Aerobic Exercise Improves Sepsis via Impairing LPS-induced Lactate and HMGB1 Release in Mice

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Aerobic exercise improves sepsis via impairing LPS-induced lactate and HMGB1 release in mice

Running title: Aerobic exercise prevents sepsis

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Abstract

Purpose: In the present study, we attempted to investigate whether aerobic exercise (AE) could prevent sepsis and its complications.

Methods: Forty ICR mice were divided into four groups: Control (Con), Lipopolysaccharide (LPS), Exercise (Ex), and Exercise + LPS (Ex + LPS) groups. Ex and Ex + LPS mice were performed with low-intensity AE for 4 weeks. LPS and Ex + LPS mice received 5 mg/kg LPS intraperitoneally for induction of sepsis. Histopathological micrographs showed the organ injury. This study examined the effects of AE on LPS-induced changes in systemic inflammation, pulmonary inflammation, lung permeability, oxidative stress-related indicators in the lung, blood glucose levels, plasma lactate levels, plasma high-mobility group box 1 (HMGB1) levels, and bronchoalveolar lavage fluid (BALF) cell count. Sixty mice were used to perform survival rate analysis.

Results: An 4-week AE pretreatment improved survival rates, multiple organ dysfunction syndrome (MODS), and aortic injury in mice with sepsis. AE decreased LPS-induced oxidative stress injury in lung tissue. AE reduced the infiltration of neutrophils in the lung, liver, kidney, and heart tissues. AE suppressed CXCL-1, CXCL-8, IL-6, and TNF- α mRNA expression but activated IL-1RN, IL-10, Sirt-1, and Nrf-2 mRNA expression in the lung. AE decreased the serum levels of lactate and HMGB1 but increased blood glucose levels during sepsis.

Conclusions: AE improves sepsis-associated lung, liver, kidney, heart, and aortic injury and death. AE modulates the inflammatory-anti-inflammatory and

oxidative-antioxidative balance in the lung. AE, which impairs LPS-induced lactate and HMGB1 release, is a novel therapeutic strategy for sepsis targeting aerobic glycolysis.

Keywords: exercise; host response; organ failure; Warburg effect; inflammation; oxidative stress.

Abbreviations: AE, aerobic exercise; ALI, acute lung injury; BG, blood glucose; BALF, bronchoalveolar lavage fluid; CXCL, C-X-C motif chemokine ligand; COPD, chronic obstructive pulmonary disease; HMGB1, High Mobility Group Box 1; LPS, lipopolysaccharide; MDA, malondialdehyde; MODS, multiple organ dysfunction syndromes; MPO, myeloperoxidase; Nrf-2, nuclear factor erythroid 2-related factor 2; GSH, glutathione; Sirt-1, silent information regulator 1; SOD, superoxide dismutase; IL, interleukin; TNF, tumor necrosis factor; PFA, paraformaldehyde; W/D, wet weight/dry weight ratio.

Noteworthy:

1. AE improves sepsis-associated lung, liver, kidney, heart, and aortic injury.
2. AE reduces pulmonary inflammation, oxidative stress injury, and lung permeability.
3. AE inhibits neutrophil inflammation by suppressing CXCL-1, CXCL-8, and TNF- α expression.
4. AE improves LPS-induced lung injury by activating Sirt-1/Nrf-2 signaling.
5. AE impairs LPS-induced lactate and HMGB1 release during sepsis.

1. Introduction

Sepsis, which has many complications, such as MODS, hypotension, and hypoglycemia, exhibits a high mortality rate stemming from a systemic infection that involves alterations in inflammatory parameters and oxidative status [1-3]. The pathogenesis of sepsis is complex and inflammation and oxidative stress play critical roles in the pathogenesis of sepsis [4,5]. Sepsis is characterized by an uncontrolled inflammatory response involving inflammatory mediators, including IL-1RN, IL-6, IL-10, and TNF- α , and effector cells, among which neutrophils play a key role [6,7]. We attempt to identify the physiological functions of these factors in response to regular AE in mice with sepsis.

Exercise has emerged as a tool to treat many autoimmune and inflammatory diseases, such as chronic obstructive pulmonary disease (COPD), asthma, and atherosclerosis because aerobic exercise had immunomodulatory effects and modulated redox balance [8-10]. Previous researches demonstrated that LPS injection led to an excessive inflammatory response and oxidative stress injury and LPS injection generally accepted as somewhat modeling the septic condition [11,12]. Strikingly, limited research has demonstrated that exercise was a novel tool to prevent sepsis and its complications [11-14]. Exercised mice showed a survival benefit compared to unexercised mice in the septic model [15,16]. Exercise reduced acute lung injury (ALI) in mice subjected to LPS-induced sepsis [17]. Regular exercise reduced liver and kidney injury during severe polymicrobial sepsis [18]. MODS was a common complication of sepsis [19]. However, previous studies ignored the effects of

sepsis and exercise on the aorta in a septic model. Besides, the underlying molecular mechanisms involved in the preventive effect of exercise in sepsis-induced MODS have not yet been fully elucidated.

The ‘Warburg effect’, which was first found in cancer cells, played an essential role in innate and adaptive immunity [20,21]. Increasing evidence has demonstrated that activated immune cells, including macrophages, neutrophils, and T cells, switched from oxidative phosphorylation to aerobic glycolysis in a manner similar to tumor cells [20]. This alteration may contribute to the regulation of innate immune functions and represent a novel target for inflammatory diseases [21]. LPS injection induced a switch from oxidative phosphorylation to aerobic glycolysis in the immune cells including dendritic cells and macrophages [22]. Increased aerobic glycolysis consumed a large amount of glucose and produces a large amount of lactate. Increased serum lactate levels were a biomarker of mortality and organ failure during sepsis and that lactate clearance was a potential therapy for sepsis [23-25].

Increased aerobic glycolysis released a large amount of lactate, which can effectively stimulate macrophages to release HMGB1 [26]. HMGB1 played a prominent role in the development of the inflammatory response and was a promising therapeutic target for sepsis treatment [27,28]. The Warburg effect could be used as a therapeutic target for inflammatory diseases, including sepsis and ALI [29,30]. We attempted to demonstrate whether AE can regulate the Warburg effect and impair LPS-induced lactate and HMGB1 release during sepsis.

2. Materials and Methods

2.1. Animal

All protocols used in this study were approved by the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China). All experiments were performed in accordance with the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. The authors have read the ARRIVE guidelines and the study was carried out in compliance with the ARRIVE guidelines. All mice were anesthetized via intraperitoneal injection of pentobarbital (0.2 mg/kg). The mice were euthanized with isoflurane. Thirty male ICR mice (6 weeks old, 18-21 g) were provided by Beijing HFK Biotechnology Co., Ltd. (Beijing, China). The conditions of the husbandry room were temperature at 22 ± 5 °C, relative humidity at $50\% \pm 10\%$, and the light cycle was 12 h/day. The body weight and health status of the mice were recorded.

2.2. Experimental design and sepsis-induced protocol

The ALI model was produced by intraperitoneal injection of 5 mg/kg LPS (O55:B5, Sigma-Aldrich, St. Louis, MO, USA). Forty mice were randomly assigned into one of four groups: (1) control group (Con), mice were injected with a volume of normal saline equivalent to the volume of LPS; (2) LPS group (LPS), mice were injected with 5 mg/kg LPS via intraperitoneal injection; (3) exercise group (Ex), mice were submitted to low-intensity AE for 4 weeks; and (4) exercise plus LPS group (Ex + LPS), mice were submitted to AE for 4 weeks and injected with 5 mg/kg LPS via

intraperitoneal injection.

Forty-eight hours after the last exercise, LPS and Ex + LPS mice were injected intraperitoneally with 5 mg/kg LPS. LPS were dissolved and diluted with 0.9% normal saline. The mice were killed at 6 h after LPS injection. Each group contained 10 mice.

2.3. Exercise protocol

The mice were forced to train using a treadmill. Ex and Ex + LPS mice were submitted to aerobic exercise with the same treadmill protocol. After adaptive training for 3 days, the maximal exercise capacity test of each mouse was performed. The mean maximal speed reached by each animal was 23.6 m/s. Hence, the maximal aerobic capacity (100%) was 23.6 m/s. Mice from the AE and COPD + AE groups were trained in low-intensity exercise (50% maximal speed, 11.8 m/s). Treadmill aerobic training lasted for 8 weeks, once a day, and 60 min per session.

2.4. Collection of tissue samples

Blood samples of mice were obtained and put into blood collection vessels. After centrifugation, the upper serum layer was harvested and frozen at -20 °C immediately. The remaining lung tissues were frozen with liquid nitrogen.

Two milliliters of ice-cold 0.9% normal saline was utilized to collect BALF, the whole BALF volume was flushed four times, and the output fluid was collected and centrifuged at 3000 r/min for 12 min at 4 °C. The supernatant was transferred to 1.5-mL sterile EP tubes and immediately stored at -20 °C.

2.5. Histopathology

For histological analysis, 4% PFA-fixed lung, heart, liver, kidney, and aorta tissue samples were embedded in paraffin. Using the paraffin section method, 5 μ m-thick sections were stained with hematoxylin and eosin (HE). Photographs taken by an inverted microscope (Olympus Corp., Japan) were utilized to identify the degree of organ injury and inflammatory cell infiltration. The histopathologic injury was scored with a semi-quantitative analysis as previously described [45-51].

2.6. Detection of pulmonary permeability

To quantify the magnitude of pulmonary permeability, Wet dry weight ratio (W/D) was detected. Blotting papers were utilized to absorb liquid and blood on the surface of the lung tissues, and then the wet weight of the left lower lobe of lung tissue was determined. The lung tissues were dried in a drying case until a stable dry weight was obtained. W/D was calculated according to the following formula: $W/D = \text{wet weight of the lung} / \text{dry weight of the lung}$.

2.7. Measurement of the levels of inflammatory factors in BALF and serum

BALF levels of the proinflammatory factors CXCL-1, CXCL-8, IL-6, and TNF- α and the anti-inflammatory factors IL-1RN and IL-10 were detected using mouse ELISA kits (Neobioscience, Neobioscience Technology Company, Shenzhen, China). Similarly, serum levels of inflammatory factors IL-6, IL-10, and TNF- α were detected using mouse ELISA kits (Neobioscience, Neobioscience Technology Company, Shenzhen, China).

2.8. Detection of the Warburg effect

Following the reagent manufacturer's specifications, the blood glucose levels in

serum were measured with a blood glucose meter (Johnson Company). Lactate in serum was detected with a colorimetric L-lactate assay kit (Abcam, Cambridge, MA, USA). The levels of HMGB1 in serum were detected using a mouse ELISA kit (Shino Test Corporation, Tokyo, Japan). In addition, the gene expression levels of HMGB1 in lung tissue were detected.

2.9. Determination of gene expression levels in lung tissue

Total RNA was extracted from DNase I-treated cells using TRIzol as previously described. Total RNA (2.0 µg) was used as a template for cDNA synthesis. Real-time fluorescence quantitative PCR was used for quantitative analysis. GAPDH was served as the housekeeping gene. All primers were designed and synthesized by the Shanghai Sangon Biotech Company. [Table 6](#) shows the murine PCR primer sequence information.

2.10. Assessment of survival rates

For survival analyses, 60 male mice (6 weeks old, 20-22 g) were randomly divided into three groups: (1) in the control group (Con), the mice were injected with equal volumes of saline via intraperitoneal injection; (2) in the LPS group (LPS), the mice were injected with 12 mg/kg LPS (O55:B5, Sigma-Aldrich, St. Louis, MO, USA) via intraperitoneal injection; (3) in the Ex + LPS group (Ex + LPS), the mice were trained at low-intensity exercise (50% maximal speed, 11.8 m/s) for 4 weeks. The exercise intervention lasted for 4 weeks, 5 days/week, and 1 hour/day. After the last training of 48 h, the mice were intraperitoneally injected with 12 mg/kg LPS. The number of dead mice was recorded every 6 h for 48 h. Each group included 20 mice.

2.11. Data processing

SPSS 21.0 software was used for data processing. The experimental results were indicated by the mean $\pm$ standard deviation ($x \pm SD$). Statistical differences among groups were assessed using one-way analysis of variance (ANOVA). The significance level was set at $P < 0.05$. GraphPad Prism 6 software was used to draw figures.

3. Results

3.1. AE improved mortality

Sham-operated mice exhibited 100% survival. Compared with the LPS group, a 4-week pretreatment showed dramatically improved survival ($P < 0.01$; **Fig 1A**).

3.2. Exercise regulated the Warburg effect during sepsis

Compared with the Con group, LPS administration markedly decreased blood glucose levels ($P < 0.001$; **Fig 1B**) but increased serum lactate levels ($P < 0.001$; **Fig 1C**), serum HMGB1 levels ($P < 0.001$; **Fig 1D**), and the gene expression levels of HMGB1 ($P < 0.001$; **Fig 1E**) in lung tissue. A 4-week exercise pretreatment markedly increased blood glucose levels ($P < 0.01$) but markedly decreased serum lactate levels ($P < 0.05$), serum HMGB1 levels ($P < 0.05$), and the gene expression levels of HMGB1 ($P < 0.001$) in lung tissue.

3.3. AE relieved pulmonary edema

Compared with the Con group, the W/D of lung tissues increased significantly after LPS administration ($P < 0.001$). A 4-week exercise pretreatment exerted a prominent role in preventing the upregulation of W/D in lung tissues ($P < 0.01$) (**Fig. 1F**).

3.4. AE prevented acute lung injury

Histologic assessment of the lung parenchyma in the four groups revealed evidence of the degree of lung injury, inflammatory cell infiltration, and interstitial edema. Compared with the Con group (**Fig. 2A**), LPS administration significantly increased lung injury, inflammatory infiltrates, and interstitial edema (**Fig. 2B**). There were no differences between the Con group and the Ex group (**Fig. 2C**). Compared with the LPS group, a 4-week exercise pretreatment significantly decreased the degree of lung injury, inflammatory cell infiltration, and interstitial edema (**Fig. 2D**). Compared with the LPS group, a 4-week exercise pretreatment significantly decreased the lung injury score ($P < 0.001$) (**Table 4**).

3.5. AE attenuated neutrophil content in lung tissue

Compared with the Con group (**Fig. 3A**), there was a large amount of neutrophil infiltration (black arrow) after LPS administration (**Fig. 3B**). There was no difference between the Con and Ex groups (**Fig. 3C**). Compared with the LPS group, a 4-week exercise pretreatment prevented the upregulation of the neutrophil infiltration (**Fig. 3D**). Compared with the Con group, the number of neutrophils increased significantly after the administration of LPS ($P < 0.001$). Compared with the LPS group, a 4-week exercise pretreatment exerted a prominent role in preventing the upregulation of the number of neutrophils ($P < 0.001$) (**Fig. 3E**).

3.6. AE attenuated the number of total cells, neutrophils, and macrophages in the BALF

After LPS administration for 6 h, the number of total cells ($P < 0.001$), neutrophils ($P < 0.001$), and macrophages ($P < 0.001$) were significantly increased. A 4-week

exercise pretreatment prevented the increase in the number of total cells ($P < 0.01$), neutrophils ($P < 0.01$), and macrophages ($P < 0.01$) compared to the LPS group. Treatment with LPS and AE did not influence the number of lymphocytes ($P > 0.05$) or eosinophils ($P > 0.05$) in the BALF ([Table 1](#)).

LPS injection increased neutrophil content in the liver ($P < 0.001$), kidney ($P < 0.001$), and heart tissues ($P < 0.001$) compared to the Con group, while exercise attenuated neutrophil content in the liver ($P < 0.01$), kidney ($P < 0.01$), and heart tissues ($P < 0.01$) compared to the LPS group ([Table 5](#)).

3.7. The effects of AE on pulmonary inflammation and systemic inflammation

We observed an effect of LPS in increasing BALF levels of CXCL-1 ($P < 0.001$), CXCL-8 ($P < 0.001$), IL-6 ($P < 0.001$), IL-10 ($P < 0.001$), and TNF- α ($P < 0.001$) but decreasing BALF levels of IL-1RN ($P < 0.001$). Compared with the LPS group, a 4-week exercise pretreatment increased BALF levels of IL-10 ($P < 0.05$) and IL-1RN ($P < 0.05$) but decreased BALF levels of CXCL-1 ($P < 0.05$), CXCL-8 ($P < 0.01$), IL-6 ($P < 0.05$), IL-10 ($P < 0.01$), and TNF- α ($P < 0.001$) ([Table 2](#)).

We observed an effect of LPS in increasing the serum levels of IL-6 ($P < 0.001$), IL-10 ($P < 0.001$), and TNF- α ($P < 0.001$). The serum levels of IL-6, IL-10, and TNF- α were no difference between the LPS and Ex + LPS groups ([Table 3](#)).

3.8. AE relieved liver injury

The liver lobules of the mice in the Con group ([Fig. 4A](#)) and the Ex group ([Fig. 4C](#)) were intact and clear, the cells were neatly arranged, the intercellular substance was free of edema, the liver stripes were clear and regular, and there were no symptoms of

injury. The liver lobules of the mice in the LPS group were severely damaged, the liver cells swelled, the intercellular substance disappeared, and there was a large amount of neutrophil (black arrow) infiltration (**Fig. 4B**). The liver lobules of the mice in the Ex + LPS group had significantly less liver tissue structural damage, with clearer liver lobules and a small amount of neutrophil infiltration (**Fig. 4D**).

LPS administration upregulated the markers of liver disease ALT ($P < 0.001$) and AST ($P < 0.001$) levels compared to the Con group. AE downregulated the ALT ($P < 0.05$) and AST ($P < 0.05$) levels compared to the LPS group. Compared with the LPS group, a 4-week exercise pretreatment significantly decreased the liver injury score ($P < 0.001$) (**Table 4**).

3.9. AE relieved kidney injury

The kidney tissues of the mice in the Con group (**Fig. 5A**) and in the Ex group (**Fig. 5C**) were intact and clear, the cells were neatly arranged, the intercellular substance was free of edema, and there were no symptoms of injury. Cortical tubular epithelial cells were well-shaped, and almost every epithelial cell owned intact nuclei. The kidneys of the mice in the LPS group were severely damaged, the cells swelled, and the intercellular substance disappeared, accompanied by a large amount of neutrophil (black arrow) and hemocyte infiltration, severe epithelial vacuolization, flattening of the tubular epithelium (white arrow), and the appearance of an atypical shape with almost no nuclei (yellow arrow) (**Fig. 5B**). The kidneys of the mice in the Ex + LPS group had significantly less kidney tissue structural damage than those of the LPS group, with clearer nephrons and a small amount of inflammatory cell infiltration, less

flattening of tubular epithelium, and less vacuolization than those of septic mice, and the degree of damage was significantly reduced (**Fig. 5D**).

Compared with the Con group, LPS administration increased the levels of markers of kidney injury Cre ($P < 0.001$) and BUN ($P < 0.001$), while Cre ($P < 0.05$) and BUN ($P < 0.05$) levels were significantly decreased in the Ex + LPS group compared with those in the LPS group. Compared with the LPS group, a 4-week exercise pretreatment significantly decreased the liver injury score ($P < 0.001$) (**Table 4**).

3.10. AE prevented septicemic cardiomyopathy

In the Con group (**Fig. 6A**) and the Ex group (**Fig. 6C**), the myocardial tissue was uniformly stained, the myocardial fibers were arranged regularly, the cell morphology, size, and arrangement were normal, and the interstitial spaces were normal. In the LPS group, myocardial tissues were disordered, myocardial degeneration occurred, dissolution occurred (white circle), and a large number of inflammatory cells infiltrated the muscle space (**Fig. 6B**). Compared with the LPS group, the inflammatory cells in the Ex + LPS group were less infiltrated, and the myocardial fiber tissue structure was normal. The distribution of muscle fibers was improved, but it did not completely return to the normal form (**Fig. 6D**). Compared with the LPS group, a 4-week exercise pretreatment significantly decreased the myocardial injury score ($P < 0.001$) (**Table 4**).

3.11. AE prevented aortic injury

In the Con group (**Fig. 7A**) and the Ex group (**Fig. 7C**), the aorta was uniformly stained and arranged regularly, the endothelium was smooth and orderly, and the

elastic fibers had a regular wavy-like shape. After LPS administration, the endothelium was not smooth and regular, the elastic fibers of the media became sparse, elastic fibers lost a regular wavy-like shape, and LPS administration significantly increased the aortic media thickness and decreased the area ratio of elastic fibers (**Fig. 7B**). Compared with the LPS group, a 4-week exercise pretreatment significantly increased the area ratio of elastic fibers and increased the aorta media thickness (**Fig. 7D**).

LPS increased the medial thickness of the aorta ($P < 0.001$) and decreased the medium membrane elastic fiber area ratio ($P < 0.001$), which was reserved by a 4-week AE. The medial thickness of the aorta ($P < 0.01$) was significantly decreased and the medium membrane elastic fiber area ratio ($P < 0.01$) was significantly increased in the Ex + LPS group compared with those in the LPS group (**Table 4**).

3.11. AE prevented oxidative stress injury in lung tissue

LPS administration increased the levels of MDA (**Fig. 8A**) ($P < 0.001$) and MPO (**Fig. 8B**) ($P < 0.001$) but decreased the levels of GSH (**Fig. 8C**) ($P < 0.001$) and SOD (**Fig. 8D**) ($P < 0.001$). 4 weeks of exercise intervention decreased the levels of MDA ($P < 0.001$) and MPO ($P < 0.001$) but increased the levels of SOD ($P < 0.05$) and GSH ($P < 0.05$) in lung tissues of septic mice.

3.12. AE prevented lung injury via activating the Sirt-1/Nrf-2 pathway

Compared with the Con group, LPS administration increased the gene expression levels of CXCL-1 (**Fig. 9A**; $P < 0.001$), CXCL-8 (**Fig. 9B**; $P < 0.001$), IL-6 (**Fig. 9D**; $P < 0.001$), and TNF- α (**Fig. 9H**; $P < 0.001$) but decreased the gene expression levels

of IL-1RN (**Fig. 9C**; $P < 0.01$), IL-10 (**Fig. 9E**; $P < 0.01$), Nrf-2 (**Fig. 9F**; $P < 0.01$), and Sirt-1 (**Fig. 9G**; $P < 0.01$). Compared with the LPS group, a 4-week exercise pretreatment activated the gene expression levels of IL-1RN ($P < 0.001$) and IL-10 ($P < 0.001$), Nrf-2 ($P < 0.001$), and Sirt-1 ($P < 0.001$) and inhibited the gene expression levels of CXCL-1 ($P < 0.001$), CXCL-8 ($P < 0.001$), IL-6 ($P < 0.001$), and TNF- α ($P < 0.001$) in lung tissue.

4. Discussion

Previous studies have demonstrated that the Warburg effect played a vital role in innate and adaptive immunity [21]. However, it was not clear whether AE can regulate the Warburg effect during sepsis. Increased aerobic glycolysis consumed a large amount of glucose and produces a large amount of lactate. Strikingly, our data demonstrated that LPS administration markedly increased serum levels of lactate and markedly decreased blood glucose levels. However, a 4-week exercise pretreatment markedly decreased lactate serum levels and markedly increased blood glucose levels. These data demonstrated that AE regulated the Warburg effect during sepsis.

Some studies found that LPS administration results in hyperglycemia [32,33]. However, other studies found that LPS administration results in hypoglycemia [34-36]. Our data demonstrated that LPS administration resulted in hypoglycemia, in part because increased aerobic glycolysis consumed a large amount of blood glucose during sepsis. Hypoglycemia was a common complication of sepsis. Our data demonstrated that LPS administration significantly decreased blood glucose levels, which was consistent with previous experimental results [34,35]. If sepsis-induced

hypoglycemia was not treated promptly, patients can deteriorate further and fall into a coma, which was easily confused with coma caused by infection, leading to delayed diagnosis and treatment or even death. Hence, septicemia patients should be monitored, and the blood glucose should be supplemented in a timely manner. Our data showed that AE prevented the decrease in blood glucose levels. Our data demonstrated that AE can be used as a preventive tool for LPS-induced hypoglycemia.

Previous researches identified that increased serum lactate levels were a biomarker of mortality and organ failure during sepsis and lactate clearance as a therapy for sepsis [23-25]. Our data demonstrated that LPS administration increased serum lactate levels, while a 4-week exercise pretreatment decreased serum lactate levels during sepsis. Hence, AE protected against sepsis via suppressing serum lactate levels. Previous researches identified that HMGB1 played a prominent role in the development of sepsis and was a promising therapeutic target for sepsis treatment [26, 27]. Increased aerobic glycolysis released a lot of lactate which can effectively stimulate macrophages to release HMGB1 [28]. Our data demonstrated that LPS markedly increased serum levels of HMGB1 and the gene expression levels of HMGB1 in lung tissue, while AE markedly decreased serum HMGB1 levels and the gene expression levels of HMGB1 in lung tissue. Hence, a 4-week exercise pretreatment impaired LPS-induced HMGB1 release. Based on these results, AE improved sepsis via regulating the Warburg effect and impairing LPS-induced lactate and HMGB1 release. Our research found a new mechanism through which AE

prevented sepsis.

In the present study, we demonstrated that a 4-week exercise pretreatment reduced sepsis-induced liver, kidney, and heart injury. The experimental results were consistent with previous conclusions [15-19]. Strikingly, we found that AE prevented sepsis-induced aortic injury. The aortic injury occurred before the onset of inflammatory infiltration and organ injury. Previous studies have overlooked the effects of sepsis on the aorta. Aorta could regulate arterial blood pressure and arterial hypotension was one of the most frequent complications of sepsis. Sepsis-induced arterial hypotension led to organ dysfunction and septic shock, which were the most severe complications of sepsis and deadly disease [20]. Our data identified that LPS administration increased aortic media thickness and reduced the area ratio of elastic fibers, which was improved by a 4-week AE pretreatment. These results demonstrated that AE could be used as a preventive tool for LPS-induced aortic injury.

Previous researches demonstrated that neutrophils played a prominent role in the development of sepsis [37]. We found that LPS administration led to a deleterious accumulation of neutrophils in lung tissue, our experimental results were consistent with previous conclusions. Strikingly, we found that 4 weeks of AE inhibited neutrophil infiltration in lung tissue. These results demonstrated that AE improved sepsis-induced lung injury in part by decreasing neutrophil content. Besides, deleterious accumulation of neutrophils within remote vital organs led to collateral tissue damage and ultimately multiple organ failure [38]. Hence, deleterious activation of neutrophils was a critical reason leading to host tissue injury and organ

damage during sepsis. Our data identified that LPS administration led to abnormal accumulation of neutrophils in the lung, liver, kidney, and heart tissues. A 4-week exercise pretreatment could prevent abnormal accumulation of neutrophils in the lung, kidney, liver, and heart tissues in septic mice. A 4-week exercise pretreatment prevented MODS partly because AE reduced the abnormal accumulation of neutrophils. It was well documented that CXCL-1, CXCL-8, and TNF- α were chemotactic for neutrophils. In the present study, we demonstrated that AE reduced the levels of CXCL-1, CXCL-8, and TNF- α in BALF as well as the gene expression levels of CXCL-1, CXCL-8, and TNF- α in lung tissue. These results demonstrated that AE inhibited neutrophil infiltration in lung tissue in part by suppressing CXCL-1, CXCL-8, and TNF- α expression.

We demonstrated that LPS injection led to an excessive inflammatory response, pulmonary edema, and the infiltration of inflammatory cells, which were the three main features of ALI [6]. A 4-week exercise pretreatment exerted a prominent role in improving the degree of pulmonary edema, the degree of neutrophil infiltration, and acute lung injury. Hence, AE could be used as a preventive tool for LPS-induced lung injury. Inflammatory cells, including neutrophils, attack blood vessels first, and resulting vascular injury leads to increased lung permeability, inflammatory cell infiltration, and pulmonary edema. In the present study, the protective effects of AE on the aorta mice were demonstrated. We found that AE prominently prevented the LPS-induced increase in the total protein concentration in BALF and the increase in the lung tissue W/D ratio. Based on these results, AE protected against LPS-induced

vascular injury.

Previous studies have found that the oxidant/antioxidant balance played a prominent role in the pathogenesis of sepsis and LPS administration led to oxidative stress injury [5]. In the present study, we identified the antioxidant effects of exercise. We demonstrated that AE downregulated the levels of MDA and MPO while upregulating the levels of SOD and GSH in mice with sepsis. Hence, AE attenuated oxidative stress injury and modulated the oxidative/antioxidative balance. AE was a preventive tool for sepsis, and one of the mechanisms involved in this process was linked to the increase in antioxidant capacity.

Sirt-1 exerted the effects of promoting lung cell proliferation and vitality and modulating the inflammatory/anti-inflammatory and oxidation/antioxidation balance [39,40]. Activation of Sirt-1 by agents, such as resveratrol, protected against sepsis because Sirt-1 modulated the inflammatory/anti-inflammatory and oxidation/antioxidation balances [41,42]. Previous studies have demonstrated that Sirt-1 protected against sepsis through Sirt-1/Nrf-2 signaling [42]. Previous studies showed that exercise activated Sirt-1 in muscle tissue because exercise increased the level of cellular NAD⁺ and the NAD/NADH ratio [44]. Sirt-1/Nrf-2 signaling was established as a crucial mechanism underlying lung protection, but its physiological roles in the response to AE were unknown. In the present study, we determined whether AE could activate Sirt-1/Nrf-2 signaling and modulate the inflammatory/anti-inflammatory and oxidation/antioxidation balances in lung tissue during sepsis. Sirt-1/Nrf-2 signaling may be a new molecular mechanism through which regular AE protected against

LPS-induced lung injury.

In contrast to traditional therapeutic methods, AE was a comprehensive intervention treatment. AE improved sepsis through multiple mechanisms simultaneously. The protective effects of 4 weeks of exercise pretreatment on LPS-induced changes MODS, aortic injury, tissue edema, neutrophilic inflammation, pulmonary inflammation, and oxidative stress injury, Sirt-1/Nrf-2 signaling, serum HMGB1 levels, serum lactate levels, blood glucose levels were detected. All of these factors cooperated to improve the survival rate of septic mice.

5. Conclusions

In conclusion, our results showed that AE improved sepsis-induced lung, liver, kidney, heart, and aortic injury. An 4-week AE pretreatment modulated the inflammatory/anti-inflammatory and oxidation/antioxidation balance in mice with sepsis. AE improved lung injury by alleviating neutrophilic inflammation, oxidative stress injury as well as activating Sirt-1/Nrf-2 signaling. AE inhibited neutrophil infiltration in part by decreasing CXCL-1, CXCL-8, and TNF- α levels. AE, which impaired LPS-induced lactate and HMGB1 release, was a novel therapeutic strategy targeting aerobic glycolysis for sepsis.

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Data Availability Statement

The datasets used or analyzed in the current study were available from the corresponding author TD on reasonable request.

Author Contributions statement

WX and TD planned and designed the work. WX also designed the figures and performed the statistical analysis. WX performed the experiments and processed the experimental data. WZ aided in the experimental work and animal handling. WX and TD drafted the manuscript. All authors approved the submitted version.

Conflicts of Interest

The authors declare no conflict of interest. No conflict of interest exists in the submission of this manuscript, and the manuscript has been approved by all authors for publication.

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Figure legends

Figure 1. 4 weeks of exercise pretreatment improved mortality and regulated the Warburg effect. **(A)** Survival rate analysis, **(B)** Detection of blood glucose levels, **(C)** Detection of serum lactate levels, **(D)** Detection of serum HMGB1 levels, **(E)** Detection of the gene expression levels of HMGB1 levels in lung tissue, **(F)** Detection of W/D in lung tissue. @, $P < 0.05$, the difference is significant between the Con and LPS groups; #, $P < 0.05$, the difference is significant between the Con and Ex groups; *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups; &, $P < 0.05$, the difference is significant between the Ex and Ex + LPS group. Values are expressed as the means $\pm$ SD. HMGB1 = High Mobility Group Box 1, W/D = wet weight/dry weight ratio.

Figure 2. Morphological analysis of lung parenchyma. **(A)** Con group, **(B)** LPS group, **(C)** Ex group, **(D)** Ex + LPS group. (magnification, x 100).

Figure 3. The density of neutrophils in the lung tissues. The microphotograph shows the density of neutrophils in the lung tissue. (A) Con group, (B) LPS group, (C) Ex group, (D) Ex + LPS group, (E) the density of neutrophils in lung tissue. @, $P < 0.05$, the difference is significant between the Con and LPS groups; #, $P < 0.05$, the difference is significant between the Con and Ex groups; *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups; &, $P < 0.05$, the difference is significant between the Ex and Ex + LPS group. Values are expressed as the means $\pm$ SD. (magnification, x 400).

Figure 4. Morphological analysis of liver tissues. **(A)** Con group, **(B)** LPS group, **(C)** Ex group, **(D)** Ex + LPS group. (magnification, x 100).

Figure 5. Morphological analysis of kidney tissues. **(A)** Con group, **(B)** LPS group, **(C)** Ex group, **(D)** Ex + LPS group. (magnification, x 400).

Figure 6. Morphological analysis of heart tissues. **(A)** Con group, **(B)** LPS group, **(C)** Ex group, **(D)** Ex + LPS group. (magnification, x 100).

Figure 7. Morphological analysis of the aorta. **(A)** Con group, **(B)** LPS group, **(C)** Ex group, **(D)** Ex + LPS group. (magnification, x 100).

Figure 8. Detection of oxidative stress injury. The levels of MDA (**A**), MPO (**B**), GSH (**C**), and SOD (**D**) as indicators of oxidative stress injury were detected. @, $P < 0.05$, the difference is significant between the Con and LPS groups; #, $P < 0.05$, the difference is significant between the Con and Ex groups; *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups; &, $P < 0.05$, the difference is significant between the Ex and Ex + LPS group. Values are expressed as the means $\pm$ SD. MDA = malondialdehyde, MPO = myeloperoxidase, GSH = glutathione, SOD = superoxide dismutase.

Figure 9. Detection of gene expression levels in lung tissues. The gene expression levels of CXCL-1 (A), CXCL-8 (B), IL-1RN (C), IL-6 (D), IL-10 (E), Nrf-2 (F), Sirt-1 (G), and TNF-α (H) were detected. @, $P < 0.05$, the difference is significant between the Con and LPS groups; #, $P < 0.05$, the difference is significant between the Con and Ex groups; *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups; &, $P < 0.05$, the difference is significant between the Ex and Ex + LPS group.

Tables

Table 1. Total and differential cell counts in BALF (cells/ml).

	Con	Ex	LPS	Ex + LPS
Total cells	1.33 ± 1.11	1.26 ± 1.41	10.88 ± 2.14 ^{@@@}	8.58 ± 0.94*
Neutrophils	0.04 ± 0.02	0.03 ± 0.02	7.55 ± 1.23 ^{@@@}	5.84 ± 2.38*
Lymphocytes	0.04 ± 0.03	0.05 ± 0.04	0.16 ± 0.11	0.14 ± 0.16
Macrophages	1.06 ± 0.48	0.96 ± 0.39	3.14 ± 0.60 ^{@@@}	2.64 ± 0.19*
Eosinophils	0.02 ± 0.04	0.02 ± 0.03	0.01 ± 0.03	0.04 ± 0.05

LPS administration increased the number of total cells and neutrophils in BALF, while a 4-week exercise pretreatment prevented the up-regulation of the number of total cells and neutrophils in BALF. There was no effect of LPS or exercise on the number of lymphocytes, macrophages, or eosinophils in BALF. @, $P < 0.05$, the difference is significant between the Con and LPS groups. *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups. Values are expressed as the means ± SD.

Table 2. Cytokine levels in BALF (pg/ml).

	Con	Ex	LPS	Ex + LPS
CXCL-1	9.99 ± 5.65	6.88 ± 4.25	594.82 ± 128.67 ^{@@@}	466.94 ± 103.79*
CXCL-8	12.38 ± 3.54	7.62 ± 2.65	365.45 ± 82.66 ^{@@@}	255.45 ± 76.51**
IL-6	8.68 ± 2.36	50.57 ± 15.47	1226.56 ± 97.69 ^{@@@}	1064.44 ± 30.75*

IL-1RN	54.15 ± 3.88	47.86 ± 4.11	20.45 ± 10.79@@@	38.52 ± 15.17*
IL-10	9.56 ± 1.6	56.37 ± 38.01	495.83 ± 136.6@@@	660.74 ± 174.17**
TNF-α	18.34 ± 16.43	25.19 ± 14.45	343.28 ± 97.7@@@	251.64 ± 59.62***

The BALF levels of CXCL-1, CXCL-8, IL-6, IL-1RN, IL-10, and TNF-α were detected. @, $P < 0.05$, the difference is significant between the Con and LPS groups. *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups. **, $P < 0.01$, the difference is significant between the LPS and Ex + LPS groups. Values are expressed as the means ± SD.

Table 3. Cytokine levels in serum (pg/ml).

	Con	Ex	LPS	Ex + LPS
IL-6	10 ± 5.65	6.88 ± 4.25	1419.98 ± 580.11@@@	1669.21 ± 216.26
IL-10	36.44 ± 32.23	50.57 ± 15.41	249.89 ± 97.68@@@	254.44 ± 110.75
TNF-α	14.15 ± 3.88	17.86 ± 4.11	130.45 ± 36.79@@@	138.51 ± 58.17

We observed an effect of LPS in increasing the serum levels of IL-6, IL-10, and TNF-α. Compared with the LPS group. There was no difference between the LPS and Ex + LPS groups. @@@, $P < 0.001$, the difference is significant between the Con and LPS groups. Values are expressed as the means ± SD.

Table 4. The degrees of organ injury.

	Con	Ex	LPS	Ex + LPS
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Alanine aminotransferase (U/L)	33.82 ± 8.87	30.54 ± 7.12	137.33 ± 28.59@@@	64.27 ± 15.48***
Aspartate aminotransferase (U/L)	119.64 ± 14.96	102.29 ± 12.57	180.67 ± 26.45@@@	140.25 ± 25.31*
Liver injury score	0.15 ± 0.02	0.13 ± 0.01	6.78 ± 0.71@@@	3.51 ± 0.48***
Cre (μmol/L)	55.91 ± 11.98	61.38 ± 17.65	214 ± 10.35@@@	134 ± 15.49**
BUN (mmol/L)	7.99 ± 1.64	6.82 ± 1.93	13.56 ± 2.31@@@	10.59 ± 2.16*
Kidney injury score	0.52 ± 0.06	0.43 ± 0.03	6.54 ± 0.89@@@	3.25 ± 0.72***
Lung injury score	2.16 ± 0.29	2.44 ± 0.27	7.45 ± 0.87@@@	4.52 ± 0.63***
Myocardial injury score	0.13 ± 0.01	0.18 ± 0.01	2.79 ± 0.27@@@	2.16 ± 0.17@
Medial thickness of aorta	51.4 ± 10.12	50.46 ± 15.6	65.46 ± 11.8@@	57.46 ± 11.8*
Medium membrane elastic fiber area ratio	48.93 ± 9.24	50.61 ± 9.15	27.7 ± 4.01@	44.5 ± 8.34***

The markers of liver damage (ALT and AST) and markers of kidney damage (Cre and BUN) were detected. The medial thickness of the aorta and the medium membrane elastic fiber area ratio were measured. The lung injury score, liver injury score, kidney injury score, and myocardial injury score were measured. @, $P < 0.05$, the difference is significant between the Con and LPS groups. *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups. Values are expressed as the means ± SD.

Table 5. The density of neutrophils in liver, kidney, and heart tissues (cells/mm²).

	Con	Ex	LPS	Ex + LPS
The density of neutrophils in liver tissue	41.54 ± 8.65	37.93 ± 5.07	655.32 ± 113.22@@@	541.47 ± 58.56**
The density of neutrophils in kidney tissue	53.11 ± 8.96	45.86 ± 8.08	587.74 ± 103.64@@@	481.63 ± 52.99**
The density of neutrophils in heart tissue	38.54 ± 6.47	32.72 ± 6.04	379.78 ± 59.62@@@	227.81 ± 24.46**

LPS injection increased neutrophil content in liver, kidney, and heart tissues ($P < 0.01$), while exercise attenuated neutrophil content in liver, kidney, and heart tissues ($P < 0.01$). @, $P < 0.05$, the difference is significant between the Con and LPS groups. *, $P < 0.05$, the difference is significant between the LPS and Ex + LPS groups. Values are expressed as the means ± SD.

Table 6. PCR primer sequence information of mouse.

Gene name	Primer sequence
CXCL-1	CTGGGATTCACCTCAAGAACATC
	CAGGGTCAAGGCAAGCCTC
CXCL-8	ATGCCCTCTATTCTGCCAGAT
	GTGCTCCGGTTGTATAAGATGAC
IL-1RN	GCTCATTGCTGGGTACTTACAA
	CCAGACTTGGCACAAGACAGG
IL-6	CCAAGAGGTGAGTGCTTCCC
	CTGTTGTTCACTCTCTCCCT
IL-10	GCTCTTACTGACTGGCATGAG
	CGCAGCTCTAGGAGCATGTG

TNF- α	ATGTCTCAGCCTCTTCTCATTC GCTTGTCACTCGAATTTTGAGA
Sirt-1	GCTGACGACTTCGACGACG TCGGTCAACAGGAGGTTGTCT
Nrf-2	TCTTGGAGTAAGTCGAGAAGTGT GTTGAAACTGAGCGAAAAAGGC
GAPDH	AGGTCGGTGTGAACGGATTG TGTAGACCATGTAGTTGAGGTCA