Activation of PPARγ Protects Obese Mice from Acute Lung Injury by Inhibiting Endoplasmic Reticulum Stress and Promoting Mitochondrial Biogenesis

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Received 10 June 2022; Revised 20 August 2022; Accepted 6 September 2022; Published 2 September 2022

Objective. Obesity-induced endoplasmic reticulum (ER) stress plays a role in increased susceptibility to acute lung injury (ALI)/acute respiratory distress syndrome (ARDS). The activation of peroxisome proliferator-activated receptor-γ (PPARγ) is associated with lung protection and is effective in ameliorating ER stress and mitochondrial dysfunction. The aim of this study was to investigate the expression of PPARγ in the lung tissues of obese mice and explore whether the PPARγ-dependent pathway could mediate decreased ALI/ARDS by regulating ER stress and mitochondrial biogenesis.

Methods. We determined PPARγ expression in the lung tissues of normal and obese mice. ALI models of alveolar epithelial cells and of obese mice were used and treated with either PPARγ activator rosiglitazone (RSG) or PPARγ inhibitor GW9662. Lung tissue and cell samples were collected to assess lung inflammation and apoptosis, and ER stress and mitochondrial biogenesis were detected.

Results. PPARγ expression was significantly decreased in the lung tissue of obese mice compared with that in normal mice. Both in vivo and in vitro studies have shown that activation of PPARγ leads to reduced expression of the ER stress marker proteins 78-kDa glucose-regulated protein (GRP78), C/EBP homologous protein (CHOP), and Caspase12. Conversely, expression of the mitochondrial biogenesis-related proteins peroxisome proliferator-activated receptor γ coactivator 1 (PGC-1α), nuclear respiratory factor-1 (NRF-1), and mitochondrial transcription factor A (TFAM) increased. Furthermore, activation of PPARγ is associated with decreased levels of lung inflammation and epithelial apoptosis and increased expression of adiponectin (APN) and mitofusin2 (MFN2). GW9662 bound to PPARγ and blocked its transcriptional activity and then diminished the protective effect of PPARγ on lung tissues.

Conclusions. PPARγ activation exerts anti-inflammation effects in alveolar epithelia by alleviating ER stress and promoting mitochondrial biogenesis. Therefore, lower levels of PPARγ in the lung tissues of obese mice may lead to an increased susceptibility to ALI.

1. Introduction

The dramatic rise in the global prevalence of obesity is a growing health concern that seriously affects the quality of life and reduces life expectancy [1]. A series of characteristic comorbidities have been linked with obesity, including sleep apnea, diabetes, hypertension, hyperlipidemia, and heart disease [2]. It was also recognized that obesity is a chronic inflammatory state, which is associated with increased risk of acute lung injury (ALI) or even acute respiratory distress syndrome (ARDS) [3, 4]. The mechanism underlying the aggravation of ALI in obese individuals has not yet been clarified. Current evidence has attributed this susceptibility to ALI due to disturbances in adipokine secretion or aberrant endoplasmic reticulum (ER) stress [5, 6].

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that are members of the nuclear receptor superfamily. The PPAR superfamily comprises three subtypes, PPARα, PPARγ, and PPARβ/δ, with differential tissue distribution [7]. As one of the three
PAR subtypes, PPARγ can be expressed in alveolar epithelial cells, vascular endothelial cells, and macrophages [8–11]. PPARγ is involved in processes such as adipogenesis, insulin sensitivity, mitochondrial biogenesis, anti-inflammation, and neuroprotection [12–15]. In addition, PPARγ plays a protective role in ALI [16], lung cancer [17], chronic obstructive pulmonary disease, and other respiratory diseases [18]. PPARγ ligands include synthetic and natural ligands. Synthetic ligands include thiazolidinedione antidiabetic drugs such as RSG, pioglitazone, and troglitazone. Among them, RSG is a representative PPARγ agonist [19]. PPARγ is present in both the cytoplasm and the nucleus. Ligand-activated PPARγ regulates target genes through heterodimerization with retinoid X receptor (RXR) [20]. GW9662 is a specific antagonist of PPARγ that covalently binds to the PPARγ ligand-binding pocket, preventing the activation of ligand binding and disrupting PPARγ signaling [21].

Activation of PPARγ can reduce the release of inflammatory factors, but its expression is always inhibited during ALI [22]. To date, the level of PPARγ in the lung tissues of obese mice is still unknown, nor is its role in the progression of ALI. PPARγ is beneficial for promoting mitochondrial biogenesis and inhibiting ER stress, a prominent feature associated with diabetes, obesity, and chronic inflammation [23–25]. Previous studies have suggested that ER stress and mitochondrial dysfunction play key roles in mediating lung injury in obese mice [6, 26]. In this study, we hypothesized that PPARγ activation can protect obese mice against ALI by regulating ER stress and mitochondrial biogenesis. Accordingly, we investigated the expression of PPARγ in the lung tissues of obese mice and explored the role of PPARγ in LPS-induced injury in lung tissues and pulmonary epithelial cells.

2. Methods

2.1. Experimental Animals. The animal experimental protocol was approved by the Animal Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (2021-527). This experiment was performed in accordance with the Guidelines for the Care and Use of Experimental Animals. Six-week-old male C57BL/6J mice, weighing 15–18 g, were purchased from the Experimental Animal Center of Chongqing Medical University. During the study, all mice were kept on a 12/12 h light/dark cycle. The animals were fed either a normal chow diet or a high-fat diet with 60% calories from fat (TP2330055A, Trophic Animal Feed High-tech Co., Ltd., China) for 12 weeks. In this study, every effort was made to minimize distress in mice.

The mice were anesthetized by sodium pentobarbital (50 mg/kg) (Sigma-Aldrich), injected intraperitoneally [16]. ALI was induced by administering 100 μg (1 mg/mL) of lipopolysaccharide (LPS, L8880, Solarbio, Beijing, China) into the trachea of anesthetized mice and gently pulling out the tongue with forceps to facilitate fluid entry into the lungs [6]. Both PPARγ activator RSG (HY-17386, MedChemExpress, Shanghai, China) and PPARγ inhibitor GW9662 (HY-116578, MedChemExpress) were dissolved in 10% dimethyl sulfoxide (DMSO; D8371, Solarbio, Beijing, China). RSG and GW9662 were injected intraperitoneally at respective doses of 10 mg/kg and 1 mg/kg. The RSG and GW9662 doses were determined based on previous studies [16, 27, 28]. An equal volume of DMSO was injected intraperitoneally as a vehicle control for all mouse experiments.

2.2. Animal Experimental Design. According to our experimental plan, the mice were divided into the following 8 groups (n = 5): (1) lean group (normal diet mice): 100 μL sterile saline administered intratracheally; (2) DIO group (diet-induced obese mice): 100 μL of sterile saline administered intratracheally; (3) lean-ALI group: LPS (100 μg) administered intratracheally; (4) in the DIO-ALI group, mice were administered 100 μg LPS intratracheally; (5) DIO-DMSO group: mice were injected intraperitoneally with 10% DMSO, and 30 min later, 100 μL sterile saline was introduced in the mouse trachea; (6) in the DIO-ALI-DMSO group, mice were intraperitoneally injected with 10% DMSO, and 30 min later, 100 μg of LPS was introduced in the mouse trachea; (7) in the DIO-ALI-RSG group, mice were intraperitoneally injected with RSG (10 mg/kg), and 30 min later, 100 μg of LPS was introduced in the mouse trachea; and (8) DIO-ALI-RSG-GW9662 group: mice were treated the same as in the DIO-ALI-RSG group, but were intraperitoneally injected with GW9662 (1 mg/kg) 30 min before administration of RSG, while the other groups received the same amount of normal saline. A schematic of the experimental protocol is shown in Figure 1(a).

2.3. Collection of Bronchoalveolar Lavage Fluid (BALF). The mice were euthanized at 24 h after LPS treatment, and the right main bronchus was ligated. The left lung was doused by administering sterile saline (3 × 0.5 mL) into the trachea. The collected BALF was centrifuged at 200 × g for 10 min to separate the pellet and supernatant, and the supernatant so obtained was stored at −80°C. The levels of tumor necrosis factor-alpha (TNF-α) and Interleukin-1β (IL-1β) in BALF were determined using an enzyme-linked immunosorbent assay (ELISA) kit (Neobioscience Technology Company, Shenzhen, China) according to the manufacturer’s instructions.

2.4. Lung Wet/Dry (W/D) Weight Ratio. The right parietal lobe lung tissue was weighed to determine the wet weight. The sample was dried in an oven at 80°C for 48 h, and was weighed again to obtain the dry weight. Finally, the W/D of the lung was obtained.

2.5. Histopathology. The lower lobe of the right lung was fixed in 4% paraformaldehyde and embedded in paraffin. Paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E). Alveolar edema, hemorrhage, alveolar septal thickening, and infiltrating leukocyte count were recorded to assess the extent of lung injury. Each of these histopathological elements was allotted four grades from 0 to 3 (0 = normal, 1 = mild, 2 = moderate, and 3 = severe) [29].

2.6. Immunohistochemical Staining. Immunohistochemical staining was performed as follows. Paraffin-embedded specimens were sectioned at 5 μm, deparaffinized, and hydrated. The sections were then incubated with 3% H₂O₂ for 10 min and rinsed with phosphate-buffered saline (PBS). Primary antibodies against GRP78 (ab21685, Abcam) and PGC-1α...
(ab191838, Abcam) were used. The slides were then washed and incubated with secondary antibodies at room temperature for 30 min. The sections were observed under an optical microscope.

2.7. TUNEL Staining. To evaluate the LPS-induced apoptosis of alveolar epithelial cells in obese mice, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was performed according to the manufacturer’s instructions (G1507, Servicebio, Wuhan, China).

2.8. Cell Culture and Treatment. A549 human lung epithelial cells were used to study the effects of PPARγ on alveolar epithelial cells. The A549 cell line is commonly used to study inflammation and apoptosis induced by LPS [30, 31], and PPARγ has been shown to be expressed in this cell line [32, 33]. A549 cells were seeded into plastic petri dishes with RPMI 1640 medium (C11875500BT, Gibco, USA) containing 10% fetal bovine serum (10099-141C, Gibco, USA), 100 U/ml penicillin, and 0.1 mg/ml streptomycin (SV30010, HyClone, USA) and placed at 37°C with 5% CO₂ in the incubator. The cells were divided into four groups: control, LPS, LPS-RSG, and LPS-RSG-GW9662. The LPS group was treated with 10 μg/ml LPS. In the LPS-RSG group, 10 μM RSG was added 30 min before the addition of LPS. In the LPS-RSG-GW9662 group, 20 μM GW9662 was added 30 min before adding RSG. An equal volume of PBS was added to the control group. Doses of these drugs were determined based on previous studies [12, 23, 34, 35]. RSG and GW9662 were incubated in vitro in a final concentration of 0.1% DMSO. At 24 h after LPS treatment, the cells were collected for further experiments.

2.9. Cytokines Assays. Cell culture supernatants were collected, added to sterile tubes, and centrifuged at 560×g for

![Figure 1: PPARγ expression was decreased in the lung tissue of obese mice. (a) Schematic diagram of experimental protocol, (b) Body weight of C57BL/6J mice fed with either high fat diet or normal diet for 12 weeks, (c) Relative mRNA expression of PPARγ. (d) Western blotting and relative protein expression of PPARγ. Data are presented as mean ± SD. ***p < 0.001, ****p < 0.0001 vs. Lean, ###p < 0.001 vs. DIO, *p < 0.05, **p < 0.01 vs. Lean-LPS.](image)
10 min. The supernatant so collected was stored at −80 °C. The levels of TNF-α and IL-1β in cell culture supernatants were determined using an ELISA kit.

2.10. Immunofluorescence. The expression of PGC-1α and GRP78 in A549 cells was detected using immunofluorescence. Coverslips bearing A549 cells were collected from each group. The cells were fixed and permeabilized with 4% paraformaldehyde and 0.5% Triton X-100. Cells were blocked with 1% normal goat serum (Beyotime Biotechnology, Shanghai, China) for 30 min at room temperature and then incubated with primary antibodies PGC-1α (1 : 400) and GRP78 (1 : 200) overnight at 4 °C. Cy3-conjugated antibody (SA00009-2, Protein-tech, Wuhan, China) was used as a secondary antibody and incubated for 1 h at room temperature in the dark. The cells were then fixed with an antifade mounting medium containing DAPI (P0131, Beyotime Biotechnology, Shanghai, China). Photomicrographs were obtained using a confocal microscope (ZEISS, Oberkochen, Germany).

2.11. Flow Cytometric Analysis. The cells were harvested by trypsinization and washed twice with cold PBS. Apoptotic cells were quantified using an annexin V-FITC detection kit (Elabscience, Wuhan, China). We performed flow cytometry (Cytoflex, USA) and analyzed the results using the Cytoflex software. The total proportion of apoptotic cells was calculated by adding the numbers of late and early apoptotic cells.

2.12. Quantitative Real-Time PCR. Total RNA was isolated from lung tissue and A549 cells using TRIzol reagent (Takara Biotechnology). Total RNA was reverse-transcribed into cDNA using a reverse transcription kit (HY-K0511A; MedChemExpress, Shanghai, China). PCR amplification was quantified using SYBR Green qPCR Master Mix (HY-K0523; MedChemExpress, Shanghai, China). β-Actin was used as an internal control. Primers used in this study are listed in Table 1.

2.13. Western Blotting. A549 cells and lung tissue samples were collected, homogenized, and lysed using RIPA lysis buffer (P0013B, Beyotime Biotechnology, Shanghai, China) containing protease and phosphatase inhibitors (P1045, Beyotime Biotechnology, Shanghai, China) for 30 min on ice. After centrifugation at 12000 × g for 15 min at 4 °C, the supernatant was collected, and the protein concentrations were detected using the BCA method (P0010, Beyotime Biotechnology, Shanghai, China). Proteins were separated on SDS-PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes.
2.14. Statistics Analysis. All data are expressed as the mean ± SD. Statistical analyses were performed using the GraphPad Prism 8.0 software (GraphPad Software, USA). The Student’s t-test was used for two-group comparisons, and one-way ANOVA was used for multiple-group comparisons. In all analyses, statistical significance was set at \( p < 0.05 \).

3. Results

3.1. Activation of PPARγ Attenuated LPS-Induced Apoptosis and Inflammation in Alveolar Epithelial Cells. To confirm the effect of PPARγ on alveolar epithelial cells, we selected human type II alveolar epithelial cells A549 for the in vitro experiments. Our results showed that LPS significantly increased inflammation and apoptosis in alveolar epithelial cells (Figure 2). Treatment with RSG significantly decreased the LPS-induced expression of TNF-α and IL-1β in alveolar epithelial cells (Figures 2(a) and 2(b)). Western blotting showed that RSG treatment reduced the cleavage of caspase 3 (Figure 2(c)), and flow cytometry results also showed that RSG administration significantly reduced the rate of apoptosis and necrosis in A549 cells (Figure 2(d)). However, we found that RSG-stimulated changes were prevented by GW9662 (Figure 2),...
indicating that these effects were mediated by the PPARγ signaling pathway. Our results demonstrate that PPARγ activation attenuates LPS-induced apoptosis and inflammation in alveolar epithelial cells.

3.2. Activation of PPARγ Inhibited LPS-Induced ER Stress in Alveolar Epithelial Cells. To investigate whether PPARγ directly reduced ER stress in alveolar epithelial cells, we measured the expression of ER stress-related indicators in alveolar epithelial cells. The results showed that LPS significantly upregulated the expression of ER stress-related proteins (Figure 3). Administration of RSG suppressed the mRNA and protein expressions of GRP78, CHOP, and Caspase12 in alveolar epithelial cells (Figures 3(a) and 3(b)). Immunofluorescence analysis showed that RSG decreased the expression of the ER stress marker protein GRP78 in alveolar epithelial cells

![Figure 3](image-url)

**Figure 3**: Activation of PPARγ inhibited LPS-induced ER stress in alveolar epithelial cells. (a) Relative mRNA expressions of GRP78, CHOP, and Caspase12 in A549 epithelial cells. (b) Western blotting and relative protein expressions of GRP78, CHOP, and Caspase12 in A549 epithelial cells. (c) Immunofluorescence staining and mean fluorescence intensity of GRP78 in A549 epithelial cells (original magnification, ×200). Data are presented as mean ± SD.

$^\times p < 0.05$, $^\times\times p < 0.01$, $^\times\times\times p < 0.001$, $^\times\times\times\times p < 0.0001$ vs. Control, $^\# p < 0.05$, $^\#\# p < 0.01$, $^\#\#\# p < 0.001$, $^\#\#\#\# p < 0.0001$ vs. LPS, $^+ p < 0.05$, $^++ p < 0.01$, $^+++ p < 0.001$, $^++++ p < 0.0001$ vs. LPS-RSG.
The PPARγ-specific blocker, GW9662, prevented the effects of RSG (Figure 3). These results indicate that PPARγ activation could inhibit LPS-induced ER stress in alveolar epithelial cells.

3.3. Activation of PPARγ Promoted Mitochondrial Biogenesis in Alveolar Epithelial Cells. To investigate the effect of PPARγ on mitochondrial biogenesis in alveolar epithelial cells, we examined the expression of mitochondrial biogenesis-related indicators in alveolar epithelial cells. The results showed that LPS significantly decreased the expression of mitochondrial biogenesis-related proteins (Figure 4). However, the administration of RSG suppressed the damaging effects of LPS on mitochondrial biogenesis. RSG upregulated the mRNA and protein expressions of PPARγ, PGC-1α, NRF-1, and TFAM (Figures 4(a) and 4(b)). Immunofluorescence also showed that RSG increased the expression of the mitochondrial biogenesis marker protein PGC-1α in alveolar epithelial cells.

(Figure 3(c)).
3.4. Activation of PPARγ Promoted MFN2 Expression in Alveolar Epithelial Cells. Mnfn2 is embedded in the mitochondrial outer membrane. It mediates mitochondrial fusion by the tethering of two adjacent mitochondria and is involved in the connection between mitochondria and the ER. Our study showed that LPS inhibited the expression of MFN2 in alveolar epithelial cells. Administration of RSG can promote the expression of MFN2, but the effect of RSG was prevented by GW9662 (Figure 5). Our results suggested that MFN2 expression is regulated by a PPARγ-dependent pathway.

3.5. PPARγ Expression Was Decreased in the Lung Tissue of Obese Mice. To determine the expression of PPARγ in the lung tissue of obese mice, we constructed an obese mouse model and detected the expression of PPARγ in the lung tissue. After 12 weeks, both the groups gained weight. Mice fed the high-fat diet had a significantly higher body weight than that of mice fed the normal diet (Figure 1(b)). Our results showed that the mRNA and protein expression levels of PPARγ in the DIO and DIO-LPS groups were lower than those in the lean and LPS groups. Compared to those in the lean and DIO groups, there was a significant decrease in lung PPARγ mRNA and protein expression in the lean-LPS and DIO-LPS groups (Figures 1(c) and 1(d)). Our study showed that obesity can lead to decreased PPARγ expression in lung tissue and LPS intervention can aggravate this decrease in PPARγ expression.

3.6. Activation of PPARγ Attenuated LPS-Induced Lung Injury in Obese Mice. To evaluate the protective effect of PPARγ on ALI in obese mice, we performed HE staining of lung tissue, lung W/D weight ratio measurements, and detection of inflammatory factors in lung tissue and BALF. Our results showed that the administration of RSG attenuated LPS-induced lung tissue damage. HE staining results showed that RSG decreased LPS-induced inflammatory cell infiltration, hemorrhage, interstitial edema, and alveolar septal thickening in the lung tissue of obese mice (Figure 6(a)). At the same time, RSG diminished the lung W/D weight ratio of obese mice (Figure 6(b)), and the mRNA expression and levels of TNF-α and IL-1β in lung tissue and BALF decreased (Figures 6(c) and 6(d)). However, the effects of RSG were inhibited by GW9662 treatment (Figure 6). These observations suggested that PPARγ activation protects obese mice from ALI.

3.7. Activation of PPARγ Inhibited LPS-Induced ER Stress in the Lung Tissue of Obese Mice. To investigate the effects of PPARγ on ER stress in ALI obese mice, we detected the expression of ER stress-related proteins in lung tissue. The results showed that LPS significantly upregulated the expression of ER stress-related proteins (Figure 7). Administration of RSG decreased the mRNA and protein expression of GRP78, CHOP, and Caspase12 (Figures 7(a) and 7(b)). At the same time, immunohistochemistry showed that RSG reduced the expression of GRP78 in lung tissue (Figures 7(c)). However, GW9662 prevented these effects of RSG (Figure 7). These results are consistent with those of the cell studies. Therefore, our results confirmed that PPARγ activation could inhibit LPS-induced ER stress in the lung tissue of obese mice.

3.8. Activation of PPARγ Promoted Mitochondrial Biogenesis in the Lung Tissue of ALI Obese Mice. Since the activation of PPARγ promotes mitochondrial biogenesis in alveolar epithelial cells, we speculated that PPARγ also has a positive effect in ALI obese mice. We examined the expression of mitochondrial biogenesis-related markers in the lung tissue. The results showed that, compared with the DIO-LPS-DMSO group, the mRNA and protein expression levels of PPARγ, PGC-1α, NRF-1, and TFAM were significantly increased in the DIO-LPS-RSG group (Figures 8(a) and 8(b)). Immunohistochemistry also showed that RSG upregulated the expression of the mitochondrial biogenesis marker protein PGC-1α in lung tissue (Figure 8(c)). The effects of
RSG were prevented by GW9662 treatment (Figure 8). These results were also consistent with those of the cell studies, and they suggest that PPARγ activation can promote mitochondrial biogenesis in the lung tissue of mice with ALI.

3.9. Activation of PPARγ Inhibited LPS-Induced Apoptosis in Lung Tissue of Obese Mice. We explored the protective effects of PPARγ activation on lung tissue cells in obese mice. LPS significantly upregulated the cleavage of caspase 3 in lung tissue, and RSG administration reduced the cleavage of caspase 3; however, GW9662 prevented this effect (Figures 9(a)). TUNEL staining showed that LPS-induced apoptosis of alveolar epithelial cells was significantly increased in obese mice. Administration of RSG reduced alveolar epithelial cell apoptosis in TUNEL-stained lung sections, but this effect was prevented by GW9662 (Figure 9(b)). This is consistent with our in vitro study on the protective effect of PPARγ on alveolar epithelial cells. Our results demonstrated that PPARγ activation inhibited LPS-induced apoptosis of alveolar epithelial cells and protected against ALI in obese mice.

3.10. Activation of PPARγ Promoted APN and MFN2 Expression in Lung Tissue of Obese Mice. To investigate the effect of PPARγ on APN and MFN2 expression, we examined APN and MFN2 expression in the lung tissues of obese mice. Our study showed that LPS reduced the mRNA and protein expression of APN and MFN2 in the lung tissue of obese mice. Administration of RSG promoted APN and MFN2 expression; however, the effect of RSG was prevented by GW9662 (Figure 10). Our results suggest that APN and MFN2 expression are regulated by a PPARγ-dependent pathway.

4. Discussion
In this study, several observations were made to improve our current understanding of the role of PPARγ-dependent pathway in ALI. We found that obesity was associated with
lower PPARγ expression in lung tissues and its level further decreased during ALI. Our study in alveolar epithelial cells suggests that activation of PPARγ/PGC-1α is beneficial for relieving inflammation and apoptosis, accompanied by improved mitochondrial biogenesis and reduced ER stress. In vivo studies in obese mice have also confirmed the protective effect of PPARγ against ALI. Moreover, PPARγ can induce the elevated production of APN and MFN2 in lung tissues, which may facilitate its anti-inflammatory efficacy.

PGC-1α is a master regulator of mitochondrial biogenesis and was originally discovered in brown adipose tissue as a co-activator of PPARγ [12]. It can activate PPARγ target genes by inducing the binding of PPARγ ligands to PPARγ [36]. In addition, PGC-1α gene expression is a direct target
Figure 8: Activation of PPARγ promoted mitochondrial biogenesis in lung tissue of ALI obese mice. (a) Relative mRNA expressions of PPARγ, PGC-1α, NRF-1, and TFAM in lung tissue. (b) Western blotting and relative protein expressions of PPARγ, PGC-1α, NRF-1, and TFAM in lung tissue. (c) Immunohistochemical staining and positive area ratio of PGC-1α in lung tissue (original magnification, ×200). Data are presented as mean ± SD. ***p < 0.001, ****p < 0.0001 vs. DIO-DMSO, #p < 0.05, ##p < 0.01, ###p < 0.001, ####p < 0.0001 vs. DIO-LPS-DMSO, †p < 0.05, ‡p < 0.01, ‡‡p < 0.001, ‡‡‡p < 0.0001 vs. DIO-LPS-RSG.
of PPARγ activation. The presence of a PPARγ-responsive element in the distal region of the PGC-1α gene promoter binds PPARγ/RXR heterodimers [37]. Previous studies have demonstrated the role of the PPARγ/PGC-1α pathway in inhibiting obesity, delaying the progression of chronic obstructive pulmonary disease, and attenuating pulmonary edema [18, 38, 39]. The present study confirmed the role of PGC-1α in mediating the protective effects of PPARγ on alveolar epithelial cells. It is reasonable to assume that the decreased activity of PPARγ/PGC-1α in obese individuals may prompt the lung to exacerbate injuries.

Impairment of mitochondrial biogenesis and function has been linked to aging, neurodegenerative diseases, and metabolic diseases such as type 2 diabetes and obesity [40]. Previous studies have documented in varied pathogenesis that the activation of PPARγ and downstream proteins can induce improved mitochondrial function [41, 42]. Our study confirmed the protective effects of PPARγ/PGC-1α against LPS-induced mitochondrial dysfunction in alveolar epithelial cells. Moreover, PPARγ also contributes to reducing the impairment of MFN2 production during ALI, which is affected by the interactions between the ER and mitochondria and plays a key role in regulating mitochondrial dynamics.

Previous studies on macrophages and pancreatic β-cells have demonstrated that the activation of PPARγ significantly attenuates ER stress [23, 34]. Our in vitro and in vivo studies in pulmonary alveolar cells also confirmed PPARγ-dependent modulation of ER stress. Reduced PPARγ is considered upstream of the enhanced ER stress [43]. However, activation of CHOP following ER stress can lead to the downregulation of PGC-1α and increased cell apoptosis [44]. These results indicate the existence of reverse signal transduction in the progression of cellular inflammatory response. Thus, an impaired balance between PPARγ/PGC-1α and ER stress may play a role in exacerbating lung injury.

Obesity is also associated with lower APN levels. Studies have confirmed that APN expression in the lung tissue of obese mice is lower than that in normal mice [45]. APN is an adipokine with prominent anti-inflammatory properties [46]. It has been observed that mice with targeted deletion of the APN gene exhibited spontaneous activation of pulmonary vascular endothelial cells and were more susceptible to

**Figure 9: Activation of PPARγ inhibited LPS-induced apoptosis in lung tissue of obese mice.** (a) Western blotting and relative protein expression of cleaved-caspase3/caspase3 in lung tissue (cleaved-caspase3 was an activated form of caspase3). (b) TUNEL staining was used to detect apoptosis and apoptotic index of alveolar epithelial cells (original magnification, ×200). Data are presented as mean ± SD. ****p < 0.0001 vs. DIO-DMSO; ####p < 0.0001 vs. DIO-LPS-DMSO; ++++p < 0.0001 vs. DIO-LPS-RSG.
In the present study, upregulation of APN was detected following the activation of PPARγ, suggesting a potential role of adipokines in mediating PPARγ-induced protection against ALI. A link between PPARγ and APN has been proposed in previous studies. APN, a target gene of PPARγ, is induced by PPAR-γ ligands via direct binding of the PPAR-γ/RXR heterodimer to the PPAR response elements (PPRE) in the APN promoter [47]. PPARγ activation can regulate APN production at transcriptional and translational levels [48–50]. However, how they contribute to the anti-inflammation efficacy in the alveolar epithelia requires further investigation.

The present study has several limitations. First, A549 cells were used instead of primary alveolar type II cells obtained from obese mice. The A549 cell line is widely used as an in vitro model for type II pulmonary epithelial cells. Our study confirmed that the activation of PPARγ/PGC-1α induces protective effects against LPS-induced epithelial injury by relieving ER stress and improving mitochondrial biosynthesis. However, the level of PPARγ in the lung epithelia of obese mice and its changes during inflammation remain unclear. Second, although current evidence suggests that low levels of PPARγ and APN in obese mice may contribute to susceptibility to ALI, it is not sufficient to demonstrate the impact of APN on ER stress and mitochondrial function. Further studies are required to elucidate the interactions between PPARγ/PGC-1α and APN in mediating lung protection. Third, our experiments confirmed that PPARγ activation has a preventive effect on ALI/ARDS in obese mice. But it is also important to determine the ability of PPARγ activation in reverting a pre-existing inflammatory state in lung. Therefore, we will continue to study this issue in the future.

5. Conclusion

In conclusion, the present study demonstrated that obesity-induced downregulation of PPARγ may increase susceptibility to ALI. The anti-inflammatory efficacy of PPARγ in
alveolar epithelia is mediated by the alleviation of ER stress and promotion of mitochondrial biogenesis.

**Data Availability**

All data used in this study are available from the corresponding author on request.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**Acknowledgments**

The authors thank Professor Guo Shuliang’s research group (Department of Respiratory Medicine, The First Affiliated Hospital of Chongqing Medical University) for donating the A549 cell line and for the experimental conditions provided by the Laboratory Research Center, The First Affiliated Hospital of Chongqing Medical University. This work was supported by grants from Chongqing Science & Technology Bureau (Project no. CSTC2019jcx-sxmx20214) and Chongqing Health Commission (Project no. 2017HBRC001).

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