NOVEL ASPECTS OF DIABETIC COMPLICATIONS: ALTERATIONS IN THE RESPIRATORY FUNCTION AND CEREBRAL INTEGRITY

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INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous disease characterised by hyperglycaemia, leading to micro and macrovascular complication. DM results in diabetic retinopathy, nephropathy and neuropathy, atherosclerosis and increases the risk of cardiovascular diseases such as coronary heart disease and myocardial infarction. Moreover, the long-term hyperglycaemia and diabetes might alter the cerebral and respiratory function as well. This multiorgan impairment is a result of a complex molecular and cell dysfunction.

The persistent hyperglycaemia leads to intracellular oxidative and osmotic stress and overproduction of reactive oxygen species (ROS) and enhanced advanced glycation end products (AGEs) formation along with several activated pathophysiological signalling pathways.

The development of the AGEs leads to intracellular IC and extracellular (EC) protein damage and dysfunction. The hyperglycaemia induced protein kinase C (PKC) activation changes gene expression. The increased transforming growth factor-β (TGF-β) level results in capillary occlusion due to the higher amount of collagen and fibronectin in the vasculature. In addition, the balance between the vasodilatory and vasoconstrictor factors are interrupted; the endothelial nitric oxide synthase (eNOS) production is low, concurrently the production of endothelin-1 (ET-1) is increased. Furthermore, vascular permeability and angiogenesis are augmented due to the increased vascular endothelial grow factor (VEGF) level in smooth muscle cells.

Respiratory complication

The pathophysiological consequences of diabetes may also damage the respiratory system as well. However, the reported lung function results are conflicting, and the mechanisms has not been completely characterised yet. The long-term hyperglycaemia induces changes in the pulmonary vascular smooth muscle cells, resulting in pulmonary hypertension and pulmonary vascular damage, leading to decreased pulmonary blood flow and pulmonary capillary blood volume. Moreover, the bronchial smooth muscle cells and pulmonary and low-grade inflammation might play an important role in respiratory damage, and lung function deterioration. The endothelial and epithelial dysfunction, ROS overproduction contributes to the remodelling of the airways and the lung parenchyma and thickening the alveolar wall. The altered pathways can manifest as sustained bronchial smooth muscle cell contraction. Since elastin and collagen fibres are abundant in the lung, the ECM is a target for diabetes as well. Thus, alterations in the elastin–collagen matrix in the lung parenchyma might occur.

However, the effect of diabetes on the lung is described previously diverging results have been reported on lung function. The effect of DM on airway function and the viscoelastic mechanics of

respiratory tissue remains unclear. It is unknown whether DM influences the changes in mechanical parameters that occur with changes in lung volume.

Cerebral complication

The brain has a high metabolic activity without reserved energy capacity and uses about 20% of the available oxygen. Therefore, it depends on constant energy supply to meet the needs of the demand. Constant blood flow is required to maintain the balance. In diabetes, cerebral autoregulation, neurovascular coupling might impair as a result of long-term hyperglycaemia and compromised insulin signalling. Increased cerebrovascular resistance and compromised flow occur due to the impaired dilatory mechanism and myogenic response. Diabetes leads to structural changes in the cerebrovasculature such as arteriogenesis and vascular remodelling. Phenotype-changing, hypertrophy and proliferation of the vSMCs along with collagen deposition, plays a key role in the cerebral diabetic complication. Diminished response to CO_2 and thickened intima layer were observed in diabetes resulting in diminished cerebrovascular reactivity. The reduced vasodilatory reserve capacity increases the risk for a reduced cerebral tissue oxygen supply in T2DM patients, which may be responsible for the higher incidence of postoperative adverse neurocognitive dysfunction and stroke in the diabetic population. Thus, it is particularly important to characterise the differences in the cerebral tissue ogine saturation ($CrSO_{2)}$ between the diabetic and non-diabetic patient population and follow the changes in $CrSO_2$ during the surgery. Moreover, In the perioperative period, the oxygen balance is routinely estimated from the oxygen saturation of central venous blood ($ScvO_2$). However, $ScvO_2$ reflects the overall oxygen balance substantial heterogeneity exists in oxygen extraction of various organs. Thus, central venous oxygen saturation is not able to reflect regional changes in the tissue oxygenation.

HYPOTHESIS AND AIMS

Effect of diabetes on respiratory function: experimental study

The effect of diabetes on lung function is not completely characterised yet. Therefore, our aims of the first study were to elucidate the effects of long-term hyperglycaemia in the respiratory system. Thus, we investigated the structural functional changes in the respiratory system in well-established animal models of type 1 (T1DM) and type 2 DM (T2DM).

I. Since the magnitude of the insults varies in the two groups with diabetes, we hypothesise differences in the respiratory outcomes between the type1 and type2 DM groups.
II. Deteriorations develop both in the dissipative and elastic components of respiratory tissue viscoelasticity.

III. The deteriorated airway function in diabetes results in changes in the airway responsiveness.

IV. The detrimental changes in respiratory function in T1DM and T2DM affect their changes with lung volume.

V. DM-induced functional and structural changes in the respiratory system are expected to result in loss of the lung volume, compromised gas exchange, and histological alteration. Thus, we investigated the functional residual capacity (FRC), fraction of arterial partial pressure of oxygen to the inspired fraction of oxygen (PaO_2/FiO_2), intrapulmonary shunt (Qs/Qt) and the amount of collagen in lung tissue samples.

Effects of diabetes on cerebral integrity: clinical study

Since the oxygen extraction rate of cerebral tissue is one of the highest in the body under physiological conditions, the mixed venous oxygen saturation ($ScvO_2$) might not be able to reflect regional changes in the tissue oxygenation. Diabetic complications could compromise the cerebral tissue oxygen balance, hence deduce the intraoperative brain tissue oxygenation is particularly challenged in diabetic patients. Therefore, online monitoring of regional cerebral tissue oxygen saturation ($CrSO_2$) would have an advantage in T2DM patients in the intraoperative period to manage local hypoxemic episodes.

I. We hypothesise that the initial $CrSO_2$ measured by NIRS differs between patients with and without diabetes.

II. We hypothesise that the gap between $ScvO_2$ and $CrSO_2$ (gSO_2) is significantly widened in T2DM patients as a cardiovascular consequence of diabetes. Accordingly, $ScvO_2$ is not suitable to infer $CrSO_2$ in diabetic patients. To test this hypothesis, we aimed at comparing direct measurements of $CrSO_2$ using near-infrared spectroscopy (NIRS) to simultaneously obtained $ScvO_2$ data in patients with and without T2DM.

III. $ScvO_2$, $CrSO_2$ and gSO_2 are expected to exhibit intraoperative changes during cardiac surgery depending on the patient management with or without cardiopulmonary bypass (CPB). Thus, measurements were made during anaesthesia in two groups of cardiac surgery patients with or and without T2DM: those undergoing CPB and those scheduled for off-pump coronary artery bypass (OPCAB) grafting procedure.

MATERIALS AND METHODS

Methods in the experimental study

Inducing diabetes

Five weeks old male Wistar rats were assigned randomly to three protocol groups: model of type 1 DM (DM1 group, n = 14), model of type 2 DM (DM2 group, n = 14), and control group (n = 14). The rats in the DM1 and control groups received a normal diet (fat and protein contents of 3.9% and 20.1%, respectively), whereas those allocated to the DM2 group were fed a high-fat diet (47% fat, 18% protein, 35% carbohydrate). After a 3-week period, the DM1 group rats were treated with a single high dose of streptozotocin (STZ, 65 mg/kg) the DM2 group rats were treated with a low dose STZ (30 mg/kg), and the controls received the vehicle of the STZ (citrate buffer, pH 4.4). Fasting glucose levels were measured from the tail vein. Four rats in the DM2 group were administered a second injection of 30 mg/kg STZ treatment because their fasting glucose level was <7.8 mmol/l one week after the STZ injection. One animal in the DM1 group was sacrificed after 7 weeks due to isolation and its unsatisfactory health condition. The study was approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary (no. XXXII./2098/2018), on September 24, 2018.

Experimental protocol

Following a 12-week housing period after the induction of DM (at the age of 20 weeks), each rat was anesthetised, and the trachea was secured. The FRC was measured, followed by the surgical insertion of the arterial and venous catheters. Animals were initially ventilated with a positive end-expiratory pressure (PEEP) of 3 cmH_2O. Arterial and venous blood gas samples were taken simultaneously and the set of Zrs data was collected at PEEP3. Measurements were repeated at PEEP0 and PEEP6, in random order. After completing the study on characterising the PEEP-dependence, the PEEP was fixed at 3 cmH_2O and a set of Zrs data was collected to establish the baseline for the bronchoprovocation tests with doubling doses (2, 4, 8, 16, and 32 μg/kg/min) of iv. methacholine (MCh). Anaesthesia was maintained with pentobarbital (5 mg/kg, intravenous (iv.), every 30 min). Neuromuscular blockade was achieved by repeated iv. administration of pipecuronium (0.1 mg/kg every 30 min). After, completing the measurement protocol, each animal was euthanised by an overdose of pentobarbital and the lungs were removed for histological analysis.

Measurement of functional residual capacity

The FRC was measured as described previously. Briefly, the rat was tracheostomised and placed in a whole-body plethysmography box. The trachea and box were closed at end-expiration and the

measurements were made while the animal generated breathing efforts against the closed trachea. The FRC was calculated from the simultaneously measured tracheal and box pressure signals by applying the Boyle–Mariotte law.

Measuring airway and respiratory tissue mechanics

The input impedance of the respiratory system (Zrs) was measured by the forced oscillation technique. The tracheal cannula was connected to a loudspeaker-in-box system, while ventilation was suspended at end-expiration. A small-amplitude pseudorandom signal was applied to the tracheal cannula through a wave tube. To maintain uniform transpulmonary pressure during the measurements, the pressure in the loudspeaker box was set to be equivalent to the actual PEEP (0, 3, or 6 cmH_2O). During 8-s measurement periods, pressures were measured simultaneously at the loudspeaker and tracheal ends of the wave tube with miniature differential pressure transducers. Zrs was calculated as the load impedance of the wave tube. The mechanical properties of the respiratory system were characterised by fitting a well-validated model to the averaged Zrs spectra. The model comprised frequency-independent airway resistance (Raw) and airway inertance in series with a viscoelastic constant-phase tissue unit, and incorporated tissue damping (G) elastance (H). Tissue histeresivity (η) was calculated as G/H.

Measurement of intrapulmonary shunt fraction and oxygenation

For the blood gas analyses, arterial and central venous blood samples were collected simultaneously. The capillary (CcO_2), arterial (CaO_2), and venous (CvO_2) oxygen contents were evaluated from the blood gases and used to calculate the intrapulmonary shunt fraction (Qs/Qt) by applying the modified Berggren equation. To characterise the oxygenation efficiency PaO_2/FiO_2 was calculated from the arterial blood gas assessments.

Lung tissue histology

After completing the experimental protocol, the lungs were fixed in 4% buffered formalin, embedded in paraffin, and 5-μm tissue sections were stained with Picro Sirius Red. The sections were scanned at a magnification of ×20. The collagen was segmented and quantified in representative regions of interest (0.15-mm^2) by using the Trainable Weka Segmentation plugin in Fiji software.

Methods in the clinical study

Patients

Hundred thirty-nine consecutive patients with T2DM (n=48) and control (C) subjects without T2DM (n=91) undergoing cardiac surgery were enrolled in this prospective descriptive cohort study. Patients were defined as having T2DM if their medical history included a diagnosis of T2DM and haemoglobin A1c (HbA1c) > 6.4%. All patients underwent either OPCAB (C-

OPCAB, n=31 and T2DM-OPCAB, n=24) or CPB (C-CPB, n=60, T2DM-CPB, n=24). Patients older than 80 years of age or with poor ejection fraction (<40%), unilateral internal carotid stenosis (>75%), or medical history of smoking, chronic obstructive pulmonary disease or stroke were excluded from the study. The protocol was approved by the Human Research Ethics Committee of Szeged University, Hungary (no. WHO 2788), and the patients gave their informed consent to participation in the study. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments between January and August 2017.

Anaesthesia

Before induction of anaesthesia, the NIRS sensors were positioned on both sides of the forehead. Sensors to detect depth of anaesthesia were also mounted on the forehead to monitor EMG and EEG activities. These signals were used to calculate response (RE) and state entropy (SE), respectively. Induction of anaesthesia was achieved by iv midazolam (30 μg/kg), sufentanil (0.4-0.5 μg/kg), and propofol (0.3–0.5 mg/kg), and iv propofol (50 μg/kg/min) was administered to maintain anaesthesia. Intravenous boluses of rocuronium (0.6 mg/kg for induction and 0.2 mg/kg every 30 minutes for maintenance) was administered iv to ensure neuromuscular blockade. After tracheal intubation patients were mechanically ventilated in volume-controlled mode with decelerating flow with a tidal volume of 7 ml/kg and a positive end-expiratory pressure of 4 cmH_2O and ventilation frequency was adjusted to 12–14 breaths/min. The fraction of inspired oxygen was maintained 0.5 during the entire OPCAB procedure and before CPB, and it was increased to 0.8 after CPB. As a standard part of the cardiac anaesthesia procedure, oesophageal and rectal temperature probes were introduced, and a central venous line was inserted into the right jugular vein. The left radial artery was also cannulated to monitor systolic, diastolic and mean arterial (MAP) blood pressures and arterial blood gas samples.

Measurement of cerebral-tissue oxygen saturation

The spatially resolved continuous-wave near-infrared spectroscopy (NIRS) technique was applied to estimate $CrSO_2$. Two adult sensors were placed on the left and right sides of the patient's forehead symmetrically, more than 3 cm from the superior rim of the orbit. The cerebral-tissue oxygen saturation was monitored continuously during the surgical procedures and the data were registered in each protocol stage. The mean value of the $CrSO_2$ measured by the sensors was calculated for each protocol stage and used for further analyses.

Measurement of central venous oxygen saturation

The $ScvO_2$ was measured from central venous blood samples. The partial pressures of oxygen (PaO_2) and carbon-dioxide ($PaCO_2$), haemoglobin, pH and oxygen content (CaO_2) were determined from arterial blood gas samples at each protocol stage.

Measurement protocol

After securing arterial and peripheral venous lines and placement of NIRS and entropy sensors, data collection was initiated immediately before anaesthesia induction in all groups of patients. Since catheterization of the jugular vein was scheduled after anaesthesia induction, $ScvO_2$ and gSO_2 data were not available at the first protocol stage. After anaesthesia induction and muscle relaxation, all measurements were repeated before surgical incision. For the patients undergoing CPB procedures, the whole data set was registered at the beginning of CPB after clamping the aorta and 5 min before the end of CPB. For the patients undergoing OPCAB procedures, collection of the full set of data was performed during performance of the first proximal anastomosis between the aorta and saphenous vein graft. The final stage of the protocol was allocated to the end of the operation after sternal closure. All invasive (i.e. arterial and venous blood gas) and non-invasive data were registered simultaneously at each protocol stage after ensuring a 3 min stead-state condition.

RESULTS

Effects of diabetes on the respiratory system: results in the experimental study

The mean body weight, of the rats in the DM1 group was significantly lower than those in the DM2 and control groups at the end of the 12-week treatment period. Significant elevations in blood glucose level were observed in the rats of the DM1 and DM2 groups compared to the control animals. The FRC measurements showed a significant reduction in static lung volume in the DM1 group rats; however, when the FRC values were normalised to body weight, they were significantly higher for the DM1 group rats than for those in the other two groups. Specific airway and respiratory tissue parameters were significantly higher in the DM1 and DM2 groups than in the control group.

Changes in the respiratory mechanical properties

The airway and viscoelastic parameters of the respiratory tissues varied with the PEEP for the three groups. Raw was significantly greater in both groups of diabetic animals than in the control group at all PEEP levels ($p<0.001$). The dissipative properties of the respiratory tissues reflected by G were significantly compromised in the DM1 group at PEEP0. The respiratory tissue stiffness was elevated in both the DM1 ($p<0.001$) and the DM2 ($p=0.039$) groups at all PEEP levels, with more pronounced differences at low lung volumes. The dissociated PEEP dependences of G and

H resulted in changes in η that varied between the groups, with significant decreases observed in the DM1 group for all three levels of PEEP ($p<0.001$) and in the DM2 group when the PEEP was 3 or 6 cmH_2O ($p=0.014$) compared to the control group.

Airway response to Metacholine

Significant elevations in the basal Raw values were observed in the DM1 ($p<0.001$) and DM2 ($p<0.05$) groups before MCh-provocation. These differences remained at the lower doses of MCh, whereas the absolute value of Raw became significantly lower in the DM1 rats at the highest dose of MCh ($p<0.05$). In the control and DM2 groups, elevations in Raw were statistically significant from the 8 μg/kg/min MCh dose ($p<0.05$), whereas in the DM1 group this increase was only observed after 16 μg/kg/min ($p<0.05$). At the highest MCh dose, the MCh-induced relative increases in Raw were significantly lower in both DM1 and DM2 groups compared to control ($p=0.001$). The characteristic shifts in the dose-response curves showed that the PD_{50} for MCh was significantly higher for the DM1 group than for the control group ($p=0.001$) and the DM2 group ($p=0.026$).

The effect of PEEP applying on the gas exchange parameters

Rats in the control group exhibited moderate values of intrapulmonary shunt (<9.3%) and physiological PaO_2/FiO_2 (>438 mmHg); these indices exhibited systematic improvements with increasing PEEP to 3 and 6 cmH_2O. In the DM2 group, there was a near-significant tendency for Qs/Qt to be reduced compared to the control group at a PEEP of 6 cmH_2O ($p=0.065$), whereas PaO_2/FiO_2 was reduced at a PEEP of 3 cmH_2O ($p=0.05$). In the DM1 group at all three PEEP levels, there were significant increases in the intrapulmonary shunt ($p<0.001$) associated with markedly compromised PaO_2/FiO_2 ($p<0.001$). In addition, changes in the PEEP dependence of the gas exchange parameters were observed in the DM1 group, with no monotonous improvements in Qs/Qt and PaO_2/FiO_2 with increasing PEEP.

Histological consequences

The percentage area of collagen was significantly higher in both DM groups than in the control group ($p<0.001$). The collagen content of the lung parenchyma was greater in the DM1 group than in the DM2 group ($p<0.001$). There were significant correlations between the collagen area and G and H, with correlation coefficients of 0.67 ($p<0.001$) and 0.63 ($p<0.001$), respectively.

Effects of diabetes on the cerebral integrity: results in the clinical study

Patient characteristics

HbA1c was significantly higher in diabetic patients while there was no significant difference in the other parameters (i.e. weight, height, age or ejection fraction).

Effects of T2DM on central venous and cerebral oxygen saturation during CPB and OPCAB procedures

No significant difference was observed in $ScvO_2$ between patients with and without T2DM at any protocol stage. Conversely, $CrSO_2$ was significantly lower in the T2DM-CPB and T2DM-OPCAB groups than in the corresponding controls ($p<0.001$ for both), and this difference endured in all phases of the surgery. This result was reflected in significant differences in gSO_2 between patients with and without T2DM in the CPB and OPCAB patients ($p<0.001$ for both). During the surgical procedure, prominent and significant increases in $ScvO_2$ ($p<0.001$) with smaller but statistically significant decreases in $CrSO_2$ ($p<0.05$) were observed at the beginning of CPB, resulting in marked elevations in gSO_2 ($p<0.001$). In both CPB groups, the $ScvO_2$ and gSO_2 reversed by the end of CPB, and these parameters decreased below their initial levels in C-CPB patients ($p<0.005$). No significant intraoperative changes were observed in the oxygen saturation parameters in the OPCAB patients.

Effects of T2DM on clinical parameters affecting cerebral oxygen supply and demand

Between-group differences were observed only in the CaO_2 before the surgical procedure ($p<0.05$) in the blood glucose levels throughout the surgery ($p<0.05$) and in the arterial oxygen saturation $CrSO_2$ differences ($p<0.05$). Considerable within-group intraoperative changes were observed primarily during the CPB procedure. The onset of CPB was associated with small but significant decreases in core body temperature in both T2DM-CPB and C-CPB patients ($p<0.05$), which subsequently returned to the initial value by the end of the surgery. Furthermore, MAP and CaO_2 decreased significantly in both groups of patients when CPB was established ($p<0.05$) and returned to their initial values following chest closure. RE and SE decreased significantly after anaesthesia induction in all groups of patients with no difference between patients with and without T2DM.

Relationship between central venous and cerebral oxygen saturation: effects of T2DM

Significant correlation was observed between $ScvO_2$ and $CrSO_2$ in the control group ($r=0.52$, $p<0.0001$). In contrast, no significant correlation was found between these global and regional oxygen saturation parameters in patients with T2DM ($r=0.13$, $p=0.34$).

DISCUSSION

In diabetes, detrimental changes on molecular, cell and tissue level lead to multiorgan damage. While the effects of diabetes are studied excessively, there are still lack of knowledge how diabetes affects distinct organs. Thus, we investigated the impact of diabetes on two vital organs, the lung and the brain. Chronic hyperglycaemia lead to pathophysiological pathway activation resulting in *i.e.* ROS overproduction, PKC-activation, TGF-β overexpression, glycation. The resulted IC and EC protein, endothelial and epithelial damage manifest in vascular and bronchial smooth muscle

cell and connective tissue dysfunction, leading to deteriorations both in the respiratory system and in the brain. The respiratory consequences of DM were studied in well-established rodent models, which allowed the use of complex measurement techniques without the influence of other potentially confounding factors (i.e. age, the onset of the disease, or comorbidities). The cerebral effects were investigated in a patient population in which the presence of diabetes is more frequent and susceptible to further organ damage. Since diabetes affects approximately one third of the patients undergoing cardiac surgery, a surrogate of the tissue oxygenation, the $CrSO_2$ was measured before and during cardiac surgery to elucidate the effect of diabetes on cerebral tissue oxygenation.

Effect of diabetes on the respiratory system: experimental study

The characterization of the pulmonary effects of DM in this study revealed detrimental changes in airway function, viscoelastic tissue mechanics, gas exchange, and collagen expression in the lung, with more severe manifestations in the rat model of type 1 DM than in that for type 2 DM. The increase in the basal airway tone with DM was associated with compromised dissipative and elastic tissue mechanics. The model of type 1 DM also showed diminished airway responsiveness to an exogenous cholinergic stimulus. These adverse mechanical and functional changes were accompanied by an increased intrapulmonary shunt and impaired PaO_2/FiO_2. Increasing the lung volume had a beneficial effect on the lung mechanics in both diabetic groups, whereas it had no benefit on gas exchange.

Type 1 DM was induced by a single high dose of STZ to cause the destruction of the pancreas, whereas type 2 DM was induced by a low dose of STZ to cause diffuse degeneration of the pancreatic cells, combined with a high-fat diet. As a result of these treatments, the blood glucose levels in both DM groups were markedly higher after 12 weeks than those of the control group. The body weight of the type 1 DM group rats was reduced, which can be explained by insulin deficiency.

Changes in the mechanical properties of the respiratory system

Forced oscillatory assessment of airway mechanics showed that DM caused a deterioration of basal Raw, with more severe changes occurring in the type 1 DM model rats. As these changes were also apparent in the specific airway resistance values of both DM groups, the differences in lung size cannot solely be accounted for by the compromised airway mechanics. Instead, these pathological changes in the airways may be explained by the decreased vagal tone, excessive mucus production, low-grade chronic inflammation, activation of inflammatory pathways, or bronchial smooth muscle cell proliferation.

The effects of DM on the mechanical properties of the lung tissue has been studied in an *ex-vivo* experiment, and the results were limited to assess the elastic behaviour of the lung parenchyma However, dissipative properties of the lung tissue play an important role in determining the physiological lung mechanics and the changes of this component is characteristic in various lung diseases. In the present study using an *in-vivo* setting, significant deteriorations were observed in the viscoelastic mechanical parameters of the respiratory tissues in both groups of diabetic rats, and this adverse change affected both the dissipative and elastic properties. These differences compared to the control group remained after normalization of the values to the FRC, indicating that intrinsic changes to the dissipative and elastic properties of the respiratory tissues can be anticipated. Collagen is the main determinant of overall lung tissue viscoelasticity. In the present study, the volume of collagen increased in the DM models, in agreement with results reported for patients with DM. There were significant correlations between the histological and mechanical findings, which suggested that the overexpression of collagen may be a primary cause of the compromised tissue damping and elastance observed in DM. The underlying pathophysiological mechanisms responsible for this extracellular matrix remodelling may be related to the activation of pathological pathways that contribute to the formation of AGEs, which also stimulate the production of extracellular matrix components, including collagens, thereby affecting the interactions of the extracellular matrix.

Effect of PEEP on respiratory function

Our findings demonstrated that the between-group differences in the airway resistance and in the tissue mechanical parameters representing viscoelastic dissipation and elastance disappeared at a moderately elevated PEEP (6 cmH_2O). The excessive PEEP dependence of the respiratory mechanical parameters was consistent with the diminished surfactant function reported previously in models of DM. It suggests that applying PEEP can have a beneficial effect on respiratory mechanics in this metabolic disease. The mechanical improvements were reflected in the decreased intrapulmonary shunt and increased PaO_2/FiO_2 in the DM rats when a PEEP of 3 cmH_2O was applied. Nevertheless, substantial differences remained even with elevated PEEP in both groups of DM rats; this can be attributed to the persistent alveolar-capillary barrier damage observed in DM. The type-II pneumocyte and surfactant layer damage along with the low-grade inflammation observed in DM can contribute to the alveolo-capillary dysfunction. Noticeably, there was recurrent deterioration of PaO_2/FiO_2 and Qs/Qt in the type 1 DM rats at a PEEP of 6 cmH_2O. This group would be expected to develop the well-established adverse pulmonary vascular consequences of hyperglycaemia. Accordingly, formation of AGEs as detailed above contributes to a proliferation of pulmonary endothelial and vascular smooth muscle cells and

thicker basal lamina, which may have resulted in the intra-acinar and alveolar arterioles becoming prone to collapse when the PEEP exerted an external mechanical load on the capillary network.

Changes in airway response to exogenous stimuli

An important physiological feature of airways is the adaptation of their caliber in response to exogenous stimuli. The results for the type 1 DM group demonstrated diminished airway responsiveness to MCh, which indicated that the adaptation ability of the bronchomotor tone had been severely compromised. Pathophysiological mechanisms that may have been involved in the decreased airway responsiveness include compromised vagal tone development due to autonomic diabetic neuropathy, smooth muscle cell dysfunction, and/or epithelial damage. Hyperinsulinemia, insulin resistance and hyperglycemia can lead to hyperproliferation and phenotype changing of the airway and bronchial smooth muscle cells and induce tracheal wall thickening. The smooth muscle cell damage might occur due to various factors, for instance disturbances in the nitric oxide synthesis, TGF-β and Rho-associated protein kinase pathway activation. Nonetheless, conflicting results have been reported for the effects of DM on airway responsiveness. The findings of the present study are consistent with earlier findings of a reduction in the bronchial response to cholinergic stimuli in DM. Previous reports of no change in airway responsiveness or the development of airway hyperresponsiveness may be explained by the insensitivity of the methods to assess airway function, the lack of airway innervation, or the involvement of confounding factors, such as, smoking, genetic differences and/or phenotype, and the duration of DM. Furthermore, in previous clinical studies, diabetes was often associated with comorbidities affecting the respiratory system, which may cause variation in the manifested pulmonary effects, and can contribute to the discordant results in the literature.

Effect of diabetes on the cerebral integrity: clinical study

Significant reduction of brain tissue oxygen saturation was obtained in the present study in T2DM patients. Since this finding was not reflected in the central venous oxygen saturation, the gap between $ScvO_2$ and $CrSO_2$ widened significantly and the relationship between these two values became uncoupled in patients with diabetes. In the T2DM patient, the oxygen saturation gap remained elevated throughout the cardiac surgery procedure.

Differences in the initial parameters between patients with and without diabetes

The lower initial $CrSO_2$ and the associated widening of gSO_2 in patients with T2DM cannot be attributed to differences in demographic, anthropometric and clinical characteristics between the two groups. In both groups of patients, the oxygen demand of the cerebral cortex was expected to be in a uniformly low range throughout the surgery, as suggested by the therapeutic entropy levels. The presence of anaemia and lower CaO_2 in the T2DM patients may contribute to the differences

in $CrSO_2$ between control and diabetic patients. However, $ScvO_2$ did not differ between the protocol groups, suggesting sufficient oxygen supply in all patients under general anaesthesia with muscle relaxation when the oxygen demand of the paralyzed skeletal muscle decreases markedly. Accordingly, the diminished overall oxygen extraction can be supplied both in patients with normal or compromised microcirculation (such as T2DM). Moreover, the widened gap in diabetic patients remains after CPB despite the lack of difference in CaO_2 and MAP between diabetic and non-diabetic patients. Most probably, the compromised $CrSO_2$ can rather be explained by the adverse cerebrovascular consequences of T2DM. The pathologic metabolic milieu is characterised by hyperglycaemia, insulin resistance and elevated level of free fatty acids. Blockade of the vasodilatory insulin signalling pathway diminishes endothelial nitric oxide (NO) synthesis. Endothelial NO production is further compromised by the advanced glycation end-products and by the increased inactivation of NO by oxidative stress. The reduced NO-mediated endothelium-dependent vasodilation increases the vascular tone. The elevated arterial tone and/or vascular remodelling facilitated by these mechanisms are associated with low-grade inflammatory, prothrombotic proliferative processes, resulting in atherosclerotic plaque formation. All these mechanisms converge to an impaired microcirculation, which may be reflected in impaired cerebral-tissue oxygen saturation.

Intraoperative changes

Remarkable intraoperative changes were observed in $ScvO_2$, $CrSO_2$ and gSO_2 at the beginning of CPB. The increases in $ScvO_2$ can be attributed to a decreased core temperature and subsequent decrease in the systemic oxygen demand. The concomitant slight, but significant, decrease in $CrSO_2$ in the diabetic patients should be interpreted in terms of a decreased MAP and haemoglobin concentration associated with an elevation in arterial pH. The opposite changes in $ScvO_2$ and $CrSO_2$ after the onset of CPB are reflected in the striking elevations in gSO_2. It is of note that in diabetic patients the gSO_2 may rise to a value threefold greater than physiologically normal (i.e. from around 10 to around 30 %). The compromised $CrSO_2$ observed in the present study may be responsible for the increases in postoperative adverse neurocognitive outcomes and stroke in T2DM patients. In contrast with the CPB patients, no intraoperative changes in oxygen saturation indices were detected in the patients with OPCAB procedure. This more stable pattern of cerebral-tissue oxygen saturation may explain the lower incidence of postoperative stroke and cognitive dysfunction after cardiac surgery with OPCAB.

Due to the unique regulation of the cerebrovascular circulation, the high oxygen demand and low hypoxic tolerance, the oxygen saturation of this organ can distinctly differ from the rest of the systemic circulation. NIRS offers a simple, non-invasive, real-time monitoring tool to quantify the

oxygen status of brain tissue. Hence, this technique has a great potential to reveal disturbances in the regional oxygen saturation promptly, even in the perioperative period. In non-diabetic patients, there is a significant difference between global and cerebral-tissue oxygen saturations, although they exhibit significant correlations. Nevertheless, there is a scatter in this relationship due to recognised interindividual variability in even in the control patients. Nonetheless, the associations between these regional and global oxygenation indices suggest the possibility to predict of the brain oxygen status from the $ScvO_2$ value when the cerebral circulation is intact. Conversely, our results also demonstrate that in addition to the gap between $ScvO_2$ and $CrSO_2$, these parameters became uncoupled in diabetic patients. The lack of a clear relationship between these indices impedes the assessment of cerebral-oxygen saturation from $ScvO_2$ in the T2DM population.

SUMMARY AND CONCLUSION

The studies included the present thesis emphasise the diabetic consequences both on the respiratory and cerebral function and contribute to better understanding the effect of diabetes on these organs. We demonstrated that diabetes deteriorates respiratory function, but PEEP elevation is beneficial for the mechanical properties, whereas it was not advantageous on the gas exchange parameters and oxygenation. As a consequence of the diabetic milieu, the cerebral regional oxygen saturation was compromised and the gap between $CrSO_2$ and $ScvO_2$ widened suggesting that the cerebral autoregulation is impaired. These deteriorations originate from the complex yet common pathophysiological changes in diabetes.

As a summary of the experimental study, distinct measurements of airway and respiratory tissue viscoelastic parameters in models of T1DM and T2DM showed evidence of detrimental changes in both compartments. The decline in airway function was reflected in elevated airway resistance and abnormal adaptation of the airways to exogenous constrictor stimuli. Lung tissue remodelling was manifested in compromised viscoelastic tissue mechanics, which affected dissipative and elastic properties, and the concurrent overexpression of collagen fibers in the extracellular matrix. These detrimental mechanical defects were overcome by applying PEEP, which demonstrated alveolar collapsibility in DM. However, even with the application of PEEP, gas exchange parameters remained compromised in the models of type 1 and type 2 DM, even deteriorated further in the type 1 DM model, indicating alveolo-capillary dysfunction.

Damages in the cerebral function is well known, however there were no study previously comparing the $CrSO_2$ between diabetic and control in anaesthetised patients during cardiac surgery. Furthermore, the relationship between the cerebral regional and the central venous saturation was not clarified earlier. The clinical study included in the present dissertation demonstrated that diabetes mellitus worsens the oxygen saturation of the cerebral tissue and uncouples indices reflecting regional cortical and global central venous oxygenation. Consequently, disturbances in the cortical oxygen saturation in diabetic patients become unpredictable from the well-established global clinical parameter of central venous oxygen saturation. Thus, diabetic patients may benefit from the continuous intraoperative measurement of regional brain-tissue oxygen saturation to optimise tissue oxygenation with adjusting cerebral perfusion pressure, arterial oxygen content and/or avoiding alkalosis.

In conclusion, the studies included in the present thesis are contributing to better understanding the detrimental effects of diabetes mellitus. Thus, the results foster better clinical practice contributing, advanced therapeutic intervention, and safer perioperative patient care

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LIST OF SCIENTIFIC PUBLICATIONS INCLUDED IN THE PRESENT THESIS:

I. Differences between central venous and cerebral tissue oxygen saturation in anaesthetised patients with diabetes mellitus.
Roberta Südy, Ferenc Peták, Álmos Schranc, Szilvia Agócs, Ivett Blaskovics, Csaba Lengyel, Barna Babik
Scientific reports, 2019 24;9(1):19740, doi: 10.1038/s41598-019-56221-4.

II. 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, doi: 10.1186/s12931-020-01334-y.

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

III. Diabetes mellitus: endothelial dysfunction and changes in hemostasis
Babik Babik, Peták Ferenc, Agócs Szilvia, Blaskovics Ivett, Alács Endre, Bodó Kinga, Südy Roberta
Orvosi Hetilap, 2018 159(33): 1335-1345, doi: 10.1556/650.2018.31130