho), TG, and LDL-C level. These analyses were performed by Kotobiken Medical Laboratories (Tokyo, Japan). The measurement of blood fluidity with an MC-FAN (MC Healthcare Software, San Diego, CA, USA) rheometer was the same as that described by Kikuchi Inc., Tokyo, Japan). The MC-FAN – microchannel method [20,21]. Briefly a V-shaped groove (width, 7 μm; length, 30 μm; depth, 4.5 μm) was made on a 15 × 15 × 0.5 mm silicon single crystal substrate of an integral circuit, using an anisotropic etching technique. Blood samples were collected via cardiac puncture under anesthesia. Heparin solution (1000 IU/mL, 0.1 mL) was added to 2 mL of the collected blood samples and used for the measurement.

2.5. Measurement of blood fluidity

The measurement of blood fluidity with an MC-FAN (MC Healthcare Inc., Tokyo, Japan) rheometer was the same as that described by Kikuchi’s microchannel method [20,21]. The measurement of blood fluidity was performed in GraphPad Prism 6 for Windows, version 6.05 (GraphPad Software, San Diego, CA, USA).

2.6. Statistical analysis

Group data are expressed as mean ± standard error of the mean. Data were analyzed using Dunnnett’s test, Tukey–Kramer test, or Student’s t-test at a confidence level of 95%. One-way analysis of variance was used for statistical comparisons of the recorded observational data, followed by pairwise post hoc comparisons. Statistical analysis was performed in GraphPad Prism 6 for Windows, version 6.05 (GraphPad Software, San Diego, CA, USA).

**Table 1**

Influence of IH 4 days on hematology, body weight, and the heart weight.

|                | SD n = 8 | SD n = 6 | SD + IH n = 8 | SD + ZnPPIX n = 4 | HFD n = 6 | HFD + IH n = 8 | HFD + IH n = 15 | HFD + IH + ZnPPIX n = 4 | HFD + IH + ZnPPIX n = 6 |
|----------------|----------|----------|--------------|-----------------|-----------|--------------|----------------|-------------------------|-------------------------|
| HW(g)          | 1.0 ± 0.039 | 0.92 ± 0.028 | 0.91 ± 0.041* | 0.85 ± 0.023** | 0.87 ± 0.023 | 0.94 ± 0.11    | 0.94 ± 0.11    | 232.5 ± 10.2**          | 4.0 ± 0.28               |
| BW(g)          | 278.8 ± 6.22 | 251.2 ± 10.2** | 249.1 ± 6.67** | 230.9 ± 4.18** | 258.5 ± 1.94* | 232.5 ± 10.2** | 4.0 ± 0.28               | 232.5 ± 10.2**          | 4.0 ± 0.28               |
| HW/BW(%)       | 3.5 ± 0.12  | 3.7 ± 0.12  | 3.7 ± 0.15    | 3.7 ± 0.074    | 3.4 ± 0.077  | 3.4 ± 0.077    | 3.4 ± 0.077    | 3.4 ± 0.077    | 3.4 ± 0.077               |
| RBC (× 10^12 μL^-1) | 661 ± 20.2 | 655 ± 18.7 | 741 ± 14.8 | 746 ± 20.5*  | 699 ± 4.77  | 824 ± 47.3**   | 824 ± 47.3**   | 17.4 ± 1.15**          | 52.9 ± 3.10**             |
| Ht(%)          | 44.6 ± 1.32 | 42.6 ± 1.40 | 46.8 ± 1.03  | 49.5 ± 1.15*  | 46.4 ± 0.525 | 52.9 ± 3.10**  | 52.9 ± 3.10**  | 17.4 ± 1.15**          | 52.9 ± 3.10**             |
| T-cho(mg/dL)   | 63.5 ± 3.41 | 74.2 ± 3.08 | 63.9 ± 3.85  | 76.0 ± 5.51   | 52.5 ± 4.03  | 77.5 ± 3.52*   | 77.5 ± 3.52*   | 77.5 ± 3.52*          | 77.5 ± 3.52*              |
| TG(mg/dL)      | 114 ± 9.45  | 149 ± 21.5  | 82.3 ± 11.4  | 91.9 ± 5.99   | 90.3 ± 10.1  | 82.8 ± 32.3    | 82.8 ± 32.3    | 82.8 ± 32.3            | 82.8 ± 32.3               |
| LDL-C(mg/dL)   | 8.13 ± 0.581 | 12.8 ± 0.792* | 8.00 ± 0.535 | 11.7 ± 0.796* | 10.0 ± 1.08 | 14.0 ± 1.41*   | 14.0 ± 1.41*   | 14.0 ± 1.41*          | 14.0 ± 1.41*              |

*p < 0.01 vs. SD, **p < 0.01 vs. SD. Data are mean ± SEM.
Influence of IH for 2 weeks on hematology, body weight, and the heart weight.

|                         | SD            | HFD           | SD + IH        | HFD + IH       | SD + ZnPPIX     | HFD + IH + ZnPPIX |
|-------------------------|---------------|---------------|---------------|---------------|-----------------|-------------------|
| Hb (g/dL)               | 14.0 ± 0.009  | 14.2 ± 0.007  | 16.0 ± 0.005  | 17.0 ± 0.003  | 17.2 ± 0.004    | 17.7 ± 0.005      |
| LVAW (mm)               | 30.0 ± 0.004  | 30.0 ± 0.004  | 30.0 ± 0.004  | 30.0 ± 0.004  | 30.0 ± 0.004    | 30.0 ± 0.004      |
| LVDd (mm)               | 54.7 ± 0.013  | 54.9 ± 0.016  | 57.0 ± 0.018  | 57.5 ± 0.021  | 57.7 ± 0.023    | 57.9 ± 0.025      |
| FS (%)                  | 54.7 ± 0.013  | 54.9 ± 0.016  | 57.0 ± 0.018  | 57.5 ± 0.021  | 57.7 ± 0.023    | 57.9 ± 0.025      |
| LVAm (mm)               | 1.65 ± 0.016  | 1.65 ± 0.016  | 1.65 ± 0.016  | 1.65 ± 0.016  | 1.65 ± 0.016    | 1.65 ± 0.016      |
| LVAm (mm)               | 0.0277 ± 0.002 | 0.0277 ± 0.002 | 0.0277 ± 0.002 | 0.0277 ± 0.002 | 0.0277 ± 0.002 | 0.0277 ± 0.002 |
| LVAm (mm)               | 0.0442 ± 0.004 | 0.0442 ± 0.004 | 0.0442 ± 0.004 | 0.0442 ± 0.004 | 0.0442 ± 0.004 | 0.0442 ± 0.004 |
| LVAm (mm)               | 0.0139 ± 0.001 | 0.0139 ± 0.001 | 0.0139 ± 0.001 | 0.0139 ± 0.001 | 0.0139 ± 0.001 | 0.0139 ± 0.001 |
| LVAm (mm)               | 0.0817 ± 0.006 | 0.0817 ± 0.006 | 0.0817 ± 0.006 | 0.0817 ± 0.006 | 0.0817 ± 0.006 | 0.0817 ± 0.006 |
| LVAm (mm)               | 0.0436 ± 0.003 | 0.0436 ± 0.003 | 0.0436 ± 0.003 | 0.0436 ± 0.003 | 0.0436 ± 0.003 | 0.0436 ± 0.003 |
| LVAm (mm)               | 0.108 ± 0.006  | 0.108 ± 0.006  | 0.108 ± 0.006  | 0.108 ± 0.006  | 0.108 ± 0.006    | 0.108 ± 0.006      |
| HR (bpm)                | 9.90 ± 0.45   | 9.90 ± 0.45   | 9.90 ± 0.45   | 9.90 ± 0.45   | 9.90 ± 0.45      | 9.90 ± 0.45        |

*p < 0.01 vs. SD. **p < 0.01 vs. SD. Data are mean ± SEM.

Table 3: Influence of IH for 4 days on Echocardiographic studies.

| SD    | HFD  | SD + IH | HFD + IH | HFD + ZnPPIX | HFD + IH + ZnPPIX |
|-------|------|---------|----------|--------------|-------------------|
| FS (%)| 54.7 ± 0.013 | 54.9 ± 0.016 | 57.0 ± 0.018 | 57.7 ± 0.023 | 57.9 ± 0.025 |
| LVAWd | 30.0 ± 0.004 | 30.0 ± 0.004 | 30.0 ± 0.004 | 30.0 ± 0.004 | 30.0 ± 0.004 |
| LVDd  | 54.7 ± 0.013 | 54.9 ± 0.016 | 57.0 ± 0.018 | 57.7 ± 0.023 | 57.9 ± 0.025 |
| BW    | 0.0292 ± 0.001 | 0.0292 ± 0.001 | 0.0292 ± 0.001 | 0.0292 ± 0.001 | 0.0292 ± 0.001 |
| HR    | 37.7 ± 0.017 | 37.7 ± 0.017 | 37.7 ± 0.017 | 37.7 ± 0.017 | 37.7 ± 0.017 |

*p < 0.01 vs. SD. **p < 0.01 vs. SD. Data are mean ± SEM.

ventricular heart rate increased in the HFD, SD + IH, and HFD + IH groups.

Echocardiographic data of 2 weeks IH exposure (Table 4) showed a significant increase in FS in the HFD + IH + ZnPPIX group alone. Additionally, narrowing of the left ventricular cavity was observed. The anterior and posterior walls of the left ventricle thickened only in the HFD + IH + ZnPPIX group during contraction and diastole. The heart rate increased in the SD + IH, HFD + IH, and HFD + IH + ZnPPIX groups.

Fig. 2 shows the M-mode echocardiography. Left ventricular hyperconstriction was observed in the HFD + IH and HFD + IH + ZnPPIX groups on day 4 of IH exposure (Fig. 2A), and left ventricular hyperconstriction was observed on day 2 in HFD + IH + ZnPPIX group (Fig. 2B). This is consistent with the results for FS and left ventricular stenosis in Tables 3 and 4.

Changes in FS were plotted over time at 0 day, 4 days, 1 week, and 2 weeks of IH exposure (Fig. 3). No changes in FS were observed in the SD, HFD, SD + IH, or SD + ZnPPIX groups (Fig. 3A). Comparing the IH + HFD group (long dashed line) and the IH + HFD + ZnPPIX group (short dashed line) with SD as the control, the FS hyperconstriction in the IH + HFD group peaked on day 4 and gradually became the same. FS hyperconstriction persisted in the ZnPPIX group.

3.3. Western blots analysis of HO-1 in myocardium

The expression of HO-1 in the myocardium was analyzed by western blotting (Fig. 4A). HO-1 was induced 4 days after IH in HFD rats, as detected by Western blot analysis. Similar results were obtained after 2 weeks of IH exposure (Fig. 4B). The expression of HO-1 was suppressed with the administration of ZnPPIX (Fig. 4B). Administration of ZnPPIX had no effect on HO-1 expression compared with that in the SD group (Fig. 4B).

3.4. Measurement of blood flow rate using the MC-FAN

Blood flow was measured 2 weeks after IH exposure using the MC-FAN. It is a device that measures the time it takes for a certain amount of blood (100 μL) to pass through, by applying a certain pressure to the collected blood (whole blood), through the groove of a silicon chip that has been finely processed to the size of a capillary vessel (7 μm). Simultaneously, the state of blood cells passing through the groove of the silicon chip can be magnified approximately 2000 times with a microscope, and the image can be displayed on the monitor via the CCD (Charge Coupled Device) camera (Fig. 5A). It can be seen that blood flow is stagnant in the HFD + IH and HFD + IH + ZnPPIX groups compared to the SD group (Fig. 5A and B).

Four SD rats (SD = 4, IH = 4, HFD + IH = 4, HFD + IH + ZnPPIX = 4) were included in the study.

*p < 0.01 vs. SD. **p < 0.01 vs. SD. Data are mean ± SEM.
4. Discussion

There have been clinical cases of SAS and sudden death. However, the underlying mechanisms remain unclear. In addition to sleep apnea, there are several factors associated with sudden death. The present study considered sleep apnea and a HFD as factors.

To the best of our knowledge, this is the first study to explain the mechanism of IH-induced hypercontraction in an animal model loaded with an HFD. The present study explains that hypercontraction is caused by the viscosity of blood and is ameliorated by the release of HO-1. Hypercontraction continued when the release of HO-1 was suppressed by ZnPPIX.

4.1. Cardiac effects of IH exposure due to high fat load

IH exposure caused weight loss (Tables 1 and 2). HFD loading increased the heart weight per body weight in the second week of IH exposure. One of the causes of cardiac hypertrophy is repeated excessive contraction of the heart muscle due to high blood pressure, which is caused by the thickening of the heart muscle. Evaluation of myocardial thickness revealed that the anterior and posterior walls of the myocardium thickened during contraction on day 4 of IH exposure due to HFD loading. In the second week, thickening was seen only in the anterior wall during contraction in the HFD + IH + ZnPPIX group (Figs. 3 and 4). This is consistent with the fact that the myocardium was excessively thickened during contraction due to the hypercontraction of the heart on the 4th day, but the hypercontraction was released in the 2nd week, and at the same time, the heart muscle thickening reduced. The thickness of the diastolic ventricular wall was not significantly different between the groups on days 4 and 14 after IH exposure. Excessive contraction of the heart is represented in "Takotsubo syndrome," caused by sudden stress. This was accompanied by partial hypercontraction of the apex of the heart [22, 23]. In our model, hypercontraction peaks and gradually returns to normal 4–5 days after intermittent hypoxic exposure to a HFD. Therefore, intermittent hypoxic stress alone did not cause hypercontraction. Therefore, it differs from the commonly known stress response [24].

Hypercontraction is thought to be the pumping action that pumps blood flow throughout the body owing to stagnation of blood flow. It is widely known that hypoxia causes hemoconcentration [25–28]. Hypercholesterolemia also reduces nitric oxide (NO) production from vascular endothelial cells and reduces vascular protective function.
but in addition to NO production, blood viscosity elevates blood concentration, white blood cell attachment, increases platelet aggregation [32,33], and decreases tissue circulation. Hypercholesterolemia reduces the ability of red blood cells to deform [34]. In particular, changes in the lipid composition of cell membranes are known to reduce erythrocyte deformability [35,36], which is an important factor in controlling organ microcirculation. Furthermore, erythrocytes are not only affected by NO supplied from the vascular endothelium [37–39] but also by endothelial NO synthase (eNOS) [40,41], which is involved in microcirculation by producing NO. Experimental results also showed stagnation of blood flow due to blood concentration (Tables 1 and 2) and erythrocyte deformability (Fig. 5).

Therefore, we used MC-FAN [42–44] to assess the blood flow. As shown in the results, blood flow can be seen through a slit with a width of 7 μm that mimics capillaries; however, blood flow usually flows like SD or SD + IH. However, when blood flow is stagnant, blood components accumulate on the slit, such as with HFD + IH and HFD + IH + ZnPPIX, and do not flow smoothly from top to bottom (Fig. 5A). Blood flow and stagnation were observed with HFD + IH and HFD + IH + ZnPPIX (Fig. 5A and B), but echocardiography did not show hypercontraction with HFD + IH. This will be explained in the next section.

### 4.2. Release of HO-1 with vasodilatory effect

As shown in the previous section, HFD + IH at 2 weeks of IH exposure did not cause myocardial hypercontraction despite the stagnation of blood flow. This is probably due to the improved blood flow.

As shown in Fig. 4, an increase in HO-1 was observed in the HFD + IH group after both 4 and 2 weeks of intermittent hypoxic exposure. A protein called HO-1 is used to break down heme to biliverdin. Biliverdin...
releases iron and carbon monoxide and acts on vasodilation [45]. In addition, HO-1 expression was suppressed by ZnPPIX [46]. As shown in Fig. 4, intraperitoneal administration of ZnPPIX in the HFD + IH group inhibited HO-1 expression. Hypercontraction persisted without vasodilation owing to HO-1 inhibition (Fig. 3B). Regarding blood flow, as explained in the previous section, stagnation occurred in the HFD + IH and HFD + IH + ZnPPIX groups during the second week of intermittent hypoxic exposure. In other words, there was a delay in the blood flow time in both groups, and echocardiography did not cause myocardial hypercontraction in the HFD + IH group, but it did cause myocardial hypercontraction in the HFD + IH + ZnPPIX group. The results of this experiment showed that vasodilation improved blood flow. This is considered to be due to the biocompatibility of the individual.

Heme oxygenase is induced by hemodynamic forces of the vascular smooth muscle and endothelial cells. Using the mesenteric artery ligation model [47], Mohamed Lamine Freidja et al. reported the involvement of HO-1 in flow (shear force) dependent remodeling using the mesenteric artery ligation model [47]. It depends on NO produced by shear stress and mitochondrial hydrogen peroxide [47]. In our experiments, we speculated that the addition of a HFD induced HO-1, stagnating red cell flow, and dilating blood vessels. Stagnation of blood flow can induce thrombosis but was not found during the observation period.

4.3. Clinical significance

In patients with SAS, sleep apnea attacks are said to cause sudden cardiac death due to myocardial infarction and heart failure [48–50]. As SAS may be associated with a variety of underlying disorders, we also investigated its relationship with a HFD. Experiments have shown that the combination of hypoxia, which affects blood levels, and a HFD, which interferes with the ability of red blood cells to deform, causes hypercontraction of the heart, resulting in the release of HO-1. Dilatation of the blood vessels normalizes cardiac function. However, if the release of HO-1 is blocked, hypercontraction continues. Hypercholesterolemia not only weakens blood vessels, but also affects cardiac function when hypoxia is present. Persistent hypercontraction can cause cardiac hypertrophy. However, the heart function of patients with hypercholesterolemia appears normal.

HO-1 may be released by biological adaptation, suggesting the need for treatment of the underlying hypercholesterolemia.

5. Summary

Intermittent hypoxic exposure in a rat model of intermittent hypoxia supplemented with a HFD causes peak myocardial hypercontraction on days 4–5. Myocardial hypercontraction is caused by an increase in the blood viscosity. The heart contracts strongly, pumping viscous blood to every corner of the body and causing myocardial hypercontraction. Therefore, HO-1 is released as part of a biological defense mechanism. HO-1 has a vasodilatory effect and suppresses hyper contractions. Therefore, IH exposure to HFD causes and promotes transient hypercontraction, releasing HO-1 as a biological response. We conclude that the blood vessels were dilated, and the EF returned to normal. However, it needs to be stressed that even if EF returns to normal levels, it is not a radical cure.

6. Novelty and significance

6.1. What is known

- HFD has been thought to promote heart failure due to pressure loading, ischemia-reperfusion, etc., but recently, the heart failure-suppressing effect of HFD has been attracting attention.

6.2. “What new information does this article contribute?”

Rats model of IH supplemented with a HFD causes cardiac hypercontraction in a few days, but the release of HO-1 dilates blood vessels and suppresses cardiac hypercontraction. Apparently, EF returns to normal, but it is not a radical cure.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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