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Noninvasive assessment of alveolar microvascular recruitment in conscious nonsedated rats

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Abstract

Recruitment of alveolar microvascular reserves, assessed from the relationship between pulmonary diffusing capacity (DL_{CO}) and perfusion (Q_c), is critical to maintenance of arterial blood oxygenation. Leptin-resistant ZDF fatty diabetic (fa/fa) rats exhibit restricted cardiopulmonary physiology under anesthesia. To assess alveolar microvascular function in conscious, non-sedated, non-instrumented, and minimally restrained animals, we adapted a rebreathing technique to fa/fa and control non-diabetic (+/+) rats (4-5 and 7-11 mo old) at rest and mild spontaneous activity. Measurements included O_2 uptake, lung volume, Q_c , DL_{CO} , membrane diffusing capacity (DM_{CO}), capillary blood volume (V_c) and septal tissue-blood volume. In older fa/fa than +/+ animals, DL_{CO} and DM_{CO} at a given Q_c were lower; V_c was reduced in proportion to Q_c . Results demonstrate the consequences of alveolar microangiopathy in metabolic syndrome: lung volume restriction, reduced Q_c , and elevated membrane resistance to diffusion. At a given Q_c , DL_{CO} is lower in rats and guinea pigs than dogs or humans, consistent with limited alveolar microvascular reserves in small animals.

Keywords

Lung diffusing capacity; pulmonary blood flow; obesity; type-2 diabetes mellitus; metabolic syndrome; alveolar microangiopathy

1. Introduction

The metabolic syndrome is a state of chronic lipo-oxidative stress characterized by obesity and type-2 diabetes mellitus (T2DM) that afflict some 34% of adults in the United States (Ervin, 2009). We previously reported a range of restrictive defects in lung volume, pulmonary perfusion (Q_c) and alveolar microvascular reserves in patients with T2DM that correlate with glycemia and extrapulmonary microangiopathy and are exacerbated by obesity (Chance et al., 2008). To understand the structure basis of pulmonary dysfunction in this condition, we explored an animal model that phenotypically resembles human metabolic syndrome, i.e., male fatty diabetic (fa/fa) ZDF rats with genetic leptin insensitivity leading

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to hyperphagia and obesity, followed by age-related development of T2DM, multiple end-organ dysfunction and a shortened life span. In fa/fa animals, we observed age-exaggerated alveolar structural abnormalities, including thickened blood-gas diffusion barrier, septal lipid infiltration and connective tissue deposition (Foster et al., 2010). Lung function measured under anesthesia via a tracheostomy show age-related reductions in lung volume, compliance, lung diffusing capacity (DL_{CO}) and pulmonary blood flow (Q_c) compared to matched lean nondiabetic (+/+) male ZDF rats (Yilmaz et al., 2010). Owing to a low Q_c , the measurements under anesthesia were unable to adequately assess diffusion-perfusion (DL_{CO}/Q_c) relationships that index functional recruitment of alveolar microvascular surface area and volume for gas exchange, a critical process necessary for the maintenance of gas exchange efficiency and normal arterial blood oxygenation in the conscious active subject (Hsia, 2002).

To address the need for noninvasive assessment of alveolar microvascular function under realistic *in vivo* (conscious, non-sedated, non-instrumented, and minimally restrained) conditions in rats, we adapted a multi-gas rebreathing technique that was initially developed for human subjects (Chance et al., 2008; Phansalkar et al., 2004), and subsequently applied to smaller species including dogs (body weight 10-35 kg) (McDonough et al., 2006) and guinea pigs (~1 kg) (Yilmaz et al., 2008; Yilmaz et al., 2005; Yilmaz et al., 2010). The rebreathing technique allows simultaneous measurement of lung volume, Q_c , DL_{CO} , and septal gas exchange tissue/blood volume (V_{tissue}) in one maneuver. By repeating the same maneuver while rebreathing test gas mixtures containing one of two O_2 concentrations, the components of DL_{CO} – membrane (DM_{CO}) and pulmonary capillary blood volume (V_c) – may be estimated. Compared to the breath-hold technique, the rebreathing method yields more uniform pulmonary distribution of the test gas (Jansons et al., 1994), an advantage in conditions associated with uneven distribution of ventilation. To improve tolerance of the apparatus, we devised a low dead space respiratory mask-body vest assembly that allows the animal limited mobility. As long as the animal tolerates the respiratory mask, the rebreathing technique need not require special respiratory maneuvers or interfere with normal breathing pattern. Here we report the adaptation of this noninvasive rebreathing technique to the rat and its application to test the hypothesis that alveolar microvascular reserves are diminished in this model of the metabolic syndrome.

2. Materials and methods

2.1. Animals

The Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center approved the protocols. Male obese diabetic rats with genetic leptin insensitivity (fa/fa) and control nondiabetic (+/+) ZDF rats were bred in the Unger laboratory. Separate cohorts were studied at 4 to 5 mo (fa/fa n=22, +/+ n=18) or 7 to 11 mo (fa/fa n=11, +/+ n=12) of age (**Table 1**). When fed rodent chow containing 6.5% fat (Formulab diet 5008, Purina, St. Louis, Missouri), the male fa/fa animals developed hyperphagia, progressive obesity, and diabetes mellitus around 12-14 wk of age. The +/+ animals, fed regular rodent chow (Teklad 2016 Global 16% Protein Rodent Diet, Harlan Teklad, Madison, WI), did not develop diabetes mellitus. After ~30 wk of age, the untreated fa/fa animals begin losing weight due to systemic complications of diabetes.

2.2. Apparatus

The apparatus for measurement in the conscious animal is shown in **Figure 1**. A plastic conical respiratory mask was constructed with a double-layer rubber diaphragm that provided a seal around the neck. A thin layer of soft latex covered the inner surface of the mask. Via a small port, an adjustable amount of air was introduced into the space between

the latex and inner surface of the mask to provide an inflatable cushion around the animal's head, which improved the level of comfort and tolerance of the mask, and minimized mask dead space. The mask was attached to a body vest made of light stretchable fabric and fastened by Velcro™; care was taken to avoid chest wall constriction. The breathing orifice of the mask was attached to a latex rebreathing bag and a two-way non-rebreathing valve (Model 2384A, Hans Rudolph, Kansas City, MO) via a stopcock (apparatus dead space ~1.0 mL). During open circuit breathing the inspiratory port opened to room air or a reservoir. The expiratory port connected to a heated pneumotachometer (Hans Rudolph, Series 8311) and a collection bag for measuring expired ventilation. Tidal respiratory pressures were detected at the mouth during open-circuit breathing before rebreathing maneuvers. Pneumotachometer signals were amplified (Hans Rudolph Amplifier 1, Series 1100) and acquired by a data acquisition card (PCM-DAS08, Computer Boards, Middleboro, MA) on a PC laptop (Pentium III, Dell Inspiron 8000) running LabVIEW 5.0 (National Instruments, Austin, TX) and Universal Library (Measurement Computing Co, Norton, MA) acquisition software for real time measurement of respiratory rate and minute ventilation. The entire mask-valve assembly was counter-weighted to allow some degree of mobility.

Expired gas collected in the anesthetic bag and the rebreathing bag was sampled and the concentrations of O₂, CO₂, N₂, CO, C₂H₂, and Ne measured by a gas chromatograph (CP-4900 Micro GC, Varian Inc, CA) with 2 columns (M5AHIBF and PPUHI) and a thermal conductivity detector using 100% high purity helium as a carrier gas. Linearity of the gas chromatograph was checked under dry as well as humidified conditions by generating calibration curves using gases of known concentrations. Water vapor was eliminated using a moisture trap (Model MT120-2, Agilent Technologies, Palo Alto, CA) at the insertion port of the gas chromatograph. The pneumotachometer was calibrated before each study by delivering 60 strokes of room air or 100% O₂ at different flow rates using a 20 ml syringe. Because 100% O₂ generated 11% higher voltage than room air at the same flow rate, the pneumotachometer was calibrated using the same inspired O₂ concentration as that used during each study. Ventilatory measurements were verified by timed collection of expired air in an anesthetic bag (pneumotachometer=1.006·bag, R²=0.98). The volume of the collected gas was measured using a calibrated syringe, and the gas concentrations were measured by the gas chromatograph.

2.3. Rebreathing measurements in conscious rats

The animals were trained to wear the respiratory mask-body vest assembly twice a week for 3 weeks prior to making any measurement. Ventilation, O₂ uptake and CO₂ output were measured during open circuit breathing by timed collection of the expired gas. The rebreathing test gas mixture (0.3% CO, 0.5% Ne, 0.8% C₂H₂, 40 or 90% O₂ in balance of N₂) was drawn into a Mylar reservoir bag. A volume of the test gas equal to the animal's tidal volume plus 4.0 mL (total 7.0-8.0 mL) was drawn into the latex rebreathing bag immediately before each maneuver. Of this test gas volume, 2.0 mL was drawn to measure initial gas concentration by the gas chromatograph. The rebreathing maneuver was conducted by turning the stopcock from open circuit to the latex rebreathing bag such that the animal rebreathed from the bag for 3, 4, 5, 6, or 7 s, in separate maneuvers. The rebreathing duration was precisely determined (to 0.01 s) from the tidal respiratory pressure tracing, which was flat during a rebreathing maneuver; the actual rebreathing duration was generally within ±0.25 s of the target duration. Final gas concentrations in the rebreathing bag were measured immediately. The minimum required sample volume was 2.4 mL (1.4 mL for the gas chromatograph and 1.0 mL to clear the dead space). If the final rebreathing bag volume was less than 2.4 mL, the rebreathing maneuver was repeated. Duplicate measurements were obtained on consecutive days at each of the two O₂ tensions in random order and the results were analyzed separately as well as averaged. The interval between

successive rebreathing maneuvers was at least 2 minutes. An entire study could be completed in about 1.5 hour. At the end, hematocrit and blood glucose concentration were measured from tail vein blood.

2.4. Rebreathing measurements under anesthesia

In older animals (7-11 mo of age) following termination of the above studies, rebreathing measurements were performed under anesthesia as described previously (Yilmaz et al., 2010). The animal was anesthetized by an intraperitoneal injection of ketamine (100 mg/kg), xylazine (10 mg/kg) and acepromazine (2 mg/kg). The trachea was cannulated via a midline neck incision. The animal was mechanically ventilated (Inspira, Harvard Apparatus, Holliston, MA, tidal volume 8 ml/kg, 65 breaths per minute) and the airway pressure-lung volume relationship was measured using a calibrated syringe and transducer (Amplifier 1 model 1100, Hans Rudolph, Kansas City, MO). Before each measurement, the animal was ventilated with 25% or 100% O₂ for at least 2 min. Volume of rebreathing gas was determined as that corresponding to 30 cmH₂O airway pressure. The test gas mixture (40 or 90% O₂, 0.3% CO, 0.5% Ne, 0.8% C₂H₂, balance N₂) was drawn into a glass syringe, delivered to the animal's lungs, and rebreathed manually for 5, 7 or 9 s at 60 strokes/min. Following each maneuver gas concentrations in the syringe were immediately measured by the gas chromatograph. Duplicate measurements were made at two inspired O₂ concentrations (40% and 90%). At the end, CO backpressure was measured by rebreathing 30 ml of 100% O₂ for 60 s, followed by euthanasia via an intracardiac injection of Euthasol™ overdose. Open circuit O₂ uptake and CO₂ output were not measured.

2.5. Data analysis

Lung volume was estimated from inert gas (Ne) dilution (ratio of initial/final Ne concentrations). Pulmonary blood flow, DL_{CO} and O₂ uptake were calculated from the exponential disappearance of C₂H₂, CO and O₂, respectively, during rebreathing (Yilmaz et al., 2005). Complete gas mixing was achieved rapidly; Ne concentration stabilized within 3 s during rebreathing, while C₂H₂, CO and O₂ concentrations declined in a log linear fashion with respect to time (**Figure 2**). DM_{CO} and V_c were estimated from DL_{CO} measured at two alveolar O₂ tensions using the Roughton-Forster relationship (Roughton and Forster, 1957):

$$\frac{1}{DL_{CO}} = \frac{1}{DM_{CO}} + \frac{1}{\theta_{CO} \cdot V_c} \quad \text{Eq. 1}$$

where θ_{CO} is the empirical rate of CO uptake by whole blood at 37°C in [mL CO·(min·mmHg·mL blood)⁻¹] estimated from mean alveolar O₂ tension (P_{AO2} in mmHg) during rebreathing. Since the relationship between θ_{CO} and O₂ tension is not available for rodents, we used the values obtained by Holland (Holland, 1969) for dog blood ($\lambda=1.6$):

$$\frac{1}{\theta_{CO}} = (0.929 + 0.00517 \cdot P_{AO2}) \cdot \frac{0.45}{Hematocrit} \quad \text{Eq. 2}$$

For each measured Q_c and P_{AO2}, we calculated 1/DL_{CO} and 1/θ_{CO}. DM_{CO} and V_c were directly obtained from the reciprocals of the intercept and slope, respectively, of the relationship between 1/DL_{CO} and 1/θ_{CO} (Eq. 1). Using the estimates of DM_{CO} and V_c, and the θ_{CO} calculated at a constant P_{AO2} (120 mmHg) and hematocrit (0.45), DL_{CO} may be expressed and compared under standardized conditions (DL_{CO-std}). The time zero point from the CO disappearance curve was applied to the C₂H₂ curve to extrapolate the intercept of C₂H₂ disappearance and to estimate septal tissue volume (Yilmaz et al., 2005).

Group mean results (±SD) were compared between ages and genotypes by unpaired t-test and analysis of variance. DL_{CO} measurements were analyzed with respect to Q_c using least

squares linear regression analysis. The slopes and intercepts of pooled relationships were compared among groups as described by Zar (Zar, 2009). $P < 0.05$ was considered significant.

3. Results

Group mean data are summarized in **Table 1**. In both age groups, blood glucose concentration was grossly elevated in fa/fa compared to +/+ animals. With age, fa/fa animals began losing weight as end organ complications such as visual and renal impairment developed; therefore, body weight was similar in younger animals (4-5 mo) but paradoxically lower in older fa/fa than +/+ animals (7-11 mo). Because the weight reduction mainly reflected a loss of body fat, we did not normalize the results by body weight. Hematocrit was not significantly different between conscious fa/fa and +/+ animals, although we have consistently found a lower hematocrit in anesthetized fa/fa than +/+ animals (Yilmaz et al., 2010). The difference probably reflects fluid shift and splenic sequestration of red blood cells between the two states of consciousness. In both genotypes, mean alveolar PO_2 during rebreathing was lower in conscious animals due to the higher O_2 uptake and ventilation compared to anesthetized animals.

Comparing conscious animals to that under anesthesia and mechanical ventilation, minute ventilation more than doubled, respiratory rate was three-fold higher while tidal volume was ~25% lower in both genotypes (**Table 1**). Mean lung volume during rebreathing was unchanged while pulmonary blood flow was about three-fold higher. DL_{CO} , DM_{CO} , and V_c were two to three folds higher while septal tissue/blood volume was not significantly different.

Repeat measurements on consecutive days are shown in **Figure 3**. Because these animals were mobile and no attempt was made to standardize their activity level, the measurements reflected a range of spontaneous activities from rest to moving about. Minute ventilation and CO_2 output during open circuit breathing showed intra- as well as inter-animal variations in accordance with activity level (**Figure 3, upper panels**). Similarly, the measured DL_{CO} varied along a consistent linear relationship with respect to Q_c (**Figure 3, middle panels**). The mean lung volume measured during rebreathing (**Figure 3, lower panels**) also showed intra- and inter-animal variation but group mean and $\pm SD$ values were highly reproducible.

Differences related to genotype and age

Mean values of ventilation, tidal volume, O_2 uptake, CO_2 output, lung volume, Q_c , DL_{CO} , DM_{CO} , V_c and septal tissue volume were significantly lower in conscious fa/fa than +/+ animals; the reductions were accentuated in older animals (**Table 1**). In 4-5 mo old fa/fa animals, the direct relationships of minute ventilation with respect to CO_2 output (**Figure 3**) as well as DL_{CO} before and after standardization to a constant P_{AO_2} and hematocrit (**Figures 3 and 4**, respectively), DM_{CO} and V_c (**Figure 4**) with respect to Q_c , were not significantly different from that in +/+ animals. In 7-11 mo old fa/fa compared to the corresponding +/+ animals, these relationships shifted to a lower range (**Figures 3 and 4**). In addition, DL_{CO-std} at a given Q_c was reduced mainly due to a reduction in DM_{CO} in the lower range of Q_c while V_c was reduced in proportion to the reduction in Q_c (**Figure 4**).

Inter-species comparison

The relationship between DL_{CO} and Q_c in conscious +/+ rats was compared to that reported in other species using a similar rebreathing technique in conscious non-sedated state from rest to light exercise (**Figure 5**). When expressed per kg of body weight, Q_c was systematically higher in dogs and rats than guinea pigs and untrained humans.

At a given $\dot{Q}_c$, body mass-specific DL_{CO} was higher in human subjects (Chance et al., 2008) and dogs (McDonough et al., 2006) than guinea pigs (Yilmaz et al., 2008) and rats. The inert gas dilution factor was similar among species but systematically higher in the conscious condition than under anesthesia due to a higher mean lung volume in the conscious animal (Table 2).

4. Discussion

4.1. Summary of results and interpretation

These are the first non-invasive measurements of alveolar microvascular function in conscious non-sedated, non-instrumented and minimally restrained rats. This study contributes new technical advance, and pathophysiological as well as comparative physiological insight.

4.1.1. Technical advance—We extended an established rebreathing technique to a new species of a body size (400–500 g) 50% smaller than that was previously possible, to examine the $DL_{CO}/\dot{Q}_c$ relationship, which could not be adequately assessed under anesthesia due to the very low $\dot{Q}_c$. We validated the measurements in 4 ways: 1) by demonstrating the expected increases in ventilatory, perfusion and diffusion indices from anesthetized to conscious conditions, 2) by demonstrating the characteristic linear $DL_{CO}/\dot{Q}_c$ relationships of alveolar microvascular recruitment with spontaneous activity up to a $\dot{Q}_c$ above 400 $\text{mL} \cdot (\text{min} \cdot \text{kg})^{-1}$, 3) by detecting significant age-exaggerated dysfunction in conscious fa/fa animals that mirror the limited observations under anesthesia, 4) by demonstrating consistent repeat measurements of lung volume as well as key physiological relationships within and among animals, and in comparison with other species of very different body sizes.

4.1.2. Pathophysiological insight—In young fa/fa animals, the $DL_{CO}/\dot{Q}_c$ relationship from rest to light activity was preserved, indicating normal alveolar microvascular recruitment. In older fa/fa animals, mean $\dot{Q}_c$ was 45% lower than in age-matched +/- controls; this could be due to the fact that fa/fa animals were chronically ill, less active, and may have primary cardiac pathology. In addition, DL_{CO} at a given $\dot{Q}_c$ was reduced, largely due to a disproportionally lower DM_{CO} at a given $\dot{Q}_c$. These data suggest diminished alveolar reserves caused not only by a low $\dot{Q}_c$ but also by impairment at the alveolar-capillary barrier leading to increased resistance to diffusion. These physiologic data from active animals agree with published morphometric analysis in postmortem fixed lung of fa/fa animals that show a thickened alveolar-capillary basement membrane, increased septal interstitial connective tissue content and lipid infiltration with age (Foster et al., 2010). Combined structure-function data support the interpretation that membranous abnormalities of alveolar membrane are largely responsible for the alveolar microangiopathy in the metabolic syndrome.

4.1.3. Comparative physiological insight—The $DL_{CO}/\dot{Q}_c$ relationship in rats exhibits a similar slope and intercept as that measured by a similar method in conscious non-sedated guinea pigs. On the other hand, there are also inter-species differences. Per kg of body weight, DL_{CO} at a given $\dot{Q}_c$ is systematically lower in rats and guinea pigs compared to large species (dogs and humans) while the slopes of DL_{CO} recruitment with respect to $\dot{Q}_c$, and the inert gas dilution factors, are similar across species. This comparison suggests that small animals possess limited alveolar microvascular reserves, an important observation with implications for the differential inter-species utilization of adaptive mechanisms in pathological states. For example, in dogs and humans following the loss of functioning lung units, the remaining lung compensates via greater utilization of its existing microvascular

reserves to increase DL_{CO}/Q_c up to a functionally adequate level necessary for maintaining arterial blood oxygenation (Hsia et al., 1993a; Hsia et al., 1993b; Hsia et al., 1992). Structural sources of adaptation – alveolar remodeling and regrowth - are much more costly and invoked only following severe stimuli and depletion of the physiological reserves (Hsia, 2004; Hsia et al., 1994a; Hsia et al., 1994b). In comparison, small animals with limited easily exhausted physiological reserves must rely heavily on the metabolically expensive structural mechanisms of compensation. The limited physiological reserves could explain the rapid induction of alveolar tissue-capillary growth and regeneration seen in small animals in response to even moderate loss of lung units (Cagle and Thurlbeck, 1988; Fehrenbach et al., 2008; Sekhon et al., 1993) or exposure to ambient hypoxia (Hsia et al., 2005; Lechner and Banchero, 1980; Sekhon and Thurlbeck, 1996).

4.2. Critique of the technique

In addition to a small body size, adaptation of the rebreathing technique to rats presents unique challenges due to the difficulty for these animals to remain still while confined in a body chamber. Therefore, we designed the mask-vest apparatus to allow measurements to be made during spontaneous activities. Our results reflect a range of conscious activities from rest to moving about. Collectively, these data characterize key physiological relationships in each experimental group. Reproducibility of the results is shown by the tight relationships between DL_{CO} and Q_c upon repeat measurements (R^2 value of 0.98 to 1.0).

To minimize apparatus dead space and the risk of overestimating mean lung volume during rebreathing, we improved the inner surface of the mask with a soft inflatable lining. Resistance of the breathing circuit was up to $0.094 \text{ cmH}_2\text{O}\cdot\text{s}\cdot\text{mL}^{-1}$ at flow rates up to 700 mL/min, which contributed up to 1.1 cmH₂O increase in airway pressure, a magnitude similar to that reported in conscious guinea pigs (Yilmaz et al., 2008). The beginning of a rebreathing maneuver was manually approximated to the end-expiratory mouth pressure signal. Errors in synchronization would cause over-estimation of end-expiratory and under-estimation of end-inspiratory lung volume. Precision in the timing of switch could theoretically be improved by using a solenoid valve, but the benefit is offset by the added bulk and dead space. Therefore, we reported a “mean lung volume” during rebreathing instead of separate end-inspiratory or end-expiratory lung volume. The latex rebreathing bag selectively absorbs acetylene at a slow constant rate ($7.5\times 10^{-6} \text{ mL/s}$) (Yilmaz et al., 2008). Because the rebreathing bag was filled immediately before each rebreathing maneuver, this source of error is negligible.

During rebreathing in conscious rat, the inert gas dilution factor was similar to that in the conscious guinea pig, dog and human subject, but systematically higher than that measured under anesthesia (**Table 2**). The inter-species similarity lends support to the validity of this technique in the rat. The higher dilution factor measured in the conscious animal is likely related to a higher functional residual capacity.

With training all animals tolerated the respiratory mask-body vest assembly. The animals clearly prefer wearing the mask-vest assembly to being confined in a body chamber, because our apparatus afforded some degree of mobility. Owing to a high respiratory rate, complete mixing of the inspired bolus was achieved within 3 s of rebreathing (**Figure 2**). The mean Q_c in our conscious $+/+$ rats ($318\pm 87 \text{ mL}\cdot[\text{min}\cdot\text{kg}]^{-1}$) agrees well with the mean cardiac output estimated from the ratio of O_2 uptake to arteriovenous O_2 content difference in catheterized conscious Sprague-Dawley rats ($307\pm 7 \text{ mL}\cdot[\text{min}\cdot\text{kg}]^{-1}$) (Favret et al., 2006). The average O_2 uptake and CO_2 output in our conscious $+/+$ rats (21.2 ± 3.7 and $23.4\pm 3.7 \text{ mL}\cdot[\text{min}\cdot\text{kg}]^{-1}$ respectively) also agree with the values previously measured by an open-circuit flow-through box or a mask in conscious Wistar or Sprague-Dawley rats (21 to 31, and 16 to 24 $\text{mL}\cdot[\text{min}\cdot\text{kg}]^{-1}$ respectively) (Fregosi and Dempsey, 1984; Kirkton et al., 2009;

Musch et al., 1988). The agreement among independent techniques supports the validity of these measurements.

4.3. Conclusion

We established a rebreathing technique to noninvasively assess cardiopulmonary function including alveolar microvascular recruitment in conscious non-sedated non-instrumented, minimally restrained rodents in the size range of 400–550 g. This technique significantly extends the scope of pathophysiological assessment to provide new tools for cardiopulmonary research using rodent models, especially in the longitudinal monitoring of disease evolution and the response to intervention in chronic preparations. Using this technique, we characterized the age-related disproportional reductions in lung volume, pulmonary blood flow and membrane conductance in fatty diabetic rats. Collectively, these abnormalities indicate a loss of alveolar-capillary reserves in the metabolic syndrome. Functional impairment is consistent with the structural microangiopathy reported elsewhere (Foster et al., 2010). In addition, an inter-species comparison of the diffusion-perfusion relationship suggests that even normal rodents possess limited alveolar-capillary physiological reserves, which in turn could explain the greater propensity for these animals to resort to structural mechanisms of adaptation, e.g., initiation of compensatory alveolar-capillary growth, in pathological states following the destruction of lung units.

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Highlights

- A noninvasive rebreathing method is developed to measure lung function in conscious rats.
- Alveolar microvascular reserves are assessed from the pulmonary diffusing capacity-to-perfusion relationship.
- Small animals show reduced microvascular reserves compared to large species.
- Rats with metabolic syndrome show greater age-exacerbated loss of alveolar microvascular reserves

Figure 1.
Schematic diagram of the experimental apparatus.

Figure 2. Sample disappearance curves during conscious rebreathing from one rat showing the typical exponential disappearance of C₂H₂, CO and O₂, and stabilization of Ne dilution. The y-axis is the natural log of alveolar concentration of the indicator gas at a given time (F_A) relative to its initial concentration (F_{A0}).

Figure 3.

Gas exchange data. *Upper row:* The relationship between minute ventilation and CO₂ output was obtained during open circuit breathing in conscious animals. *Middle row:* The relationship between DL_{CO} and pulmonary blood flow was measured while breathing 40% inspired O₂ concentration in conscious animals compared to the mean ($\pm$ SD) values obtained while breathing 40% inspired O₂ under anesthesia (7-11 mo group only). *Lower row:* Mean lung volume during rebreathing in conscious animals. Individual lines connect two data points each representing the average of duplicate measurements obtained from the same animal on consecutive days. *Left panels:* 4-5 mo old rats. *Right panels:* 7-11 mo old rats. Open symbols: +/+. Closed symbols: fa/fa. P < 0.05 vs. corresponding +/+: * by unpaired t-test or repeated measures ANOVA, † by regression line analysis.

Figure 4.

Relationships between standardized DL_{CO} (DL_{CO-std} , expressed at P_{AO_2} of 120 mmHg and hematocrit of 0.45, upper panels), DM_{CO} (middle panels) and V_c (lower panels) with respect to pulmonary blood flow (Q_c) are shown in conscious fa/fa and $+/+$ animals studied at age 4-5 mo (*left panels*) and 7-11 mo (*right panels*). Each data point in conscious animals represents the average of duplicate measurements obtained from the same animal on consecutive days. Data from anesthetized animals (7-11 mo old only) are shown as mean ($\pm$ SD). Regression equations: 4-5 mo old, $+/+$: $DL_{CO-std}=0.0025 \cdot Q_c - 0.0184$, $R^2=0.91$; $DM_{CO}=0.0049 \cdot Q_c - 0.211$, $R^2=0.15$; $V_c=0.0072 \cdot Q_c - 0.074$, $R^2=0.50$; fa/fa : $DL_{CO-std}=0.0021 \cdot Q_c - 0.005$, $R^2=0.81$; $DM_{CO}=0.0048 \cdot Q_c + 0.027$, $R^2=0.19$; $V_c=0.0059 \cdot Q_c + 0.053$, $R^2=0.47$. 7-11 mo old, $+/+$: $DL_{CO-std}=0.0012 \cdot Q_c + 0.1661$, $R^2=0.59$; $DM_{CO}=0.0014 \cdot Q_c + 0.77$, $R^2=0.01$; $V_c=0.0073 \cdot Q_c - 0.113$, $R^2=0.39$; fa/fa : $DL_{CO-std}=0.0024 \cdot Q_c - 0.0392$, $R^2=0.80$; $DM_{CO}=0.0041 \cdot Q_c + 0.090$, $R^2=0.31$; $V_c=0.0053 \cdot Q_c + 0.003$, $R^2=0.60$. $P < 0.05$ vs. corresponding $+/+$: * by unpaired t-test, † by regression line analysis.

Figure 5.

Comparison of the relationship between DL_{CO-std} (expressed at a standardized P_{AO2} of 120 mmHg and hematocrit of 0.45) and pulmonary blood flow (Q_c) in conscious ZDF +/+ rats (4-5 mo old) with that measured by a similar rebreathing technique from rest to light exercise in normal human subjects (Chance et al., 2008), dogs (McDonough et al., 2006), and guinea pigs (Yilmaz et al., 2008). Regression equations: Guinea pig: DL_{CO}=0.0024·Q_c-0.0106, R²=0.82; dog: DL_{CO}=0.0023·Q_c+0.225, R²=0.58; human: DL_{CO}=0.0034·Q_c+0.060, R²=0.71; rat: DL_{CO}=0.0024·Q_c-0.017, R²=0.88.

Table 1

Cardiopulmonary function.

Age	4-5 mo		7-11 mo			
Condition	Conscious non-		Conscious non-sedated		Anesthetized,	
Genotype	+/+	fa/fa	+/+	fa/fa	+/+	fa/fa
Number	18	22	12	11	12	11
Age (mo)	4.4±0.3	4.7±0.3	8.6±1.6	8.2±1.4	8.8±1.7	8.8±1.0
Body Weight (g)	424±38	440±45	544±26 [†]	439±73 [*]	544±24	432±91 [*]
Hematocrit (%)	48.7±1.0	47.5±2.2	43.8±4.6 [†]	41.9±5.5	45.7±2.4	34.1±8.8 [*]
Blood glucose (mg·dL ⁻¹)	122±29	429±165 [*]	130±20	421±195 [*]		
Ventilation (mL·min ⁻¹)	535±77	412±69 [*]	736±85 ^{§†}	501±77 ^{*§†}	294±17	218±23 [*]
Respiratory rate	152±21	172±53 [*]	179±27 ^{§†}	201±19 ^{*§†}	59±1	64±2 [*]
Tidal volume (mL)	3.5±0.4	2.5±0.5 [*]	4.2±0.6 ^{§†}	2.5±0.4 ^{*§}	5.0±0.4	3.4±0.5 [*]
O ₂ uptake (mL·min ⁻¹)	11.1±1.4	9.3±1.6 [*]	11.5±2.0 [§]	7.8±2.1 ^{*§†}		
CO ₂ output (mL·min ⁻¹)	9.6±1.5	6.8±1.4 [*]	12.7±2.1 ^{§†}	6.8±1.9 ^{*§}		
Mean P _{AO2} (mmHg)						
Rebreathing 40% O ₂	131±8	123±4 [*]	128±2 [§]	125±6 [§]	207±6	226±15 [*]
Rebreathing 90% O ₂	494±23	469±18 [*]	501±23 [§]	569±17 ^{*§}	584±12	600±18 [*]
Mean Lung volume	10.4±1.9	7.3±1.5 [*]	12.2±1.4 ^{§†}	9.4±3.2 ^{*†}	13.8±1.0	10.4±1.4 [*]
Pulmonary blood flow	119±41	104±37 [*]	173±44 ^{§†}	96±37 ^{*§}	53±13	35±7 [*]
DL _{CO-std}	0.285±0.	0.229±0.0	0.379±0.08	0.216±0.09	0.241±0.0	0.108±0.045 [*]
DM _{CO} (mL·[min·mmHg] ⁻¹)	0.805±0.	0.540±0.3	0.854±0.27	0.517±0.29	0.467±0.1	0.199±0.133 [*]
Capillary blood volume	0.82±0.3	0.69±0.32	1.26±0.52	0.51±0.24	0.58±0.24	0.33±0.16 [*]
Septal tissue/blood	2.19±0.9	1.78±0.79	3.59±1.50 [†]	2.24±1.48	3.22±1.08	2.67±0.97 [*]

Mean±SD.

* p 0.05 vs. +/+ under the same condition

§ vs. anesthetized in the same genotype and age group

† vs. 4-5 mo of age in the same condition and genotype by unpaired t-test. DL_{CO-std} is expressed at P_{AO2} = 120 mmHg and hematocrit = 0.45.
 Open circuit O₂ uptake and CO₂ output were not measured under anesthesia.

Table 2

Inert gas dilution factor during rebreathing

Condition	Conscious non-	Anesthetized and
Rat	2.83±0.48	1.29±0.04
Guinea pig (Yilmaz et al., 2008;	2.75±0.45	1.37±0.09
Dog (McDonough et al., 2006)	2.39±0.31	1.64±0.15
Human (Chance et al., 2008;	2.48±0.52	N/A

Mean±SD.