Eur Respir J, 1994, 7, 2000–2004
DOI: 10.1183/09031936.94.07112000

European Respiratory Journal
ISSN 0903 - 1936

Changes in respiratory resistance to low dose carbachol inhalation and to pneumatic trouser inflation are correlated

AM. Lorino, F. Lofaso, H. Lorino, A. Harf

Changes in respiratory resistance to low dose carbachol inhalation and to pneumatic trouser inflation are correlated. A.M. Lorino, F. Lofaso, H. Lorino, A. Harf. ©ERS Journals Ltd 1994.

ABSTRACT: Inflation of the leg compartments of pneumatic trousers increases thoracic blood volume. The resultant response in respiratory impedence was investigated in nine normal volunteers, and compared with the response to increasing doses of inhaled carbachol.

Respiratory impedance was measured by the forced oscillation technique (4–32 Hz), and respiratory resistance at zero frequency (R_0) was extrapolated from linear regression analysis of resistive impedance *versus* frequency.

The mean increase in R_0 was 31% after inhalation of 125 µg carbachol, and 21% after inflation of pneumatic trousers. The percentage changes in R_0 following pneumatic trouser inflation highly correlated those induced by inhalation of 125 µg carbachol (r=0.98)

Our data demonstrate that, even in normal subjects, pneumatic trouser inflation causes an increase in respiratory resistance, which can be predicted by the response to a low dose of carbachol. These results support the assumption that cholinergic agents might not only be bronchoconstrictors but also vasodilators of the bronchial vessels. At a low dose, the vasodilating action of carbachol could be the major factor involved in the respiratory response.

Eur Respir J., 1994, 7, 2000–2004.

INSERM U296 and Département de Physiologie, Hôpital Henri Mondor, Créteil, France.

Correspondence: A.M. Lorino, Service d'Explorations Fonctionnelles Hôpital Henri Mondor 94010 Créteil France

Keywords: Airway resistance
blood shift
bronchopulmonary responsiveness
resistive impedance
thoracic blood volume

Received: December 17 1993
Accepted after revision July 5 1994

This study was supported by a grant from Caisse Nationale d'Assurance Maladie des Travailleurs salariés.

The effects of an increase in thoracic blood volume (TBV) on the forced expiratory volume in one second (FEV_1) appear to vary among subjects, according to their bronchial responsiveness and to experimental conditions. In response to a slight increase in TBV induced by inflation of a pneumatic trouser, no change in FEV_1 was observed in normals [1], whereas a slight fall was observed in asthmatics [2]. By contrast, in response to a larger increase in TBV induced by vascular volume expansion, a fall in FEV_1 was observed both in normals and asthmatics [3]. Compared to the normals, a larger capillary bed has been reported within the airway walls of the asthmatics [3], which might explain their bronchial response to even a small increase in TBV.

As regards the effects of a slight increase in TBV on the airway response to bronchial challenge, enhanced responses to bronchoconstrictive agents have been documented not only in asthmatics but also in normals [1, 2]. Thus, one wonders whether a slight increase in TBV may affect airway calibre without resulting in a detectable change in FEV_1. Therefore, we investigated, separately, the respiratory responses to inflation of pneumatic trouser and to inhalation of carbachol, an agent which is not only a constrictor of the bronchial smooth muscle but also a dilator of the bronchial and pulmonary vessels [1, 4]. In order to evaluate these responses, we used the forced oscillation technique, which has proved to be more sensitive than forced expiration for the detection of subclinical airway abnormalities [5].

Materials and methods

Subjects

After the experimental protocol had been approved by the Research Ethics Committee of our Medical School, the study was performed in 9 healthy young volunteers (1 female and 8 males), aged 19–22 yr. All were non-smokers and had normal lung function and no history of pulmonary or cardiovascular disease, or of atopy. They were fully informed of the nature of the experiment and gave informed consent.

Respiratory Impedance

Respiratory impedance (Zrs) was measured by the forced noise technique. The forced pseudorandom noise used in this study was composed of 29 harmonics (4–32 Hz) of the fundamental (1 Hz), with enhanced amplitudes at the lower frequencies to limit the influence of spontaneous breathing. The phases were calculated in

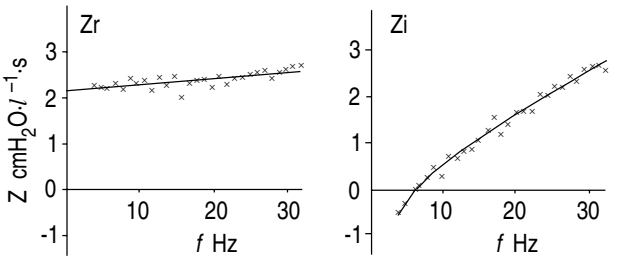

Fig. 1. – Typical data of respiratory impedance (Z). Real part (Zr) and imaginary part (Zi) are plotted as functions of frequency (*f*). Cross: measured values; solid lines: fit of models $Zr = R_0 + Sf$ and $Zi = 2\pi f\,Irs - 1/(2\pi f\,Crs)$. R_0: respiratory resistance extrapolated at zero frequency; S: frequency dependence of resistance; Irs: respiratory inertance; Crs: respiratory compliance.

order to minimize the peak-to-peak amplitude of the excitation signal. The forced signal, generated by a digital-to-analogue converter, excited, through a power amplifier, two 60 W loudspeakers attached to a 12 *l* rigid chamber. The amplitude of the resulting pressure oscillations was limited to 2 cmH_2O peak-to-peak, which resulted approximately in 0.2–0.5 $l \cdot s^{-1}$ peak-to-peak amplitudes of superimposed flow. The forced pressure excitation was applied at the mouth of the subject, who was wearing a noseclip and supporting his cheeks. Mouth pressure was measured by means of a differential pressure transducer (Sensym SCX 01D, ±70 cmH_2O), and mouth flow by means of a screen pneumotachograph (Jaeger Lilly, Rp=0.35 $cmH_2O \cdot s \cdot l^{-1}$) connected to a similar transducer. Pressure and flow signals were low-pass filtered (Butterworth, 8th order, cut-off frequency=32 Hz), and sampled at 128 Hz for 16 s. The data were then high-pass filtered (3rd order, cut-off frequency=3.5 Hz) to eliminate the low harmonics of the breathing noise. A Fast Fourier Transform algorithm was applied to adjacent 4 s periods. Impedance data were calculated from the auto- and cross-spectra obtained by averaging the spectra of 3 consecutive manoeuvres. Impedance data corresponding to a coherence value higher than 0.9 were retained for analysis, and fitted by a four parameter model. The real part of impedance was submitted to linear regression analysis, which yielded the respiratory resistance extrapolated at 0 Hz (R_0), and the slope (S) of the linear relationship of resistive impedance *versus* frequency (fig. 1). The imaginary part of impedance was submitted to multilinear regression analysis which yielded the respiratory compliance (Crs) and inertance (Irs) (fig. 1).

The quality of the fit was assessed by calculating the mean relative difference (RD) between the response of the model and that of the subject, according to the following equation proposed by OOSTVEEN *et al*. [6]:

$$RD = \frac{1}{n} \sum_{i=1}^{n} \frac{[(Re,m,i-Re,s,i)^2 + (Im,m,i - Im,s,i)^2]^{1/2}}{[(Re,s,i)^2 + (Im,s,i)^2]^{1/2}}$$

where n is the number of data points, and Re and Im are the real and imaginary parts of the impedance of the model (index m) and of the subject (index s).

Experimental protocol

All subjects were studied on two different days, at about the same time. The order of the investigations was randomized.

On one study day, bronchopulmonary responsiveness was assessed from the changes of R_0 in response to inhalational bronchial challenges of increasing doses of carbachol (125, 250 and 500 µg) following an initial challenge with physiological saline. Saline and carbachol were aerosolized with an ultrasonic nebulizer (De Vilbiss 5610 D), which delivers particles with a mean mass-median aerodynamic diameter of 3 µm. Aerosols were administered at 5 min intervals by means of an electronic breath-operated dosimeter (Médiprom FDC 88). At the end of the test, 200 µg of salbutamol, a $ß_2$-adrenergic agonist, were administered in two puffs (Ventoline, Glaxo Laboratory, France), and Zrs was measured 10 min later, to verify recovery. For each administration, Zrs was measured in the erect posture, 2 min after cessation of aerosol delivery. R_0 was expressed as a percentage of the basal value assessed after saline administration.

On the other study day, with the subject in the erect posture and wearing uninflated medical pneumatic trousers (Gladiator Airpants, Jobst Institute Inc., Toledo, USA), control values of the mechanical parameters derived from Zrs were estimated. Control values of transfer factor of the lungs for carbon monoxide (T_{LCO}), alveolar volume (V_A), and functional residual capacity (FRC) were then measured with the single-breath technique, using a water-sealed spirometer (Volugraph Diffusimat, Mijnhardt, Holland). With the subject in the supine position, the leg compartments were slowly inflated up to a pressure of 8 kPa. The subject resumed the erect position, and after a 20 minute period, measurements of Zrs, T_{LCO} and lung volumes were repeated. Pressure in both compartments was periodically checked during the experiment and readjusted if necessary. Arterial blood pressure and heart rate were monitored throughout the study (Finapress BP Monitor, Ohmeda 2300, BOC Group Inc, USA).

Statistical analysis

Statistical analysis was performed using analysis of variance, paired Student's t-test and linear regression analysis. A value of $p<0.05$ was considered as statistically significant.

Results

No significant difference was observed between the basal values obtained for any respiratory parameter on the two days of the experiment (tables 1 and 2).

Mean values of R_0, S, Crs and Irs obtained during carbachol bronchial challenge are given in table 1. Carbachol inhalation increased R_0 and decreased Irs in a dose dependent way. Decreases in S and Crs were observed only at the highest dose of carbachol. Respiratory response

Table 1. – Effects of carbachol inhalation on respiratory mechanics

	Carbachol µg			
	0	125	250	500
R_0 $cmH_2O \cdot s \cdot l^{-1}$	2.18 (0.51)	2.93* (1.18)	3.13** (1.14)	3.83*** (1.68)
S $cmH_2O \cdot s \cdot l^{-1} \cdot Hz^{-1}$	0.018 (0.14)	0.018 (0.14)	0.011 (0.022)	-0.004** (0.036)
Irs $cmH_2O \cdot s^2 \cdot l^{-1}$	0.014 (0.002)	0.013* (0.002)	0.012** (0.003)	0.011*** (0.003)
Crs $l \cdot cmH_2O^{-1}$	0.031 (0.010)	0.031 (0.010)	0.030 (0.011)	0.024** (0.007)

Data are presented as mean±SD (n=9) obtained after inhalation of increasing doses of carbachol. R_0: resistive impedance extrapolated at zero frequency; S: slope of the linear relationship between respiratory inertance and frequency; Irs: respiratory inertance; Crs: respiratory compliance. *, **, and ***: significantly different from basal value corresponding to 0 µg carbachol ($p<0.05$, 0.01 and 0.001 respectively).

Table 2. – Effects of trouser inflation on respiratory mechanics

	BI		AI	
R_0 $cmH_2O \cdot s \cdot l^{-1}$	2.21	(0.38)	2.73*	(0.94)
S $cmH_2O \cdot s \cdot l^{-1} \cdot Hz^{-1}$	0.017	(0.015)	0.015	(0.019)
Irs $cmH_2O \cdot s^2 \cdot l^{-1}$	0.014	(0.002)	0.014	(0.04)
Crs $l \cdot cmH_2O^{-1}$	0.032	(0.011)	0.031	(0.010)

Data are presented as mean±SD (n=9) obtained before inflation (BI) and after inflation (AI) of the pneumatic trousers. *: significantly higher than before inflation ($p<0.05$). For abbreviations see legend to table 1.

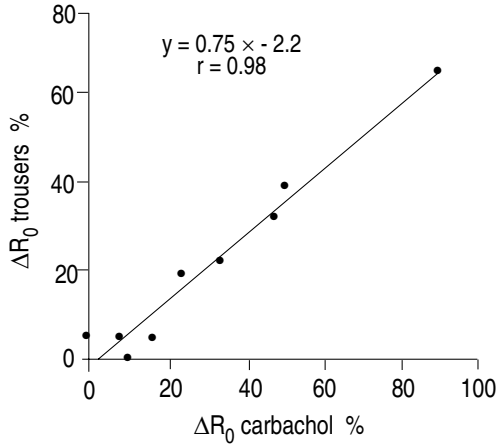

Fig. 2. – Increases in respiratory resistance at zero frequency (ΔR_0) following inflation of pneumatic trouser, plotted in relation to ΔR_0 following inhalation of 125 µg carbachol. ΔR_0 are expressed as a percentage of the corresponding R_0 basal values. Circles: data from individual subjects; straight line: regression line.

to carbachol inhalation did not reveal hyperresponsiveness in any subject, *i.e.* none of the volunteers doubled his basal value of respiratory resistance (R_0), even at the highest dose of carbachol.

Table 3. – Effects of trouser inflation on alveolar diffusion parameters

	BI		AI	
TLCO $mmol \cdot min^{-1} \cdot kPa^{-1}$	11.19	(1.52)	10.73	(1.37)
VA l	6.54	(1.24)	6.48	(1.23)
KCO $mmol \cdot min^{-1} \cdot kPa^{-1} \cdot l^{-1}$	1.74	(0.25)	1.69	(0.24)
FRC l	3.29	(0.63)	3.22	(0.61)

Data are presented as mean±SD (n=9) obtained before inflation (BI) and after inflation (AI) of the pneumatic trousers. TLCO: transfer factor of the lungs for carbon monoxide; VA: alveolar volume.

Mean values of R_0, S, Crs and Irs obtained with the uninflated pneumatic trousers are listed in table 2. Inflation of the leg compartments caused a significant increase in R_0 ($p<0.05$) but did not affect the other mechanical parameters (table 2). When expressed as percentage of basal values, the mean increase in R_0 was about 21% ($p<0.02$), with a wide range of responses (0–66%). After pneumatic trouser inflation, FRC remained unchanged and no significant change was observed in either TLCO or the KCO (TLCO/VA) ratio (table 3). Similarly, arterial blood pressure and heart rate remained statistically unchanged.

As shown in figure 2, a highly significant correlation was found between the percentage changes in R_0 following pneumatic trouser inflation and the percentage changes in R_0 in response to the lowest dose of carbachol ($r=0.98$, $p<0.0001$). The response to 125 µg inhaled carbachol was significantly greater than that to trouser inflation ($p<0.02$). By contrast, no significant correlation was found between the percentage changes in R_0 following pneumatic trouser inflation and those induced by higher doses of carbachol.

Discussion

During the past few years, several studies relating to normals and asthmatics have stressed that the vasculature might influence both basal values of airway resistance and airway response to cholinergic agonists [1–3, 7, 8]. However, in response to an increase in TBV, no systematic increase in airway resistance was demonstrated when airway calibre was assessed by the FEV_1 parameter. The small increase in TBV induced by pneumatic trouser inflation did not change FEV_1 in normals [1], whereas it slighty decreased baseline FEV_1 in asthmatics [2]. By contrast, a larger increase in TBV induced by vascular volume expansion resulted in a fall in FEV_1 in both normals and asthmatics [3]. Lastly, as regards the airway response to cholinergic agonists in normals, even a small increase in TBV has been shown to enhance the decrease in FEV_1 induced by methacholine [1]. All these results suggest, either that a minimum increase in TBV is necessary to induce an airway response, or that the FEV_1 is not sensitive enough to detect slight changes in airway calibre, such as those possibly induced by a small increase in TBV.

In the present study, the effects of carbachol inhalation and of pneumatic trouser inflation on airway resistance were assessed by the changes in the mechanical parameters derived from respiratory impedance. Impedance data were described by a four parameter model (R_0, S, Irs, Crs), which has proved sufficiently sensitive to detect early airway abnormalities [5]. The adequacy of the model to describe respiratory impedance is illustrated by the mean relative difference, which was always less than 7%. Over the 4–32 Hz frequency range, pulmonary resistance represents 70–80% of respiratory resistance [9], and the airways are responsible for most of pulmonary resistance [10]. Therefore, in the following account, the changes in resistive impedance are interpreted in terms of airway resistance.

In this study, only the bladders of the lower limbs of the pneumatic trouser were inflated, to ensure that lung volumes, particularly FRC, remained unchanged. Respiratory impedance was always measured in the standing posture. In this way, it could be assumed that the variations in airway resistance did not originate either from a decrease in FRC, nor from a postural change [11]. The fact that the basal values for R_0 were the same on the two days of the experiment allowed comparisons to be made between percentage changes.

Respiratory response to pneumatic trouser inflation

Firstly, our data demonstrate that, even in nonhyperreactive subjects, pneumatic trouser inflation results in a significant increase in airway resistance, which varies widely among individuals, ranging 0–66% of the baseline value in our nine normal subjects. These findings differ from those of REGNARD *et al*. [1] who did not demonstrate any effect of trouser inflation in their normal subjects. This apparent discrepancy may be attributed to the methods used to assess the bronchial response. Indeed, it is likely that the FEV_1 parameter derived from the forced expiration technique, and the respiratory resistance R_0 derived from the forced oscillation technique, do not provide comparable assessments of airway resistance. For example, FEV_1 has been found to be unchanged when shifting from the sitting to the supine position [12], whereas resistive impedance has been reported to significantly increase [11, 13]. In contrast to the R_0 parameter which corresponds to airway resistance assessed during quiet breathing, *i.e*. at a mean level of lung volume close to the FRC, the FEV_1 is measured during an expiratory effort, over a wide pulmonary volume range, and at a higher mean level of lung volume. Furthermore, the forced expiration is preceded by a deep inflation, which has been observed to temporarily decrease flow resistance in normals whose airways are constricted [14]. In addition, the forced expiration itself generates high alveolar pressures, which may affect pulmonary and tracheobronchial blood distribution in the same way as positive end-expiratory pressure [15, 16]. Lastly, the forced oscillation technique has proved to be more sensitive than the forced expiration technique by allowing detection of early airway abnormalities in subjects exposed to respiratory irritants, at a stage when the FEV_1 was not yet modified [5].

The increase in TBV induced by inflation of the leg compartments of pneumatic trousers has been estimated at about 200–250 ml in the erect posture [4, 2]. The small amplitude of this increase may explain why we observed no changes in TLCO, VA or KCO (table 3). Our results are in accordance with those of ABRAHAM *et al*. [17] who did not detect change in diffusing capacity of the lungs for carbon monoxide after inflation of all the bladders of pneumatic trousers. In the same way, we did not demonstrate any effect of pneumatic trouser inflation on Crs. This may be due to the fact that either the resulting increase in pulmonary blood volume was too small to induce stiffness in lung tissues, or the sensitivity of our Crs parameter was too low to detect small change in lung compliance. The frequency dependence of resistive impedance (S), which reflects distribution of flow among intrathoracic parallel inhomogeneities [18], was not affected by pneumatic trouser inflation (table 2). Thus, it is likely that the airway narrowing induced by trouser inflation was quite homogeneous.

The fact that Irs was not affected by pneumatic trouser inflation could appear inconsistent with the observed increase in Rrs. In fact, as tissue inertance is less than 10% of Irs [6], Irs mainly reflects airway inertance, which is proportional to the reciprocal of cross-section. Thus, the dependence of Irs on the airway radius (r) is weaker than the dependence of airway resistance (r^{-2} *vs* r^{-4}). Furthermore, airway inertance and resistance may be differently affected by the velocity profile. Indeed, when the airway calibre is reduced, the velocity profile tends to become blunter. Airway inertance then tends to decrease, whereas airway resistance tends to increase. Thus, the influence of the velocity profile on Irs may counterbalance, or even overbalance the influence of changes in cross-section, whereas both these influences tend to increase Rrs. This might explain why Irs remained unchanged after pneumatic trouser inflation (table 2) but decreased after carbachol inhalation (table 1).

Relationship between respiratory responses to pneumatic trouser inflation and to carbachol inhalation

The most interesting finding of this study lies in the strong correlation found in our subjects between the respiratory response to pneumatic trouser inflation and the respiratory response to the lowest dose of 125 µg carbachol. A plausible, though highly speculative, explanation of these results is that both responses originate from a modification in the airway vasculature. Carbachol, besides its direct action on the cholinergic receptors of the tracheobronchial smooth muscle, also has vasodilating effects on the bronchial and pulmonary vessels [2, 19]. This vasodilatation would be mediated by the release of endothelium-derived relaxing factor, caused by the activation of muscarinic receptors [20].

Pneumatic trouser inflation induces transient haemodynamic changes [21, 22]. At the time of respiratory impedance measurement, *i.e*. 20 min after inflation, a

steady hemodynamic state was achieved, with decreased blood volume in the lower limbs and increased TBV. The fact that values for arterial blood pressure and heart rate were then similar to their preinflation values, suggests a sympathetic reflex systemic vasodilatation evoked by cardiopulmonary receptor stimulation in response to the increase in venous return [21, 23]. The tracheobronchial vessels, which belong to the systemic vasculature, were probably affected by this vasodilatation.

Thus, although originating from different mechanisms, tracheobronchial vasodilatation might be the main factor involved in the increase in airway resistance observed after inhalation of a low dose of carbachol or inflation of pneumatic trousers. The fact that no correlation was found between the respiratory responses to the higher doses of carbachol and to the pneumatic trouser inflation, suggests that the respective contributions of the vasodilation and of the direct action on the muscarinic receptors of airway smooth muscle to the airway response could vary with the dose of cholinergic agents.

The effects of an increase in TBV and of carbachol inhalation could be cumulative. This might explain the enhanced airway response to methacholine observed in normals either after trouser inflation [1], or when shifting from the sitting to the supine position [12], and the hyperresponsiveness observed in patients with impaired left ventricular function [7].

In summary, our study demonstrates that, even in normal subjects, inflation of the lower limbs of a pneumatic trouser induces an increase in respiratory resistance comparable to the one resulting from the inhalation of a low dose of carbachol. These results suggest that the airway vasculature plays a major role in both these types of bronchoconstriction. However, further investigations are still required to verify this assumption and specify the mechanisms involved in each of these responses.

Acknowledgements: The authors gratefully acknowledge J. Régnard for thoughtful discussion, and E. Dahan for helpful technical assistance.

References

1. Regnard J, Baudrillard P, Salah B, Dinh Xuan AT, Cabanes L, Lockhart A. Inflation of antishock trousers increases bronchial response to methacholine in healthy subjects. *J Appl Physiol* 1990; 68: 1528–1533.
2. Regnard J, Dinh Xuan AT, Similowski T, Marsac J, Lockhart A. Increase in thoracic blood volume aggravates bronchial response to inhaled histamine in asthmatic subjects. *Am Rev Respir Dis* 1989; 139: A134.
3. Gilbert IA, Regnard J, Lenner KA, Nelson JA, McFadden Jr ER. Pulmonary mechanics following vascular volume expansion with intravenous saline or dextran in normals and asthmatics. *Clin Res* 1990; 38: 484S.
4. Adnot S, Kouyoumdjian C, Defouilloy C, *et al*. Hemodynamic and gas exchange responses to infusion of acethylcholine and inhalation of nitric oxide in patients with chronic obstructive lung disease and pulmonary hypertension. *Am Rev Respir Dis* 1993; 148: 310–316.
5. Brochard L, Pelle G, de Palmas J, *et al*. Density and frequency dependence of resistance in early airway obstruction. *Am Rev Respir Dis* 1987; 135: 579–584.
6. Oostveen E, Peslin R, Gallina C, Zwart A. Flow and volume dependence of respiratory mechanical properties studied by forced oscillation. *J Appl Physiol* 1989; 67: 2212–2218.
7. Cabanes LR, Weber SN, Matran R, *et al*. Bronchial hyperresponsiveness to methacholine in patients with impaired left ventricular function. *N Engl J Med* 1989; 320: 1317–1322.
8. Lockhart A, Dinh-Xuan AT, Regnard J, Cabanes L, Matran R. Effect of airway blood flow on airflow. *Am Rev Respir Dis* 1992; 146: S19–S23.
9. Nagels J, Landser J, Van Der Linden L, Clément J, Van de Woestijne KP. Mechanical properties of lung and chestwall during spontaneous breathing. *J Appl Physiol: Respirat Environ Exercise Physiol* 1960; 49: 408–416.
10. Ferris Jr, BG, Mead J, Opie LH. Partitioning of respiratory flow resistance in man. *J Appl Physiol* 1964; 19: 653–658.
11. Lorino AM, Atlan G, Lorino H, Zanditenas D, Harf A. Influence of posture on mechanical parameters derived from respiratory impedance. *Eur Respir J* 1992; 5: 1118–1122.
12. Shardonofsky FR, Martin JG, Eidelman DH. Effect of body posture on concentration-response curves to inhaled methacholine. *Am Rev Respir Dis* 1992; 14: 750–755.
13. Navajas D, Farre R, Rotger MM, Milic-Emili J, Sanchis J. Effect of body posture on respiratory impedance. *J Appl Physiol* 1988; 64: 194–199.
14. Fish JE, Ankin MG, Kelly JF, Peterman VI. Regulation of bronchomotor tone by lung inflation in asthmatic and nonasthmatic subjects. *J App1 Physiol: Respirat Environ Exercise Physiol* 1981; 50: 1079–1086.
15. Baile EM, Albert RK, Kirk W, Lakshaminarayan S, Wiggs BJR, Pare PD. Positive end-expiratory pressure decreases bronchial blood flow in the dog. *J Appl Physiol: Respirat Environ Exercise Physiol* 1984; 56: 1289–1293.
16. Cassidy SS, Haynes MS. The effects of ventilation with positive end-expiratory pressure on the bronchial circulation. *Respir Physiol* 1986; 66: 269–278.
17. Abraham E, Gong H, Tashkin DP, Baraff LJ, Geehr E. Effect of pneumatic trousers on pulmonary function. *Crit Care Med* 1982; 10: 754–757.
18. Fredberg JJ, Mead J. Impedance of intrathoracic airway models during low-frequency periodic flow. *J Appl Physiol Respirat Environ Exercise Physiol* 1979, 47: 347–351.
19. Laitinen LA, Laitinen MVA, Widdicombe JG. Parasympathetic nervous control of tracheal vascular resistance in dog. *J Physiol (Lond)* 1987; 385: 135–146.
20. Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989; 3: 2007–2018.
21. Eich RH, Smulyan H, Chaffee WR. Hemodynamic response to G-suit inflation with and without ganglionic blockade. *Aerospace Med* 1966; 35: 247–250.
22. Gray III S, Shaver JA, Kroetz FW, Leonard JJ. Acute and prolonged effects of G-suit inflation on cardiovascular dynamics. *Aerospace Med* 1969; 40: 40–43.
23. Mancia G, Mark AL. Cardiopulmonary baroreflexes in humans. *In:* Shepherd JT, Abboud M, eds Handbook of Physiology: Cardiovascular System. Vol. 3. Bethesda, 1983 pp. 794–813.