Original Paper

Leptin Downregulates LPS-Induced Lung Injury: Role of Corticosterone and Insulin

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Key Words

Leptin • Insulin, corticosterone, acute lung injury • Lipopolysaccharide • Lung inflammation

Abstract

Background/Aims: We investigated the effects of leptin in the development of lipopolysaccharide (LPS)-induced acute lung inflammation (ALI) in lean mice. **Methods:** Mice were administered leptin (1.0µg/g) or leptin (1.0µg/g) followed by LPS (1.5µg/g) intranasally. Additionally, some animals were given LPS (1.5µg/g) or saline intranasally alone, as a control. Tissue samples and fluids were collected six hours after instillation. **Results:** We demonstrated that leptin alone did not induce any injury. Local LPS exposure resulted in significant acute lung inflammation, characterized by a substantial increase in total cells, mainly neutrophils, in bronchoalveolar lavages (BAL). We also observed a significant lymphocyte influx into the lungs associated with enhanced lung expression of chemokines and cytokines (KC, RANTES, TNF-α, IFN-γ, GM-CSF and VEGF). LPS-induced ALI was characterized by the enhanced expression of ICAM-1 and iNOS in the lungs. Mice that received LPS showed an increase in insulin levels. Leptin, when administered prior to LPS instillation, abolished all of these effects. LPS induced an increase in corticosterone levels, and leptin potentiated this event. **Conclusion:** These data suggest that exogenous leptin may promote protection during sepsis, and downregulation of the insulin levels and upregulation of corticosterone may be important mechanisms in the amelioration of LPS-induced ALI.

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Introduction

The development of acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), a more severe form of ALI, are important health conditions in critically ill patients; these conditions are associated with increased mortality rates. ALI involves intrapulmonary neutrophil accumulation and loss of barrier function of the alveolar epithelial and pulmonary capillary endothelial cells with excessive production of inflammatory mediators, resulting in respiratory failure [1, 2].

Lipopolysaccharide (LPS) is a component of Gram-negative bacterial cell walls [3] and may be responsible for exacerbating inflammatory responses in ALI and ARDS [4]. The induction of ALI by LPS in mice is accepted as a model of severe lung injury [5] because in experimental animals, LPS administration mimics the pathological condition of ongoing sepsis and ARDS-like lung injury characterized by polymorphonuclear sequestration in the pulmonary microcirculation and an increase in microvascular permeability [3].

Leptin is a 16 kDa adipocyte-derived protein identified as a product of the obesity gene (*ob*). It is a member of the IL-6 family of cytokines and has proinflammatory effects [6]. Leptin was originally identified as a key factor in the regulation of food intake and body weight [7]. However, it is now known that leptin influences a wide variety of biological functions, including hematopoiesis, angiogenesis, phagocytic activity and reactive oxygen species production, endothelial cell activation and production of Th1 cytokines in T cells [8, 9].

Previous work showed that LPS induced leptin mRNA in adipose tissue and rapidly increased serum leptin [10]. Although white adipose tissue is the major source of leptin, this hormone is also found in lung tissue [11]; the leptin receptor (Ob-R) is expressed by bronchial and alveolar epithelial cells, bronchial smooth muscle cells and in the bronchial submucosa [12]. It also observed increased leptin levels in BAL from humans exposed to LPS [13]; together, these findings confirm that the lung is a likely target organ for leptin signaling [12].

Leptin is recognized as a proinflammatory molecule; however, studies that define a role for leptin in sepsis have yielded conflicting results. Leptin-deficient mice (ob/ob) seem to be unaffected [14] or protected [15] from the deleterious effects of endotoxin. On the other hand, Gultekin et al. [16] demonstrated that exogenous leptin may be protective role in cerulein-induced acute pancreatitis associated to ALI, and Dong et al. [17] showed that leptin-deficient ob/ob mice were more susceptible to LPS-related lethality than lean mice. Therefore, the present study was designed to investigate the effects of acute exogenous leptin treatment in the development of LPS-induced acute lung inflammation in mice. We hypothesized that exogenous leptin may modulate LPS-induced acute lung inflammation, interfering with the production of hormones, cytokines and chemokines.

Materials and Methods

Animals

Male C57Bl/6 mice weighing 20-25 g (6-8 weeks old) were obtained from our animal facilities and were housed in a room with 12 h light-dark cycle with water and food ad libitum. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by Ethical Committee for Animal Research of the Biomedical Sciences Institute, University of São Paulo.

Animal Procedures

Mice were anesthetized with intraperitoneal ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively; Agibrands do Brazil, São Paulo, São Paulo, Brazil) and received an intranasal inoculation of either 50 µl of *Escherichia coli* LPS 026:B6 (Sigma, St Louis, MO, USA) in 0.9% NaCl (nonpyrogenic saline, Baxter Healthcare Corp., Deerfield, IL, USA) at a concentration of 1.5 µg/g [4] or 50 µl of saline as a control.

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Mice were also treated intranasally with leptin (1.0 µg/g, 50 µL, Sigma, St Louis, MO, USA) or leptin (1.0 µg/g) followed by LPS (1.5 µg/g). The experiments were performed 6 h after treatment.

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed 6 h after the nasal treatment to evaluate cell infiltration and leukotriene B_4 (LTB_4) levels. The animals were anesthetized with intraperitoneal ketamine and xylazine; a tracheal cannula was inserted via a midcervical incision, and the airways were flushed twice with 1 ml of phosphate-buffered saline (PBS, pH 7.4 at 4°C).

Total and Differential Cell Counts

The bronchoalveolar lavage fluid was centrifuged at 170 X g for 10 min at 4°C, the supernatant was collected, and the cell pellet was resuspended in 0.5 ml of PBS. One volume of a solution containing 0.5% crystal violet dissolved in 30% acetic acid was added to nine volumes of the cell suspension. The total number of cells was determined by counting in a hemocytometer. Differential cell counts were performed after cytocentrifugation and staining with hematoxylin–eosin (Hema 3).

Protein assay

BAL protein levels were measured by Bradford assay (Bio-Rad).

Flow Cytometry Analysis of Lymphocytes

Phenotypic analysis of lymphocyte subpopulations were assessed by three-color FACS using a FACScalibur Cytometer equipped with Cell Quest software (Becton and Dickinson, San Jose, CA, USA) with gates defined by forward and side light scattering properties. To isolate cells from lung tissue, the tissue was minced, incubated with collagenase D (Roche Diagnostics, Indianapolis, IN, USA) for 20 min at 37°C and then dissociated using either a BD Medimachine (BD Biosciences, San Jose, CA, USA) or a gentleMACs Dissociator (Miltenyi Biotec, Cambridge, MA, USA), according to the manufacturers' recommendations. The cells isolated from lung tissue were then incubated with fluorescein isothiocyanate (FITC), R-phycoerythrin (PE) or cychrome-labeled monoclonal antibodies. The following antibodies were used: anti-CD4 (clone H129.19), anti-CD8 (clone 53–6.7), anti-CD45R/B220 (clone RA3-6B2) and Ly-6G (clone RB6-8C5). All antibodies were purchased from BD Pharmingen (San Diego, CA, USA). The cells were then adjusted to a concentration of 5 x 10^5 cells mL^{-1} in PBS supplemented with 5% fetal bovine serum and sodium azide (0.1%).

Lung histology

Lungs of all groups (n=5) were harvested after bronchoalveolar lavage collection, immersed in 10% phosphate buffered formalin for 24 h and then kept in 70% ethanol until embedding in paraffin. Tissues were sliced into 5µm thickness, and stained with hematoxilin-eosin. The number of neutrophils present in the lung parenchyma (mice each group) were quantified (expressed as mean ± S.E.M.) by an investigator blinded to the various groups in approximately 20 different histologic regions per animal (high-power fields).

Cytokines and Insulin assay

Bio-Plex assay kits (Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used to measure tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), keratinocyte-derived chemokine (KC) and regulated on activation, normal T-expressed and secreted (RANTES) in the lung samples. Insulin was measured in serum samples. The kits were used according to the manufacturer's instructions. The assays were read on the Bio-Plex suspension array system; the data were analyzed using Bio-Plex Manager software version 4.0 (Bio-Rad Laboratories, Hercules CA, USA). Standard curves ranged from 1.95 to 32,000 pg/mL. The total protein in lung tissue samples was measured, and the results are indicated as pg of specific molecule/µg of total protein.

Quantification of Leukotriene B4 (LTB4)

The LTB_4 concentration in BAL was determined with an EIA kit (Cayman Chemical Co., MI, USA) according to the method of Pradelles et al. [18]. The sensitivity of the LTB4 assay was 4.0 pg/mL.

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Corticosterone assay

Serum corticosterone levels were measured using an enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA) according to the manufacturer′s instructions. The detection limit was 24 pg/mL.

Immunohistochemistry for ICAM-1

Lung sections (5 μm thick) were deparaffinized, hydrated and subjected to antigenic retrieval by incubation in sodium citrate buffer (10 mM) at 90°C for 20 minutes. Sections were treated with 3% H_2O_2 in PBS for 30 minutes to block endogenous peroxidase activity. Non-specific staining was blocked by incubating the sections for 30 minutes with 10% bovine serum albumin in PBS. The sections were incubated overnight at 4°C with monoclonal primary antibody (biotinylated anti-mouse CD54, 1:200, Abcam, Cambridge, MA, USA) in 0.3% Tween 20 in PBS. After washing in PBS, the sections were incubated in streptavidin-peroxidase using the ABC kit (Vector Laboratories, Burlingame, CA, USA) for 1 hour at RT. Peroxidase was visualized using 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma, Saint Louis, MO, USA) in PBS with 0.01% H_2O_2. Sections were counterstained with Mayer's hematoxylin, dehydrated in ethanol and mounted in VectaMount mounting medium (Vector Laboratories, Burlingame, CA, USA). The brown staining intensity of the immunohistochemical reaction was analyzed with an Image Analysis System; the results were processed using Image Pro Plus software, version 4.1 (Media Cybernetics, Silver Spring, MD, USA).

Western Blot

Lung cells were lysed in RIPA buffer, run on 10% SDS-polyacrylamide electrophoresis gels and transferred onto nitrocellulose membranes (Hybond C Extra, Amersham Biosciences, Little Chalfont, UK). The membranes were incubated with primary antibodies for iNOS (Cell Signaling, Danvers, MA, USA), using the manufacturer-recommended dilutions, followed by peroxidase-conjugated monoclonal anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, USA). HRP activity was detected using enhanced chemiluminescence. The membranes were stripped, and the densitometric values for the phosphorylated forms of iNOS were normalized to the values for β-actin, (Sigma, St. Louis, MO, USA). We used the software GeneSnap (Syngene, USA) and Gene Tools (Syngene, USA) to analyze the bands.

Statistical Analysis

All results are described as the means ± S.E.M. Statistical evaluation of the data was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-test. A p value that was lower than 0.05 was considered to be significant. All statistical analyses were performed with the aid of GraphPad software (San Diego, CA, USA).

Results

Leptin reduced LPS-induced cells influx into the lung in BAL

We observed a substantial increase in total cells and neutrophils harvested from BAL fluid from LPS-treated mice when compared with those treated with saline or leptin (Figs. 1A and 1B). Leptin pre-treatment inhibited the LPS-induced influx of neutrophils and other cells in the BAL (Figs. 1A and 1B). Leptin treatment alone did not increase the cell number in BAL samples (Figs. 1A and B).

Cell phenotype

We examined the lymphocyte population (CD4+, CD8+, B cells and granulocytes) in the lung tissue of mice treated with saline, leptin, LPS or leptin+LPS. Leptin administration did not alter the lymphocytes number in lung tissue, whereas a significant increase in CD4+, CD8+, B cells and granulocytes was observed in LPS-challenged mice (Figs. 1C to 1F). Leptin administered prior to LPS attenuated the influx of lymphocytes into the lung tissue.

BAL protein

BAL protein content, an indicator of lung vascular permeability, was markedly increased in LPS-treated group when compared to control

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Fig. 1. Total cells and neutrophils in bronchoalveolar lavage (BAL) and lymphocyte infiltration in lung tissue. BAL was performed 6 h after the challenge. The cells (A) were counted in a hemocytometer, and neutrophils (B) were counted after cytocentrifugation and staining with hematoxylin/eosin. Lung tissue was harvested 6 h after the challenge, and lymphocyte infiltration [(C) CD4^{+}, (D) CD8^{+}, (E) B cells and (F) granulocytes] in lung tissue was evaluated by flow cytometry, as described in the methods. The results are the means ± S.E.M. of 6 animals/group. * $P< 0.01$, # $P<0.05$.
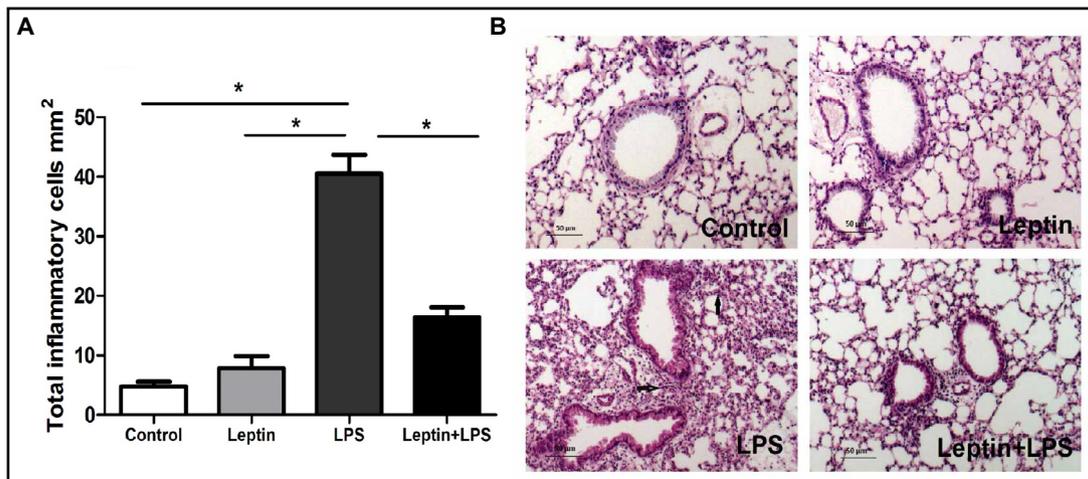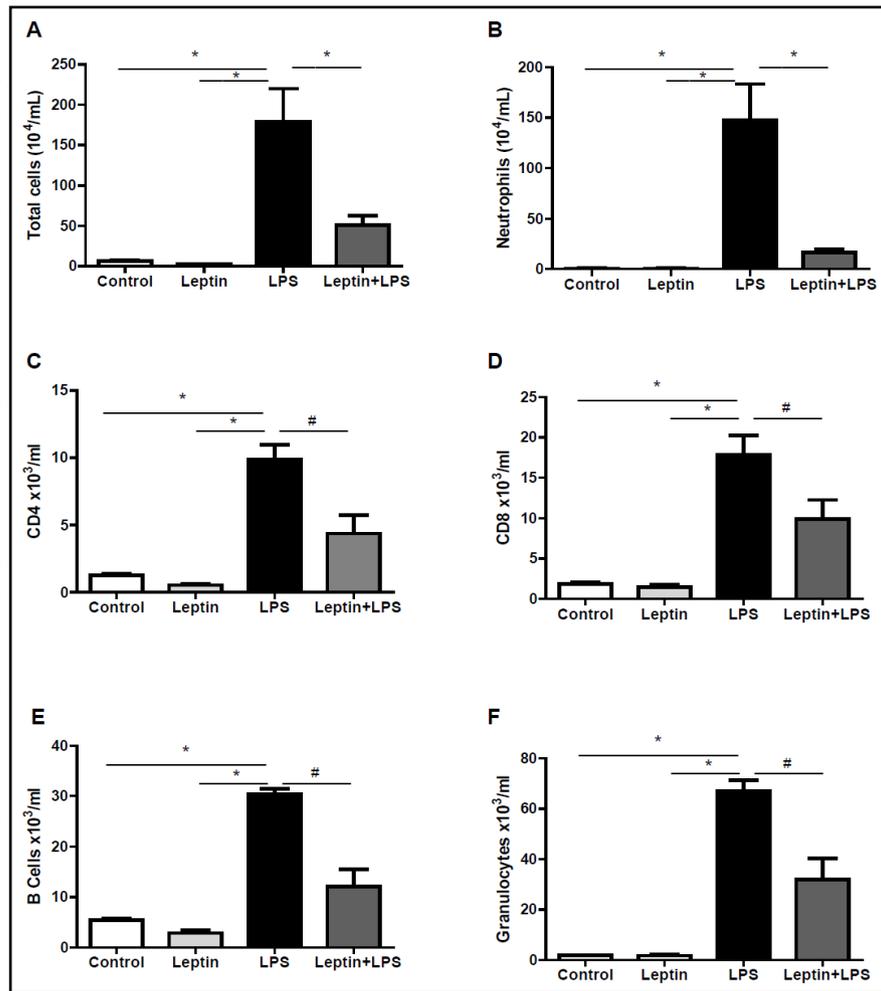

Fig. 2. Effect of leptin on LPS-induced neutrophil infiltration into the lung interstitium. Lungs were harvested after bronchoalveolar lavage collection, and embedding in paraffin. Tissues were sliced into 5μm thickness, and stained with hematoxilin-eosin. The inflammation at 6h post-LPS treatment is reduced in LPS-treated mice given leptin prior to LPS compared with those given LPS (A, B). LPS-treated mouse lung showed robust accumulation of neutrophils in the perivascular compartment (B, open arrow), accompanied by a *significant* increase in alveolar septal thickness (B, black arrow). The results are the means ± S.E.M. of 5 animals/group; * $P< 0.05$.

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Fig. 3. Effect of leptin on LPS-induced cytokines and chemokines in the lung tissue. (A) keratinocyte-derived chemokine (KC), (B) RANTES, (C) tumor necrosis factor-alpha (TNF-α), (D) interferon-gamma (IFN-γ), (E) granulocyte-macrophage colony-stimulating factor (GM-CSF) and (F) vascular endothelial growth factor (VEGF) were quantified in lung tissue 6 h after the challenge by Bioplex, as described in the methods. The total protein of the lung tissue was measured, and the results are indicated as pg of specific molecule/μg of total protein. The results are the means ± S.E.M. of 6 animals/group, *P<0.05.
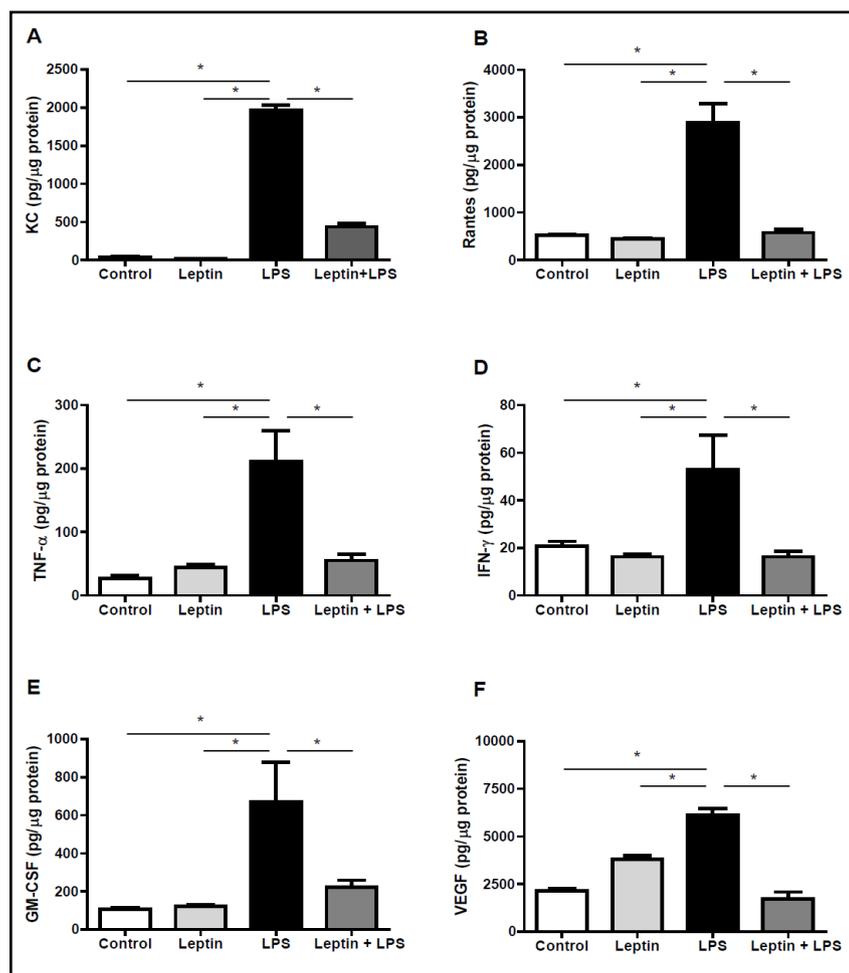
(320.7 ± 26.2 μg/mL vs 148.4 ± 15.4 μg/mL, respectively; *P<0.05) and leptin (320.7 ± 26.2 μg/mL vs 139.6 ± 37.1 μg/mL, respectively; *P<0.05) groups. Leptin administration prior to LPS prevented this effect (320.7 ± 26.2 μg/mL vs 232.8 ± 18.5 μg/mL, respectively; *P<0.05).

Leptin prevented LPS-induced neutrophil infiltration into the lung tissue
In contrast to saline and leptin instillation, LPS instillation produced significant infiltration of neutrophils into the lung interstitium; and this effect was abolished when leptin was given before LPS treatment (Figs. 2A and 2B).

Leptin lowered LPS-induced inflammatory cytokines and chemokines in pulmonary tissue
BioPlex assay kits were used to measure chemokine and cytokine levels in pulmonary tissue. We did not observe a difference in the expression of TNF-, IFN-γ, GM-CSF, VEGF, KC or RANTES in pulmonary tissue between leptin-treated and control mice (Fig. 3). Mice treated with LPS showed an increase in the expression of all the chemokines and cytokines analyzed; leptin administration prior to LPS prevented this effect (Fig. 3).

Leptin inhibited LPS-induced LTB4 production in BAL
The LTB_4 concentration in BAL was determined using an EIA kit. As shown in Fig. 4, neither saline nor the leptin treatment affected the production of LTB_4. The production of LTB_4 was enhanced by LPS treatment, and leptin administration prior to LPS reversed this effect (Fig. 4).

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Fig. 4. Effect of leptin on LPS-induced leukotriene B_4 (LTB_4) in bronchoalveolar lavage fluid. LTB_4 was measured in the bronchoalveolar lavage fluid using an EIA kit 6 h after the challenge, as described in the methods. The results are the means ± S.E.M. of 6 animals/group, *P<0.05.
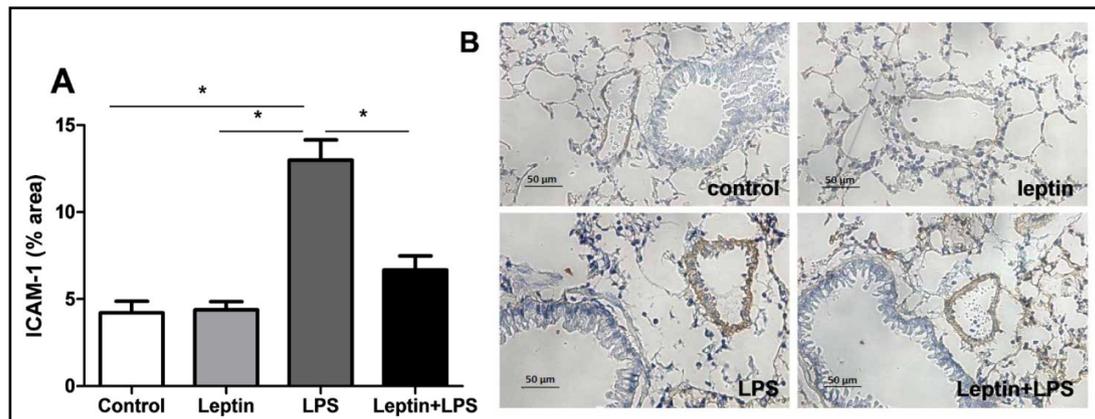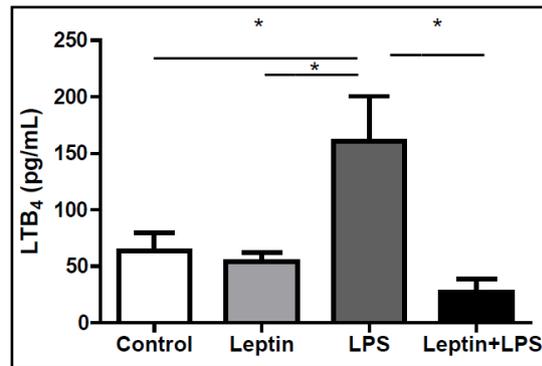

Fig. 5. Effect of leptin on LPS-induced ICAM-1 expression in lung tissue. Immunohistochemical analysis (A) and representative images of immunohistochemical staining (B) of ICAM-1 in lung tissue. The results are the means ± S.E.M. of 6 animals/group. Magnification, 20X. *P<0.05.

Fig. 6. Effect of leptin on LPS-induced iNOS expression in lung tissue. Lungs were harvested 6 h after challenge to quantify the expression levels of iNOS by Western blot. The graphs represent the band intensities, determined by densitometric analysis and normalized to the total β-actin present in each lane. The results are the means ± S.E.M. of 6 animals/group. * P< 0.01.
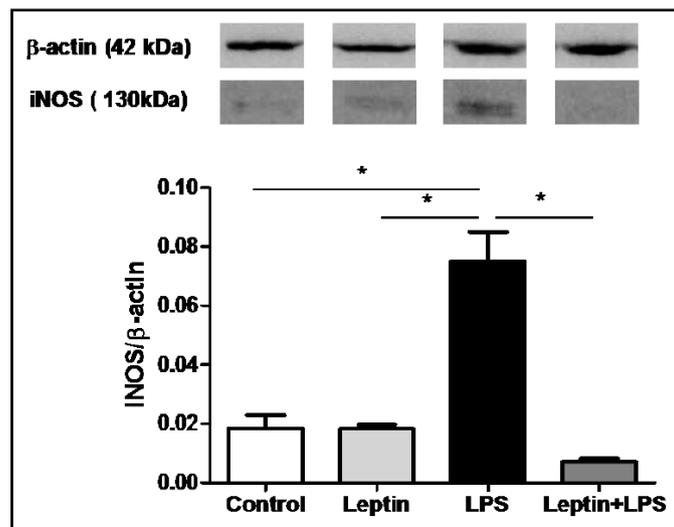
Prior leptin administration decreased LPS-induced ICAM-1 expression in pulmonary tissue
ICAM-1 expression in lung tissue was not altered by leptin treatment compared with the control group. ICAM-1 expression was markedly increased in the LPS-treated group. In contrast, this effect was abolished when leptin was given before LPS treatment (Figs. 5A and 5B).

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Fig. 7. Effect of leptin on LPS-induced insulin / corticosterone in serum. Insulin and corticosterone were quantified in serum 6 h after the challenge by Bioplex, as described in the methods. The results are the means ± S.E.M. of 5 animals/group, *P<0.05.
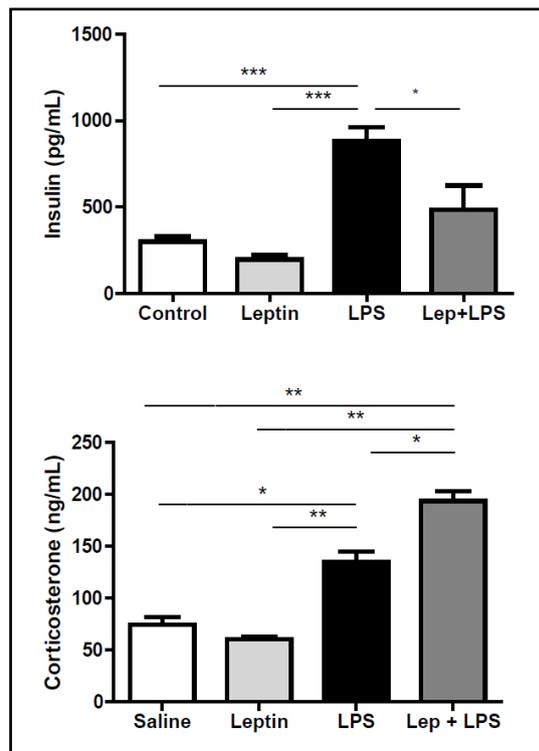
Leptin decreased LPS-induced iNOS expression in pulmonary tissue

iNOS was quantified in lung tissue using Western blotting. Leptin treatment did not alter the expression of iNOS in pulmonary tissue. The LPS-treated group showed increased iNOS expression, whereas prior leptin administration to LPS abolished this effect (Fig. 6).

Effect of leptin on LPS-induced insulin and corticosterone levels

The Bio-Plex assay kit was used to measure insulin and an enzyme immunoassay kit was used to measure corticosterone levels in the serum. As observed in Fig. 7, leptin administration did not alter hormone levels. LPS treated-mice had increased levels of both insulin and corticosterone. Prior leptin administration attenuated the action of LPS on insulin levels and increased the action of LPS on corticosterone levels.

Discussion

The major objective of this study was to evaluate the role of exogenous leptin treatment in the development of acute lung injury (ALI) induced by LPS. Our results suggest that exogenous leptin may limit the development of ALI in mice. This conclusion is supported by the observation that LPS elicited a significant pulmonary inflammatory response, characterized by intense cell infiltration into the lungs, increased inflammatory mediators, lung vascular permeability and increased expression of insulin and ICAM-1. Although leptin treatment did not stimulate an inflammatory response, the administration of leptin prior to LPS reversed the LPS-induced effects.

LPS, or endotoxin, is the major component of the outer membrane of Gram-negative bacteria. LPS inhalation in mice mimics human Gram-negative acute lung injury (ALI), stimulating the recruitment of leukocytes. Lymphocytes and neutrophils play a key role in the host defense against pathogens, but they are also responsible for pulmonary injury [19]. Lymphocytes have been shown to synthesize a variety of cytokines, which may then stimulate endothelial cells to express higher levels of adherence molecules, further facilitating the migration of inflammatory cells into tissue [20]. The recruitment of neutrophils is related

to plasma leakage, deterioration of oxygenation [2], degradation of surfactant proteins, epithelial cell apoptosis [21] and generation of reactive oxygen and nitrogen species, leading to tissue injury in ALI [22]. In our study, we found that leptin pre-treatment decreased the severity of LPS-induced lung injury, in part by diminishing the infiltration of neutrophils and lymphocytes into the lung.

The migration of neutrophils into the lungs is a complex process and involves rolling, adhesion and subsequent chemokine-directed tissue migration to the alveolar space. Therefore, to understand the basis for the reduction of LPS-induced cell infiltration in leptin-treated mice, we assessed the expression of key cytokines, chemokines and adhesion molecules involved in the recruitment of inflammatory cells into the lung.

Even though LPS is not an effective chemoattractant for neutrophils, it can trigger an inflammatory cascade, inducing the synthesis of cytokines, chemokines, eicosanoids, and other proinflammatory mediators by resident alveolar macrophages and other cells, thereby playing a crucial role in contributing to ALI [23]. KC, a murine neutrophil-chemoattractant chemokine, and RANTES, which recruits eosinophils, lymphocytes and neutrophils, play a critical role in ALI, augmenting LPS-induced cell recruitment into the lung [23]. LPS is the most potent inducer of TNF-α. TNF-α not only amplifies the inflammatory cascade and causes inflammatory injury but also recruits neutrophils into the lung [24] and, similar to leukotriene B$_4$ (LTB$_4$) and IFN-γ, this cytokine is associated with recruitment of neutrophils [24-26]. GM-CSF enhances the functional responsiveness and extends the life of mature polymorphonuclear leukocytes in addition to augmenting leukotriene production and aggravating tissue injury [27, 28]. VEGF plays a crucial role in a variety of diseases by promoting angiogenesis and affecting vasopermeability [29]. ICAM-1 is expressed on vascular endothelial and lung epithelial cells and can be induced there by the administration of LPS in vivo [30], thereby participating in leukocyte migration out of the blood in response to pulmonary inflammation. These inflammatory molecules act together to modulate neutrophilic inflammation and are associated with the development of lung injury.

The conclusions derived from studies of the effect of leptin on inflammatory mediators are conflicting. Several lines of evidence suggest that leptin has proinflammatory effects; for example, monocytes/macrophages incubated with leptin alone or coincubated with leptin and endotoxin produced cytokines [31-33], and lung inflammatory response to hyperoxia is attenuated in mice with leptin resistance [34]. In contrast, leptin infusion downregulated the LPS-induced neuroendocrine response in primates [35] and high dose of leptin resulted in neutrophil inhibition [16]. In our work, we evaluated the impact of leptin on expression of the inflammatory molecules. Leptin downregulated the expression of LPS-induced chemokines, cytokines and ICAM-1, suggesting that the protective effects of leptin on LPS-induced ALI may be at least partly related to the inhibition of these inflammatory factors. These studies indicate the beneficial role of leptin on severe inflammatory diseases.

Inducible nitric oxide synthase (iNOS) and nitric oxide (NO), produced by iNOS, are elevated in many inflammatory diseases of the respiratory tract. Although NO is also involved in antimicrobial, anti-inflammatory and antioxidant effects, overproduction of NO may be harmful in conditions such as sepsis and infection [36]. Excessive NO production may also enhance the generation of reactive nitrogen species (RNS), including peroxynitrite (ONOO-) and nitrogen dioxide (NO$_2$), and the formation of such RNS is thought to be the mechanism underlying the role of NO in the etiology of inflammatory lung disease [36]. One of the potential mechanisms by which leptin pretreatment abolished LPS-induced lung inflammation might be a decrease in iNOS expression, given that the inducible isoform of NOS is responsible for the excess production of NO in LPS-induced sepsis in animals and NO also increases lung chemokine expression, facilitating cell influx in to the airways [37, 38].

A role for insulin in the regulation of LPS-induced acute pulmonary inflammation has been identified. Diabetic rats exhibited a reduction in the production of proinflammatory cytokines and cell infiltration [39] and a reduction in prostaglandin E$_2$ production [40]. Treatment of the animals with insulin completely reverses these abnormalities. However, studies of the modulation of insulin levels by LPS have yielded conflicting results. De Oliveira

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Martins et al. [39] observed no difference in serum insulin in LPS-treated rats, while, similarly to Cornel [41] and Creely et al. [42], we observed augmented serum insulin levels in LPS-treated mice. Fehmann et al. [43] suggested the existence of an "adipo-insular axis" based on the fact that insulin increases leptin expression, and elevated leptin concentrations decrease insulin secretion by a negative feedback mechanism. We demonstrated that, in mice treated with leptin prior to LPS, downregulation of insulin levels may exert an anti-inflammatory effect, reinforcing the importance of a regulatory loop in the adipo-insular axis in the development of acute lung injury induced by LPS.

Acute inflammation response is regulated by immune and endocrine systems because there is a regulatory loop between the HPA axis and circulating leptin. Nevertheless, studies examining the effects of leptin on the corticosterone levels have been controversial. Central administration of leptin has been shown to increase plasma corticosterone levels in rats [44], and systemic administration of leptin also increased corticosterone levels 60 and 120 min postinjection [45]. However, leptin was found to decrease the preparturient increase in adrenocorticotropic hormone and cortisol in the sheep fetus [46]; additionally, in diabetes, in which there is a well-documented decrease in leptin levels, there is a concurrent increase in HPA activity [47]. In agreement with Spinedi and Gaillard [48], who demonstrated a positive correlation between leptin and corticosterone, our study showed that acute leptin treatment potentiated the LPS-induced increase in corticosterone secretion. The observed increase in serum corticosterone may be associated with the reduction in insulin levels [49] and may have led to the downregulation of LPS-induced lung injury [50] because corticosterone inhibits the action of various inflammatory mediators and reduces the synthesis of chemokines and cytokines and the activation of leukocytes [15, 41]. By sharing structural and functional similarities with cytokines, such as interleukin (IL)-6, IL-1 and TNF-α, leptin is classified as a type I cytokine [4, 5], and, as such, it could activate the hypothalamo-pituitary-adrenal axis [6] and increase corticosterone levels [7]. Taken together, these results demonstrated the strong influence of leptin on LPS-induce lung injury.

We conclude that acute leptin treatment prior to LPS exhibited protective effects in lung injury, reducing the following: a) cell infiltration in lung tissue and BAL; b) lung vascular permeability; c) the expression of cytokines, chemokines and inflammatory mediators in lung tissue; and d) ICAM-1 and iNOS expression in lung tissue. We also conclude that the modulation of insulin and corticosterone levels by leptin is an important mechanism to ameliorate LPS-induced ALI.

Acknowledgements

Conflicts of Interest

The authors confirm that there are no conflicts of interest.

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