Bull. Egypt. Soc. Physiol. Sci. Vol. (45), Issue (4), 449- 463

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

The Effectiveness Of Whole-Body Plethysmography In evaluating Ventilatory Parameters In lung fibrosis and After Resveratrol Treatment In Conscious Unrestrained Rats

Seham Z Nassar$_1$, Rania Gaber Ali 2, Noha Mohamed Badae$_1$

1. *Department of Medical Physiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt*
2. *Department of Pathology, Faculty of Medicine, Alexandria University, Alexandria, Egypt*

Submit Date : 18 Feb. 2025
Revised Date :14 April 2025
Accept Date : 28 April 2025

Keywords

- Whole-body Plethysmography
- Pulmonary fibrosis
- Resveratrol
- Ventilatory function
- Tidal volume
- Expiratory flow rates

Abstract

Background: Pulmonary fibrosis (PF) is among the highly fatal respiratory diseases worldwide. The establishment of good experimental evaluation technique is essential to study the disease and evaluating possible treatment modalities. The Classical methods used to evaluate spirometry like parameters in animal model has its limitations such as the use of anesthesia and animal immobilization. In contrast, unrestrained whole-body plethysmography (WBP) offers a non-invasive alternative for obtaining spirometric-like parameters in conscious, freely moving animals, providing a more accurate reflection of physiological respiratory function. Resveratrol, a natural polyphenol, has emerged as a promising therapeutic agent. This study aimed to validate the utility of WBP as a non-invasive method to assess ventilatory function in a bleomycin-induced rat model of PF and evaluated the efficacy of resveratrol treatment. ***Methods:*** PF was induced in 20 Wistar male rats by single endotracheal injection of bleomycin (2.5 mg/kg), those were randomly allocated into two groups (10 rats each): a disease control group (PF) and a treatment group (PF + Resveratrol; 10 mg/kg/day, orally). Lung function was serially assessed using WBP. ***Results:*** Induction of PF resulted in a distinct restrictive pattern, confirmed by significant increase in respiratory rate, reductions in tidal volume (Vt) and minute respiratory volume (MRV), while the FEV1/FVC ratio and flow rates remained normal consistent with a restrictive pathophysiology. Resveratrol treatment markedly improved these parameters, demonstrating a significant reversal of the bleomycin-induced ventilatory impairment. ***conclusion:*** The study confirms that WBP is a sensitive, non-invasive tool for detecting the functional hallmarks of restrictive lung disease in conscious rats. Furthermore, it provides compelling functional evidence for the therapeutic potential of resveratrol in alleviating PF. WBP proves highly effective for evaluating both disease progression and treatment response in pre-clinical PF research.

Corresponding author: Dr. Seham Zakaria Nassar , Department of Medical Physiology, Faculty of Medicine, Alexandria University, Alexandria, Egypt , Tel: +201006184850, E-mail:seham.hassan@alexmed.edu.eg ; drsehamn2001@yahoo.com

1. Introduction:

Pulmonary fibrosis (PF) is a progressive and often fatal interstitial lung disease characterized by irreversible scarring, leading to severe respiratory compromise. Animal studies has been used as an effective tool for the study of the pulmonary pathophysiology to prevent, control and cure these diseases [i]. A significant challenge in these studies is how to assess the respiratory function in rat models particularly in conscious, unrestrained rats.

Existing methods for measuring pulmonary function in rodents *in vivo* are divided into invasive and non-invasive approaches, each with distinct advantages and disadvantages. At one extreme, unrestrained plethysmography (Penh) in conscious animals is highly convenient but less specific [ii]. At the other extreme, the measurement of input impedance in anesthetized, paralyzed, tracheostomized mice is precise and specific but requires studying the animal under conditions far removed from its natural state [iii].

Whole-body plethysmography (WBP), a technique for the assessment of lung function, has been widely recommended for the characterization of respiratory physiology in mice and rats. WBP providing a non-invasive approach for the assessment of the respiratory function in conscious animals meanwhile avoiding technical limitations such as the use of anesthesia and animal immobilization. This contrasts with other methods, such as double-chamber plethysmography (DCP), which requires immobilization, or the forced oscillation technique (FOT), which involves invasive surgery on anesthetized animals and precludes recovery. Consequently, the respiratory parameters obtained with WBP in rat reflect basal physiological values since instrumental restraints and/or anesthesia are not applied [iv,v]. Notably, these experimental conditions are key for the use of WBP in longitudinal studies [vi,vii]. Furthermore, WBP in rat is a well-established technique that has been applied for the study of a wide range of biological aspects of the respiratory function providing new insights into neuronal network controlling respiratory rhythm [viii], sleep-related breathing disorders [ix] or the role of inflammation in the control of breathing [x]. WBP relies on the recording of pressure changes that are produced by the spontaneous breathing activity of an animal placed inside an airtight chamber. During normal respiration, pressure variation is directly proportional to the respiratory pattern of the animal allowing the measurement of the respiratory rate and tidal volume. These parameters are commonly used to evaluate pulmonary function in different physiological and disease models.

Interstitial Pulmonary Fibrosis (IPF), a specific form of PF, is characterized by early alveolar injury, inflammation, and late-stage lung fibrosis [xi]. A key feature of IPF is the accumulation of alveolar macrophages and neutrophils in the lower respiratory tract,

parenchymal cell injury and fibrosis of the alveolar walls [xii]. Fibrosis is a pathological feature of tissue remodeling that arises from dysregulated repair mechanisms, which can adversely impact lung function [xiii]. In Idiopathic Pulmonary Fibrosis (IPF), the accumulation of macrophages and neutrophils leads to the release of toxic oxidants, inducing oxidant-mediated parenchymal cell injury. Activated neutrophils further contribute to this damage by releasing myeloperoxidase (MPO), which catalyzes the formation of highly toxic hydroxyl radicals[xiv]. Therefore, it is reasonable to hypothesize that the alveolar epithelial cell injury may result, at least in part, from an enhanced oxidant burden. Thus, antioxidant and anti-inflammatory agents may have beneficial effects in the treatment of IPF.

The bleomycin-induced lung fibrosis model is widely used to study human IPF. In rats, intratracheal administration of bleomycin triggers an acute inflammatory response characterized by the release of proinflammatory cytokines, leading to initial lung injury and the subsequent development of fibrosis [xv].

Resveratrol, a polyphenol found in various plant sources such as grape skin, cranberries, peanuts, and others, possesses potent antioxidative and anti-inflammatory properties[xvi]. It has been shown that resveratrol has preventive and curative effects on cardiovascular disease, protection of vascular endothelium, regulation of lipid metabolism, increase in intracellular nitric oxide levels, and inhibiting effect of platelet aggregation [xvii, xviii]. Many studies have studied the preventive and curative effect of resveratrol on bleomycin induced pulmonary fibrosis through studying its histopathological and biochemical effects, however no study has demonstrated its effect on pulmonary function tests. This protocol describes the procedures for measurement of different ventilatory parameters in rats using noninvasive WBP. This technique will serve as a primary tool to assess pulmonary function at three critical time points: at the study's baseline, after the induction of pulmonary fibrosis with bleomycin, and following treatment with resveratrol.

2. Materials and methods

2.1. Laboratory animals

The study was conducted on 30 Wister albino male rats, ten weeks old and weighed 200-250 grams, obtained from the Physiology Department Animal House, Alexandria Faculty of Medicine. One week before and during the experiments, the animals were housed in plastic cages with an ambient temperature of (23±3) °C, stable air humidity and a natural day/night cycle. The rats had free access to standard rodent laboratory food and tap water. All experimental procedures were conducted in accordance with the institutional animal ethics committee at the Faculty of Medicine, Alexandria University (IRB NO:

00012098-FWA NO: 00018699). The serial registration number of this study 0306811

2.2. The study design

The 30 rats were divided randomly into two groups: negative control group (n=10) and pulmonary fibrosis group (n = 20)**.** Pulmonary fibrosis was induced in 20 rats by a single endotracheal injection of Bleomycin (5 mg/kg) dissolved in 0.3 ml of sterile saline [xix].Negative control rats received an equal volume of saline and served as negative controls (n = 10). Bleomycin injected rats were randomly divided into two groups (10 rats each); untreated pulmonary fibrosis group (PF group) and Resveratrol pulmonary fibrosis treated group (Res-PF group) which received Resveratrol (10 mg/kg) orally for 14 days starting after 3 weeks of Bleomycin administration [xx].

Whole body plethysmography was assessed in serial measurements T0; before bleomycin administration to exclude abnormal results, T1; 3 weeks after bleomycin injection to assure the induction of restrictive lung disease and T2; after 14 days of resveratrol treatment.

2.3. Whole Body Plethysmograph (WBP):

Whole body plethysmography measurements are non-invasive and can be performed on awake (non-anesthetized) animals (Anesthetics are known to suppress/modulate respiration). WBP afforded the possibility of performing repeated evaluations in a longitudinal manner during the trial and as an end-point for evaluating the efficacy of treatment.

The apparatus used is a custom-made chamber (40 cm long x 20 cm width x 6 cm height) made of clear plastic. The cover is fitted with air-tight gaskets and removed for access to the interior of the chamber (i.e. placing mouse inside). The chamber's final volume is 400 mL. The chamber has two inlets, of which one is used to inlet a gas mixture (N_2, O_2 and/or CO_2), and the second is used as outlet and is connected to the pneumotachometer. The chamber is placed in a cardboard box to avoid distraction and rat stress during the recordings [xxi].

2.3.1. Ventilatory parameter recording:

The ventilatory parameters are recorded using a pneumotachometer (MLT1L, AD Instruments, Colorado Springs, CO) with P1 channel end connected to the outlet of the NP/ WBP (for whole animal recordings). The other end is open for differential measurements of the spirometer. The pneumotachometer is connected to the spirometer. The analog output channel signal is connected to an analog-digital converter (Power Lab/8SP, AD Instruments). The output (digital) signal obtained is acquired by PC using Chart version 5.5.6. ventilatory parameters recorded are tidal volume (V_T), minute respiratory volume (MRV) and forced vital capacity

(FVC)]. WBP were assessed on day one of the study and after 3 and 5 weeks after Bleomycin administration [xxii].

2.3.2. Procedures:

Several parameters may critically influence WBP outcomes and reproducibility. Therefore, they were considered in our study. These include time of the day (considering rat circadian cycle), rat behavior during recording, and standardization of the habituation period [xxiii, xxiv] Furthermore, environmental conditions were kept constant during the entire procedure. Here, we maintained a constant temperature of 22 °C and a relative humidity of 55%

2.3.3. Calibration of Apparatus:

The rats were brought from the animal house to the laboratory one to two hours before the start of the training session to recover from the transportation and new environment stresses. Water and food are provided *ad libitum.* Avoiding excessive noise and manipulation was necessary. Each rat was weighted separately and introduced inside the training chamber for WBP for at least 30 minutes breathing room air for acclimatization purpose.

The calibration was done as described in the transducer manufacturers' manual (AD Instruments) by injecting 100 µL of air using a calibrated micropipette in about 1 to 2 seconds into the pneumotachometer end which is connected to the spirometer P1 channel. Calibration must be performed every time the equipment is switched on. There is no calibration between each animal in the same day.

2.3.4. WBP recording:

The rat was placed in the plethysmography device and the chamber is tightly closed and the rat can move freely. The Pneumotachometer recording head was connected to the training chamber (fig.1B, C) and the rat was allowed to breath freely for 15 minutes. The rat was visually monitored but without disturbing the equipment to avoid interferences with the analysis. After recording, the equipment must be cleaned and wiped with 70% Ethanol before recording the next rat. Recordings were automatically saved and available for data analysis [xxv].

2.3.5. Determination of Correction factor for WBP using Nose Plethysmograph (NP):

The apparatus is used to obtain a correction factor for thorax expansion during inspiration, which reduces the observed V_T value when using WBP. The NP was constructed from a 15 mL cylindrical tube with two ports. To obtain an air-tight seal, parafilm "M" (American National Can, Greenwich, CT, Cat no. PM-996) is used to form an air-tight seal between mouse nose and tube. The rats were anesthetized with ketamine xylazine mix. NP recording was done during normoxia of the

same rat. Five different segments of WBP and NP recordings were selected during normoxia exposure. The V_T values of the five WBP and NP samples were obtained and normalized by the body weight. The V_T normalized values from NP were divided by V_T normalized values from WBP to obtain correction factor (CF). Multiply the V_T from WBP by the calculated CF.

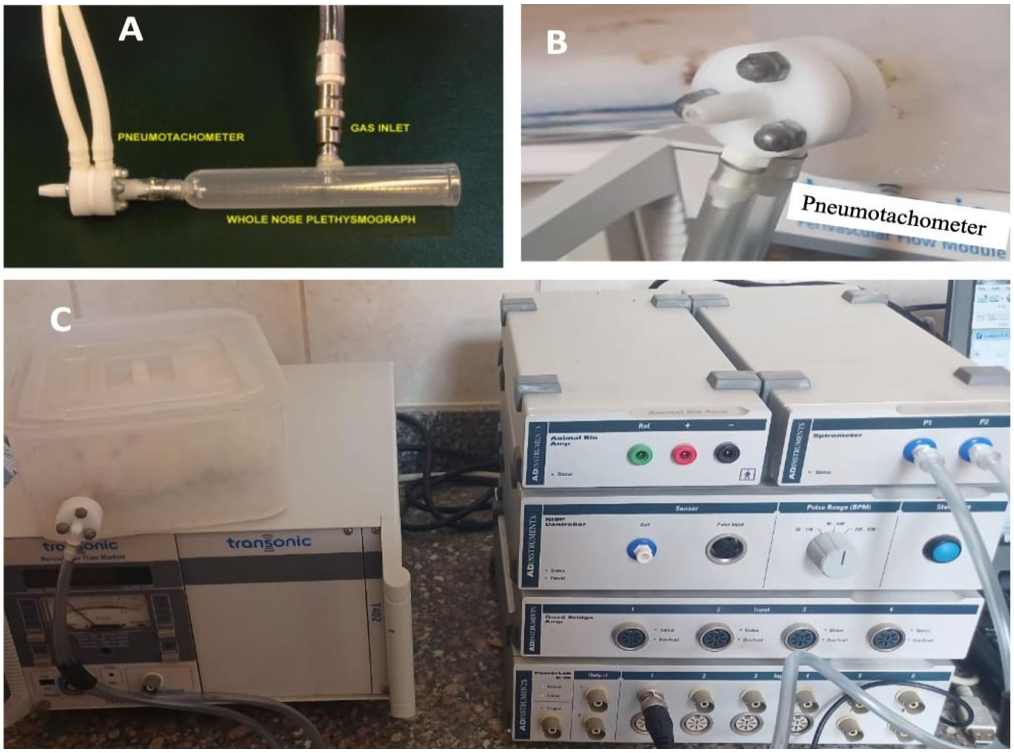

Fig.1; A: nose plethysmography for calculation of the correction factor. B: Pneumotachometer flow head MLT1L, AD Instruments. C: whole body plethysmography recording.

2.4. Wet Weight and Physical Lung Volume

At the end of the study all animals were weighted and anesthetized with ketamine/xylazine cocktail. A mid-ventral laparotomy was done, and left lungs were removed after ligation of the left main bronchus, rinsed with ice cold phosphate buffered saline (PBS) and weighted. To measure the lung volume, the lungs were immerged into a milliliter-graduated cylinder filled with Krebs solution and the change in volume displaced by the lungs was recorded.

2.5. Histological Examination:

Endotracheal tube was inserted in the rats after excision of the left lung and 10% formalin was next injected into the animal's trachea until the right lung inflated with 2-3ml formalin for histopathological examination. The right lung was then excised and fixed in 10 % formaldehyde and embedded in paraffin blocks for histopathological investigations. The lung sections were stained with hematoxylin and eosin (H&E) and examined under the light microscope (Leica, Germany)[xxvi].

2.5. Statistical analysis

Individual data are presented, together with means ± standard deviations (SD). Two-way ANOVAs were used to assess the effect of pulmonary fibrosis on each measured readout. When the interaction was significant, it was followed by multiple comparisons test Tukey. All statistical analyses were performed with SPSS 25. Differences with a $p < 0.05$ were considered statistically significant.

3. Results

3.1. The body weight, lung weight.

At the end of the study, there was no significant difference in body weight between all the studied groups, however there was a significant increase in the lung weight and lung/ body weight ratio in bleomycin induced PF group as compared to normal control rats. Concerning lung to body weight ratio (lung/BW ratio), bleomycin-injected rats showed 1.7 folds increase as compared to control rats. Meanwhile, this ratio decreased again by resveratrol treatment. Although there is an increase in lung wight of PF group however it is associated with 29% reduction in lung volume as compared to normal rats (table 1).

Table 1; Changes in body weight, lung weight and lung volume in the studied groups.

	Normal	Bleomycin induced PF	Resveratrol treated PF
Body weight (g)	242.4 ± 26.5	213.6 ± 9.4	231.6 ± 13.7
Lungs weight (g)	1.34 ± 0.16	1.96 ± 0.41 *	1.48±0.15 #
Lungs/BWratio (%)	0.553 ± 0.063	0.92 ± 0.18 *	0.64 ± 0.039 #
Lung volume (ml)	9.8 ± 1.04	6.92 ± 0.56*	8.72 ± 0.91 #

Data is represented as mean ± SD. (n = 10). □ Significantly different from normal control animals ($P < 0.05$). # Significantly different from Bleomycin induced PF animals ($P < 0.001$).

3.2. Physical examination of the lungs

The lungs were studied for physical appearance. The lungs of the control group appear reddish with a smooth surface, soft and uniform texture, and no bleeding spots on the surface. In the Bleomycin induced PF group, the lungs appeared pale with reduced volume and increased hardness. The surface was not smooth and had small, scattered nodules and grooves with bleeding spots. The physical appearance in the Resveratrol treated PF group were slightly red with old bleeding spots; there was no hardening nor consolidation on the surface of the lung (Fig.2).

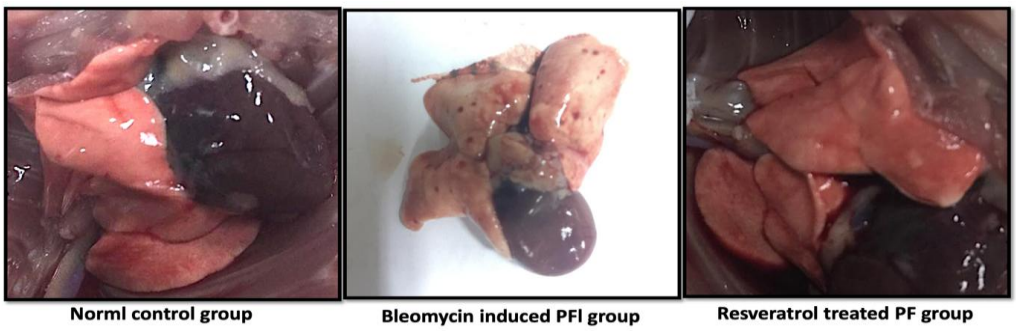

Fig.2; physical appearance of the lungs in the three studied groups

3.3. Histological Examination:

Histological examination of the lung tissue showed normal lung architecture in the normal control group with no fibrosis or inflammatory cell infiltration (Fig. 3A). The bleomycin treated group showed multiple areas of fibrosis (Ashcroft score 6) with a heavy inflammatory infiltrate formed of macrophages, lymphocytes and neutrophils arranged in the inter-alveolar spaces in addition to perivascular and peribronchiolar spaces with a mean fibrosis/HPF 60.7% ± 3.5 (Fig. 3 B). The Resveratrol treated group revealed different grades of fibrosis that causes a mild increase in the thickness of the alveolar and bronchiolar walls without damage of the lung parenchyma (Ashcroft score 2) and the mean percentage of fibrosis was 31.96% ± 8.2/HPF (fig.3 C).

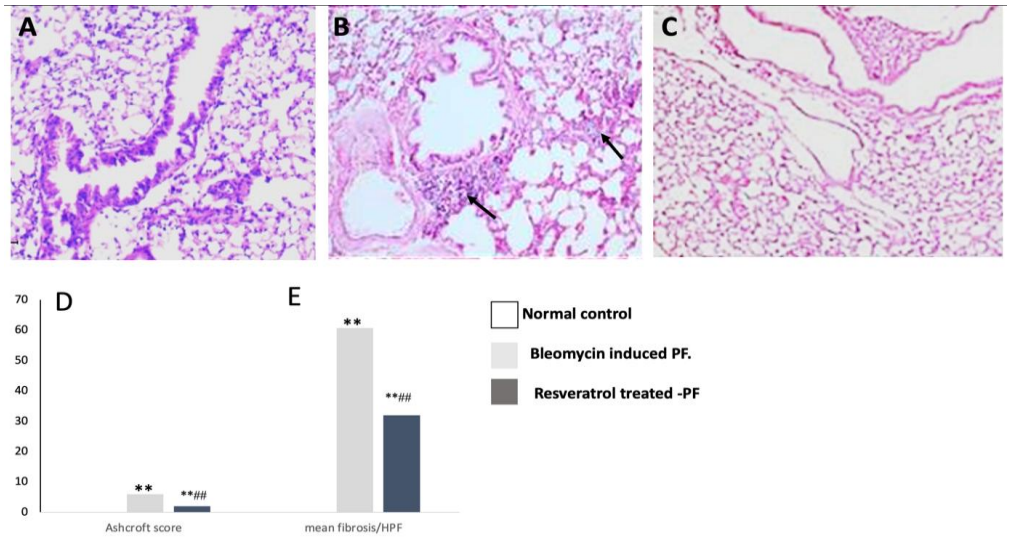

Fig 3: H&E-stained sections from different studied groups; A: normal lung parenchyma. B: the bleomycin treated group depicts increased alveolar and bronchial walls thickness with extensive inflammation. C: Resveratrol treated group reveals moderate decrease in fibrosis. X100, D: The Ashcroft score in the different studied groups. E: the percentage of fibrosis and inflammatory infiltrate. Data are presented as means ± SD. ** Significantly different from normal control rats (P < 0.001). ## Significantly different from Bleomycin induced PF animals (P < 0.001).

3.4. Changes in the ventilatory parameters measured by WBP

Baseline ventilatory parameters were tidal volume (Vt) (4.276 ± 0.427ml/kg), Respiratory rate (Rf) (129.14 ± 9.15 breath/min) and minute respiratory volume (MRV) (694.125 ± 47.31 ml/kg/min). After Bleomycin injection there was significant reduction of Vt to a mean value of (2.620 ± 0.41ml/kg), MRV (479.92 ± 72.58ml/kg/min) and increase of Rf (183.161 ± 13.42 breath/min) ($P < 0.001$). However, treatment with resveratrol had significantly increased Vt by 60% and MRV by 34 % and reduce Rf by 15.5 % as compared to PF untreated group ($P < 0.001$) (Table 2, Fig. 4).

Table 2; Changes in spirometry like pulmonary ventilatory parameters in the studied groups.

	Normal	Bleomycin induced PF	Resveratrol treated PF
Rf (Breath/Min)	127.59 ± 7.085	183.161 ± 13.412**	154.635 ± 10.375**##
VT(ml / kg)	5.525 ± 0.611	2.620 ± 0.408**	4.195 ± 0.426**##
MRV(ml/kg/min)	704.125 ± 77.91	479.917 ± 72.575**	643.438 ± 34.025##
FVC (ml/kg)	29.117 ± 2.219	17.678 ± 1.687**	24.802 ± 1.902**##
$FEV_{1\%}$ (%)	94.16 ± 1.01	96.43 ± 1.09	95.27 ± 1.18
PIF(ml/SEC)	223.045 ± 8.146	201.625 ± 35.995	206.00 ± 15.901
PEF(ml/SEC)	225.267 ± 25.051	213.00 ± 8.280	208.625 ± 8.634
Ti(sec)	0.158 ± 0.034	0.119 ± 0.02*	0.164 ± 0.012
Te(sec)	0.297 ± 0.053	0.134 ± 0.013**	0.204 ± 0.026*##

Data is represented as mean ± SD. (n = 10). Abbreviations: Vt; tidal volume, MRV; minute respiratory volume, Rf; respiratory rate, PIF; peak inspiratory flow**,** PEF; peak expiratory flow, Ti; inspiration time, Te; expiration time. ** Significantly different from normal control rats ($P < 0.001$). □ Significantly different from normal control animals ($P < 0.05$). # Significantly different from Bleomycin induced PF animals ($P < 0.001$).

Spirometry-like lung function parameters are highly dependent on age and weight, and that needs a great care to be taken when matching groups of rats of the same gender for generating this type of data. That is why all the data is expressed ml/kg to exclude the body weight variations.

Moreover, the criteria of the disease were further evaluated by the WBP results which showed 39% reduction in forced vital capacity (FVC) with maintained FEV1% which confirm the restrictive pattern of the disease. Resveratrol treatment leads to 40% increase in FVC as compared to untreated PF group also with no change in FEV1% ($P<0.001$) (Fig.4, Fig.5A). The flow rates whether PIF or PEF showed no significant difference in the three studied groups (Fig 5).

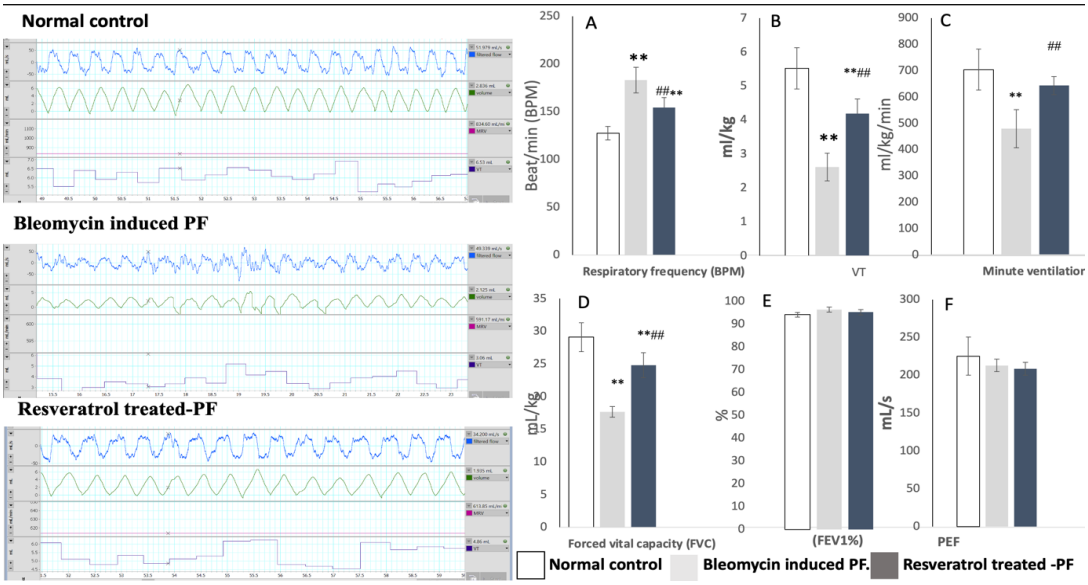

Fig. 4: Data is represented as mean ± SD. (n = 10). Abbreviations: Vt; tidal volume, MRV; minute respiratory volume, Rf; respiratory rate, FVC; forced vital capacity, FEV1%; forced expiratory volume 1%, PEF; peak expiratory flow. ** Significantly different from normal control rats (P < 0.001). ## Significantly different from Bleomycin induced PF animals (P < 0.001).

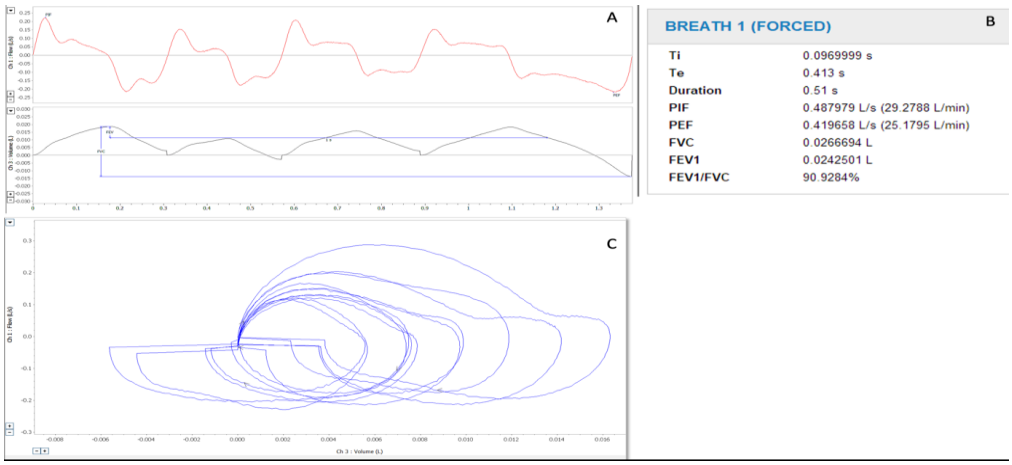

Fig.5; A: data view of a single forced breath. The upper red graph shows the PIF and PEF, the lower blue graph demonstrates the volume of FVC and FEV1. B: demonstrate the spirometry report of the single forced breath. C: demonstrate the flow volume loop.

4. Discussion

This study evaluated ventilatory function using a well-established rodent model of pulmonary fibrosis. The establishment of pulmonary fibrosis was confirmed by histological analysis and gross anatomical observations, notably an increase in lung mass coupled with a reduction in volume, both hallmark features of the disease. Assessing ventilatory function in small animal models presents significant methodological challenges that necessitate techniques that are both precise and reliable.

To this end, we employed whole-body plethysmography (WBP) to quantitatively assess lung volumes. Our measurements, taken at baseline, post-fibrosis induction, and following therapeutic intervention. The success of induction of PF was evident by the physical appearance of the lungs, increase in its weight,

decreased lung volume and histological findings. The lung volume studies revealed a clear restrictive ventilatory pattern. This was characterized by significant reductions in tidal volume (Vt), minute respiratory volume (MRV), and forced vital capacity (FVC), while the FEV1/FVC ratio (FEV1%) remained unchanged. This physiological profile is a definitive signature of restrictive lung disease, confirming the functional impact of the fibrosis induced by endotracheal bleomycin administration.

Bleomycin is a chemotherapy drug used to treat cancer. It can cause damage to the lungs leading to inflammation (interstitial pneumonitis) and scarring (fibrosis) which can cause breathing difficulties. Bleomycin forms a complex with molecular oxygen and metal ions such as iron, causing the generation of reactive oxygen species (ROS) in lung tissue and developing pulmonary fibrosis [xxvii].

Notably, Resveratrol had significantly decreased lung weight and increasing the volume with histological preservation of the lung parenchyma. Our WBP data demonstrated a marked amelioration of the functional deficits (Vt, MRV and FVC) following treatment with resveratrol. Resveratrol is a non-flavonoid polyphenol widely found in 72 seed plants [xxviii]. Because of its varying biological and pharmacological activities, such as anti-oxidation, antibacterial, anti-inflammatory, growth inhibitory, anti-platelet aggregation, pro-apoptotic, anti-tumor, anti-cardiovascular disease, liver protection, and estrogen regulation [xxix, xxx].It is known to exert a therapeutic effect on pulmonary fibrosis mainly through scavenging free radicals and activating antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GSSG-R), to maintain the dynamic balance of intracellular glutathione, thereby improving pulmonary fibrosis [xxxi]. In addition, resveratrol by increasing the SOD activity can

inhibit fibrosis caused by abnormal remodeling of the extracellular matrix. It also exerts beneficial effects in the later stages of pulmonary fibrosis by regulating the metabolism of collagen in the lung tissues and reducing its deposition in the lung interstitium. Furthermore, Resveratrol inhibits the activation of NF-κB in macrophages and lymphocytes, thus reducing the level of inflammatory mediators such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and transforming growth factor-β (TGF-β) to protect against inflammatory damage which eventually lead to fibrosis [xxxii, xxxiii].

In that context, we did not explore the mechanism of action of Resveratrol in our study. However, we proved that its therapeutic effect is well established by its effect on the results of lung volumes WBP study.

Conclusions

This study provides insight into the role of WBP for unrestrained unanesthetized animal to assess spirometry-like pulmonary function parameters and demonstrates that such parameters are reduced in pulmonary fibrosis and improved by Resveratrol. To this end, whole body plethysmography in rat is valid as a pre-clinical tool when investigating new drug targets or disease mechanisms in PF.

Limitations:

To overcome the technical limitations in WBP, adequate chamber habituation should be done to avoid animal stressors and to obtain adequate reproducible results. Also, while we considered the effect of weight in our study, other parameters must be considered as the effect of sex, age, or the influence of airway challenges, in a rat model of PF.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

None of the authors has any potential conflicts of interest to disclose.

REFERENCES

1. **Forum of International Respiratory Societies.** The Global Impact of Respiratory Disease. Second Edition. Sheffield, European Respiratory Society 2017.
2. **Lundblad, L. K. A., Irvin, C. G., Adler, A., and Bates, J. H. T.** A reevaluation of the validity of unrestrained plethysmography in mice. *J. Appl. Physiol.*2002;93: 1198–207.
3. **Bates, J. H. T., and Irvin, C**. Measuring lung function in mice: the phenotyping uncertainty principle. *J. Appl. Physiol.* 2003; 94: 1297–1306.

4. **Lim, R., Zavou, M. J., Milton, P. L., Chan, S. T., Tan, J. L., Dickinson, H., Murphy, S. V., Jenkin, G. and Wallace, E. M.** Measuring respiratory function in mice using unrestrained whole-body plethysmography. *J Vis Exp*.2014;(90): e51755.
5. **Quindry, J. C., Ballmann, G. C., Epstein, E. E. and Selsby, J. T.** Plethysmography Measurements of Respiratory Function in Conscious Unrestrained Mice. *J Physiol Sci* .2016;66(2): 157-64.
6. **Cramer, N. P., Xu, X., Christensen, C., Bierman, A., Tankersley, C. G.** and Galdzicki, Z. Strain variation in the adaptation of C57Bl6 and BALBc mice to chronic hypobaric hypoxia. *Physiol Behav.* 2015;143: 158-65.
7. **Flanagan, T. W., Sebastian, M. N., Battaglia, D. M., Foster, T. P., Cormier, S. A. and Nichols, C. D.** 5-HT2 receptor activation alleviates airway inflammation and structural remodeling in a chronic mouse asthma model. *Life Sci. 2019;* 236: 116790.
8. **Crone, S. A., Viemari, J. C., Droho, S., Mrejeru, A., Ramirez, J. M. and Sharma, K.** Irregular Breathing in Mice following Genetic Ablation of V2a Neurons. *J Neurosci* 2012; 32(23): 7895- 906.
9. **Bastianini, S., Alvente, S., Berteotti, C., Lo Martire, V., Silvani, A., Swoap, S. J., Valli, A., Zoccoli, G. and Cohen, G.** Accurate discrimination of the wake-sleep states of mice using non- invasive whole-body plethysmography. *Sci Rep* 2017; 7: 41698.
10. **Giannakopoulou, C. E., Sotiriou, A., Dettoraki, M., Yang, M., Perlikos, F., Toumpanakis, D., Prezerakos, G., Koutsourelakis, I., Kastis, G. A., Vassilakopoulou, V., Mizi, E., Papalois, A., Greer, J. J. and Vassilakopoulos, T.** Regulation of breathing pattern by IL-10. *Am J Physiol Regul Integr Comp Physiol* 2019; 317(1): R190-R202.
11. **Ley B, Collard HR, Talmadge E. King J.** Clinical Course and Prediction of Survival in Idiopathic Pulmonary Fibrosis. American Journal of Respiratory and Critical Care Medicine 2011; 183: 431- 40.
12. **Moss BJ, Ryter SW, Rosas IO.** Pathogenic mechanisms underlying idiopathic pulmonary fibrosis. Annual Review of Pathology: Mechanisms of Disease. 2022 Jan 24;17(1):515-46.
13. **Wolters PJ, Blackwell TS, Eickelberg O, Loyd JE, Kaminski N, Jenkins G, Maher TM, Molina-Molina M, Noble PW, Raghu G, Richeldi L.** Time for a change: is idiopathic pulmonary fibrosis still idiopathic and only fibrotic?. The Lancet Respiratory Medicine. 2018 Feb 1;6(2):154-60.
14. **Peter C. Dedon, Steven R.** Tannenbaum. Reactive nitrogen species in the

chemical biology of inflammationArchives of Biochemistry and Biophysics. 2004; Volume 423, Issue 1:12-22

15. .**P C Dedon , I H Goldberg.** Free-radical mechanisms involved in the formation of sequence-dependent bistranded DNA lesions by the antitumor antibiotics bleomycin, neocarzinostatin, and calicheamicin Chem Res Toxicol.1992; 5(3):311-32.
16. **L. Fremont.** Biological effects of resveratrol. Life Sciences. 2000; Volume 66, Issue 8: 663-73
17. **Dong-Im Cho, Na-Youn Koo, Woon Jae Chung, Tae-Sung Kim, Shi Yong Ryu, Suhn Young Im, Kyeong-Man Kim** .Effects of resveratrol-related hydroxystilbenes on the nitric oxide production in macrophage cells: structural requirements and mechanism of action.Life Sciences. 2002;Volume 71, Issue 17: 2071-82.
18. **Alessandro Sgambato, Raffaele Ardito, Beatrice Faraglia, Alma Boninsegna, Federica I. Wolf, Achille Cittadini**. Resveratrol, a natural phenolic compound, inhibits cell proliferation and prevents oxidative DNA damage. Mutation Research/Genetic Toxicology and Environmental Mutagenesis.2001; Volume 496, Issues 1–2: 171-80.
19. **Reinert T, Baldotto CS, Nunes FA, Scheliga AA.** Bleomycin- induced lung injury. Journal of Cancer Research. 2013;2013(1):480608.
20. **Göksel Şener , Nurhayat Topaloğlu , A. Özer Şehirli , Feriha Ercan , Nursal G edik**. Resveratrol alleviates bleomycin-induced lung injury in rats. Pulmonary Pharmacology & Therapeutics. 2007; 20 (6): 642-9.
21. **Han F, Subramanian S, Dick TE, Dreshaj IA, Strohl KP.** Ventilatory behavior after hypoxia in C57BL/6J and A/J mice. *J Appl Physiol.* 2001. 91:1962-970.
22. **N. Limjunyawong, W. Mitzner, M.R.** Horton, A mouse model of chronic idiopathic pulmonary fibrosis. Physiol. Rep. 2 (2014) e00249.
23. **Quindry, J. C., Ballmann, G. C., Epstein, E. E. and Selsby, J. T**. Plethysmography Measurements of Respiratory Function in Conscious Unrestrained Mice. *J Physiol Sci* 2016*;* 66(2): 157-64.
24. **Receno, C. N., Eassa, B. E., Cunningham, C. M**. and DeRuisseau, L. R. Young and middle-aged mouse breathing behavior during the light and dark cycles. *Physiol Rep* 2019;7(8): e14060.
25. **Thomas Glaab, Armin** Braun. Noninvasive Measurement of Pulmonary Function in Experimental Mouse Models of Airway Disease Lung 2021; 199:255–61

26. T. Ashcroft, J.M. Simpson, VJJocp Timbrell, in: Simple Method of Estimating Severity of Pulmonary Fibrosis on a Numerical Scale 41(4), 1988, pp. 467–470, https://doi.org/10.1136/jcp.41.4.467.

27. A. Moeller, K. Ask, D. Warburton, J.Gauldie, M. Kolb. The bleomycin animal model: A useful tool to investigate treatment options for idiopathic pulmonary fibrosis? Int. J. Biochem. Cell Biol. 2008; 40: pp. 362-82.

28. Tyler VE. Grape expectations compoud in grapes may fight cancer includes related articles on grapeseed extract and on Echinacea. J Pathol. 1997; 49(6):80-4.

29. Cao Z, Li Y. Potent induction of cellular antioxidants and phase 2 enzymes by resveratrol in cardiomyocytes: protection against oxidative and electrophilic injury. Eur J Pharmacol. 2004; 489:39–48.

30. Gocmez SS, Gacar N, Utkan T, Gacar G, Scarpace PJ, Tumer N. Protective effects of resveratrol on aging-induced cognitive impairment in rats. Neurobiol Learn Mem. 2016; 131:131–36.

31. Cao Z, Li Y. Potent induction of cellular antioxidants and phase 2 enzymes by resveratrol in cardiomyocytes: protection against oxidative and electrophilic injury. Eur J Pharmacol. 2004; 489:39–48.

32. Sener G, Topaloğlu N, Sehirli AO, Ercan F, Gedik N. Resveratrol alleviates bleomycin-induced lung injury in rats. Pulm Pharmacol Ther. 2007; 20:642–49.

33. Fu Y, Yan M, Xie C, Hu J, Zeng X, Hu Q. Polydatin relieves paraquat-induced human MRC-5 fibroblast injury through inhibiting the activation of the NLRP3 inflammasome. Ann Transl Med. 2020; 8:765.