Pulmonary function and disease progression in high-fat diet + bleomycin-induced and spirometry-confirmed mouse model of IPF

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BACKGROUND & AIM

Idiopathic pulmonary fibrosis (IPF) is a chronic and fatal interstitial lung disease, characterized by progressive fibrotic development within the lungs and decline in pulmonary function. Thus, preclinical models of IPF should demonstrate pulmonary dysfunction in conjunction with disease progression for translational evaluation of novel drug therapies.

The aim of the present study was to characterize pulmonary function, metabolic, biochemical, histological, and transcriptomic changes in a combined high-fat diet (HFD) + bleomycin (BLEO) -induced and spirometry-confirmed mouse model of IPF.

METHODS

10-12 weeks old C57BL/6JRj male mice were fed 60% HFD for 2 weeks prior to receiving either a single intratracheal instillation of bleomycin (1.5 mg/kg, 50 µL) or saline (CTRL) at study day 1. Animals remained on HFD for the entire study period. HFD-BLEO-IPF animals were randomized into study groups based on body weight loss at day 6 post-BLEO and terminated at specified time points. Terminal pulmonary end-points included spirometry (Flexivent) for expiratory/inspiratory capacity, biochemical analysis for hydroxyproline (HP) content, quantitative histomorphometry for markers of inflammation and fibrosis, and whole lung RNAsequencing including bioinformatic analysis. Gubra Histopathological Objective Scoring Technique (GHOST) was used for performing pathological Ashcroft scoring.

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1 Study outline

Group	Animal model	Name	Number of animals
1	Chow	CTRL	10
2	HFD	CTRL	10
3	HFD-BLEO-IPF	D7	11
4	HFD-BLEO-IPF	D21	12
5	HFD-BLEO-IPF	D28	12
6	HFD-BLEO-IPF	D35	12

2 Metabolic and biochemical parameters

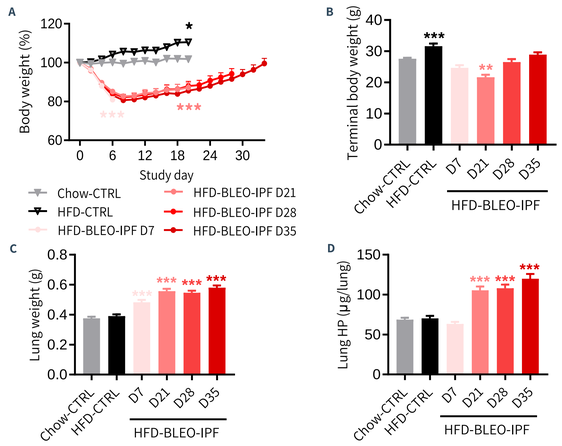

Figure 1. Metabolic and biochemical parameters in HFD-BLEO-IPF mice. (A) Body weight change relative to baseline (day 1). **(B)** Terminal body weight (g) **(C)** Terminal lung weight (g). **(D)** Terminal lung total HP. **p<0.01 and ***p<0.001 compared to Chow-CTRL group (Dunnett's test one-factor linear model).

3 Pulmonary function testing

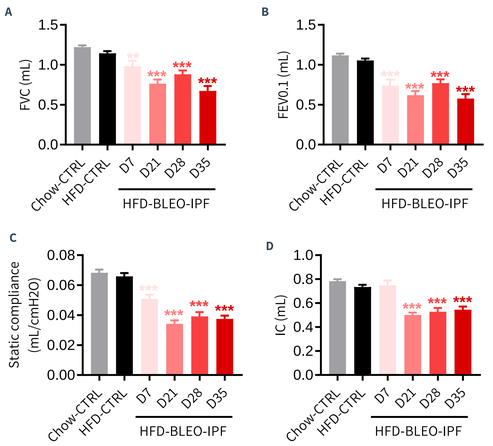

Figure 2. Pulmonary function testing in HFD-BLEO-IPF mice. (A) Forced vital capacity (FVC). **(B)** Forced expiratory volume in 0.1 seconds (FEV0.1). **(C)** Static compliance. **(D)** Inspiratory capacity (IC). *p<0.05, **p<0.01, and ***p<0.001 compared to Chow-CTRL group (Dunnett's test one-factor linear model).

4 Histopathological Ashcroft scoring

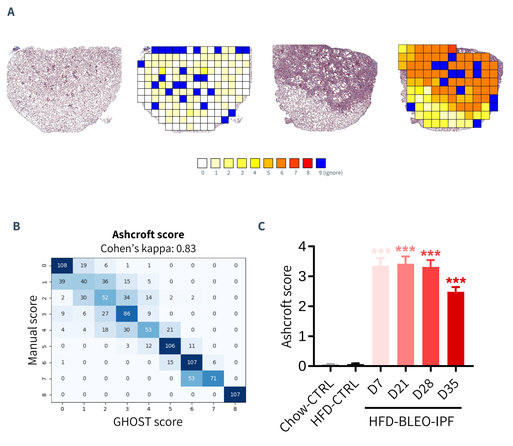

Figure 3. Ashcroft score in HFD-BLEO-IPF mice. Histopathological Ashcroft score were determined by GHOST deep learning-based image analysis. **(A)** Representative Masson's Trichome photomicrographs used for GHOST evaluation. **(B)** Ashcroft compared by GHOST assessment and manual scoring. **(C)** Ashcroft score by GHOST. Mean ± SEM. ***p<0.001 compared to Chow-CTRL group (Dunnett's test one-factor linear model).

5 Histological markers of inflammation, fibrosis, and fibrogenesis

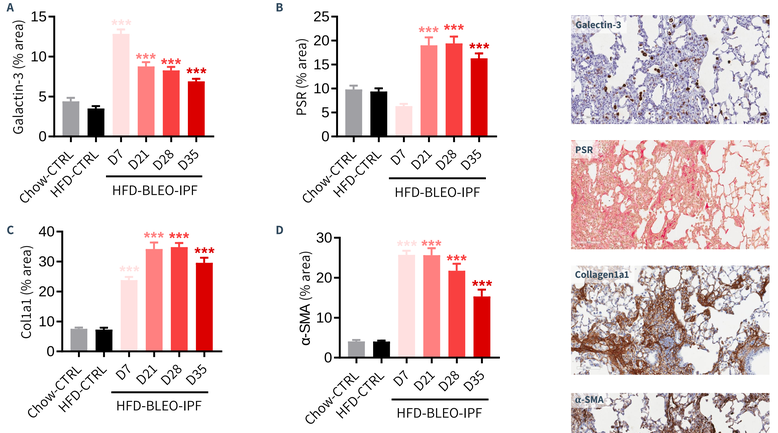

Figure 4. Lung quantitative histological markers in HFD-BLEO-IPF mice.
Histomorphometric assessments were performed by conventional IHC image analysis (panels A-D). **(A)** total Galectin- 3 content. **(B)** total PSR content. **(C)** total collagen-1a1 content. **(D)** total alpha-smooth muscle actin (α-SMA) content. Mean ± SEM. *p<0.05, *p<0.01, and ***p<0.001 compared to the Chow-CTRL group (Dunnett's test one-factor linear model). Right panels: Representative galectin-3, PSR, collagen 1a1 and α-SMA photomicrographs for BLEO-IPF D21 group (scale bar, 100 µm).

6 Transcriptomic profile for fibrosis and inflammation

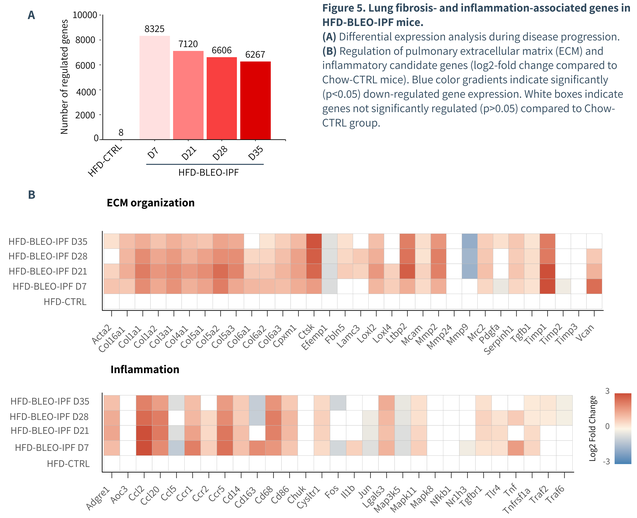

Figure 5. Lung fibrosis- and inflammation-associated genes in HFD-BLEO-IPF mice.
(A) Differential expression analysis during disease progression. **(B)** Regulation of pulmonary extracellular matrix (ECM) and inflammatory candidate genes (log2-fold change compared to Chow-CTRL mice). Blue color gradients indicate significantly (p<0.05) down-regulated gene expression. White boxes indicate genes not significantly regulated (p>0.05) compared to Chow-CTRL group.

CONCLUSION

- HFD-BLEO-IPF mice demonstrate progressive increase in lung weight and total hydroxyproline content..
- HFD-BLEO-IPF demonstrate reduced pulmonary expiratory and inspiratory function.
- HFD-BLEO-IPF mice demonstrate increased histopathological Ashcroft score.
- HFD-BLEO-IPF mice demonstrate increased lung levels of quantitative histological markers of fibrosis, inflammation and fibroblast cell activation.
- HFD-BLEO-IPF mice demonstrate marked lung transcriptomic regulation and increased fibrosis- and inflammation-associated gene expression.
- The HFD-BLEO-IPF mouse represent a translational preclinical model for exploring novel therapeutic agents for IPF.