ALTERATIONS IN RESPIRATORY MECHANICS AND GAS EXCHANGE IN MODELS OF TREATED AND UNTREATED TYPE 2 DIABETES

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I. INTRODUCTION

Long-term hyperglycaemia in T2DM leads to chronic inflammation by activating various cellular and molecular pathways, including the promotion of advanced glycation end products (AGEs) formation and oxidative stress, disturbances of the nitric oxide – endothelin-1 balance, an abnormal plasma lipid profile, cytokine recruitment, and activation of nuclear factor-κB, in addition to proinflammatory gene expression. Consequently, several organs are affected by T2DM because of endothelial and smooth muscle cell dysfunction and extracellular matrix (ECM) remodelling. The whole circulatory minute volume is received by the lungs as a single organ, so their microvascular area is large, and they contain high numbers of endothelial and smooth muscle cells. Therefore, metabolic diseases have respiratory consequences.

I.1. Skeletal muscle function in T2DM

Skeletal muscle plays an important role in glucose homeostasis, and insulin resistance of the skeletal muscle cells is the foremost cause of T2DM development. Therefore, diabetes also leads to functional and structural changes in skeletal muscle, due to elevated plasma levels of dicarbonyl metabolites leading to ECM remodelling and muscle loss by prolonged activation of receptor for AGEs. The primary cause of compromised respiratory function in long term hyperglycaemia has not been elucidated, and there is a particular lack of knowledge regarding the contribution of the lung and chest wall compartments to the overall changes in the respiratory system.

I.2. Metformin therapy

Metformin is the first-line therapeutic agent for suppressing the T2DM-related pathologies mentioned above. The main mechanism to decrease blood glucose level is due to directly affecting hepatic glucose production, as opposed to augmenting insulin secretion or increasing glucose disposal. As secondary effects, metformin increases insulin-stimulated glucose uptake and alters intestinal glucose absorption due to changes in gut microbiome composition and hormone secretion. Previous studies reported the preventing effects of metformin against the development of systemic T2DM complications. Furthermore, the control of hyperglycaemia in the presence of T2DM, in patients receiving metformin improves survival following respiratory infections and inhibits the progression of lung fibrosis. However, the mechanism of metformin treatment on the respiratory consequences of diabetes have not been explored.

I.3. Ventilator-induced lung injury

Ventilator-induced lung injury (VILI) commonly may occur due four major mechanisms. Three of them induced by physical forces such as barotrauma, volutrauma and atelectrauma and lastly

the predominantly consequent biotrauma. Ventilator-induced alveolar inflammation induces various structural and functional alterations such as epithelial-mesenchymal transformation, surfactant dysfunction, fibroproliferation, increased alveolar-capillary permeability, pulmonary oedema, overproduction of hyaline membrane and sloughing of bronchial epithelium. The consequent structural and functional changes result in increased physiological dead space, decreased compliance and deteriorated gas exchange.

II. AIMS AND HYPOTHESISES

II.1. Effect of T2DM on the PEEP-dependent respiratory mechanics

Our aim was to characterize the effects of elevated blood glucose levels on the mechanics of chest wall muscles and the lung separately at different PEEP levels. Furthermore, we investigated the related gas exchange alterations, and finally we aimed at confirming the respiratory mechanical changes with histological analysis.

I. Due to the structural changes in diabetes, the end-expiratory lung volume of the animals in T2DM group may be lower than in the control animals.
II. Since the skeletal muscles are affected by T2DM, their viscoelastic and mechanical properties may be compromised compared to those in the control animals.
III. Metformin may protect the lung from adverse structural and functional changes subsequent to T2DM, thereby the respiratory mechanical and gas exchange parameters may be preserved as measured in the control animals.
IV. The structural and functional changes in the respiratory system during diabetes can be confirmed by overexpression of collagen on lung histological samples.

II.2. Modulation of VILI in models of treated and untreated T2DM

We aimed at revealing whether T2DM modulates the development of adverse respiratory symptoms of VILI by characterizing the changes in the respiratory mechanics and gas exchange parameters. Since T2DM is treated with metformin as the first-line therapy, we also aimed at exploring the potential ability of this treatment to modify VILI in T2DM with controlled hyperglycaemia.

I. We hypothesize that following 4 hours of injurious ventilation animals with untreated T2DM will show more deteriorated gas-exchange and worse respiratory mechanics.
II. Development of VILI may be protected by metformin therapy in the presence of T2DM.
III. The lung injury induced by prolonged mechanical ventilation may be more severe in animals with T2DM than those without metabolic disorder or in the metformin-treated diabetic animals.

III. MATERIALS AND METHODS

III.1. Ethical considerations

Both experimental protocol were part of a research project approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary (no. XXXII./150/2020) on March 18, 2020.

III.2. Pretreatments

Both experimental protocols included in the present thesis were performed using male Wistar rats randomly assigned, at the age of 4 weeks, to one of the following three groups: untreated T2DM model (T2DM), metformin-treated T2DM model (MET), and a control group (CTRL). A well-validated T2DM model was adapted to induce diabetes in the T2DM and MET groups, which was based on feeding the animals with high-fat diet from the age of 5 weeks. Rats in the CTRL group received a normal diet before the experiments. At the age of 7 weeks, rats in the MET and T2DM groups were treated with a single low-dose intraperitoneal injection of streptozotocin (STZ, 30 mg/kg) to reduce insulin production by the pancreas, whereas rats in the CTRL group received the vehicle. After 4 weeks, 300 mg/kg/day metformin in drinking water was administered to rats in the MET group. All rats were housed for 15 weeks before the experiments.

III.3. Group allocations and exclusions

Investigating the effect of T2DM on the PEEP-dependent respiratory mechanics, animals were allocated randomly to the study groups as follows: T2DM group (n=7), MET group (n=6) and CTRL group (n=7).

To describe the modulation of VILI in models of treated and untreated T2DM, 24 animals (CTRL, n=8; MET, n=8; T2DM, n=8) were included in the final analysis.

III.4. Blood gas measurements and intrapulmonary shunt fraction calculation

In both experiments, arterial and venous blood samples were collected simultaneously for blood gas analysis. The arterial partial pressure of oxygen (PaO_2) and arterial oxygen saturation (SaO_2) were determined using a point-of-care blood analyser system. The capillary (CcO_2), arterial (CaO_2), and venous (CvO_2) oxygen contents were determined from the blood gas values and used to calculate the intrapulmonary shunt fraction (Qs/Qt) by applying the following modified Berggren equation:

$$\frac{\boldsymbol{Q_s}}{\boldsymbol{Q_t}} = \frac{\boldsymbol{CcO_2 - CaO_2}}{\boldsymbol{CcO_2 - CvO_2}}$$

III. 5. Measurement of respiratory mechanics

The forced oscillatory input impedance of the total respiratory system (Z_{rs}) was measured using a previously described wave tube technique. Briefly, the tracheal cannula was connected to a loudspeaker-in-box system, which generated a small-amplitude pseudorandom forcing signal. During the measurement, the ventilation was suspended for a short period (8 s) at end-expiration, and the pressure oscillations were led through a wave tube. Pressure was measured simultaneously at the loudspeaker and tracheal ends of the wave tube using miniature differential pressure transducers. Z_{rs} was calculated as the load impedance of the wave tube.

III.5.1. Separation of chest wall and lung mechanics

To characterize the chest wall and lung mechanical properties separately, the oesophageal pressure (P_{es}) as a surrogate of intrapleural pressure was measured by introducing a miniature catheter-tip pressure manometer in the lower one-third of the. The catheter tip position was verified using the modified occlusion test for subjects under muscle relaxation. The mechanical impedance of the chest wall (Z_{cw}) was determined by calculating the transfer function of P_{tr} and P_{es} as $Z_{cw} = Z_{rs}(P_{es}/P_{tr})$, as described previously. For the different frequency responses of the transducers, the pressure transfer function P_{es}/P_{tr} was compensated in the frequency domain. Lung input impedance (Z_L) was calculated as $Z_L = Z_{rs} – Z_{cw}$.

The mechanical properties of the lungs and the chest wall were characterized by fitting a well-validated constant-phase model to the averaged Z_L and Z_{cw} spectra by minimizing the relative difference between the measured and the modelled impedance data as follows:

$$Z = R_N + j\omega I + (G - jH)/\omega^{\alpha} ,$$

where $\alpha = (2/\pi)\arctan(H/G)$, j is the imaginary unit, and ω is the angular frequency. R_N reflects the frequency-independent airway resistance when the model was fitted to Z_L, whereas it represents Newtonian (i.e., frequency-independent) resistance when model fits were performed for Z_{cw}. The parameter I is related to the inertia of the intrapulmonary gas (for Z_L) or the chest wall tissue (for Z_{cw}). This parameter was negligible in the frequency range studied. The viscoelastic constant-phase tissue component of the model characterizes the damping (G) and elastic (H) properties of the lung and chest wall compartments. The tissue hysteresivity (η) characterizing the coupling between the dissipative and elastic forces within the lungs and chest wall tissues, respectively, was also calculated as $\eta = G/H$.

III.5.2. Model fitting to the total respiratory impedance spectra

Due to the high tidal volumes during the injurious ventilation, reliable estimation of intrapleural pressure was not feasible. In this protocol, therefore, the mechanical properties of the total respiratory system were characterized by fitting a well-validated constant-phase model to the

ensemble-averaged Z_{rs} spectra. The model comprises the frequency-independent airway resistance (R_{aw}) and airway inertance in series with a viscoelastic constant-phase tissue unit that incorporates tissue damping (G) and elastance (H). In this case, G_{rs} and H_{rs} represents the damping and elastic properties of the total respiratory system including the lungs and the chest wall.

III. 6. Lung section preparation and histological analysis

After completion of the experimental protocols, the lungs were fixed in 4% paraformaldehyde then 7-μm thick sections were cut from the subhilar region of each lung using a microtome.

III.6.1. Effect of T2DM on the PEEP-dependent respiratory mechanics

To quantify the collagen in the lungs, Masson's trichrome staining was performed. The collagen was segmented and quantified using the Trainable Weka Segmentation plugin in ImageJ software.

III.6.2. Modulation of VILI in models of treated and untreated T2DM

The lung injury was determined by observing the sections stained with haematoxylin and eosin under a light microscope. Lung injury score (LIS) was calculated using the following formula: LIS = [(alveolar haemorrhage points) + 2 × (alveolar infiltrate points) + 3 × (fibrin points) + (alveolar septal congestion points)]/total number of alveoli counted in the field. To evaluate oxidative DNA damage, sections were selected for the permanent immunocytochemical staining of 8-hydroxy-2′-deoxyguanosine (8-OHDG). Fields were evaluated by manual cell counting using ImageJ.

III. 7. Additional measurements

III.7.1. Thoracic gas volume

Rats were placed into a whole-body plethysmograph. Measurements were performed at PEEP of 0, 3, and 6 cmH_2O (PEEP 0, 3, and 6). The thoracic gas volume (TGV) was calculated from the simultaneously measured pressure signals by applying the Boyle–Mariotte law. To compensate for differences in body size among the animals, TGV was normalized to bodymass (nTGV = TGV/bodymass).

III.8. Experimental protocols

III.8.1. Effect of T2DM on the PEEP-dependent respiratory mechanics

Following the pretreatment period, the animals were anesthetized, tracheostomy was performed, and mechanical ventilation was initiated as detailed above. TGV was then measured at PEEP levels of 0, 3, and 6 cmH_2O using a whole-body plethysmograph. The intraoesophageal catheter was then introduced to measure P_{es}, and its position was verified using the modified occlusion test. The animals were then ventilated while maintaining PEEP of 3 cmH_2O, and a

hyperinflation manoeuvre was performed by closing the expiratory limb of the ventilator tubing until the next expiration to standardize the volume history. After 3 min, arterial and venous blood gas samples were taken simultaneously, and the first set of lung and chest wall mechanical impedance data was collected. Following a 5-min adaptation period, blood gas analyses and forced oscillatory data recordings were repeated under PEEP levels of 0 and 6 cmH_2O in random order. Electrocardiogram and systemic blood pressure were registered continuously during the whole protocol. At the end of the measurements, the animals were euthanized by an overdose of sodium pentobarbital (200 mg/kg), and the lungs were excised for histological analysis.

III.8.2. Modulation of VILI in models of treated and untreated T2DM

After the pretreatment period, the rats were anesthetized and ventilated in the volume control mode with physiological parameters (VT: 7 mL/kg, PEEP: 3 cmH2O and 55–60/min frequency) for 20 min. Airway opening pressure was monitored to evaluate the peak inspiratory pressure (PIP), and electrocardiogram and systemic blood pressure were also registered. Next, forced oscillatory measurements were performed to evaluate the respiratory mechanics, and then arterial and venous blood samples were collected for blood gas analyses. An injurious ventilation strategy was initiated to induce VILI by setting high VT (23 mL/kg) and low PEEP (0 cmH_2O), and it was maintained for 4 h. To avoid severe hypocapnia, the ventilation frequency was reduced to 25–30/min to maintain minute ventilation and the end-tidal CO_2 in the range of 25–30 mmHg. This ventilation has been demonstrated to induce VILI consistently by the simultaneous induction of barotrauma due to the high VT and atelectrauma as a consequence of low PEEP. Forced oscillatory measurement of the respiratory mechanics and blood gas analyses were conducted after a 15-min period that was allowed for the stabilization of respiratory and hemodynamic variables (0 h) and 2 and 4 h after the onset of injurious ventilation. After the completion of the measurement protocol, the animals were euthanized by an overdose of pentobarbital (200 mg/kg), and the lungs were removed for histological analysis, as detailed earlier.

IV. RESULTS

IV.1. Effect of T2DM on the PEEP-dependent respiratory mechanics

IV.1.1. Thoracic gas volumes

Whereas nTGV significantly increased with increasing PEEP ($p < 0.001$), no significant differences were observed among the study groups at any PEEP.

IV.1.2. Lung and chest wall mechanical parameters

Elevation of PEEP resulted in significant decreases in the pulmonary components of RN, G, and H (all $p < 0.05$), whereas mechanical parameters representing the chest wall mechanics were less affected by PEEP changes. The between-group differences in R_N, G, and H of the lungs were significantly larger at PEEP 0 in the T2DM group than in the other groups, whereas η was significantly smaller (all $p < 0.05$). These differences among the protocol groups were not detectable at higher PEEP levels. No significant difference was evidenced among the protocol groups in R_N, G, and H of the chest wall. Conversely, chest wall η was significantly lower in the T2DM group than in the CTRL group at PEEP 0 ($p < 0.05$).

IV.1.3. Gas exchange parameters

Qs/Qt was significantly higher and SaO_2 was significantly lower in the T2DM group than in the MET and CTRL groups at PEEP 0 and 6 (all $p < 0.001$). At PEEP 0, a significant decrease of PaO_2 was observed in the T2DM group compared to those in the CTRL and MET groups ($p < 0.001$ and $p < 0.05$, respectively), whereas these differences were not statistically detectable at higher PEEP levels.

IV.1.4. Haemodynamics

While significant decrease in the mean arterial pressure (MAP) was observed in Group CTRL when PEEP was elevated from 0 to 6 cmH_2O (-124 ± 155%), rats in the Groups T2DM or MET exhibited no significant change in MAP with increasing PEEP (-2.8 ± 10.6% and -26 ± 32%, respectively).

IV.1.5. Lung histological findings

While the mean linear intercept did not show any difference between the protocol groups, the percentage area of collagen deposition in the lung parenchyma was significantly higher in the MET and T2DM groups than in the CTRL group (both $p < 0.001$). Furthermore, significant overexpression of collagen was also apparent in the T2DM group compared to its expression in the MET group ($p < 0.001$).

IV.2. Modulation of VILI in models of treated and untreated T2DM

VI.2.1. Gas exchange parameters

Although PaO_2 decreased in the CTRL group after 4 h of injurious ventilation ($p < 0.05$), this decline was more significantly manifested in the T2DM group ($p < 0.001$). Rats in the MET and CTRL groups demonstrated only tendencies for worsening in SaO_2 and Qs/Qt, whereas rats in the T2DM group exhibited significantly compromised gas exchange due to injurious ventilation ($p < 0.05$). When the alterations in gas exchange indices were expressed as relative changes compared with the onset of injurious ventilation (0 h), significantly greater

deterioration was observed in PaO_2 and SaO_2 in the T2DM group than in the other two groups ($p < 0.05$).

IV.2.2. Respiratory mechanical alterations

Significantly higher PIP was recorded in the T2DM group than in the other study groups throughout the study period ($p < 0.05$), and the rats in the T2DM group exhibited significant increases in PIP after 4 h of injurious ventilation ($p < 0.05$). Rats in the T2DM group also showed higher Raw values than those in the CTRL group at the onset of injurious ventilation (0 h, $p = 0.013$). This difference was also observed after 4 h of injurious ventilation ($p = 0.012$), with significance also being detected in comparison with the MET group ($p < 0.05$). Respiratory tissue mechanical parameters exhibited no significant difference between the study groups at any time point of measurement.

IV.2.3. Lung injury score – results of histological analysis

LIS was significantly higher in the T2DM group than in the CTRL and MET groups ($p < 0.001$). The grades of intra-alveolar fibrin were significantly higher in the T2DM than in the CTRL group ($p = 0.018$), and the grades of intra-alveolar infiltrates were significantly higher in the T2DM group than in the MET group ($p = 0.009$), the alveolar septal congestion and alveolar hemorrhage were significantly more intense in the T2DM group than in both CTRL and MET groups ($p < 0.001$).

IV.2.4. Number of 8-OHDG positive cells – results of immunohistochemistry

Rats in the MET group exhibited significantly higher number of positive-stained nuclei than those in the CTRL group ($p = 0.037$), whereas the number of positive-stained nuclei in the lungs of T2DM rats was significantly greater than that in both CTRL and MET rats ($p < 0.001$).

V. DISCUSSION

The chronic hyperglycemia in T2DM affects various organ systems and biological processes due to alterations in molecular mechanisms, cell, and tissue functions. The environmental and genetic factors, which are responsible for the development of T2DM often increase the risk of comorbidities such as obesity, hypertension, atherosclerosis *etc.* These etiopathologies also lead to a systemic inflammation which potentially affects the respiratory system. Although, several studies attempted to investigate the respiratory consequences of T2DM, the exact mechanisms and the response of the diabetic lung to various ventilation settings are unclear. Furthermore, the studies about T2DM regularly use pure diabetes models, without treatment factors such as metformin therapy, however, our diabetic patients typically live with controlled hyperglycemia due to certain antidiabetic medications. The respiratory alterations of treated and untreated

T2DM model were investigated during the application of different ventilation scenarios. Using different PEEPs and an injurious ventilation protocol, we studied the alterations of respiratory mechanics and gas exchange in well-established rodent models of untreated and metformin-treated T2DM.

V.1. Effect of T2DM on the PEEP-dependent respiratory mechanics

Separate assessment of the mechanical properties of the lungs and chest wall in an experimental model of type 2 diabetes revealed the involvement of both compartments in the detrimental changes in global respiratory mechanics. At low PEEP, diabetes elevated mechanical parameters reflecting airflow and lung tissue resistance and stiffness of the pulmonary parenchyma. Increasing lung volume alleviated these pulmonary mechanical abnormalities. Regarding the effect of sustained hyperglycaemia on the chest wall, only tendencies of changes in the dissipative or elastic properties of chest tissues were observed. However, η reflecting the coupling of these mechanical properties was disturbed at low PEEP with a shift toward the dominance of elastic forces. Although metformin-treated diabetic animals displayed no signs of abnormal lung or chest wall mechanics, gas exchange defects or the remodelling of the extracellular fibre network was observed in the lung connective tissue in histopathological examinations.

To induce T2DM, a well-established model was created by administering a single low dose of STZ combined with high-fat diet feeding. The beta cell-damaging agent STZ induces necrosis followed by atrophy of the Langerhans islets. According to the diagnostic criteria, rats in both the T2DM and MET groups exhibited definitive blood glucose parameters characteristic of diabetes during the first IPGTT, performed before the initiation of metformin therapy. The mode of administration and the daily dose of metformin were adapted to reflect the clinical scenario under which some patients with diabetes are treated. Based on this therapeutic approach, control of hyperglycaemia was observed in the MET group during the second IPGTT, whereas hyperglycaemia was maintained in the T2DM group. Animals in the MET group exhibited lower weight than those in the other two groups, in accordance with the clinical scenario. Metformin-associated weight loss is attributable to modulation of the hypothalamic appetite-regulatory centres and alteration of the gut microbiome, further confirming the effective administration of this medication at clinically relevant concentrations. The lack of difference between the body weight of CTRL and T2DM animals is probably due to the depletion of insulin secretion by STZ, and the shifted muscle/fat ratio in the diabetic animals.

V.1.1. Respiratory mechanical consequences

Measurement of esophageal pressure using a miniature catheter-tip pressure transducer allows the separate assessment of lung and chest wall mechanics. As diabetes and metformin therapy have minor effects on chest wall mechanics regardless of the PEEP, these well-validated assessments revealed the primary contribution of the abnormal pulmonary mechanics to the pathologic changes in the respiratory system following sustained hyperglycemia. These respiratory mechanical alterations confirmed previous findings of reduced airway patency and compromised lung tissue viscoelasticity at low PEEP, whereas increased PEEP can prevent deleterious changes in the lungs, thereby overcoming the detrimental lung mechanical consequences of diabetes. The nTGV values in the T2DMgroup tended to be higher compared with the other protocol groups, which can be explained by small airway obstruction and gas trapping. However, the lack of significant differences in nTGV among the protocol groups indicates that the altered lung tissue mechanics in diabetes is of intrinsic origin, as opposed to resulting from a shift in static lung volumes. The primary involvement of intrinsic lung tissue remodeling in diabetes was further confirmed by the histological evidence of collagen overexpression in the pulmonary parenchyma. Pathological arrangement and cross-bridging of elastin-collagen fibers resulting from glycation determine the overall lung tissue viscoelasticity rather than their amounts. T2DM may affect not only the amount but also the structural rearrangement of the elastic-collagen network subsequent to lung inflation, which may explain the apparent discrepancy between the PEEP-dependent mechanical and histological findings.

Regarding the alterations of the chest wall properties in diabetes, the significantly reduced η at low PEEP suggested the development of a shift toward elasticity at the expense of energy dissipation in the tissues forming the chest wall. The pathophysiological background of these findings is not completely clear. However, the trends of changes of G and H in diabetes indicate a somewhat stiffened fiber network of ECM associated with reduced internal friction within chest wall tissues. The trend of increased chest wall elastance is in line with previous results demonstrating increased passive stiffness of single skeletal muscle fibers in older adults with T2DM. The reduced sensitivity of our measurements to detect alterations in skeletal muscle mechanics can be explained by the involvement of the cage bones and costal cartilage in our in vivo mechanical parameters. Due to the lack of an in vivo method to determine changes in the mechanical properties of these anatomical structures, the diabetes-related alterations of skeletal muscle mechanics were blunted.

V.1.2. Effects of metformin therapy

Another main finding of the present study was the ability of metformin to reduce the respiratory manifestations of T2DM. Metformin monotherapy lowers blood sugar by enhancing the

insulin-mediated suppression of gluconeogenesis and increasing insulin-mediated glucose uptake in skeletal muscle. Furthermore, the pleiotropic effects of metformin may contribute to its ability to reduce inflammation, oxidative stress, endothelial remodeling, and proliferation of pulmonary vascular smooth muscle cells. Phosphorylation of AMP-activated protein kinase influences the intracellular energy balance via lipid and glucose metabolism and inhibits transforming growth factor-β–induced collagen production by fibroblasts. All these mechanisms may have contributed to the reduced collagen accumulation in lung tissue and the normalized lung and chest wall mechanics in rats in the MET group. In accordance with previous results, T2DM increased intrapulmonary shunt and compromised lung oxygenation at low and high PEEP. The elevated Qs/Qt and compromised PaO_2 at 0 cmH_2O PEEP can be explained by the small airway obstruction and atelectasis, and these adverse phenomena were reversed to some extent by elevating PEEP to 3 cmH_2O. Further elevating PEEP with the lack of hemodynamic response for the elevated intrathoracic pressure caused the additional compression of the pulmonary capillaries in the well-ventilated alveolar regions, thereby redirecting the perfusion to lung regions with somewhat poorer aeration. The gas-exchange outcomes in the MET group did not differ from those in the CTRL group. This finding demonstrates that the beneficial effects of metformin on respiratory mechanics were also manifested in the potential of this treatment to maintain physiological gas exchange.

V.2. Modulation of VILI in models of treated and untreated T2DM

This study has revealed the potential of T2DM to worsen respiratory functions and lung injury following a biotrauma of prolonged mechanical ventilation according to decline in parameters reflecting gas exchange. Lung histological and immunochemical parameters also showed that diabetes enhanced VILI and oxidative DNA damage. Metformin treatment prevented the detrimental pulmonary consequences of long-term mechanical ventilation in the presence of diabetes.

In this study, a well-established model was adapted to induce T2DM by administering a single low dose of STZ to induce diffuse degeneration of pancreatic cells to imitate beta cell insufficiency combined with high-fat diet. The fasting glucose levels of the STZ-treated rats were consistently above the threshold of 7.0 mmol/L and their blood glucose levels were significantly higher compared to the control animals. Furthermore, the 120-min blood glucose values were above the threshold of 11.1 mmol/L in all STZ-treated animals, while none of the control animals had abnormal blood glucose values. Consequently, according to the diagnostic criteria, rats in both STZ-treated groups (T2DM and MET groups) exhibited definitive blood glucose abnormalities characteristic to diabetes one week after the STZ treatment, before the

initiation of metformin therapy. At the end of the pretreatment period, during the second tolerance test, the fasting and 120-min blood glucose values of the T2DM group exceeded both thresholds. The MET group showed a more controlled hyperglycaemia during the second test, while the blood glucose curves of the CTRL animals were identical during both tolerance tests.

V.2.1. VILI in the presence of T2DM

The translational animal model used in the present study aimed at mimicking the development of mild–moderate VILI in the absence of a pre-existing pulmonary disorder. Accordingly, we applied no intervention to induce surfactant deficiency or proinflammatory treatments before initiating the prolonged mechanical ventilation. The alveolar overdistension and enhanced lung parenchymal shear stress as key features of VILI were generated by a combined application of high VT and low PEEP to facilitate the development of barotrauma, volutrauma, and atelectrauma in the rats of all experimental groups. Irrespective of the presence of T2DM, these mechanical stresses were manifested in deterioration in gas exchange and in lung injury and oxidative DNA damage. Interestingly, although a decline in the mechanical properties of the respiratory system due to the injurious ventilation was indicated by significant elevations in PIP in the T2DM group, the changes in the forced oscillatory parameters did not reach significance during the study period. This result can be explained by the application of excessively high VTs during the injurious ventilation (more than three times the normal) that assured maximal alveolar recruitment during the study period. Moreover, the involvement of a significantly unaffected chest wall component in G and H may have blunted the sensitivity of these mechanical outcomes to detect mild–moderate lung injury. Conversely, markedly greater LIS and overexpression of 8-OHGD-positive cells in the lung tissue were observed in CTRL animals than those obtained in previous experiments in naïve rats without injurious ventilation (0.15 ± 0.12 vs. 0.49 ± 0.18 and 3.0 ± 1.0 vs. 7.0 ± 2.9, $p < 0.05$ for both). These findings confirm the development of the structural and functional pathologies characteristic of VILI even in the control animals without metabolic disorder.

The most remarkable finding of the present study is the exaggeration of VILI in rats with untreated T2DM. The more severe detrimental consequences of untreated T2DM were evidenced by the greater magnitude of deteriorations in the gas exchange ability of the lungs and parameters reflecting Qs/Qt. As the respiratory mechanical parameters did not exhibit an excessive change in rats with diabetes, atelectatic lung volume loss was not likely to play a major role in the excessively compromised gas exchange. Alternatively, the exaggerated impairment of gas exchange in the diabetic animals can be attributed to the intrinsic lung tissue remodelling and inflammation with an impaired alveolar–capillary barrier, all leading to a

reduced diffusion of gas molecules through the alveolar membrane. The involvement of this mechanism is confirmed by the histological findings evidencing alveolar septal congestion and haemorrhage associated with intra-alveolar deposition of fibrin and infiltrates. In accordance with our findings, previous studies have also demonstrated direct tissue damage in experimental models of VILI due to inflammation and oxidative damage of cellular components. These include oxidation of tissue lipid and protein components, increased levels of IL-1β, IL-6, IL-8, and TNF-α. These molecular processes enhance the proinflammatory effects of prolonged hyperglycaemia, resulting in more severe lung injury.

V.2.2. Effects of metformin therapy

A further noteworthy finding of this study is the ability of metformin to prevent the worsening of VILI subsequent to T2DM. The results of the 2nd IPGTT performed after metformin treatments demonstrated the effectiveness of this therapy in leading to controlled hyperglycaemia in the MET group through the following well-established mechanisms of action: inhibiting hepatic gluconeogenesis and reducing hepatic glucose output; increasing glucose uptake and utilization in peripheral tissues (muscle and fat); and improving energy metabolism in the muscle, fat, and liver through the activation of AMP-activated protein kinase. It has been described that the AMP-activated protein kinase down-regulated inflammatory pathways such as the NF-κB pathway, which might contribute to the beneficial respiratory effects of metformin. Consistent with previous results, metformin therapy had no significant effects on the baseline lung functional or structural parameters. However, the effects of this first-line antidiabetic therapy on the lungs were clearly manifested in the potential to prevent the T2DM-induced excessive worsening in gas exchange, the aggravation of lung injury and the oxidative DNA damage. These findings suggest the ability of metformin therapy to not only reduce hyperglycaemia and impaired glucose tolerance but also abolish the adverse pulmonary consequences of T2DM. Our results correspond to previous experimental and clinical findings demonstrating that adequate diabetes therapy prevents the development of lung injury as a complication of mechanical ventilation, although excessive hyperglycaemia results in elevated expression of pro-inflammatory cytokines leading to severe lung injury.

VI. SUMMARY AND CONCLUSIONS

The investigations detailed in the present thesis reveal novel aspects of the respiratory consequences of T2DM, with particular focus on the PEEP-dependence of lung and chest wall mechanics and on the potential of T2DM to modulate lung injury following a prolonged mechanical ventilation.

As a summary of the results on how treated or untreated T2DM affect the PEEP-dependent respiratory mechanics, our findings have led to the following conclusions:

I. Separate measurements of the lung and chest wall mechanical properties demonstrated the primary involvement of the pulmonary system in the global deterioration of total respiratory system mechanics following the development of diabetes.

II. Sustained hyperglycaemia compromised airway patency, increased lung parenchymal stiffness and energy loss along with increased atelectasis development at low PEEP.

III. These adverse pulmonary changes were associated with the overexpression of extracellular collagen fibres in lung tissue.

IV. Gas exchange was compromised due to the increased risk of atelectasis development at low PEEP levels and due to the remodelling of the alveolocapillary barrier and the augmented compressibility of intraalveolar capillaries at high PEEP.

V. The mild mechanical consequence of chronic hyperglycaemia on the viscoelastic properties of chest wall tissues was only manifested as a decrease in tissue damping at a low PEEP level, demonstrating a mechanical shift toward the dominance of elastic stresses over internal frictional forces.

The results of a further study assessing how models of treated and untreated T2DM modulate lung injury after a prolonged mechanical ventilation revealed the following main findings:

VI. Prolonged mechanical ventilation of diabetic lungs aggravates the functional and structural manifestations of mild–moderate VILI.

VII. Exaggerated lung injury in a model of T2DM results in more severe remodelling of the alveolar–capillary barrier, and this is the primary cause of the declined gas exchange following prolonged injurious mechanical ventilation.

VIII. Although major deterioration in gas exchange and respiratory mechanics has not been observed after injurious ventilation, the enhanced inflammation and the tissue damage in a model of T2DM may be warning signs of a more severe long-term consequences in the respiratory system.

Finally, the effects of a standard diabetes therapy with metformin were assessed in the respiratory consequences of T2DM in both studies included in the present thesis, with results demonstrating:

IX. Early and adequate metformin therapy effectively treats the adverse respiratory consequences of diabetes, in addition to its well-established beneficial systemic effects.

X. Although metformin therapy has no direct effect on lung function or gas exchange, controlled hyperglycaemia or euglycemia lowers the risk of developing ALI and, in severe cases, the development of ARDS subsequent to long-term mechanical ventilation.

XI. Considering these findings together further emphasize the importance of the early diagnosis and therapy in diabetes.

In conclusion, these findings have particular relevance for both improving our understanding of breathing difficulties in diabetes and optimizing anaesthesia management and intensive care requiring mechanical ventilation in this patient population, which is susceptible to respiratory complications.

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LIST OF SCIENTIFIC PUBLICATIONS INCLUDED IN THE PRESENT THESIS

I. **Lung and chest wall mechanical properties in metformin-treated and untreated models of type 2 diabetes**
   Álmos Schranc, Gergely H. Fodor, Roberta Südy, Bence Ballók, Richard Kulcsár, József Tolnai, Barna Babik, Ferenc Peták
   Journal of Applied Physiology, 2022; 132(5):1115-1124

II. **Exaggerated ventilator-induced lung injury in an animal model of type 2 diabetes mellitus: a randomized experimental study**
   Álmos Schranc, Gergely H. Fodor, Roberta Südy, József Tolnai, Barna Babik, Ferenc Peták
   Frontiers in Physiology, 2022; 13:889032

LIST OF SCIENTIFIC PUBLICATIONS RELATED TO THE SUBJECT OF THE PRESENT THESIS:

III. **Lung volume dependence of respiratory function in rodent models of diabetes mellitus**
   Roberta Südy, Álmos Schranc, Gergely H. Fodor, József Tolnai, Barna Babik, Ferenc Peták
   Respiratory Research, 2020; 21(1):82