### Original Article Protective effects of exercise on endotoxin-induced acute lung injuries in obese rats

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**Abstract:** Background: The aim of this study was to investigate the effects of exercise on endotoxin-induced acute lung injuries in obese rats. Methods: Male Sprague-Dawley rats were randomly divided into three groups: normal diet (N), high fat diet (HF), and high fat diet + exercise (HF+E). N group rats were fed with a normal diet. HF group and HF+E group rats were fed with a high fat diet for 10 weeks, while HF+E group rats were trained with daily exercise for 6-10 weeks. ALI model was established by intratracheal administration of LPS after the last physical test. Pulmonary function and histopathology were performed to evaluate the severity of ALI. Serum levels of lipid metabolism parameters and inflammatory factors were measured by automatic analyzer and ELISA, respectively. TREM1 and TREM2 expression was detected by real-time PCR and Western blot. NF-κB activation was also measured by Western blot. Results: LPS instillation resulted in severe acute lung injuries in obese rats, compared with the N group, as impaired pulmonary resistance and elastane function increased lung wet-to-dry weight ratios and histopathological injuries and elevated inflammatory factors. Exercise resulted in the decrease of lung injuries in obese rats. TREM1 expression was significantly increased in the HF group, whereas TREM2 was decreased. Exercise reversed TREMs expression. NF-κB was activated in the HF group and inhibited by exercise. Conclusion: Present findings suggest that obesity aggravated the severity of ALI, which could be protected by exercise. Exercise might be a potential preventive approach for ALI through regulating TREMs expression, NF-κB activation, and inflammatory factor release.

Keywords: Obesity, acute lung injury, exercise, TREM, NF-кB

#### Introduction

Acute lung injuries (ALI) and acute respiratory distress syndrome (ARDS), caused by various pathogenic stimulus, are progressive respiratory dysfunctions, characterized by clinically significant hypoxemia and diffuse pulmonary infiltrates [1]. These diseases show as pulmonary edema and permeability increases, resulting in severe alveolar capillary barrier disorder and local tissue destruction [2]. Although treatment and cognition of ALI continues to enhance, morbidity and mortality remain high worldwide [3]. Endotoxin-induced ALI often aggravates even with the use of antibiotics. This deterioration is associated with an imbalance of pro- and anti-inflammatory factors.

Obesity is a common complex disorder regulated by genetic, environmental, and neuroendo-

crine factors. Fat is an essential component, vital for maintaining homeostasis in the human body. Imbalances of energy intake and consumption finally cause excessive fat accumulation [4]. Obesity could decrease pulmonary compliance. Obese patients may be at higher risk of occurrence and poorer outcomes stemming from ALI [5].

Inflammation causes lung tissue injuries and organ failure if unchecked, playing an important role in the pathophysiological process of ALI [6]. Excess consumption of nutrients triggers inflammation, while specialized metabolic cells, such as adipocytes, mediate the interface between obesity and inflammation [7, 8]. With the prevalence of obesity, obesity related lung injuries have significantly risen in past decades. Exercise has been recommended as a major strategy to prevent and treat obesity [9]. However, its impact on obesity related lung injuries remains unclear.

Triggering receptor expressed on myeloid cells (TREM) receptors constitute an immunoglobulin superfamily in the innate immunity system, critical for the clearance of cellular pathogens [10]. It has been reported that TREM1 and TREM2 expression is altered in ALI [11, 12]. However, whether the effects of exercise on obesity related lung injuries are associated with TREMs remains unknown.

In this present study, rats were fed with a high fat diet to generate the obese model. ALI was induced by intratracheal administration of lipopolysaccharides (LPS) to observe inflammation and expression of TREMs in lung tissues, assessing the effects of exercise on obesity related lung injuries.

### Materials and methods

### Animals

Four-week-old male Sprague-Dawley rats were purchased from the Laboratory Animal Center of Shanxi University (Taiyuan, China). Rats were provided with standard rodent chow and water ad libitum. They were allowed illumination with 12:12 hour day/night cycles. Animal experiments conformed to Guidelines of Laboratory Animal Care and Use Committee and were approved by the Institutional Animal Ethics Committee of Changzhi Medical College (Changzhi, China).

### Experimental design

Thirty SD rats were divided into three groups (n = 10 in each group): N group (rats fed with a normal diet for 10 weeks), HF group (rats fed with a high fat diet for 10 weeks), HF+E group (rats fed with a high fat diet for 10 weeks + daily exercise between the 6th and 10th week).

### High fat diet

The high fat diet was composed of normal diet 54.6%, lard 16.9%, sucrose 14%, casein 10.2%, maltodextrin 2.2%, and premix 2.1%, as described previously [13]. Total calories of normal and high fat diets were 3,530 and 4,580 kcal/Kg, respectively.

### Exercise training and conditioning

Rats were initially adapted to exercise for three days (20 minutes, 0% inclination, 0.6 km/hour).

Afterward, exercise was performed with a 5-minute warm-up (0% inclination, 0.6 km/ hour), followed by 25-minute training (5% inclination, 0.9 km/hour). During this process, if exhaustion occurred, rats were given rest for 3-5 minutes before returning to exercise.

### LPS instillation

Twenty-four hours after the last exercise training, the rats received 1 mg/kg E. coli LPS (Sigma Aldrich, MO, USA), intratracheally, to induce the acute lung injury model.

### Growth and development indicators

Body weights and lengths were measured before and after model establishment. Body mass index (BMI) was also calculated (BMI = body weight/body length<sup>2</sup>).

### Pulmonary function test

Rats were anesthetized and intratracheally intubated to detect pulmonary function, using an animal pulmonary function instrument (YL-S-001, Buxco Electronics, USA), at 6 hours after ALI. Inspiratory resistance (RI), dynamic lung compliance (Cdyn), tidal volume (VT), and respiratory frequency (f) were used to evaluate pulmonary function.

### Lung wet-to-dry weight ratios and histopathological evaluation

Lung wet-to-dry weight ratios were performed, as previously described [14]. Right upper lung tissues were collected and weighed. The tissues were dried at  $70^{\circ}$ C for 3 days and weighed again. Lung wet-to-dry (W/D) weight ratios were then calculated.

Histopathological evaluation was performed, as previously described [15]. H&E staining was used to observe histopathological changes of the lung tissues. Each sample was evaluated through a scoring system by three pathologists blinded to the experimental groups. The scoring system (15) included four categories, edema, leukocyte infiltration, hemorrhage, and alveolar septal thickening. Each category was scored from 0 to 3.

### Serum parameters detection

Whole blood was centrifuged at a low temperature by 3,000 rpm for 20 minutes. Supernatant was collected and stored at -80°C for serum parameters detection. Lipid metabolism

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parameters, total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL-C), and lowdensity lipoprotein (LDL-C), were measured using the Automatic Biochemical Analyzer (Hitachi 7180, Tokyo, Japan). Serum free fatty acid (FFA) levels were detected using Free Fatty Acid Quantitation Kit (Sigma-Aldrich, St. Louis, MO, USA), according to manufacturer recommendations. Inflammatory cytokines, serum tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and IL-1 $\beta$ , were measured by enzyme-linked immunosorbent assay (ELISA) kits (Boster, Wuhan, China), according to manufacturer instructions.

### Bronchoalveolar lavage fluid (BALF) collection and detection

BALF was obtained by lavage of the lungs twice with 1 mL PBS. They were centrifuged at 2,000 g for 10 minutes. Supernatant was used to detect inflammatory cytokines via the ELISA kit.

TREM expression and NF- $\kappa$ B activation detection

Total RNA was extracted using TRIzol Reagent (Invitrogen, USA) and converted into cDNA using PrimeScript<sup>™</sup> cDNA Synthesis Kit (Takara, Japan). All real-time PCR reactions were performed in an ABI7500 real-time thermal cycler (Applied Biosystems, USA) using SYBR Permix Ex Taq kit (Takara, Japan). Primer sequences were as follows: The forward primer of TRE-M1 was 5'-AAGTATGCCAGAAGCAGGAAGG-5', and the reverse primer of TREM1 was 5'-GGTAGGGTCATCTTTCAGGGTTGT-5'; the forward primer of TREM2 was 5'-GGATGCTGGA- GATCTCTGGTT-5', and the reverse primer of TREM2 was 5'-ATGCAGGCCAGGAGGAGAA-5'; the forward primer of  $\beta$ -actin was 5'-AGTGCGACCTGGACAT-CCG-5', and the reverse primer of  $\beta$ -actin was 5'-TGGCT-CTAACAGTCCGCCTAG-5'. Relative fold differences of TR-EMs mRNAs were calculated with  $\beta$ -actin as a reference [16].

Total protein was extracted by RIPA lysis buffer (Beyotime, China) and quantified by BCA protein assay (Beyotime, China). Total protein (20 µg per

lane) was separated onto a 10% SDS-PAGE and transferred to PVDF membranes (Millipore, MA, USA). They were blocked by 5% skimmed milk and incubated overnight at 4°C with primary antibody TRIM1 and TRIM2 (dilution 1:1000, Sigma) and IkB and p65 (dilution 1:1000, Sigma) or  $\beta$ -actin (dilution 1:2000, Sigma). Subsequently, membranes were incubated for 1 hour at room temperature with horseradish peroxidase-conjugated goat anti-rabbit or antimouse second antibodies (1:5000, Boster, China). They were then incubated with ECL substrate reagent (Thermo Pierce) and pictures were recorded using Healthcare ImageQuant LAS 500 (GE).

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation and were analyzed using SPSS 19.0 software program (SPSS Inc., Chicago, IL, USA). One-way analysis of variance was used to analyze statistical differences in the data. *P*<0.05 values are considered statistically significant. GraphPad Prism version 5 (GraphPad Prism Software Inc, San Diego, Calif) was used for figure preparation.

### Results

## Effects of exercise on growth development indicators of obese rats

First, this study observed the effects of exercise on physiological indicators of rats with a high-fat diet, as shown in **Figure 1**. Rats showed growth throughout the study according to



**Figure 2.** Effects of exercise on pulmonary function and morphology of obese rats with endotoxin-induced ALI. Compared with the N group, HF group increased the RI and f and decreased Cdyn and VT of endotoxin-induced ALI rats (p<0.05). HF+E group improved impaired pulmonary function of obese rats with ALI, shown as a partial reversal of pulmonary function indicators (p<0.05) (A). Compared with the N group, the lungs of rats in the HF group showed marked edema, hemorrhage, leukocyte infiltration, and alveolar septal thickening after LPS instillation. HF+E group had significantly lower scores of acute lung injuries when compared with the HF group (p<0.05) (B and C). Lung W/D ratio was statistical increased in HF group compared with N group, while reduced in HF+E group (p<0.05) (C). \*p<0.05 vs. the N group, #p<0.05 vs. the HF group, (n = 10).

gained physiological indicators. Starting body weights, lengths, and BMIs did not differ between groups (p>0.05), but were significantly increased at the end of 10 weeks (p<0.05). Compared with the normal diet group, rats fed with a high-fat diet showed higher body weights, lengths, and BMIs (p<0.05). The obese rat model was established successfully. Exercise was shown to decrease the growth of body weights, lengths, lengths, and BMIs of rats with a high-fat diet (p<0.05).

### Effects of exercise on pulmonary function and morphology of obese rats with endotoxininduced ALI

Pulmonary function was impaired in endotoxininduced ALI. Inspiratory resistance (RI), dynamic lung compliance (Cdyn), tidal volume (VT), and respiratory frequency (f) were used to evaluate pulmonary function at 6 hours after ALI. Data are shown in **Figure 2A**. Compared with the normal diet, a high-fat diet increased RI and f and decreased Cdyn and VT of endotoxin-induced ALI rats (p<0.05). Exercise improved the impaired pulmonary function of obese rats with ALI, showing a partial reversal of pulmonary function indicators (p<0.05).

Lung morphology was changed in endotoxin-induced ALI as well (Figure 2B, 2C). Compared to the normal diet group, the lungs of rats in the high-fat diet group showed marked edema, hemorrhaging, leukocyte infiltration, and alveolar septal thickening after LPS instillation. All injury scores were significantly increased. However, animals in the exercise and high-fat diet group had significantly lower scores of acute lung injuries, compared with those of the high-fat diet group.

Lung W/D ratio was another indicator used to assess the severity of ALI. Lung W/D

ratios were shown as  $4.40 \pm 0.24$  in the normal diet group,  $6.95 \pm 0.35$  in the high-fat diet group, and  $5.21 \pm 0.11$  in the exercise and high-fat diet group, respectively. Ratios were statistically increased in the high-fat diet group, compared with the normal diet group, and reduced by exercise preconditioning. Results are shown in **Figure 2C**.

Results indicated that exercise could improve pulmonary function and morphology, while attenuating ALI damage in obese rats.

# Effects of exercise on hyperlipidemia and inflammation of obese rats with endotoxin-induced ALI

High-fat diets cause hyperlipidemia. Therefore, TG, HDL-C, LDL-C, and FFA were determined to assess the effects of exercise on lipid metabolism. Exercise ameliorated the dyslipidemia of rats with a high-fat diet in LPS-induced acute lung injuries, showing decreased serum levels



**Figure 3.** Effects of exercise on hyperlipidemia and inflammation of obese rats with endotoxin-induced ALI. Compared with the N group, serum levels of TC, TG, LDL-C, and FFA were increased, while HDL-C was decreased in HF group. HF+E group ameliorated the dyslipidemia of rats by decreased serum levels of TC, TG, LDL-C, and FFA, along with elevated HDL-C (p<0.05) (A). Compared with N group, serum and BALF TNF $\alpha$  and IL-1 $\beta$  concentrations were significantly increased in the HF group, but were reduced in HF+E group (p<0.05) (B). \*p<0.05 vs. the N group, #p<0.05 vs. the HF group, (n = 10).

of TC, TG, LDL-C, and FFA, along with elevated HDL-C (Figure 3A).

Inflammatory cytokines play important roles in acute lung injuries by provoking damage to the capillary endothelium and alveolar epithelium. Serum and BALF TNF $\alpha$  and IL-1 $\beta$  concentrations were measured to investigate the effects of exercise on systemic inflammatory response in high-fat diet ALI rats, shown in **Figure 3B**. Results showed that levels of TNF $\alpha$  and IL-1 $\beta$  in the high-fat diet group rats were significantly increased, compared with those of normal diet group rats (p<0.05). These levels were reduced by exercise preconditioning. Descendant serum TNF $\alpha$  and IL-1 $\beta$  indicated that exercise reduced inflammatory response in obese rats with ALI.



**Figure 4.** Effects of exercise on lung TREM expression of obese rats with endotoxin-induced ALI. A. Expression of TREMs was detected by Real-time PCR. B. Expression of TREMs and Nf– $\kappa$ B was detected by Western blot. Compared with the N group, lung TREM1 and p65 expression was significantly increased in HF group (p<0.05), whereas expression of TREM2 and I $\kappa$ B was decreased (p<0.05). TREMs expression was reversed in the HF+E group (p<0.05). \*p<0.05 vs. the N group, #p<0.05 vs. the HF group, (n = 10).

#### Effects of exercise on lung TREM expression and NF-κB activation of obese rats with endotoxin-induced ALI

To explore the mechanisms of exercise on obese rats with endotoxin-induced ALI, expression of TREMs mRNAs was determined by realtime PCR. Expression of TREMs and Nf- $\kappa$ B proteins was detected by Western blot. Compared with the normal diet group, TREM1 and p65 expression was significantly increased in highfat diet group ALI rats (p<0.05), whereas expression of TREM2 and I $\kappa$ B was decreased (p<0.05). Exercise preconditioning reversed TREMs expression (p<0.05) (**Figure 4**).

### Discussion

The present study investigated the mechanisms involved in the protective effects of exercise on endotoxin-induced acute lung injuries in obese rats. The SD rat obese model was established with a high fat diet, submitted to exercise, and induced ALI by LPS. LPS instillation resulted in impaired pulmonary function, abnormal lung morphology, and excessive inflammatory response in obese rats. It was demonstrated that five-weeks of exercise before LPS instillation decreased the severity of lung injuries. Additionally, TREM1 expression was significantly increased in the high-fat diet group ALI rats, whereas expression of TREM2 was decreased and exercise could reverse TREMs expression. Results suggest that exercise might play a pivotal role in protecting lungs of LPS induced ALI by regulating TREMs expression.

Obese rats were established successfully through high fat diet feed, confirmed by high growth development indicators and hyperlipidemia. Obesity is emerging as an important public health concern around the world [17]. A high fat diet is considered a primary contributing factor for obesity, leading to the establishment of high fat diet feed models to investigate the pathophysiological effects of obesity related diseases. Mechanisms of high fat diet induced obesity are the imbalance of fat intake and consumption due to low satiating effects. Fat is stored in the body, altering hormones such as leptin, insulin, and ghrelin, all involved in energy balance [18]. The accumulation of fat causes changes in physiology and structure, affecting system function. Obesity affects respiratory system function via several aspects, including direct structural modification and local and systemic inflammation [19]. During obesity, excess body fat accumulating in the chest and abdomen limits the action of the respiratory muscles. Structural and functional changes of respiratory muscles could reduce lung compliance as well [20]. Obesity has been associated with increased inflammation, mainly evidenced by massive inflammatory factors, contributing to the development of obesity and related diseases [21].

LPS intratracheal administration is a classical method for establishing acute lung injuries [22]. In this study, the severity of ALI was evaluated through three aspects, lung function, histopathology, and inflammatory response. Impaired lung function and morphology in obesity related lung injuries could be improved by exercise. Indicators of lung function included

inspiratory resistance, respiratory frequency, dynamic lung compliance, and tidal volume. LPS instillation decreased lung compliance, resulting in higher tidal volume, consistent with the changes of lung injuries [23]. Morphological changes of the lungs could represent the degree of ALI as well. LPS causes pulmonary structural changes with alveolar septal thickening, hemorrhage, leukocyte infiltration, edema, as well as increased W/D ratios. Exercise reduced lung injury and minimized edema formation, attenuating lung injuries in ALI rat models of obesity [24]. Excessive inflammatory response is one of the major mechanisms of ALI and aggravated lung tissue injuries. Excess fat tissue could induce the production of inflammatory factors, such as TNF $\alpha$ , IL-1 $\beta$ , and IL-6 [25]. TNF $\alpha$  takes part in the inflammatory response of the immune system and promotes release of other inflammatory factors. IL-1ß is released by monocytes when lung tissue is damaged and has been associated with the release of other inflammatory factors [26]. Present data demonstrates that exercise decreased the release of TNF $\alpha$  and IL-1 $\beta$  in obesity related lung injuries. The benefits of exercise were evaluated in a lung ischemiareperfusion rat model as well [27].

ALI is characterized by injuries in lung endothelial and alveolar epithelial cells, along with increased vascular permeability. TREM1 and TREM2 are new inflammatory molecules [28, 29]. Lungs are often the first organs with inflammation during ALI. TREMs mRNA and protein expression was measured by real-time PCR and Western blot. TREM1 expression was significantly increased in the high-fat diet group ALI rats, whereas expression of TREM2 was decreased, in accord with previous studies [11, 30]. The biological activity of TREM1 is similar with TLR and NLR, stimulating inflammatory signaling and response [31]. In contrast, TREM2 exhibits anti-inflammatory effects, provides protective effects on TREM1 mediated inflammation, and prevents infectious diseases, such as ALI [32]. Additionally, the present study observed the activation of NF-kB in high-fat diet group ALI rats, showing as decreased IkB and increased p65. NF-ĸB activation was inhibited by exercise. Observations that exercise downregulated TREM1 expression, upregulated TREM2 expression, and upregulated NF-kB

inhibition suggest that exercise is a novel intervention involved in the regulation of inflammatory processes.

In summary, exercise attenuated acute lung injuries in an obese rat model. Five-weeks of exercise in ALI rats reduced inflammatory response, TREMs expression, and NF- $\kappa$ B activation. Exercise may be a potential preventive approach for obesity related ALI via regulating TREMs and NF- $\kappa$ B, revealing a new molecular target for obesity related lung injury treatment.

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### Disclosure of conflict of interest

None.

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