

# LIRAGLUTIDE INCREASES SURFACTANT PROTEINS (SPA & SPB) AND ANGIOTENSIN-CONVERTING ENZYMES (ACE & ACE2) EXPRESSION IN A RAT MODEL OF ACUTE LUNG INJURY BY BLEOMYCIN

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## Introduction

Acute lung injury (ALI), and his most severe manifestation, Acute respiratory distress syndrome (ARDS), is a clinical syndrome caused by an excessive inflammatory response to an external insult. This excessive response causes a disruption in the lung endothelial and epithelial barrier, that leads to alveolar and interstitial oedema, reduced alveolar fluid clearance, impairment of surfactant production and respiratory function and later pulmonary fibrosis (1).

Glucagon-like peptide-1 (GLP-1) is a gut-produced hormone with potent insulinotropic effects. GLP-1 receptor is widely expressed in different tissues and organs, including the lung. GLP-1 plays an important role in the synthesis of surfactant proteins (2).

We have previously shown that liraglutide (LIR), a GLP-1 receptor agonist, restores surfactant protein-B (SPB) levels, a limiting factor for survival, in different rat models and also angiotensin converting enzymes (ACE and ACE-2) in type-1-like diabetes rat model (3).

## Objetives

❖ To study the beneficial effect of the GLP-1 receptor agonist Liraglutide, at low doses, in an animal model of Acute Lung Injury induced by bleomycin on:

- 1-Inflammatory response after bleomycin administration.
- 2-Expression of Surfactant Proteins A and B.
- 3-Expresion of Angiotensin-Converting Enzymes-1 (ACE) and -2 (ACE-2).

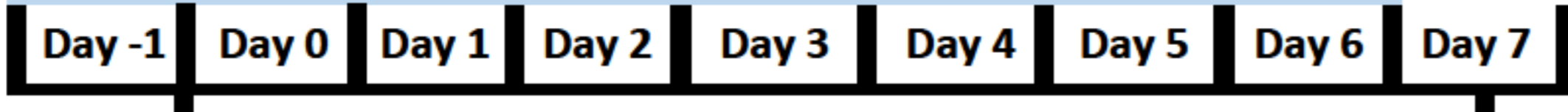
## Methods

### Experimental protocol

**Animals used**  
Adult male Sprague-Dawley rats, 225-250 g.  
n= 7 animals per group

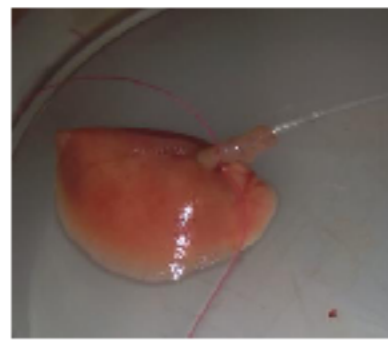
**Intratracheal instillation**  
**Bleomycin (BLM)**  
Dose 2,5 mg/kg on 200 µL  
**Saline 0,9% (CT)**  
Volume: 200 µL

**Subcutaneous treatment:**  
**Liraglutide (LIR)** 100 µg/kg, dissolved on NaCl 0,9% + 0,4% acetic acid. **Vehicle (VEH)** NaCl 0,9% + Acetic acid 0,4%



**Sacrifice**

**Left Lung**      **Right Lung**



Excised and weighted; then left primary bronchi was cannulated & broncho-alveolar lavage was performed by 2 mL of cold sterile saline. Caudal lobe was remove and stored at -80 °C.

### Broncho-alveolar lavage fluid (BALF) analysis

- Broncho-alveolar lavage fluid (BALF) was centrifuged at 100 g for 20 minutes at 4°C.
- Supernatant was aliquoted and frozen for total protein quantification by colorimetric assay (Bradford).
- Pellet was suspended on 500 µL cold sterile saline for total cell count in a Neubauer chamber.

### mRNA expression analysis

- Extracted using Chowczynsky & Sacchi single step method.
- Retro-transcribed to cDNA and running a conventional PCR for each signal.

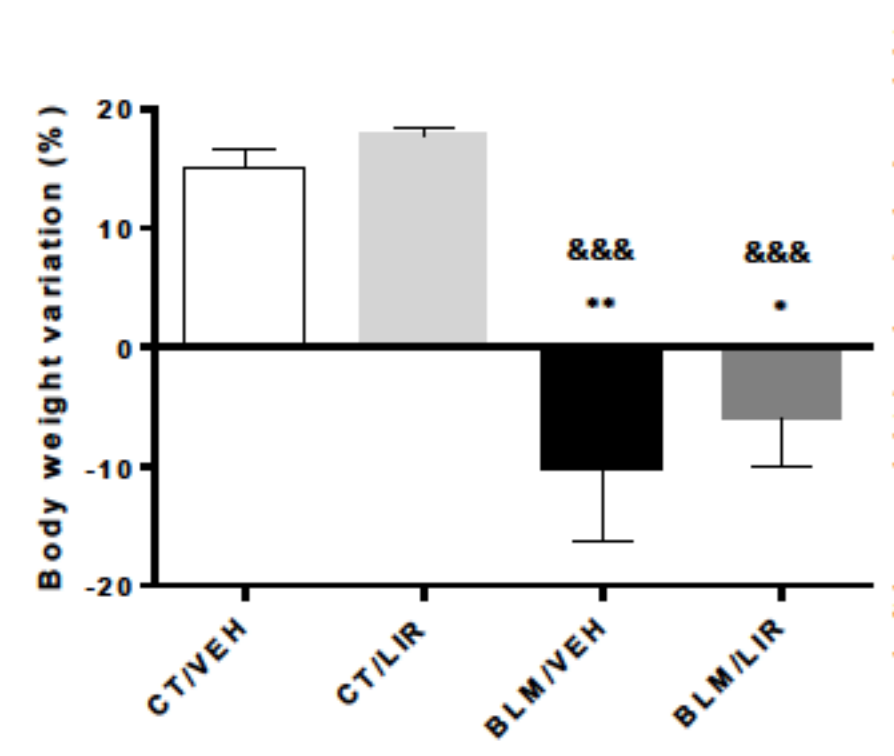
### Statistics

- Non parametric Kruskal-Wallis Test following Pairwise multiple Comparisons.
- “\*” → Statistically different with CT/VEH group.
- “&” → Statistically different with CT/LIRA group.
- “#” → Statistically different with BLM/VEH group.
- \*,&,# → p<0,05; \*\*,&&,# → p<0,01; \*\*\*,&&&### → p<0,001.

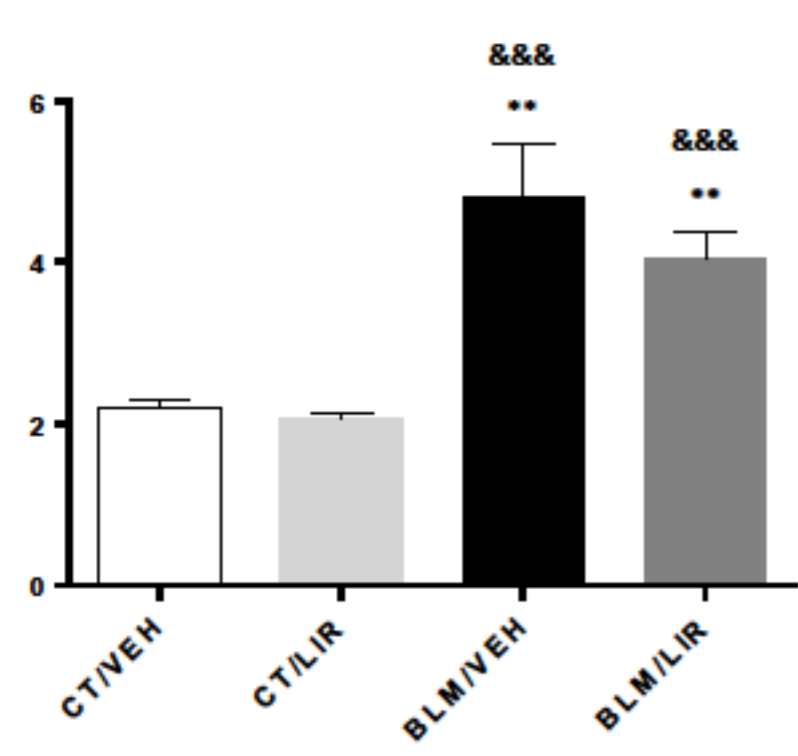
## Results

### Effects over body and left lung weight

#### Body weight



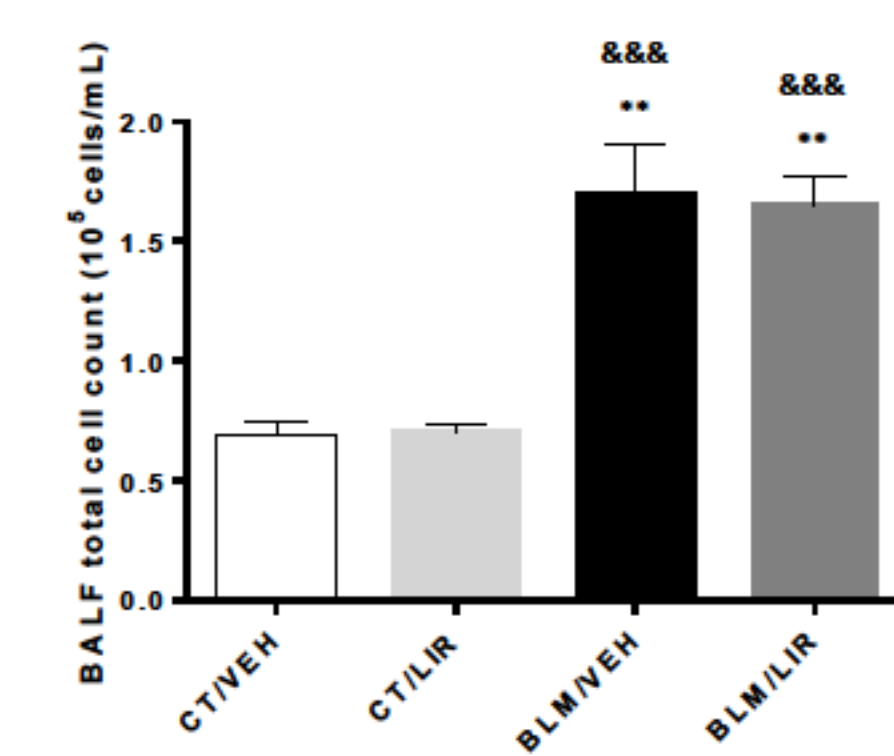
#### Lung weight



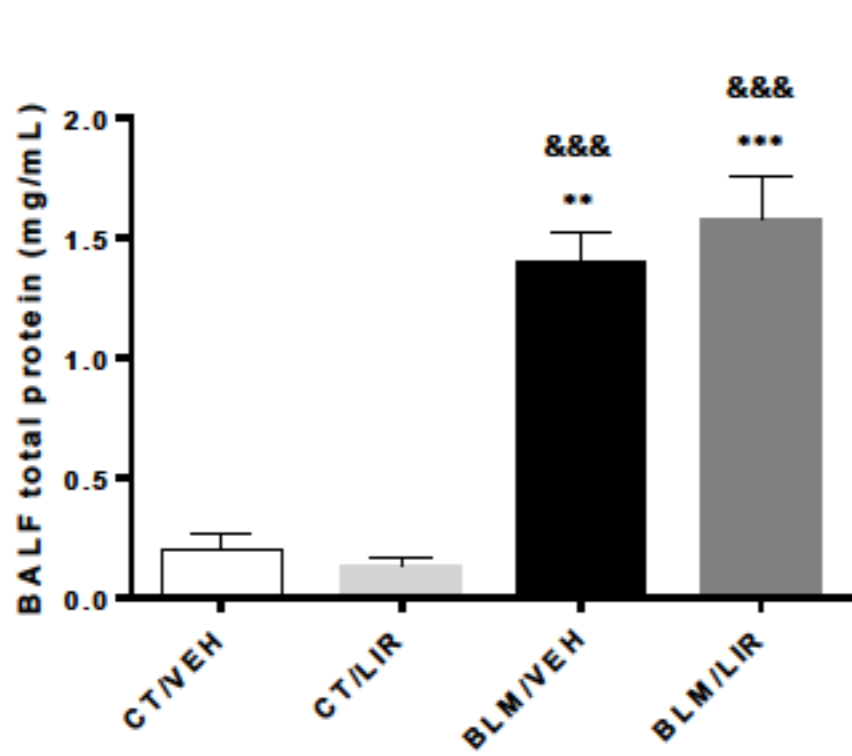
- Bleomycin administration decreases body weight gain. No differences was observed after Liraglutide treatment.
- Lung wet weight increased in bleomycin-treated rats because pulmonary inflammation. No differences was observed after liraglutide treatment.

### Effects over BALF total cells and total protein level

#### Total cells



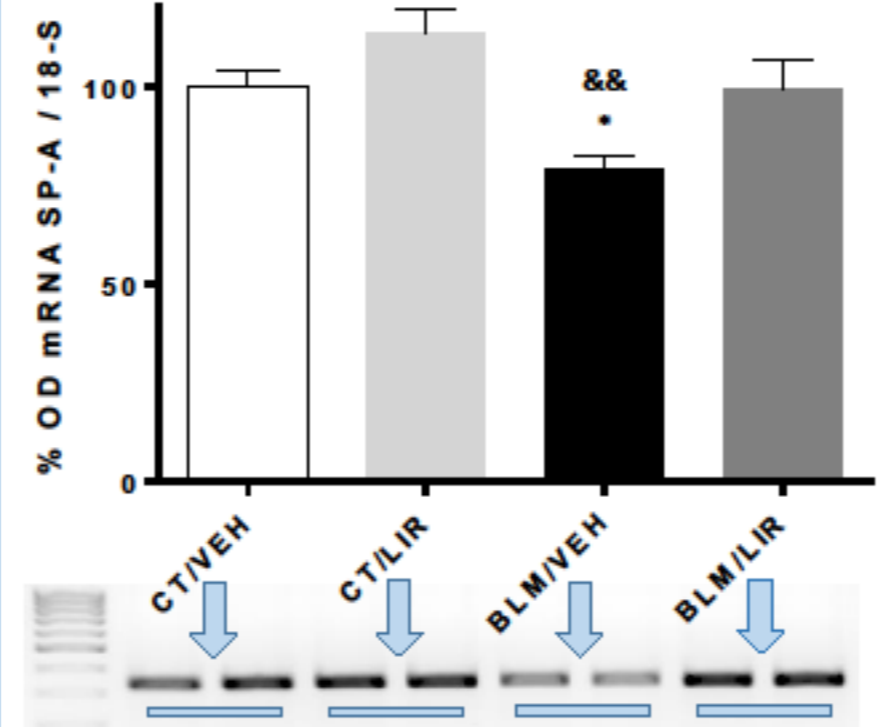
#### Total protein



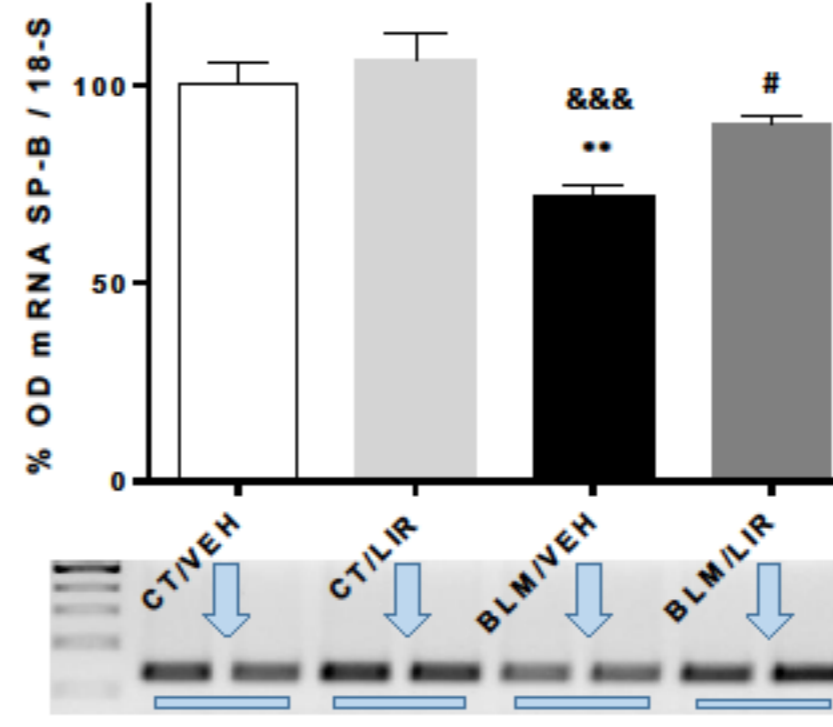
- Bleomycin administration increases total cell count and total protein levels in BALF
- No differences had been observed after liraglutide treatment.

### Effects over Surfactant Proteins mRNA expression

#### SP-A



#### SP-B



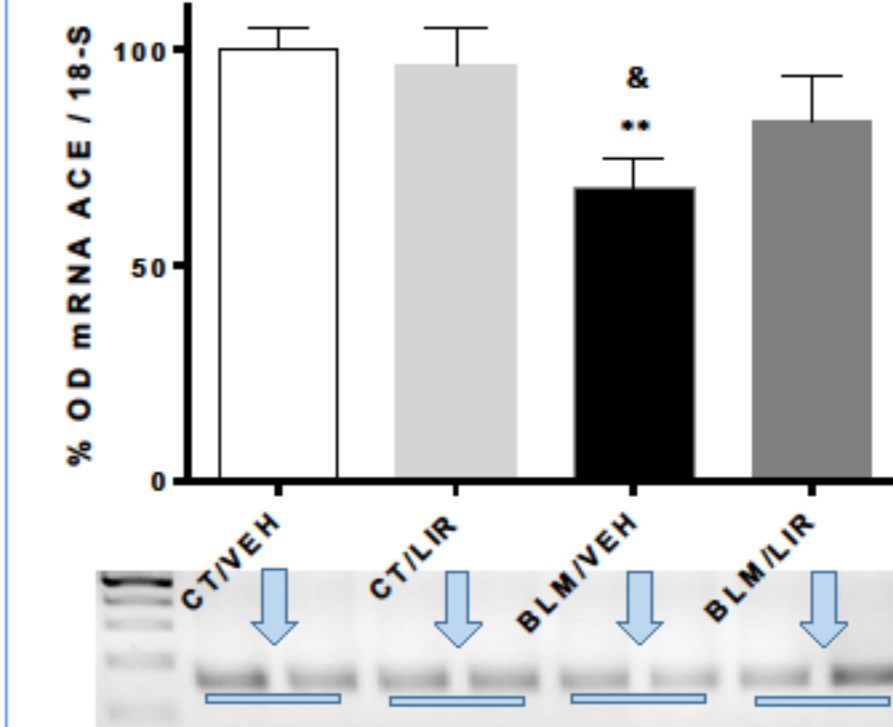
#### 18-S Housekeeping gene



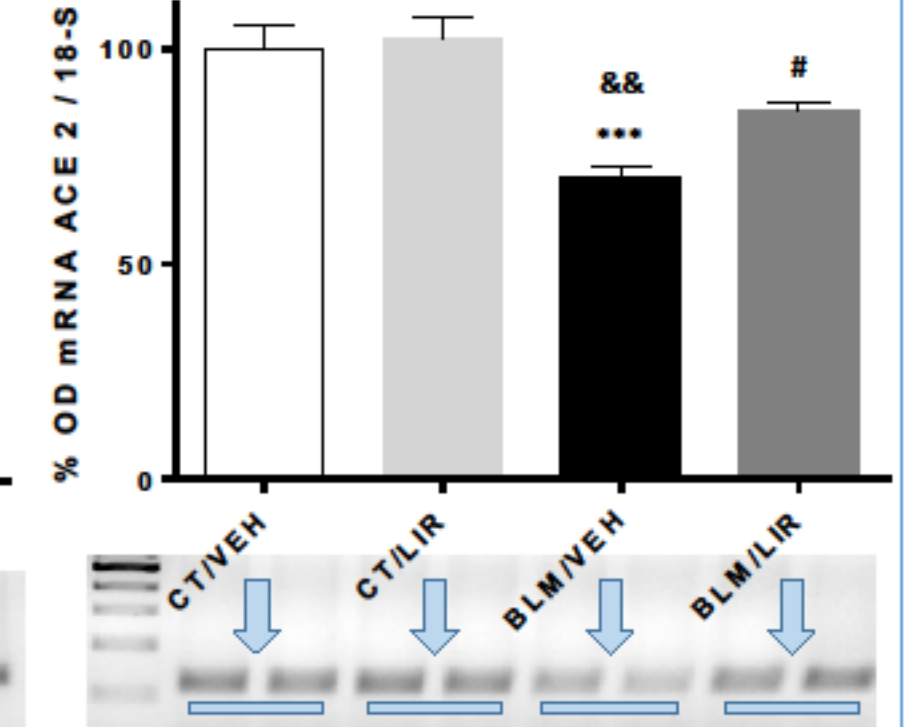
- Bleomycin administration decreases mRNA expression of Surfactant Protein A (SP-A) and Surfactant protein B (SP-B) to a 21% and 28% vs. CT/VEH group, respectively.
- Liraglutide treatment completely restores mRNA expression of both Surfactant Proteins.

### Effects over Angiotensin-Converting enzymes mRNA expression

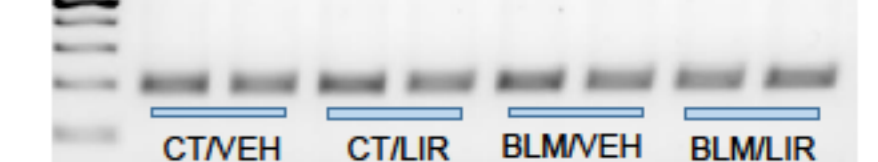
#### ACE



#### ACE 2



#### 18-S Housekeeping gene



- Bleomycin administration decreases mRNA expression levels of both vascular enzymes by a 32% (ACE) and 30% (ACE2) vs. CT/VEH group.
- Liraglutide treatment completely restores mRNA expression of both enzymes.

## Conclusion

Subcutaneous administration of liraglutide during the acute inflammatory phase, can restore surfactant proteins and angiotensin converting enzymes expression in the lung of Bleomycin-treated rats, despite liraglutide treatment can't improve initial inflammatory response in this animal model.

This implicates that GLP-1 agonist could improve pulmonary functionality in Acute lung injury animal model irrespective inflammatory responses.

## References

- 1-Matute-Bello G, Frevert C, Martin T. Animal models of acute lung injury. Animal Journal of Physiology-Lung Cellular and Molecular Physiology. 2008; 295(3):379-399.
- 2-Romaní-Pérez M, Outeiriño-Iglesias V, Gil-Lozano M, González-Matías L, Mallo F & Vigo E. Pulmonary GLP-1 receptor increase at birth and exogenous GLP-1 receptor agonists augmented surfactant-protein levels in litters from normal and Nitrofen-treated pregnant rats. Endocrinology 2013; 154 1144-1155.
- 3-Romaní-Pérez M, Outeiriño-Iglesias V, et al. Activation of the GLP-1 receptor by Liraglutide increases ACE2 expression, reversing right ventricle hypertrophy and improving the production of SP-A and SP-B in the lung of type 1 diabetes rats. Endocrinology. 2015; 156(10):3559-3569.

