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The effect of body warming on respiratory system stress recovery in the rat

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The mechanical characteristics of respiratory system tissues include visco-elastic behaviour. In particular, after mechanical unloading, i.e., a reduction in respiratory system volume, the lower stress achieved slowly increases, approaching higher constant value, due to visco-elastic stress recovery. We performed experiments in which constant deflation flow arrest was applied in rats to study the successive pressure-time course, which defines the visco-elastic stress recovery. To investigate the possible effects of temperature changes, measurements were performed at two body temperatures, 36.6 ± 0.3 and 39.0 ± 0.1 °C. We found that stress recovery is reduced by increasing body temperature. Pressure-time curves after deflation arrest were fitted by specific mathematical model, and a good agreement was found. Model parameters exhibited significant changes with body temperature variations, suggesting that temperature-dependent micro-structural rearrangement phenomena in the tissues of alveolar wall were involved in the stress recovery decrement with body temperature increase. Thus, visco-elastic phenomena in respiratory system tissues of mammals exhibit temperature dependence. The stress recovery changes with body temperature suggest that expiration is expected to be easier in condition of physiological body temperature than in the case of increased temperature.

Key words: *body temperature, rat, respiratory system mechanics, respiratory system stress recovery, stress recovery analytical model*

1. Introduction

Stress relaxation is a complex mechanical phenomenon exhibited by most tissues which influences the response of the corresponding biological structures, such as the lungs and the respiratory system. Due to their visco-elastic properties, the respiratory system tissues do not maintain a constant stress under constant deformation. Instead, the stress slowly relaxes, approaching a constant lower value [1].

The effect of stress relaxation is evaluated together with stress recovery, which implies that after a reduction in respiratory system volume, the lower stress achieved slowly increases and approaches a higher constant value [2], [3].

It was recently shown that respiratory system stress relaxation is increased by an increment of the lung's blood volume and flow [4], exhibits a decrement with increasing body temperature [5], and is increased in inflamed lung tissue [6]–[10], and as an effect of interleukin-6 [11]. These reports suggest that stress-relaxation and recovery may depend on the physical-chemical characteristics of the lung tissue and interstitial fluid, including temperature. Furthermore, the lung tissue visco-elastic behaviour has been attributed to the mechanical characteristics of the network of the fibrous elements in the alveolar septa [12]–[14], which has been demonstrated to be influenced by temperature [15], [16].

The temperature-dependence of respiratory system stress relaxation has been already demonstrated [5] and, in the present report, the effect of body warming on stress recovery was investigated.

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Our working hypothesis was that the visco-elastic behaviour of the respiratory system tissues during stress recovery may be influenced by body warming, as stress relaxation is. Results may help to increase the knowledge about the visco-elastic behaviour of the respiratory system tissues.

The stress relaxation of the respiratory system may be quantified by the end-inflation occlusion method [5], [17]–[19]: the slow decay of the tracheal pressure which is seen after a sudden occlusion following constant flow respiratory system inflation is due to stress relaxation [18]–[20]. Similarly, the pressure recovery after expiratory flow arrest subsequent to constant flow respiratory system deflation stays in a direct reciprocal correlation and quantifies stress recovery [2], [3], [21]. Thus, we applied flow arrest during constant flow deflation of the respiratory system on rats in control conditions and immediately after body warming to investigate the effect of body temperature on respiratory system stress recovery.

2. Materials and methods

2.1. Experimental procedure

The experiments were carried out on twelve albino Wistar rats of both sexes (mean weight 300 ± 26 g, six males). The animals were housed and treated in accordance with the Italian law on animal experimentation (L. 116/92), and with the EU directive 2010/63/EU. The experimental protocol was authorised by the local ethical committee for animal experimentation (C.E.A.S.A.).

The rats were anaesthetized with 50 mg/100 gr i.p. chloralose, and then placed on a heated operation table. After tracheotomy, a small polyethylene cannula (2 mm i.d., 5 cm long) was inserted through an incision in the second tracheal ring and firmly secured in place.

Positive pressure ventilation (tidal volume 10 ml/Kg, breathing frequency 60/min, Rodent Ventilator 7025, Basile, Italy) was consistently maintained throughout the experiment.

Limbs ECG probes were placed, and the rats were paralysed (cis-atracurium 1 mg/100 gr i.p.).

Positive pressure ventilation was maintained for 10 min, and deflation respiratory mechanics were then measured arresting the deflation flow [2], [3], [21].

In order to ensure a constant volume history, the respiratory system was first inflated three consecutive times up to a pressure of 25–30 cm H_2O, and then 6 ml air was introduced by a precision glass syringe. A constant flow pump (SP 2000 Series Syringe Pump sp210iw, World Precision Instruments, USA) set in aspiration mode allowed 5 ml to be subtracted at a constant flow rate (F) of 4 ml/sec. The time for the start and stop of aspiration flow was less than 30 ms. The accuracy of the flow pump settings was accurately checked by direct measurement before the beginning of the experiments. The exact aspiration volume data were computed by electronic digital integration of the imposed flow value over the time of deflations, and found to be very close to 5 ml. The lateral tracheal pressure proximal to the tracheal cannula was monitored (142 pc 01d, Honeywell, USA) and recorded (1326 Econo Recorder, Biorad, Italy). Because no abrupt changes in the diameters were present in the circuit, flow resistance measurement errors such as those reported by CHANG and MORTOLA [22] were avoided. The same was applied in the "in vitro" measurement of equipment resistance (see below). The frequency response of the transducer and the pressure measuring system were tested by sinusoidal forcing, and found to be flat up to 20 Hz. According to the literature [19], [23], [24], this frequency response was adequate to avoid mechanical artefacts in the pressure signal records.

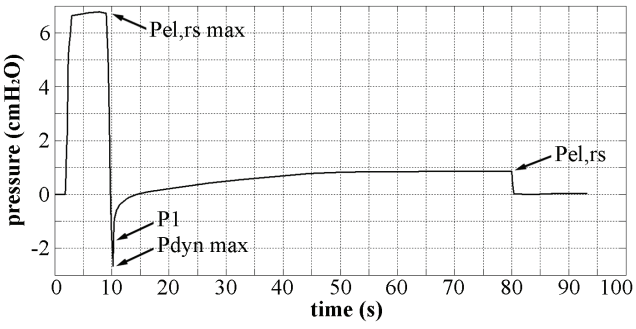

Fig. 1. Representative tracing of lateral tracheal pressure during constant flow deflation and flow occlusion. $P_{el,rs\ max}$: static elastic pressure after 6 ml air inflation. $P_{dyn\ max}$: largest pressure drop upon deflation. P_1: pressure value immediately following flow occlusion. $P_{el,rs}$: static elastic pressure after deflation

An example of the pressure tracing during constant flow deflation is reported in figure 1. The static elastic pressure of the respiratory system after deflation ($P_{el,rs}$), the total resistive pressure recoil upon deflation ($P_{max,rs}$) and the sudden resistive pressure drop upon aspiration flow interruption ($P_{min,rs}$) were digitally measured on magnified tracings. $P_{max,rs}$ was measured

as the difference between the minimum value of pressure at the end of deflation ($P_{dyn,max}$) and $P_{el,rs}$. $P_{min,rs}$ was measured as the difference between $P_{dyn,max}$ and P_1, the pressure value immediately after aspiration flow interruption (figure 1).

Thus, $P_{min,rs}$ represents the resistive pressure drop that theoretically occurs at an infinite breathing frequency [17], [18]. $P_{min,rs}$ does not include the visco-elastic pressure recoil that results from stress recovery. In contrast, the visco-elastic pressure recoil is included in the $P_{max,rs}$ value. To avoid a visco-elastic pressure component to be considered within $P_{min,rs}$, P_1 values were identified by means of the pressure evolution at the time the flow stopped [25].

The total deflation resistance of the respiratory system ($R_{max,rs} = P_{max,rs}/F$) was calculated. It includes the ohmic deflation resistance to airflow offered by the airways ($R_{min,rs} = P_{min,rs}/F$), and the pressure recoil resulting from the effects of stress recovery. This last component of $R_{max,rs}$ was isolated and quantified as the deflation viscous resistance ($R_{visc,rs} = R_{max,rs} - R_{min,rs}$).

Ohmic resistance is the normalised-to-flow pressure dissipation due to friction forces opposing the airflow in the airway, as predicted by the Poiseuille law. Viscous resistance is the normalised-to-flow pressure recoil due to stress recovery.

The equipment resistance, including the tracheal cannula and the standard three-way stop-cock, was separately measured for a flow of 4 ml/s and amounted to 0.0575 cm H_2O/ml s^{-1} (Req). Req was subtracted from the results, which therefore represent the intrinsic values.

The measurements and calculations described above were repeated for each rat after total body warming. The mean rectal temperature in control conditions was T_1 = 36.6 ± 0.3 °C. Rectal temperature rose to T_2 = 39.0 ± 0.1°C after 5–10 min warming using an infrared lamp (150 W) positioned approximately 30 cm away from the rat. The entire experimental procedure lasted less than one hour.

It was previously demonstrated that the ventilatory settings adopted here are not injurious per se to the respiratory system [11], [24]. In particular, respiratory mechanics parameters did not change at least after mechanical ventilation lasting up to one hour. Thus, it may be excluded that any change in respiratory mechanics parameters observed here might be due to a time-related effect.

Each rat was examined at two different temperatures. Thus, it was its own control, and statistical analysis of the differences observed in the mean values of respiratory mechanics parameters was performed using Student's *t* test for paired data.

2.2. Mathematical approach

For each rat, stress recovery-related pressure-time tracing was analysed by a mathematical approach. Physical models composed of ohmic resistive and volumetric terms are usually adopted to interpret the mechanical behavior of the respiratory system. The ohmic terms aim to investigate pressure drop phenomena because of gas flow, while volumetric elements specify the actual pressure-volume behavior of the system. Results from experimental activities show the time-dependence of respiratory system pressure-volume response and visco-elastic formulations should be adopted to interpret such relationship [21]. With particular regard to stress recovery processes, the trend of the viscous pressure recoil ΔP_{visc} can be interpreted by the following formulation [21]:

$$\Delta P_{visc}(t) = \sum_{i=1}^{n} a_i(\Delta V, F)\left\{1 - \exp\left[-\frac{t}{\tau_i}\right]\right\} \qquad (1)$$

where:

t – the time elapsed from the beginning of the recovery process,

ΔV – the deflated air volume,

F – air flow rate during the deflation stage,

n – specifies the number of viscous branches within the visco-elastic formulation,

τ_i – relaxation times that specify the times the viscous processes require to develop,

a_i – terms related to mechanical properties of viscous branches for the specific deflation condition.

Each viscous branch is associated to a time dependent phenomenon developing during the recovery process, such as biological tissues rearrangement phenomena, air redistribution within lung structures, etc. More details about the proposed formulation are reported in Appendix.

Experimental data from deflation tests were fitted by equation (1) to evaluate the influence of temperature on parameters a_i and τ_i. Two viscous branches were assumed within the formulation to interpret the main viscous phenomena developing during the recovery process, as micro-structural rearrangements within respiratory system tissues and air redistribution within the lung alveolar structure. The fitting was performed by an iterative stochastic-deterministic optimization procedure. The iterative process was stopped when the *R*-square, as the square of the correlation between experimental data and model results,

reached the value of 0.995. The results from the formulation proposed were found to consistently approach experimental data (figure 2).

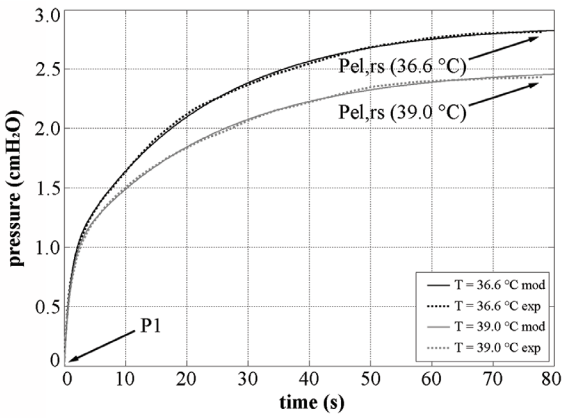

Fig. 2. Pressure-time mean experimental curves describing stress recovery observed after flow interruption at the two temperatures tested, and mathematical model-predicted exponential curves. P_1: pressure value immediately following flow occlusion. $P_{el,rs}$: static elastic pressure after deflation

3. Results

The results are summarised in table 1, table 2 and figure 2. In table 1, the mean values of $R_{min,rs}$, $R_{visc,rs}$ and $R_{max,rs}$ are reported together with the statistical indexes of the differences observed. Also indicated are the ECG-derived mean values of heart rate at the two temperatures tested.

Table 1. Mean values [±SE] of respiratory system resistances and heart rate [HR] at temperatures T_1 [36.6 ± 0.3 °C] and T_2 [39.0 ± 0.1 °C]. Statistical indexes of the differences are also reported

	T_1	T_2	p
$R_{max,rs}$ [cm H_2O/ml s^{-1}]	0.85 ± 0.2	0.66 ± 0.2	0.06
$R_{min,rs}$ [cm H_2O/ml s^{-1}]	0.19 ± 0.05	0.15 ± 0.04	n.s.
$R_{visc,rs}$ [cm H_2O/ml s^{-1}]	0.66 ± 0.1	0.5 ± 0.1	< 0.05
HR [b/min]	301 ± 38	323 ± 31	n.s.

Table 2 reports the parameters achieved from the fitting by equation (1) of pressure-time data for the different rats at the two temperatures tested, together with the mean values and the statistical indexes of discrepancy. Model results were computed by equation (1) using the mean parameters at the two temperatures and reported in figure 2 together with mean experimental data.

4. Discussion

4.1. Methodology

When modelling the respiratory system as consisting of two distinct compartments, the end-inflation occlusion method has been widely used to study respiratory mechanics in experimental animals [4], [5], [23], [24], [26] and in humans [6]–[8], [17]–[19], but very scarce data are available regarding constant flow deflations [21].

Table 2. Parameters from fitting of stress recovery data. The fitting procedure was performed by equation (1) accounting for data from tests performed on the different rats at temperatures T_1 [36.6 ± 0.3 °C] and T_2 [39.0 ± 0.1 °C]. Mean values [±SE] are reported together with statistical indexes of the differences: ** $p < 0.01$ with respect to T_1

Rat N°	T_1				T_2			
	a_1 (cm H_2O)	τ_1 (s)	a_2 (cm H_2O)	τ_2 (s)	a_1 (cm H_2O)	τ_1 (s)	a_2 (cm H_2O)	τ_2 (s)
1	1.74	7.32	0.20	0.45	1.26	4.89	0.33	0.39
2	1.73	34.68	1.26	1.41	1.06	10.39	0.34	0.55
3	1.53	12.04	0.50	0.57	1.11	10.02	1.42	1.07
4	1.18	7.03	0.50	0.40	1.16	6.81	1.09	0.75
5	1.71	15.40	0.19	0.35	1.15	11.59	0.49	1.11
6	1.49	21.40	0.87	0.84	0.21	5.54	0.30	0.71
7	6.91	32.99	2.59	0.56	6.44	27.98	2.46	1.02
8	1.63	11.09	1.05	0.73	1.19	4.69	0.46	0.34
9	2.28	14.17	0.59	0.70	0.76	2.91	0.26	0.22
10	1.40	12.97	0.66	1.08	1.46	11.25	0.63	0.80
11	0.91	10.93	0.42	0.65	1.05	9.80	0.54	0.66
12	0.72	27.62	0.79	1.90	0.79	22.31	0.71	0.93
mean ± SE	**1.94 ± 0.45**	**17.30 ± 2.66**	**0.80 ± 0.18**	**0.81 ± 0.13**	**1.47 ± 0.44****	**10.68 ± 2.06****	**0.75 ± 0.18**	**0.71 ± 0.08**

Although measured during deflations, the mean values of respiratory system resistances described here are comprised in the range of those previously reported from different laboratories in constant flow inflation experiments in the rat [4], [5], [23], [24], [26]. The results are in agreement with previous investigation on deflation experiments in the rat that reported deflation resistance values of the same order of magnitude as those presented here, particularly with respect to $R_{\min,rs}$ [21].

Ideally, the deflation flow should stop instantaneously, but this is practically impossible to achieve. However, a procedure has been proposed to correct for this technical limitation [25]. In this procedure, pressure tracings are extrapolated to account for the time that is necessary to completely halt the flow, thereby minimizing the error. This procedure was employed to analyse the deflation pressure tracings in the current study. The related corrections were found to be negligible, as previously reported in similar experiments [4], [5], [23].

We cannot exclude the possibility that some stress relaxation-related phenomena might have occurred during the time the deflations lasted, thereby affecting the subsequent $P_{\min,rs}$. Nevertheless, any effect of stress recovery would be predicted to be minor, due to the short duration of deflation compared with the stress-recovery time course (figure 1).

The mechanical ventilation settings used in these experiments were the same as those described as "non injurious" in the literature. In particular, "non injurious" ventilation lasting one hour has been shown not to alter respiratory system mechanics [11], [24]. The results here were, therefore, not influenced by the injurious effects that longer term mechanical ventilation per se might exert.

The mean values of heart rate presently observed (table 1) are comprised in the normal range for the rat and suggest, as expected, a trend to increase with body warming.

The observed $R_{\min,rs}$ mean values suggest a trend to decrease with body warming (table 1). Although not significant, probably due to a high dispersion in individual experimental data, this change is in agreement with previously reported data obtained during inflation experiments [5].

4.2. The effect of temperature change on stress recovery

The present main finding is, however, the demonstration that stress recovery exhibits temperature-dependence (table 1, table 2 and figure 2).

Confirming temperature effects on visco-elasticity, LEMPERT and MACKLEM [27] reported a temperature-dependent change in the stress-relaxation rate in excised rabbit lungs. Furthermore, it has previously been shown that the activity of various contractile cells in the lung parenchyma, including the peripheral contractile elements, affects the visco-elastic characteristics of the respiratory system [1], [13], [14]. This activity is expected to be temperature-dependent, similarly to that described for various smooth muscle cell types in the respiratory system [28], [29].

According to SUKI et al. [14], visco-elasticity combines liquid-like and solid-like characteristics of the lung tissue, and the chief mechanism responsible for the macroscopic visco-elastic behaviour is the micro-structural rearrangement processes of collagen, elastin and interstitial liquid. Interstitial liquid movements in the lung parenchyma and interaction phenomena of elastin and collagen fibres, such as reciprocal sliding, may be affected by body temperature, because these components are in coordinated thermal motions, the driving force of which is the thermal energy [14].

Experimental results reported in the literature show a reduction of both viscosity [30] and stiffness [5] of biological tissues with temperature increment. Such a coupled reduction of stiffness and viscosity suggests a decrease with temperature increment of tissue relaxation and recovery capabilities when constant stretching conditions are applied (table 1), as confirmed here.

Accordingly, it was previously shown that stress relaxation, whose molecular basis is not expected to differ from that of stress recovery, also exhibits changes with body temperature following the same direction as stress recovery changes documented here [5], both of them decreasing with increasing body temperature.

The mechanical response of the respiratory system is mainly determined by the visco-elastic behaviour of constitutive tissues and air redistribution processes within the alveolar organization. Previous experimental work demonstrated substantial temperature effects on visco-elasticity of tissues [5]. This is in keeping with previous results showing a minor contribution only of pendelluft to the total post flow interruption pressure change in comparison to the effect of true tissues visco-elasticity [17]–[19].

Accordingly, while the temperature change under investigation poorly influences air movements phenomena, because of negligible changes in air viscosity, the effects on biological tissues mechanical response appear to be consistent. The fact that significant dis-

crepancy between the parameters at temperatures T_1 and T_2 was achieved for viscous branch 1, but not for viscous branch 2, suggests that the former is to be associated with the micro-structural rearrangement phenomena within the tissues (specific tissues visco-elasticity), whereas the latter, with the air redistribution processes.

Hence, the effects of temperature on the pressure changes following the flow arrest appear to be mainly attributable to the influences on the visco-elastic characteristics of the respiratory system tissues, the effects on intrapulmonary air redistribution (pendelluft) being minor.

Our data describe the stress recovery of the respiratory system, and do not allow us to partition the relative contribution of the separated lung parenchyma and chest wall. However, it has been reported that the relative contribution of the chest wall to the visco-elastic behaviour of the respiratory system is about one third of the total in rat [31] and guinea pig [32], and even less in humans [33]–[35]. Thus, our results may be attributed mainly to the stress recovery of lung parenchyma.

5. Conclusions

The formulation of the complex visco-elastic responses of the respiratory system leads to results that, in agreement with experimental data, suggest also practical consequences, mainly regarding the elastic expiratory flow drive. In fact, the stress recovery related pressure recoil increment with time (figure 2) shows that the expiratory flow driving pressure also increases with time, approaching higher values when, for example, the breathing rate is slower. Thus, expiration is expected to be easier the lower the breathing frequency, and in condition of physiological rather than incremented body temperatures.

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Appendix

The mechanical behaviour of the respiratory system can be investigated by physical models defined as series of resistive and volumetric elements [18]. The resistive elements interpret pressure drop phenomena arising because of gas flow, while volumetric elements specify the behaviour of the system due to gas inflation or deflation. With specific regard to the characterization of the volumetric response, visco-elastic formulations are usually adopted, as deeply reported in the literature [18], [21].

Some notes about the model formulation are recalled here. Letting the pressure inside the volumetric compartment be $P(t)$, the following relationship is defined:

$$P(t) = E^0 \Delta V(t) - \sum_{i=1}^{n} q^i(t) \tag{A1}$$

where:

- t – time,
- ΔV – volume change,
- E^0 – instantaneous elastance,
- q^i – viscous variables that specify relaxed or recovered stresses after inflation or deflation phenomena, respectively, because of viscous rearrangement processes.

The evolution of viscous variables can be computed using the mechanical theory of visco-elasticity, as reported in NATALI et al. [36]:

$$q^i(t) = \frac{\gamma^i}{\tau^i} \int_{-\infty}^{t} \exp\left[\frac{-(t-s)}{\tau^i}\right] E^0 \Delta V(s) ds \tag{A2}$$

where:

- τ^i – relaxation times evaluating the time development of the viscous processes,
- γ^i – are the parameters specifying the relative contributions of the viscous processes to the overall elastance of the system, as $E^i = \gamma^i E^0$.

Equations (A.1) and (A.2), together with specific mathematical steps, as reported in RUBINI et al. [21], allow the pressure trend with time to be defined during the stress recovery stage of the deflation test:

$$P(t) = \left(1 - \sum_{i=1}^{n} \gamma^i\right) E^0 \Delta V + \sum_{i=1}^{n} \gamma^i E^0 \tau^i F \left\{1 - \exp\left[\frac{\Delta V / F}{\tau^i}\right]\right\} \exp\left[-\frac{t}{\tau^i}\right], \quad \text{(A3)}$$

where: F – flow rate during deflation. The viscous pressure recoil ΔP_{visc} is defined as the difference between pressure at the generic time t and the pressure at the beginning of the recovery process ($t = 0$):

$$\Delta P_{visc}(t) = P(t) - P(0) = \sum_{i=1}^{n} \gamma^i E^0 \tau^i F \left\{\exp\left[-\frac{\Delta V / F}{\tau^i}\right] - 1\right\} \left\{1 - \exp\left[-\frac{t}{\tau^i}\right]\right\}. \quad \text{(A4)}$$

The introduction of terms a_i leads to the equation (1):

$$a_i = \gamma^i E^0 \tau^i \left\{\exp\left[-\frac{\Delta V / F}{\tau^i}\right] - 1\right\}. \quad \text{(A5)}$$