Protective effects of aerobic exercise on survival, MOSD, and blood glucose response during sepsis

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Research Article

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Protective effects of aerobic exercise on survival, MOSD, and blood glucose response during sepsis

Running title: Aerobic exercise prevents sepsis and its complications

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Abstract

Aim: In the present study, we attempted to investigate whether aerobic exercise could prevent sepsis and its complications and explored the related mechanisms.

Methods: Forty ICR mice were divided into four groups: control (Con), LPS, exercise (Ex), and exercise + LPS (Ex + LPS). Low-intensity aerobic exercise was performed for 4 weeks. Forty-eight hours after the last exercise intervention, LPS and

Ex+LPS mice received 5 mg/kg LPS intraperitoneally for induction of sepsis. This study examined the effects of a 4-week exercise pretreatment on LPS-induced changes in blood glucose (BG) levels; inflammatory cytokine levels in BALF; bronchoalveolar lavage fluid (BALF) cell counts; the degree of pulmonary edema; neutrophil density in liver, lung, and kidney tissues; and the gene expression levels of IL-1RN, IL-8, IL-10, TNF-α, Sirt-1, and Nrf-2 in lung tissue. Sixty mice were used to perform survival rate analysis.

Results: A 4-week exercise pretreatment significantly reversed the histopathological severity of LPS-induced lung, heart, liver, kidney, and aortic injuries ($P < 0.05$) and ameliorated neutrophil inflammation in the lung, heart, liver, and kidney ($P < 0.05$). A 4-week exercise pretreatment improved survival rates ($P < 0.01$), deranged glucose homeostasis ($P < 0.01$), and pulmonary edema ($P < 0.01$) in mice with sepsis. A 4-week exercise pretreatment increased the levels of IL-6 ($P < 0.05$), IL-10 ($P < 0.05$), and IL-1RN ($P < 0.05$) and decreased the levels of IL-8 ($P < 0.05$) and TNF-α ($P < 0.05$) in BALF. A 4-week exercise pretreatment decreased the gene expression levels of IL-8 ($P < 0.01$) and TNF-α ($P < 0.01$) and increased the gene expression levels of IL-1RN ($P < 0.01$) and IL-10 ($P < 0.01$) in mice with sepsis. 4-week exercise pretreatment activated the gene expression levels of lung protective factors Sirt-1 ($P < 0.01$) and Nrf-2 ($P < 0.01$).

Conclusion: Aerobic exercise improved survival rates, multiple organ dysfunction syndromes (MODS), and deranged glucose homeostasis in mice with sepsis. Aerobic exercise alleviated lung injury partly because aerobic exercise exerted immune effects and activated Sirt-1/Nrf-2 signaling.

Keywords: aerobic exercise, sepsis, MODS, inflammation, Sirt-1/Nrf-2 signaling

Introduction

Sepsis, which has many complications, such as MODS and deranged glucose homeostasis, exhibits high mortality rates stemming from a systemic infection that involves alterations in inflammatory parameters in many tissues[1]. Exercise has emerged as a tool to treat many autoimmune and inflammatory diseases, such as

chronic obstructive pulmonary disease (COPD), asthma, and atherosclerosis because exercise has immunomodulatory effects[2-4]. However, limited research has demonstrated that exercise has emerged as a novel tool to prevent sepsis and its complications and the beneficial effects of exercise in a septic model have been demonstrated[5-9]. Exercised mice showed a survival benefit compared to unexercised mice in the septic model[8,10,11]. Exercise reduced ALI in mice subjected to LPS-induced sepsis[12-13]. Regular exercise induces specific adaptations resulting in reduced liver and kidney injury during severe polymicrobial sepsis[14-15]. Sepsis-induced MODS was a common complication of sepsis. Previous studies demonstrated that sepsis causes acute organ dysfunction, including dysfunction of the cardiovascular system[16], while previous studies ignored the effects of exercise on the cardiovascular system 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.

Deranged glucose homeostasis is a common complication of sepsis[17]. It is well documented that exercise could maintain glucose homeostasis in many animal models, including the diabetes model and obesity model[18-19]. However, it is not clear whether exercise pretreatment could maintain glucose homeostasis in the sepsis model.

Silent information regulator 1 (Sirt-1)/nuclear factor erythroid 2-related factor 2 (Nrf-2) pathway plays a pivotal role in the development of lung diseases. Sirt-1 exerts the effects of promoting lung cell proliferation and vitality and modulating the inflammatory-anti-inflammatory balance[20]. Activation of Sirt-1 by its activating agents such as resveratrol and SRT1720 protects against lung injury[21-22]. Previous studies have demonstrated that Sirt-1 played a vital role in protecting the lung system through Sirt-1/Nrf-2 signaling[23]. Sirt-1/Nrf-2 signaling was established as a crucial mechanism underlying lung protection, but its physiological roles in the response to aerobic exercise are unknown.

1. Materials and Methods

1.1. Animal

All the protocols in this study were approved by the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (IASI80321). 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 complied with the ARRIVE guidelines and every effort was made to minimize suffering. Forty male ICR mice (6 weeks old, 20-22 g) were provided by BEIJING HFK BIOSCIENCE CO., LTD. The mice were anesthetized via intraperitoneal injection of pentobarbital (50 mg/kg). The temperature of the animal room was maintained at 22 ± 5°C, the relative humidity was maintained at 50 ± 10%, and the illumination time was 12 hours/day.

1.2. Experimental design and sepsis-induced protocol

The ALI model was produced by intraperitoneal injection of 5 mg/kg LPS (L2880, Sigma-Aldrich, St. Louis, MO, USA). Forty mice were randomly assigned into one of four groups: (1) control group (Con), mice were injected with the same volume of saline via intraperitoneal injection; (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 aerobic exercise for 4 weeks; and (4) exercise plus LPS group (Ex + LPS), mice were submitted to aerobic exercise for 4 weeks and injected with 5 mg/kg LPS via intraperitoneal injection. Forty-eight hours after the last training, mice were injected with LPS, and the mice were killed 6 h after LPS injection. Each group contained 10 mice.

Forty-eight hours after the last exercise capacity test, LPS and Ex + LPS mice were injected intraperitoneally with 5 mg/kg LPS. LPS were dissolved and diluted with 0.9% saline. Forty-eight hours after the last training, mice were injected with LPS, and the mice were sacrificed at 6 h after LPS injection.

1.3. Exercise protocol

Training of mice was performed using a six-lane treadmill. Adaptive training of mice was performed for 3 days (20 minutes, 25% inclination, 3.3 m/min). After adaptive training, the maximal exercise capacity test of each mouse was performed.

The maximal aerobic capacity (100%) was determined depending on the mean maximal speed reached by each animal (23.6 m/s). Mice from the Ex and Ex + LPS groups were trained at low-intensity exercise (50% maximal speed, 11.8 m/s). The exercise intervention lasted for 4 weeks, 5 days/week, 1 hour/day. The exercise protocol was illustrated in Additional files 1 and 2.

1.4. Collection of tissue samples

Blood samples were collected from the retro-orbital sinus and put into a blood collection vessel containing anticoagulants, followed by centrifugation at 3000 r/min for 15 min. Serum was obtained and transferred to 1.5-ml sterile EP tubes and stored at -80°C immediately.

Two milliliters of ice-cold 0.9% NaCl was utilized for collecting BALF. The whole lung was flushed four times, and the returned fluid was collected, followed by centrifugation at 3000 r/min for 12 min at 4°C. The supernatant was stored at -80°C for subsequent analysis.

After sampling, lung, heart, liver, kidney, and aorta tissues were immediately placed into an incubator filled with liquid nitrogen. Then, the tissue was transferred to a liquid nitrogen tank for long-term preservation. For histological analysis, the lung, heart, liver, kidney, and aorta tissues of mice of appropriate size were fixed in 4% paraformaldehyde (PFA) for 24 hours.

1.5. Histopathology

For histological analysis, 4% PFA-fixed lung, cardiac, liver, kidney, and aorta tissues were embedded in paraffin. With the paraffin section method, 5-μm-thick sections were stained with hematoxylin and eosin (Merck, Darmstadt, Germany). Photographs taken by an inverted microscope were utilized to identify the degree of organ injury and inflammatory cell infiltration.

1.6. Detection of pulmonary permeability

To quantify the magnitude of pulmonary permeability, 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 weighed. The left lower lobes of lung tissue were dried in a drying case until a stable dry weight was obtained.

W/D was calculated according to the following formula: W/D = wet weight of the lung/ dry weight of the left lung.

1.7. Detection of BALF levels of IL-1RN, IL-6, IL-8, IL-10, and TNF-α

Following the manufacturer's specifications, the levels of the proinflammatory factors IL-8 and TNF-α and the anti-inflammatory factors IL-1RN, IL-6, and IL-10 in BALF were detected using mouse ELISA kits (Neobioscience, Neobioscience Technology Company).

1.8. Detection of glycometabolism

Following the manufacturer's specifications of the reagents, the blood glucose level in serum was measured by a blood glucose meter (Johnson Company).

1.9. Determination of gene expression levels in lung tissue

Total RNA was extracted from heart tissue using TRIzol (Promega, Madison, WI), and an Applied Biosystems 2720 Thermal Cycler was used to synthesize cDNA. Real-time fluorescence quantitative PCR was used to measure gene expression levels. GAPDH was selected as the internal reference gene, and the $2^{-\triangle\triangle CT}$ method was used for quantitative analysis. All primers were designed and synthesized by the Shanghai Sangon Biotech Company. Additional file 3 showed the PCR primer sequence information for mice.

1.10. 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 (L2880, 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, 1 hour/day. After the last training of 48h, the mice were intraperitoneally injected with 12 mg/kg LPS. The number of dead mice was recorded every 6 h for 48 h, and each group included 20 mice.

1.11. Data processing

SPSS 21.0 software was used for data processing. The experimental results are indicated by the mean ± standard deviation (x ± s). One-way ANOVA was applied in this study. The significance level was set at $P < 0.05$. * indicates that the difference in the data is significant compared with the Con group. # indicates that the difference in the data is significant compared with the LPS group. GraphPad Prism 6 software was used to draw pictures.

2. Results

2.1. Aerobic exercise improved mortality

Sham-operated mice exhibited 100% survival. After the administration of 12 mg/kg LPS, the mortality rate of mice increased significantly ($P < 0.01$). 4 weeks of exercise pretreatment ameliorated the mortality rate of mice at each point ($P < 0.01$) (Fig 1. A).

2.2. Exercise improved disturbed glucose homeostasis

Compared with the Con group, the administration of LPS markedly decreased the level of blood glucose ($P < 0.01$). A 4-week exercise pretreatment markedly increased the level of blood glucose compared with that in the LPS group ($P < 0.01$). These results suggested that LPS injection exerted detrimental effects on glucose homeostasis and that a 4-week aerobic exercise pretreatment improved disturbed glucose homeostasis in septic mice (Fig 1. B).

2.3. Aerobic exercise 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 2. a), LPS administration significantly increased lung injury, inflammatory infiltrates, and interstitial edema (Fig 2. b). There were no differences between the Con group and the Ex group (Fig 2. c). Compared with the LPS group, 4 weeks of exercise administration significantly decreased the degree of lung injury, inflammatory infiltrate, and interstitial edema (Fig 2. d), suggesting that aerobic exercise in mice subjected to LPS administration could prevent acute lung injury induced by LPS.

2.4. Aerobic exercise attenuated neutrophil content in the lung tissue

The microphotograph shows the density of neutrophils in the lung tissue. As Fig 3. e shows, compared with the Con group (Fig 3. a), the density of neutrophils increased after LPS administration (Fig 3. b) ($P < 0.01$). Compared with the LPS group, a 4-week exercise pretreatment prevented the upregulation of the density of neutrophils (Fig 3. d) ($P < 0.01$). There was no difference between the Con and Ex groups (Fig 3. c) ($P > 0.05$). These results suggested that aerobic exercise prevented acute lung injury partly because aerobic exercise attenuated neutrophil content.

2.5. Aerobic exercise relieved pulmonary edema

Compared with the Con group, the W/D increased significantly after the administration of LPS ($P < 0.01$). A 4-week exercise pretreatment exerted a prominent role in preventing the upregulation of W/D ($P < 0.01$), demonstrating that 4 weeks of exercise exerted positive effects on reducing the degree of pulmonary edema induced by LPS (Fig 1. C).

2.6. Aerobic exercise attenuated the number of total cells and neutrophils

The cell counts in BALF are shown in Table 1. LPS injection increased the number of total cells and neutrophils in BALF ($P < 0.01$), a 4-week exercise pretreatment prevented the upregulation of total cells and neutrophils in BALF($P < 0.01$). There was no effect of LPS or exercise on the number of lymphocytes, macrophages, or eosinophils in BALF ($P > 0.01$).

2.7. Aerobic exercise had immunosuppressive effects

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

2.8. Aerobic exercise relieved the acute liver injury

The liver lobules of the mice in the Con group (Fig 4. a) and in the Ex group (Fig 4.

c) 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 4. b). 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 4. d), suggesting that 4 weeks of aerobic exercise could attenuate neutrophil content and prevented the acute liver injury.

2.9. Aerobic exercise relieved kidney injury

The kidney tissues of the mice in the Con group (Fig 5. a) and in the Ex group (Fig 5. c) 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 demonstrated intact nuclei. The kidneys of the mice in the LPS group were severely damaged (Fig 5. b), the cells swelled, and the intercellular substance disappeared, accompanied by a large amount of neutrophil and hemocyte infiltration, severe epithelial vacuolization (arrowhead), flattening of the tubular epithelium (black arrow), and the appearance of an atypical shape with almost no nuclei (white arrow). The kidneys of the mice in the Ex + LPS group had significantly less kidney tissue structural damage (Fig 5. d) 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

control septic mice, and the degree of damage was significantly reduced.

2.10. Aerobic exercise prevented septicemic cardiomyopathy

In the Con group (Fig 6. a) and the Ex group (Fig 6. c), 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 6. b). 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 6. d). These results demonstrated that 4 weeks of aerobic exercise prevented septicemic cardiomyopathy.

2.11. Aerobic exercise prevented aortic injury

In the Con group (Fig 7. a) and the Ex group (Fig 7. c), 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 significantly decreased the area ratio of elastic fibers (Fig 7. b). Compared with the LPS group, a 4-week exercise pretreatment significantly increased the area ratio of elastic fibers and significantly increased the aorta media thickness (Fig 7. d).

2.12. Aerobic exercise prevented neutrophilic inflammation

As Table 4 showed, LPS injection increased neutrophil content in liver, kidney, and heart tissues ($P < 0.05$), while exercise attenuated neutrophil content in liver, kidney,

and heart tissues compared with the LPS group ($P < 0.05$).

2.13. Aerobic exercise prevented lung injury via the Sirt-1/Nrf-2 pathway

LPS injection significantly inhibited the gene expression levels of anti-inflammatory factors IL-1RN (Fig 8. A) and IL-10 (Fig 8. C) and lung-protective factors Sirt-1 (Fig 8. E), and Nrf-2 (Fig 8. F) ($P < 0.05$), and significantly increased the gene expression levels of proinflammatory factors IL-8 (Fig 8. B) and TNF-α (Fig 8. D) ($P < 0.05$). A 4-wk of exercise pretreatment activated the gene expression levels of IL-1RN, IL-10, Sirt-1, and Nrf-2 ($P < 0.05$), and inhibited the gene expression levels of IL-8 and TNF-α ($P < 0.05$).

3. Discussion

In the present study, we attempted to investigate whether aerobic exercise could prevent sepsis and its complications and investigated the potential mechanisms.

A 4-week exercise pretreatment not only played a prominent role in the restoration of the sepsis-induced liver, kidney, and heart injury but also played a prominent role in protecting against aortic injury[24]. The aortic injury occurs before the onset of inflammatory infiltration and organ injury[25]. Previous studies have overlooked the effects of sepsis on the aorta. We found that LPS injection increased aortic media thickness and reduced the area ratio of elastic fibers, which was improved by a 4-week exercise. These results demonstrated that sepsis had detrimental effects on the aorta and physical exercise could be used as a preventive tool for sepsis-induced aortic injury. The protective effects of exercise on aortic injury induced by LPS were first demonstrated.

In this study, we found sepsis was found to have detrimental effects on the aorta and the protective effects of exercise on aortic injury induced by LPS were first demonstrated.

Deranged glucose homeostasis was a common complication of sepsis. In the present study, we found that LPS injection decreased significantly the level of blood glucose, which conformed with the previous experiment results[17]. If sepsis-induced hypoglycemia is not treated promptly, patients may develop further and develop coma, which is easily confused with the coma caused by infection, leading to delayed diagnosis and treatment and even leading to death[26]. Hence, septicemia patients should monitor and supplement blood glucose timely. Strikingly, a 4-week exercise pretreatment exerted a prominent role in preventing the down-regulation of the level of blood glucose. It was first demonstrated that physical exercise could be used as a preventive tool for LPS-induced deranged glucose homeostasis.

Sepsis was an excessive and uncontrolled inflammatory response involving inflammatory mediators and effector cells in which neutrophils were crucial components[27-28]. Deleterious accumulation of neutrophils within remote vital organs led to collateral tissue damage and ultimately multiple organ failure[29]. Hence, deleterious activation of neutrophils is a critical reason leading to host tissue injury and organ damage during sepsis. In the previous study, abnormal accumulation of neutrophils in the lungs, liver, kidney, and heart tissues was observed in septic mice. 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 could prevent MODS partly because exercise could reduce abnormal accumulation of neutrophils.

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[25]. 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.

Sirt-1/Nrf-2 signaling has been established as a crucial mechanism of pulmonary protection, but little is known about the direct or indirect effects of aerobic exercise on Sirt-1/Nrf-2 signaling. In the present study, the physiological roles of Sirt-1/Nrf-2 signaling in the response to aerobic exercise were proven for the first time. This study confirmed that a 4-week aerobic exercise pretreatment could activate the Sirt-1/Nrf-2 signaling and significantly upregulate the expression of the lung-protective factors Sirt-1 and Nrf-2. We found a new signaling pathway through which regular aerobic exercise protected lung injury.

In contrast to traditional therapeutic methods, exercise is a comprehensive intervention treatment. Previous studies have mainly focused on single factors and single pathways. The idea that one or two pathways could play a decisive role in sepsis is not comprehensive. This may explain why traditional methods of sepsis are invalid. However, exercise played a positive role in sepsis through multiple mechanisms simultaneously. The protective effects of 4 weeks of exercise pretreatment on LPS-induced changes in blood glucose levels, MODS, tissue edema,

neutrophilic inflammation, pulmonary inflammation, and systemic inflammation were detected. All of these factors cooperated to improve the survival rate of septic mice.

4. Conclusions

In conclusion, our results show that aerobic exercise plays an important role in preventing sepsis-induced lung, liver, kidney, heart, and aortic injury. Exercise pretreatment plays a vital role in modulating the inflammatory-anti-inflammatory balance and glucose homeostasis in mice with sepsis.

Funding

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Author contributions

xs W designed the study, performed the research, analyzed the data, and wrote the paper. zhq W performed the research and analyzed the data, dh T developed the idea for the study.

Ethics approval

All protocols in this study were approved by the Animal Experimental Welfare of the Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China (IASI80321), in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no.85-23, revised 1996).

Consent for publication

No conflict of interest exists in the submission of this manuscript, and the

manuscript has been approved by all authors for publication.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

No conflict of interest exists in the submission of this manuscript, and the manuscript was approved by all authors for publication.

Acknowledgments

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Figure legends

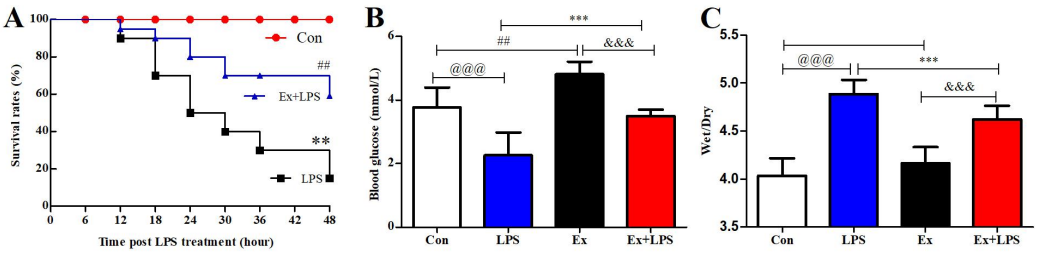

Figure 1. 4 weeks of exercise pretreatment improved mortality, glucose derangement, and lung edema in mice with sepsis.

Sham-operated mice exhibited 100% survival. After the administration of 12 mg/kg LPS, the mortality rate of mice increased significantly at 48 h (** $P < 0.01$). 4 weeks of exercise pretreatment ameliorated the mortality rate of mice at each point (## $P < 0.01$) (A). (n=20 per group).

LPS administration resulted in a decreased level of blood glucose compared to that of the Con group ($P < 0.01$). A 4-week exercise pretreatment resulted in increased levels of blood glucose compared to the LPS group ($P < 0.01$). These results suggested that sepsis exerted detrimental effects on glucose metabolism and that a 4-week exercise pretreatment maintained glucose homeostasis in septic mice (B).

Compared with the Con group, the wet/dry ratio increased significantly after LPS

injection ($P < 0.01$). Compared with the LPS group, 4 weeks of exercise pretreatment caused a significant decrease in wet/dry ($P < 0.01$). There was no difference between the Con and Ex groups ($P > 0.05$). These results demonstrated that aerobic exercise prevented lung edema induced by LPS (C).

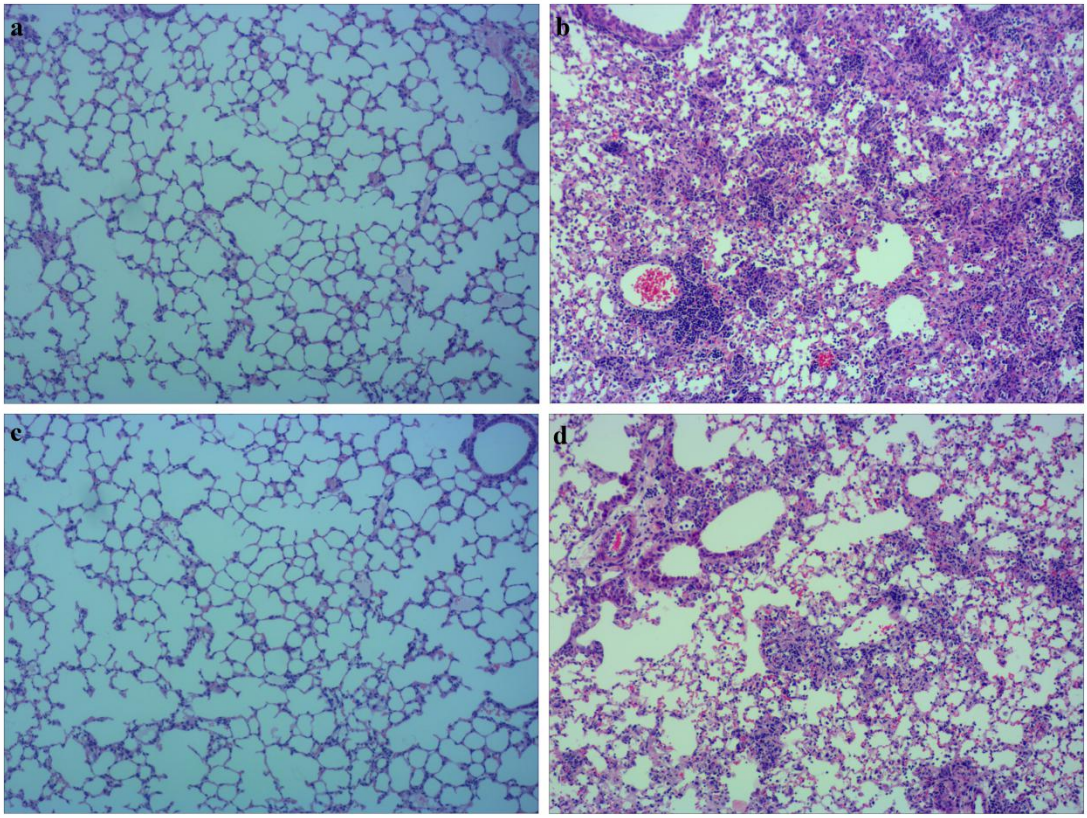

Figure 2. Morphological analysis of lung parenchyma

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 (a), LPS administration led to diffuse alveolar damage (DAD) and an increased degree of inflammatory infiltrate and interstitial edema (b). There was no difference between the Con group and the Ex group (c). Compared with the LPS group, a 4-week exercise pretreatment improved the degree of diffuse alveolar damage, inflammatory infiltrate, and interstitial edema (d), suggesting that a 4-week aerobic exercise could prevent ALI induced by LPS. (magnification, x 100).

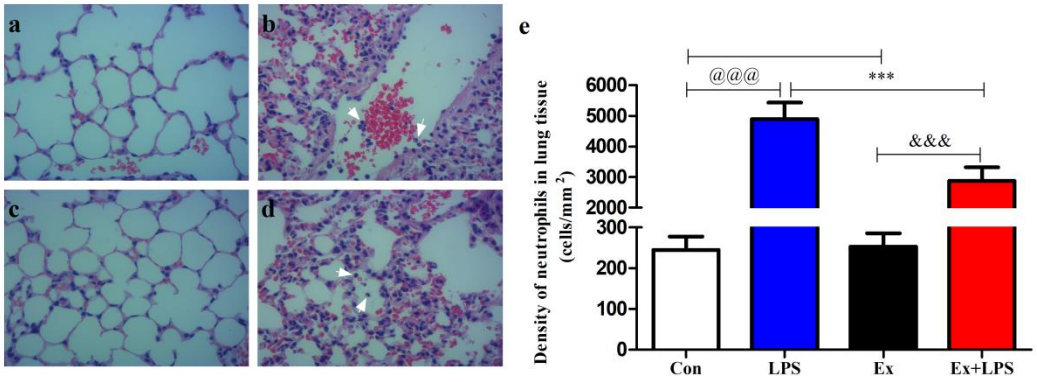

Figure 3. The density of neutrophils in the lung tissues

The microphotograph shows the density of neutrophils in the lung tissue. As figure e shows, compared with lung tissues of Con mice (a), the density of neutrophils increased significantly after LPS administration (b) ($P < 0.01$), while aerobic exercise in mice submitted to LPS administration prevented the upregulation of the density of neutrophils (d) ($P < 0.01$). There was no difference between the Con and Ex groups (c). These results suggested that aerobic exercise in mice submitted to LPS administration could prevent acute lung injury partly because aerobic exercise could attenuate the density of neutrophils. (magnification, x 400).

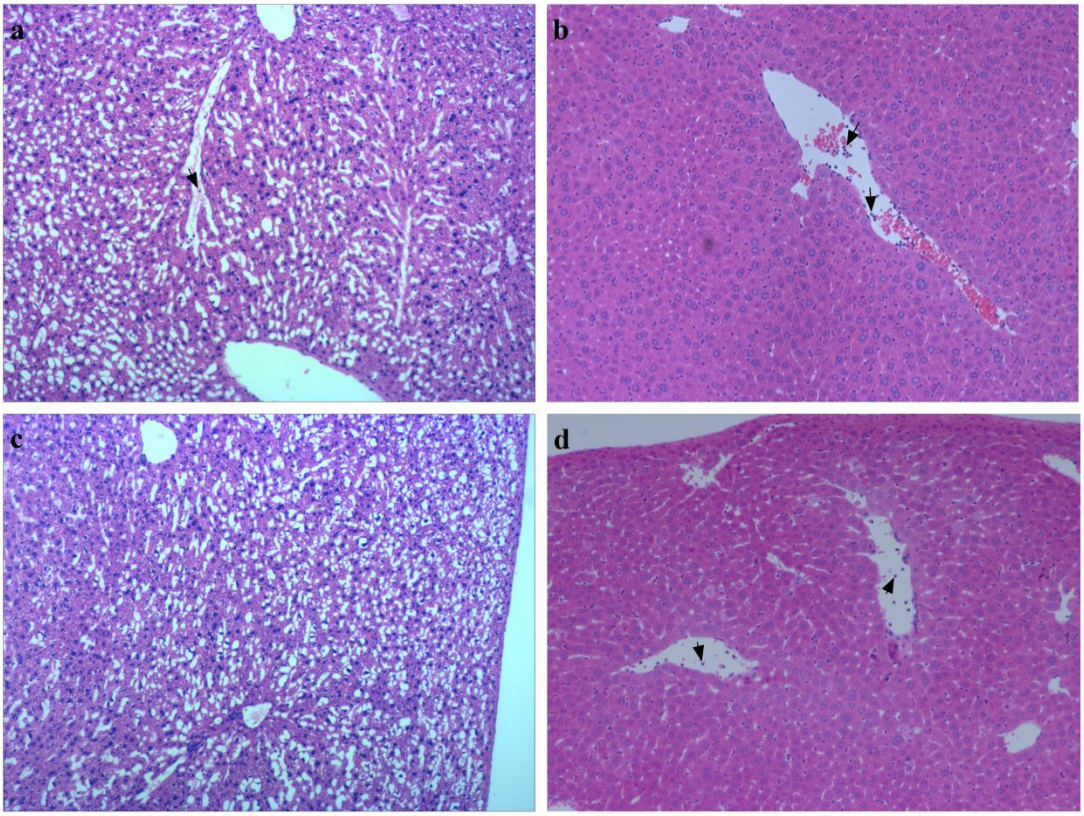

Figure 4. Morphological analysis of liver tissues

The liver lobules of the mice in the Con group (a) and the Ex group (c) 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, and the intercellular substance disappeared, accompanied by a large amount of neutrophil (black arrow) infiltration (b). 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 (d), suggesting that 4 weeks of aerobic exercise prevented acute liver injury and attenuated the neutrophil content in the liver tissue. (magnification, x 100).

As Table 3 shows, the markers of liver disease (ALT and AST) in the LPS group were significantly increased compared with those in the Con group ($P < 0.05$), while ALT and AST in the Ex + LPS group were significantly increased compared with those in the LPS group ($P < 0.05$).

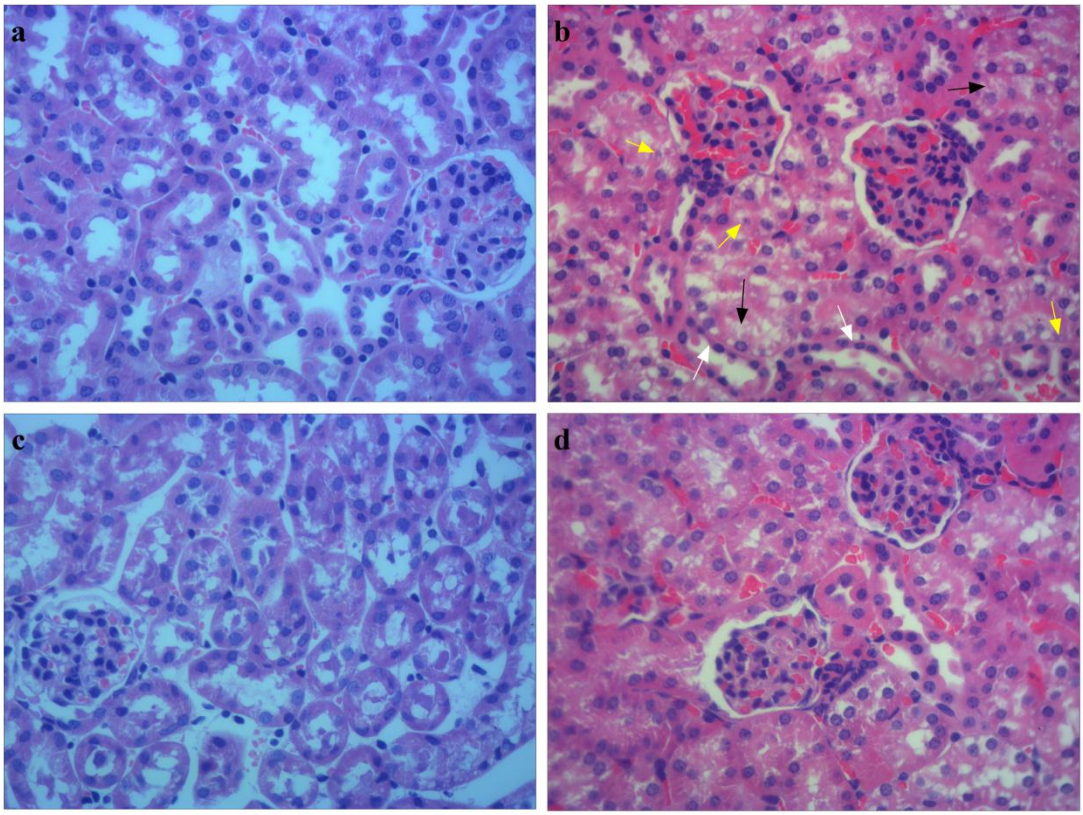

Figure 5. Morphological analysis of kidney tissues

The kidney tissues of the mice in the Con group (a) and the Ex group (c) were intact and clear, the cells were neatly arranged, the intercellular substance was free of edema, cortical tubular epithelial cells were well-shaped, and almost every epithelial cell demonstrated an intact nucleus. The kidneys of untrained septic mice were severely damaged, the cells swelled, and the intercellular substance disappeared. Untrained septic mice had severe epithelial vacuolization (black arrow), flattening of tubular epithelial cells (white arrow), and an atypical shape with almost no nuclei (yellow arrow) (b). A 4-week exercise pretreatment of septic mice had significantly less kidney tissue structural damage, with clearer nephrons, less flattening of tubular epithelium, and less vacuolization than unexercised septic mice (d), suggesting that 4 weeks of aerobic exercise prevented acute kidney injury. (magnification, x 400).

As Table 3 showed, the markers of kidney damage (Cre and BUN) in the LPS group were significantly increased compared with those in the Con group ($P < 0.05$),

while Cre and BUN in the Ex + LPS group were significantly increased compared with those in the LPS group ($P < 0.05$).

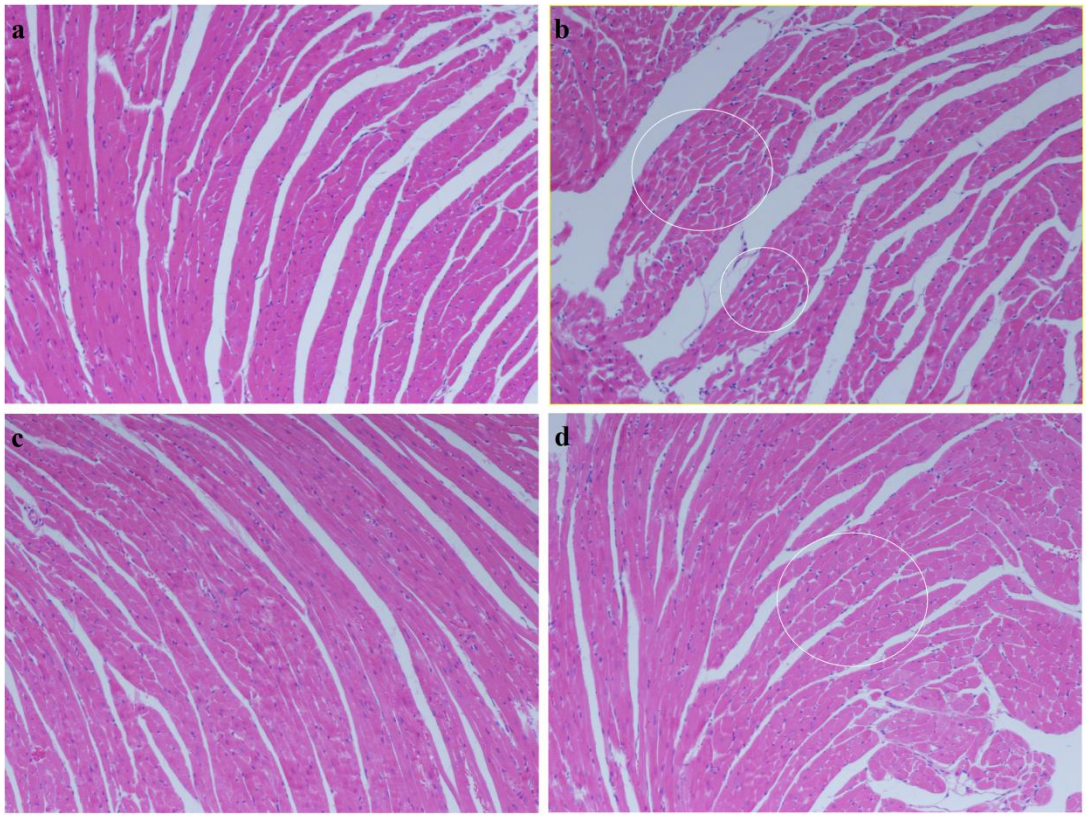

Figure 6. Morphological analysis of heart tissues

In the Con group (a) and the Ex group (c), the myocardial tissue was uniformly stained, the myocardial fibers were arranged regularly, the cell morphology, size, and arrangement were normal, the interstitial spaces were regular, and there was no inflammatory cell infiltration. In the LPS group, myocardial tissues were disordered, myocardial degeneration and dissolution were apparent, and a large number of inflammatory cells infiltrated the muscle space (b). Compared with the LPS group, the inflammatory cells in the Ex + LPS group were less infiltrated, the muscle fibers were still partially dissolved (d). Compared with the LPS group, the distribution of muscle fibers was improved, but it did not completely return to the normal form. This

suggests that the myocardial injury induced by sepsis was significantly reduced and that 4 weeks of aerobic exercise prevented septicemic cardiomyopathy (SIC). (magnification, x 100).

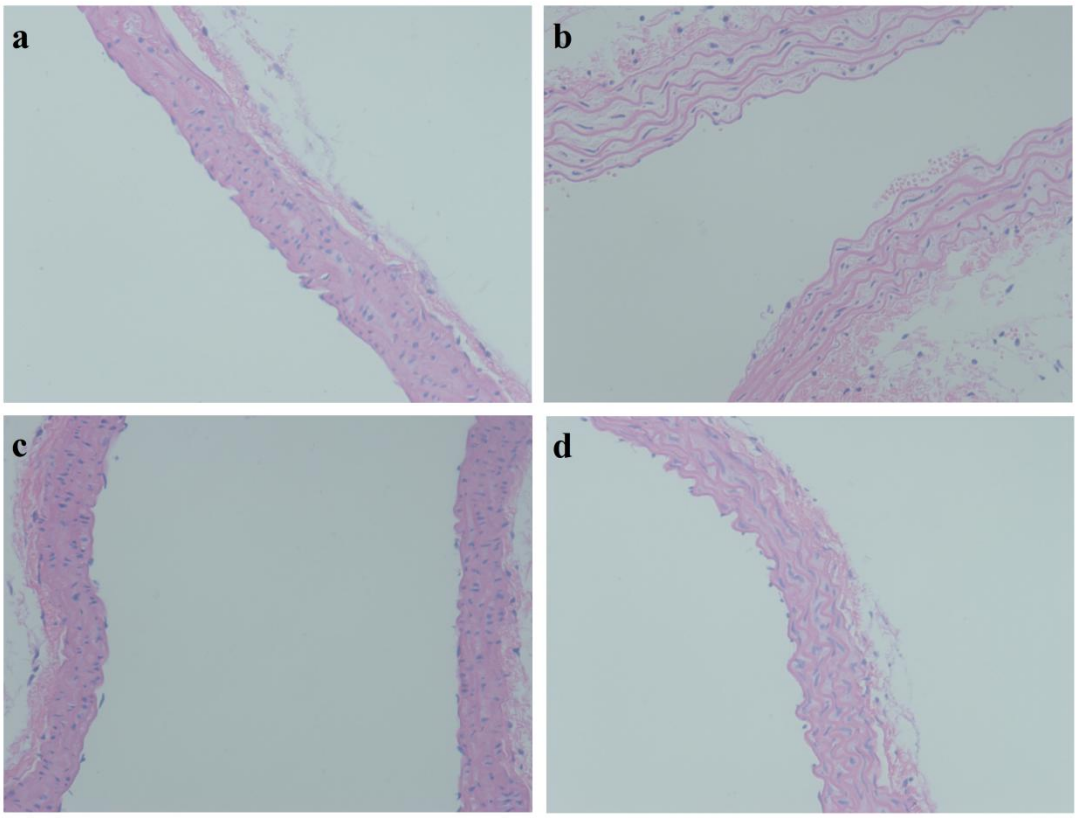

Figure 7. Morphological analysis of the aorta

In the Con group (a) and the Ex group (c), the elastic fibers of the media were compact, and the aortic media thickness was normal. In the LPS group, LPS injection increased the aortic media thickness and reduced the area ratio of elastic fibers (b). Compared to the LPS group, the elastic fibers of the media became compact, and the area ratio of elastic fibers increased in the Ex + LPS group (d). As Table 3 showed, LPS injection increased medial thickness of the aorta and decreased the medium membrane elastic fiber area ratio ($P < 0.05$), which was reserved by a 4-week exercise pretreatment ($P < 0.05$). (magnification, x 100).

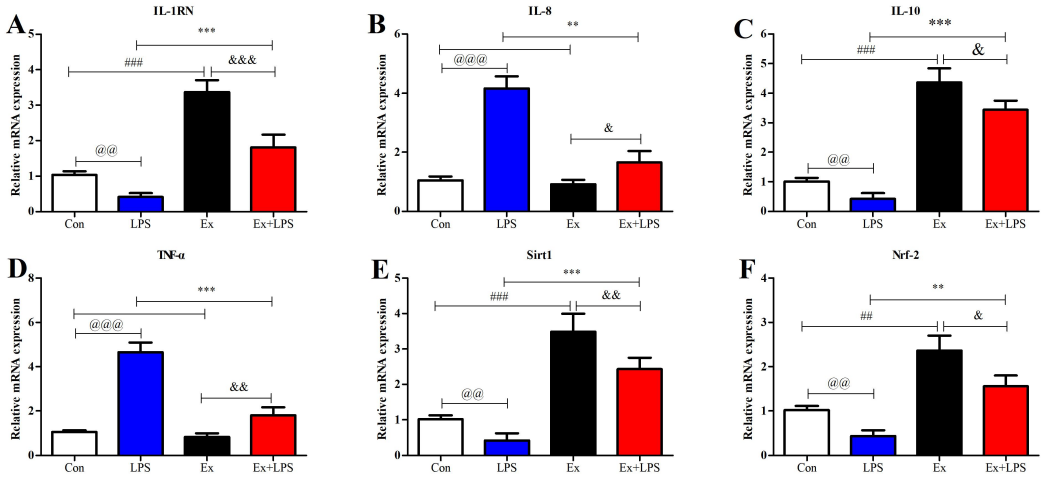

Figure 8. Aerobic exercise prevented lung injury via the Sirt-1/Nrf-2 pathway

Compared with the Con group, LPS injection inhibited the gene expression levels of IL-1RN (A) ($P < 0.01$), IL-10 (C) ($P < 0.01$), Sirt-1 (E) ($P < 0.01$), and Nrf-2 (F) ($P < 0.01$), and increased the gene expression levels of IL-8 (B) ($P < 0.01$) and TNF-α (D) ($P < 0.01$). Compared to the LPS group, a 4-week exercise pretreatment prevented the downregulation of the gene expression levels of IL-1RN ($P < 0.01$), IL-10 ($P < 0.01$), Sirt-1 ($P < 0.01$) and Nrf-2 ($P < 0.01$) and the upregulation of the gene expression levels of IL-8 ($P < 0.01$) and TNF-α ($P < 0.01$).

Tables

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

	Con	Ex	LPS	Ex + LPS	*P*
Total cells	1.33 ± 1.11	1.26 ± 1.41	8.88 ± 2.14[a]	6.58 ± 0.94[b]	< 0.001
Neutrophils	0.04±0.02	0.03±0.02	7.55 ± 1.23[a]	5.84 ± 2.38[b]	< 0.001
Lymphocytes	0.04 ± 0.03	0.05 ± 0.04	0.16 ± 0.11	0.14 ± 0.16	> 0.05

Macrophages	1.06 ± 0.48	0.96 ± 0.39	1.14 ± 0.60	0.96 ± 0.70	> 0.05
Eosinophils	0.02 ± 0.04	0.02 ± 0.03	0.01 ± 0.03	0.04 ± 0.05	> 0.05

The cell counts in BALF were shown in Table 1. LPS increased the number of total cells and neutrophils in BALF ($P < 0.001$), while a 4-week exercise pretreatment prevented the up-regulation of the number of total cells and neutrophils in BALF ($P < 0.05$). There was no effect of LPS or exercise on the number of lymphocytes, macrophages, or eosinophils in BALF.

[a] indicates that the difference in the data is significant compared with the Con group, [b] indicates that the difference in the data is significant compared with the LPS group. Values are expressed as the means ± SD.

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

	Con	Ex	LPS	Ex + LPS	*P*
IL-6	9.99 ± 5.65	6.88 ± 4.25	1289.6 ± 541.46[a]	1669.2 ± 216.26[b]	< 0.01
IL-8	9.6 ± 2.3	50.57 ± 15.4a	234.56 ± 97.69[a]	154.44 ± 30.75[b]	< 0.05
IL-1RN	54.15 ± 3.88	47.86 ± 4.11	20.45 ± 56.79[a]	68.52 ± 58.17[b]	< 0.05
IL-10	9.6 ± 1.6	56.3 ± 38	495.3 ± 156.6[a]	660.4 ± 234.1[b]	< 0.01
TNF-α	18.3 ± 16.3	25.1 ± 14.5	362.8 ± 171.7[a]	211.4 ± 159.6[b]	< 0.05

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

[a] indicates that the difference in the data is significant compared with the Con group, [b] indicates that the difference in the data is significant compared with the LPS group.

Values are expressed as the means ± SD.

Table 3. The degrees of organ injury

Group	Con	Ex	LPS	Ex + LPS	*P*
Alanine aminotransferase (U/L)	33.82 ± 8.87	30.54 ± 7.12	137.33 ± 28.59[a]	64.27 ± 15.48[b]	< 0.01
Aspartate aminotransferase (U/L)	119.64 ± 14.96	102.29 ± 12.57	180.67 ± 26.45[a]	140.25 ± 25.31[b]	< 0.05
Cre (μmol/L)	55.91 ± 11.98	61.38 ± 17.65	214 ± 10.35[a]	134 ± 15.49[b]	< 0.05
BUN (mmol/L)	7.99 ± 1.64	6.82 ± 1.93	13.56 ± 2.31[a]	10.59 ± 2.16[b]	< 0.05
Medial thickness of aorta	51.4 ± 10.12	50.46 ± 15.6	65.46 ± 11.8[a]	57.46 ± 11.8[b]	< 0.05
Medium membrane elastic fiber area ratio	48.93 ± 9.24	50.61 ± 9.15	27.7 ± 4.01[a]	44.5 ± 8.34[b]	< 0.05

LPS injection increased markers of liver damage (ALT and AST) and markers of kidney damage (Cre and BUN) ($P < 0.05$), which was improved by a 4-week aerobic exercise ($P < 0.05$). LPS increased the medial thickness of aorta and decreased the medium membrane elastic fiber area ratio ($P < 0.05$), which was reserved by a 4-week aerobic exercise ($P < 0.05$).

[a] indicates that the difference in the data is significant compared with the Con group, [b] indicates that the difference in the data is significant compared with the LPS group. Values are expressed as the means ± SD.

Table 4. The density of neutrophils in liver, kidney, and heart tissues (cells/mm^2)

	Con	Ex	LPS	Ex + LPS	*P*
The density of neutrophils in liver tissue	41.5 ± 8.6	37.9 ± 0.07	655.3 ± 113.2[a]	341.5 ± 58.5[b]	< 0.01
The density of neutrophils in kidney tissue	53.1 ± 8.9	45.8 ± 0.08	587.7 ± 103.6[a]	281.6 ± 52.9[b]	< 0.01

The density of neutrophils in heart tissue	38.5 ± 6.4	32.7 ± 0.04	379.7 ± 59.6[a]	127.8 ± 24.4[b]	< 0.01

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 ($P < 0.01$).

[a] indicates that the difference in the data is significant compared with the Con group, [b] indicates that the difference in the data is significant compared with the LPS group. Values are expressed as the means ± SD.

Additional material

Additional file 1: Treadmill for exercising mice. The picture illustrates the mice during exercise.

Additional file 2: Video of the treadmill with mice. The video illustrates the mice during exercise.

Additional file 3: PCR primer sequence information for mice.

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Figures

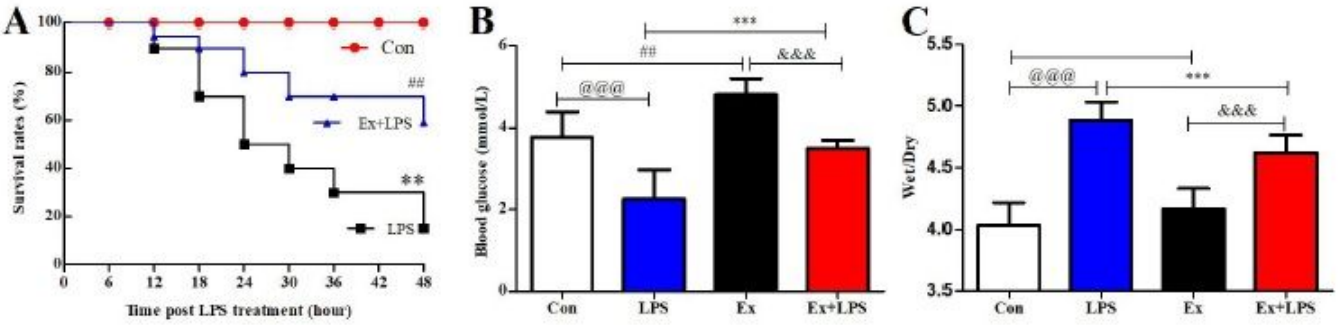

Figure 1

4 weeks of exercise pretreatment improved mortality, glucose derangement, and lung edema in mice with sepsis.

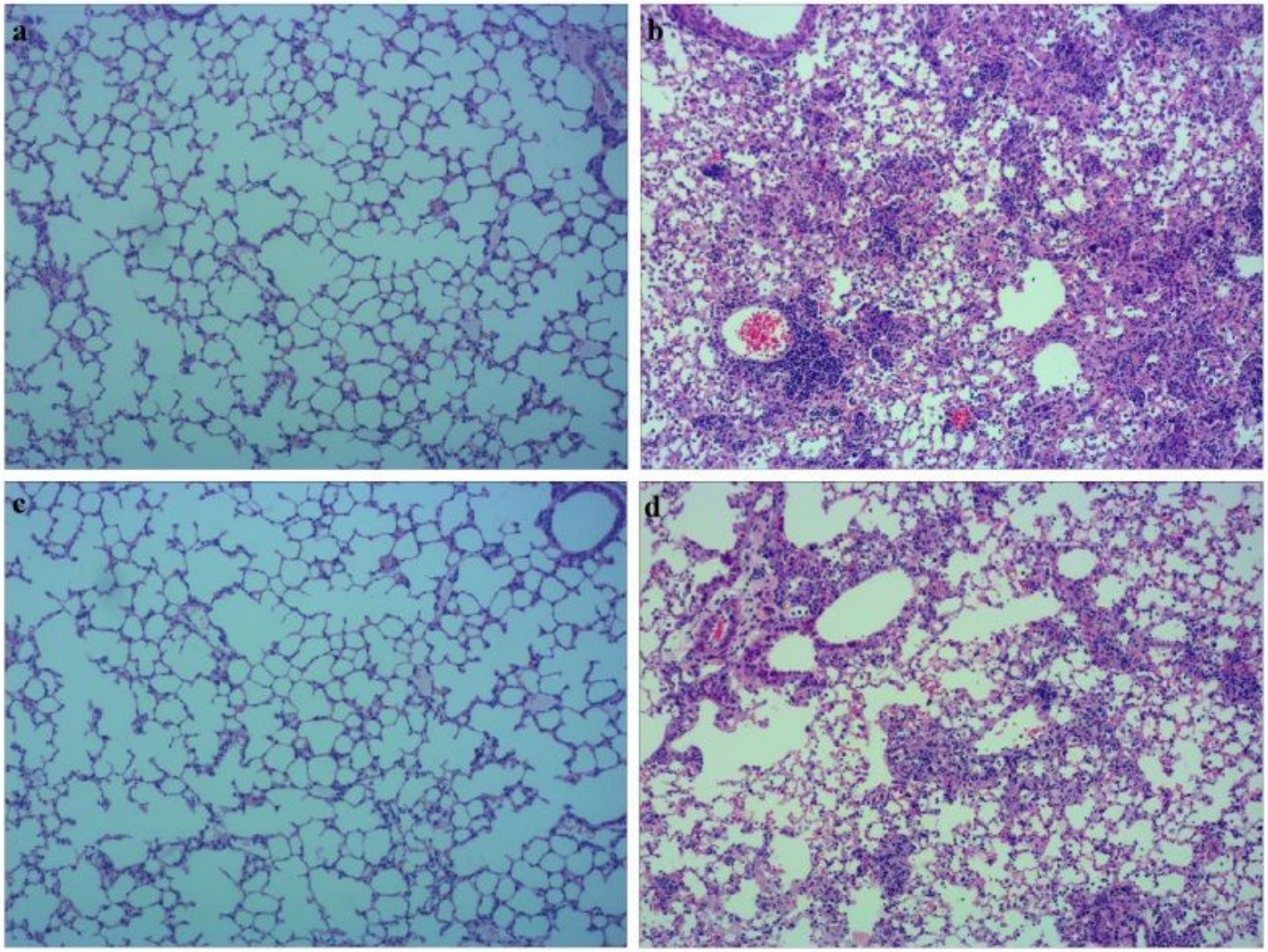

Figure 2

Morphological analysis of lung parenchyma

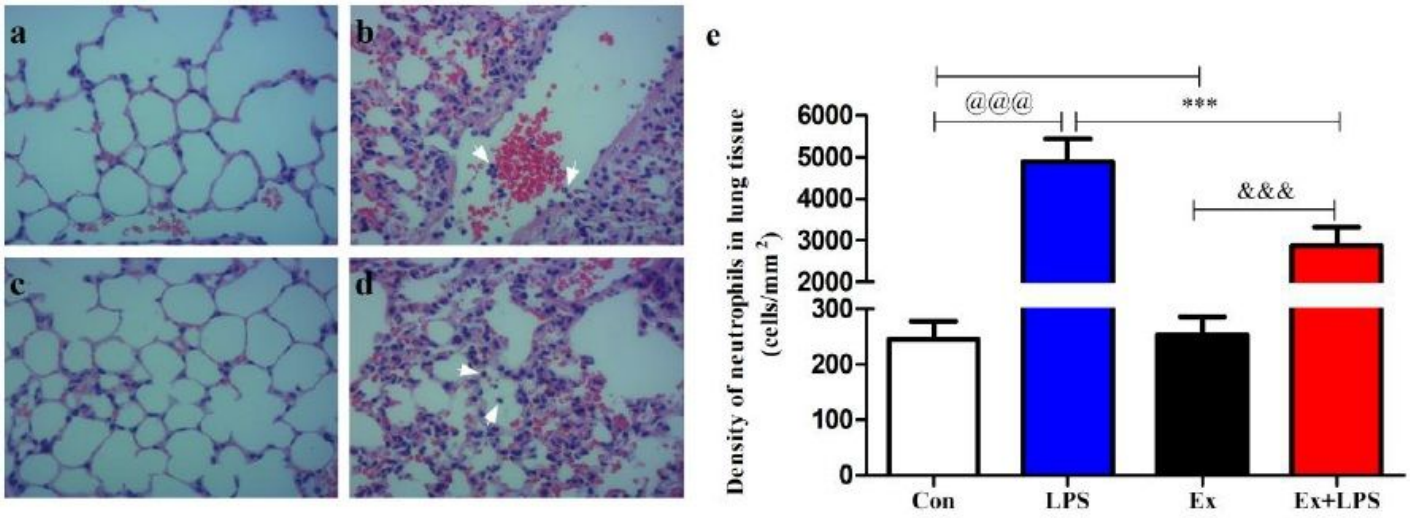

Figure 3

The density of neutrophils in the lung tissues

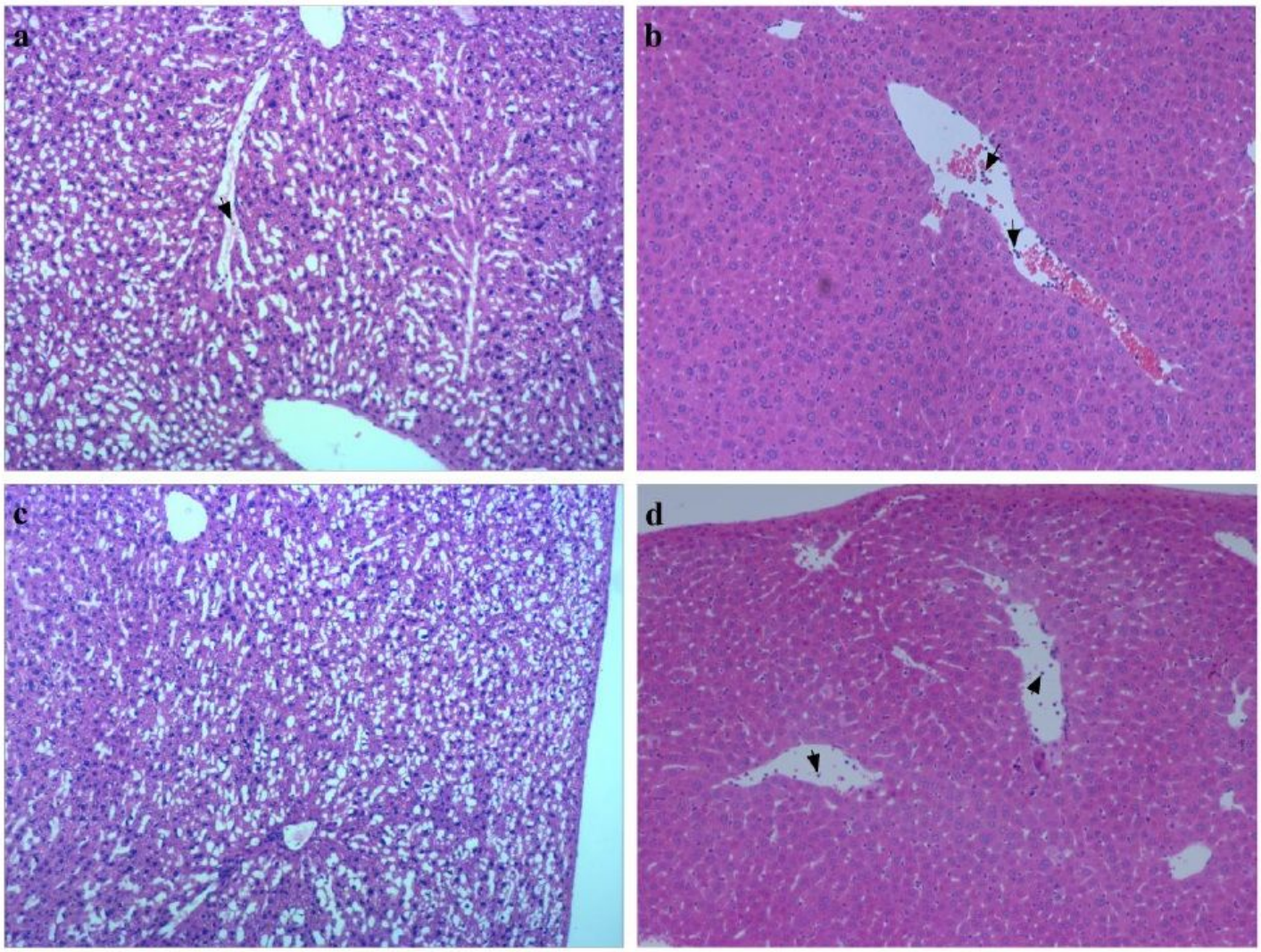

Figure 4

Morphological analysis of liver tissues

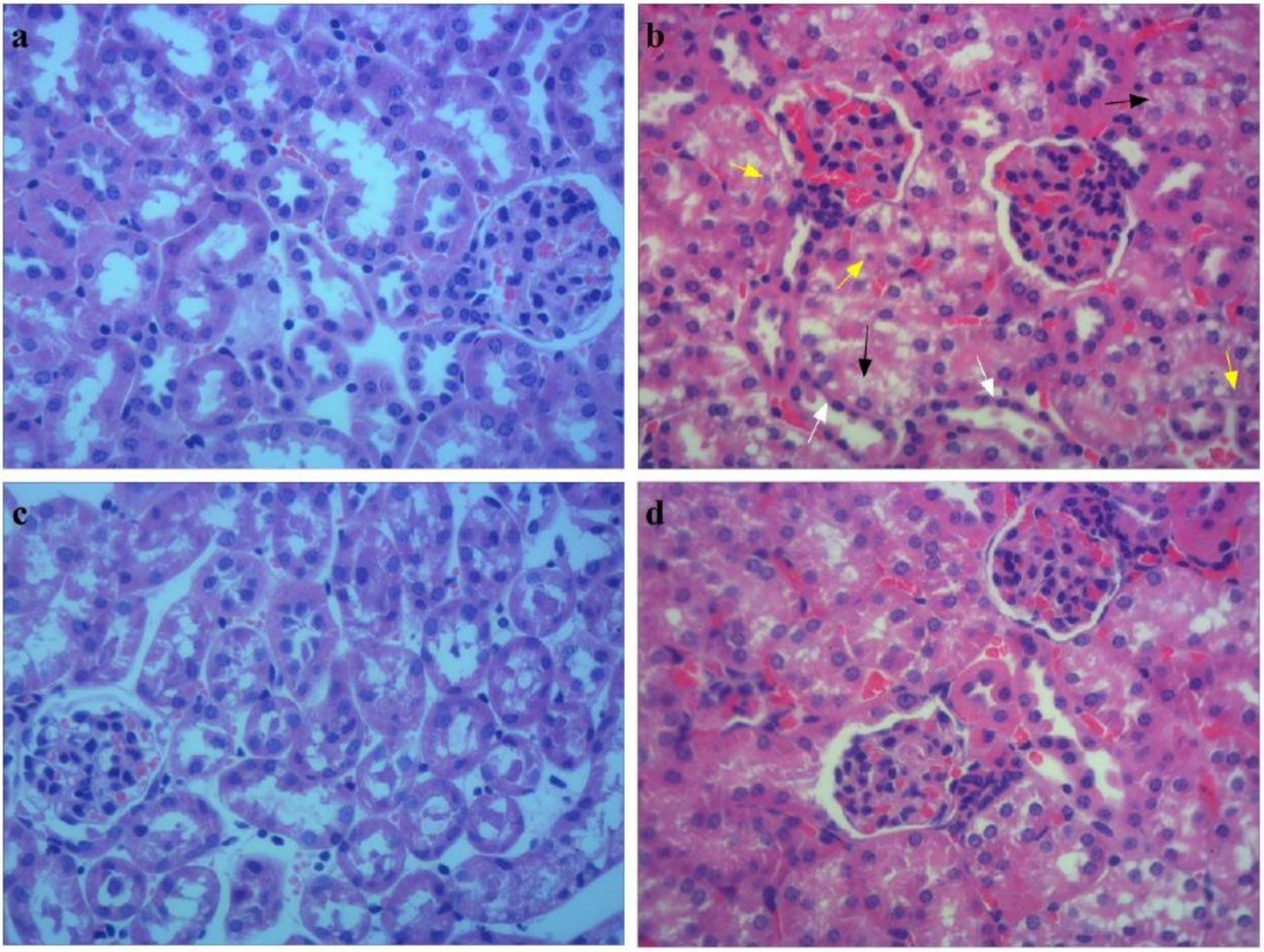

Figure 5

Morphological analysis of kidney tissues

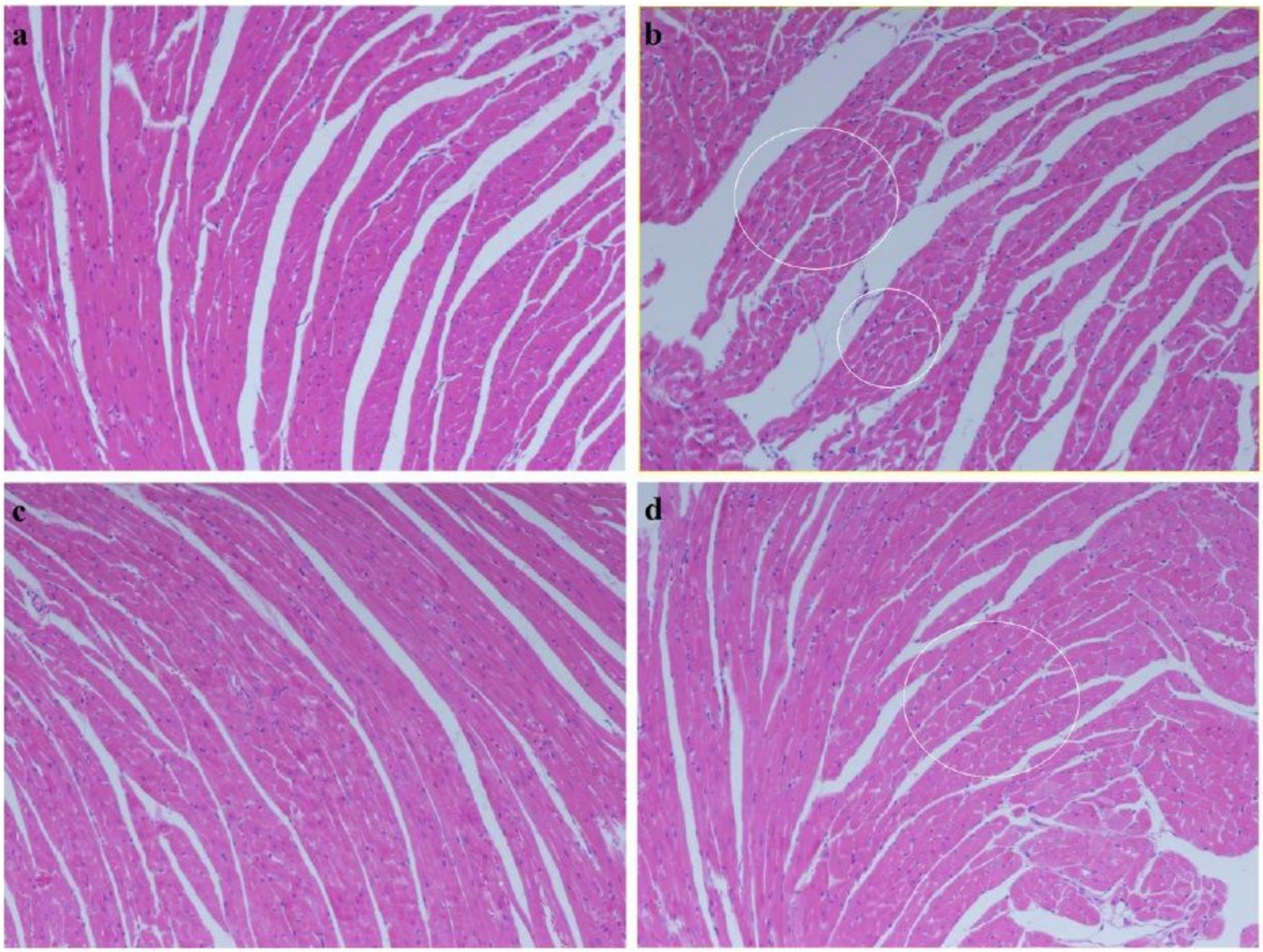

Figure 6

Morphological analysis of heart tissues

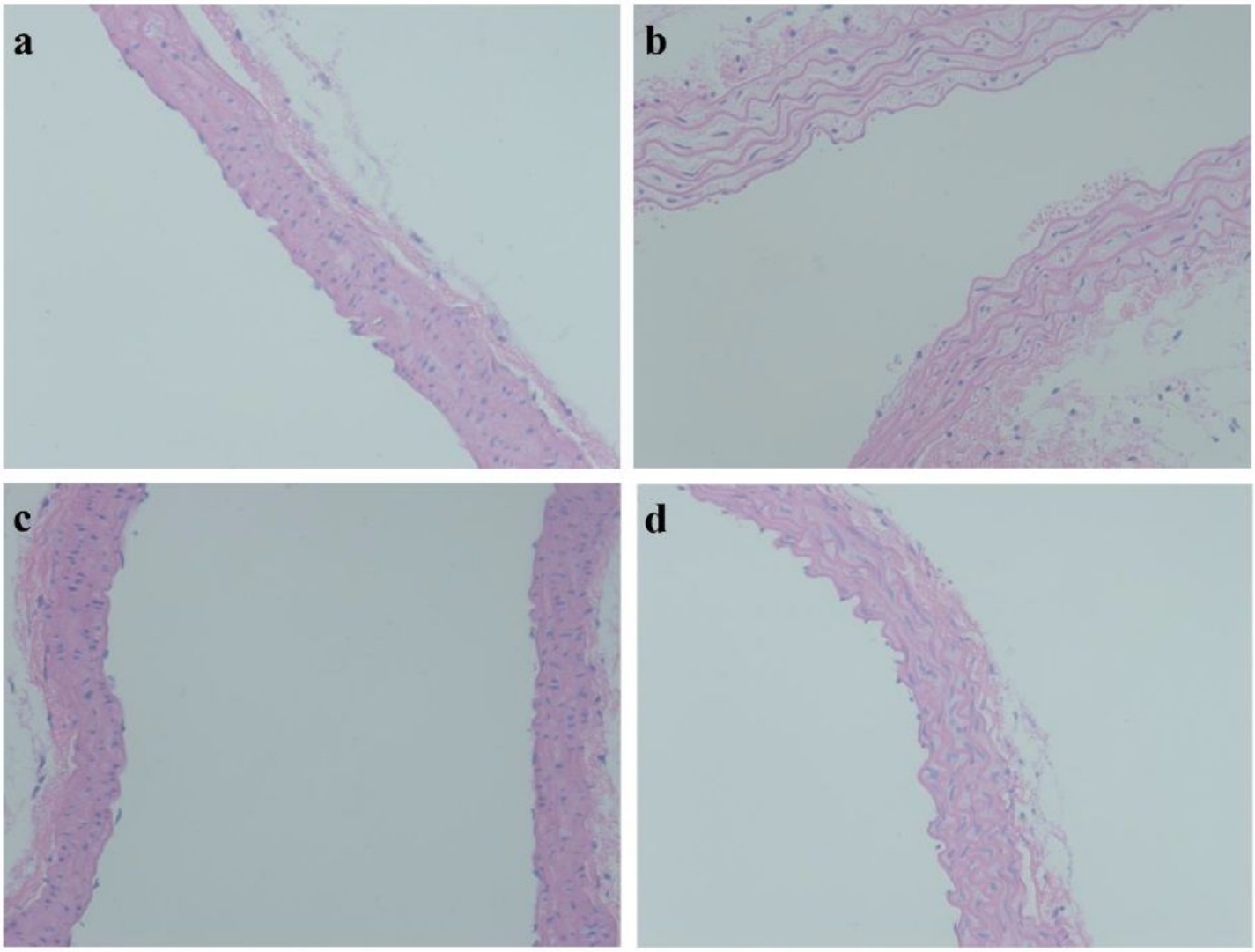

Figure 7

Morphological analysis of the aorta

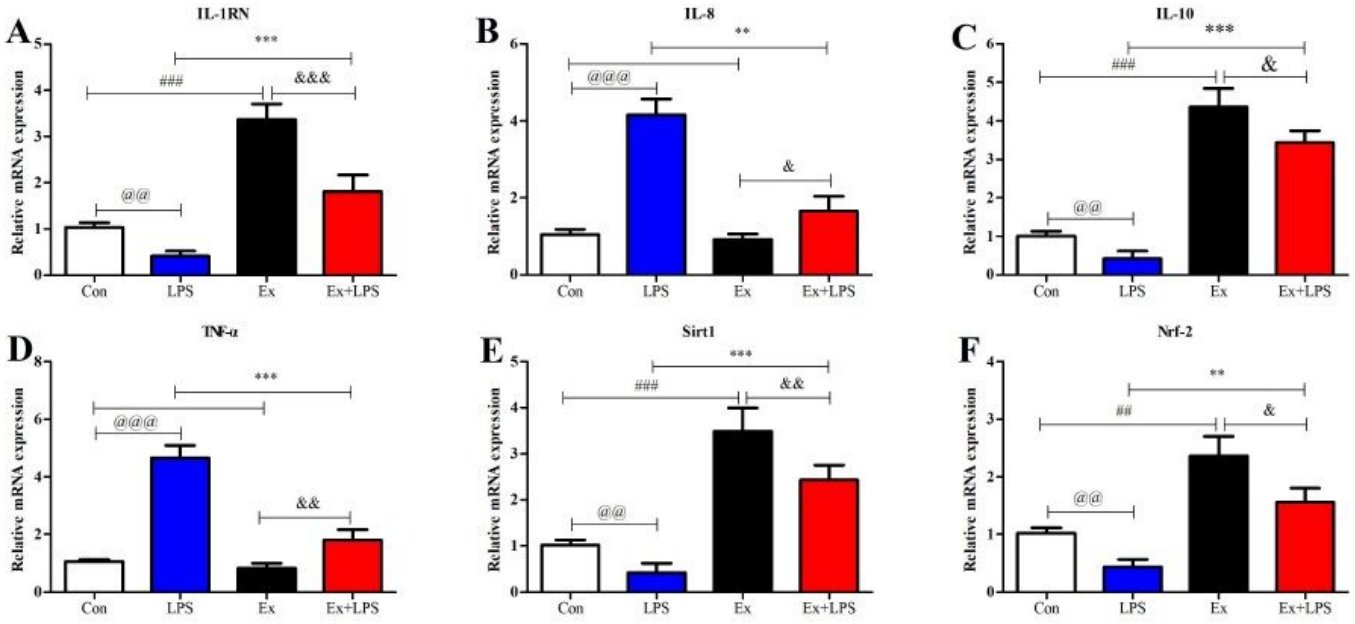

Figure 8

Aerobic exercise prevented lung injury via the Sirt-1/Nrf-2 pathway

Supplementary Files

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- Additionalfile1.tif
- Additionalfile2.avi
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