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Effects of rebreathing conditions and body size on normal human lung tissue volume

J.M. Polianski, P.D. Vivet, T.C. Chinet, S.I. Labrune, D.G. Henzel, G.J. Huchon

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ABSTRACT: To evaluate the consequences of breathing pattern variations inherent to lung disease on the rebreathing measurement of lung tissue volume (Vt), we carried out a study of ten normal human subjects in whom we assessed the effects of changes in rebreathing volume (Vreb), additional deadspace volume (AV_D), respiratory rate (RR), and body height. Vt and alveolar volume (V_A) were determined from the end-tidal concentrations of acetylene and helium. We performed Vt measurements using different combinations of Vreb (20, 30 and 50% of predicted vital capacity), of AV_D (0, 100, and 200 ml) and of RR (10, 25, and 40 br·min^{-1}). Only slow RR (10 br·min^{-1}) resulted in a higher Vt ($p<0.001$). An increase in Vreb induced an increase in V_A but not in Vt. V_A and Vt were positively correlated with the height of the subjects. We conclude that, in normal subjects, Vt increases: 1) with the height of subjects; and 2) when the respiratory rate is low. Interpretation of Vt results must take into account these two variables.

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The inert gas rebreathing method is a noninvasive technique which provides an estimate of lung tissue volume (Vt) calculated from the rate of disappearance of a soluble gas from the alveolar spaces. Vt, which comprises both parenchymal tissue and blood, has been measured using this method in animals [1–6] and humans with normal and oedematous lungs [7–13]. Computer and mass spectrometry gas analysis measures Vt faster and more accurately than previous methods, because rebreathing can be substituted for breath-holding [1, 7, 9, 10, 14, 15]. In addition to the inherent inaccuracies due mainly to inhomogeneity of ventilation [16], theoretical and animal studies [1, 15, 17, 18], and one study in man [13] have shown that Vt depends on the pattern of rebreathing. The latter study reported that Vt increases with greater penetration of the inspired gas mixture into the lungs and that the time allowed for alveolar mixing is an important determinant of Vt.

We studied a group of normal untrained subjects with a large range of body heights to determine the effect of height on Vt. Because lung diseases may induce changes in Vt measurement, not corresponding to real changes in Vt but to changes in rebreathing conditions, we also assessed the effects on Vt of varying rebreathing volumes (Vreb), additional deadspaces (AV_D) and respiratory rates (RR).

Materials and methods

Materials

The study design and technique were similar to those previously reported by OVERLAND *et al.* [10]. Subjects rebreathed through a three-way valve (Hans-Rudolph 2770, Kansas-City, MO, USA) in a bag-bottle system filled with a gas mixture containing 0.5% acetylene (C_2H_2), 9.5% helium (He), 25% oxygen (O_2) and 65% nitrogen (N_2). Gas from the valve chamber was continuously sampled at a rate of 60 ml·min^{-1} by a mass spectrometer (Centronic MGA 200, Croydon, England) with a 90% response time of 80 ms and a sampling delay of 540 ms. The rate of airflow in and out of the rebreathing bag was measured with a Fleisch No. 3 pneumotachograph (MSR, Paris, France) and a differential transducer (CH 5112/0 Enertec-Schlumberger, Vélizy-Villacoublay, France); the signal was amplified (CA 9036/0 Enertec-Schlumberger, Vélizy-Villacoublay, France) and integrated to provide a record of Vreb. The electrical outputs of the integrator and of the mass spectrometer were converted to digital data at a rate of 10 Hz per channel and stored for analysis in a PDP 11/23 computer (Digital Equipment Co., Maynard, MA, USA).

Calculations

Lung tissue volume (Vt) was calculated using the mathematical approach originally described by CANDER and FORSTER [14], as modified for the computer-assisted multiple rebreathing studies [7, 10]. "Alveolar" end-expiratory fractional concentrations of C_2H_2 relative to the concentration of the insoluble gas He were calculated for each of the first six breaths. Time zero was defined as the moment when one-half of the inspired Vreb became greater than the sum of the volumes of deadspace in the apparatus (107 ml) and the calculated deadspace in the subject's airways (body wt in kg: 2.2) [1]. The first end-expiratory fractional concentrations of each rebreathing study were discarded to minimize problems due to deadspace and mixing during the first breath; not doing so would increase variability in the measured Vt. We established a semilogarithmic plot against time of the second to sixth end-expiratory fractional concentrations of C_2H_2 in the system, and its intercept at time zero was calculated. We computed the alveolar volume (V_A) as the total gas volume at the end of inspiration during the rebreathing manoeuvres minus the apparatus deadspace and subjects' calculated deadspace. Vt was calculated using the alveolar volume, the C_2H_2 lung tissue solubility established by CANDER [19], and the intercept, which is the extrapolated end- expiratory fractional concentration of C_2H_2 relative to He at time zero [1, 14].

Experimental conditions

Ten normal healthy nonsmoking subjects agreed to participate in the study after having undergone informed consent procedures. All subjects had normal lung flows and volumes; age ranged 31–56 yrs (36±8 yrs), height 162–187 cm (171±9 cm) and weight 52–81 kg (65±10 kg). Each subject was studied under seven experimental conditions to evaluate the effects of varying Vreb, AV_D, and RR on Vt measurement. The "standard" experimental condition was defined as the following: Vreb equal to 35% of the subject's predicted vital capacity (VC), AV_D equal to 0 ml (no additional deadspace), and RR equal to 25 br·min^{-1}. The six other experimental conditions were obtained by changing one of these three variables whilst keeping the other two constant. Vreb was, respectively, 20, 35 or 50% of VC (with RR equal to 25 br·min^{-1} and AV_D equal to 0 ml), AV_D was 0, 100 or 200 ml (with Vreb equal to 35% VC and RR equal to 25 br·min^{-1}) and RR was 10, 25 or 40 br·min^{-1} (with Vreb equal to 35% VC and AV_D equal to 0 ml). AV_D was increased by adding rubber tubing between the three-way valve and the mouthpiece.

Prior to rebreathing, the bag was filled with a volume of gas mixture equal to the selected Vreb. The seated subject first breathed ambient air through the mouthpiece. After a few seconds, recording of the rebreathing bag volume and He and C_2H_2 concentrations began. Between 10–20 s later, at the end of a tidal expiration, the subject changed the position of the three-way valve from room air to the bag. Rebreathing manoeuvres started at or near functional residual capacity; subjects rebreathed the predetermined Vreb by emptying and filling the bag with each breath in time with a metronome set to the chosen RR. Each of the seven experimental conditions was repeated six times for each subject.

Statistical analysis

Results were expressed as mean±1 SD. Comparisons between V_A and Vt were made by repeated measurements from analysis of variance (Vreb, RR and AV_D) and covariance (height). Correlations and regression lines were calculated using the method of least squares [20]. We considered a value of $p<0.05$ as significant.

Table 1. – Mean±SD of alveolar volume (V_A) and lung tissue volume (Vt) under various rebreathing conditions

Rebreathing condition	Vreb % pred VC	RR br·min^{-1}	AV_D ml	V_A ml STPD	Vt ml
1	35	25	0	3782 ±499	503 ±79
2	20	25	0	3110 ±463	466 ±96
3	50	25	0	4081 ±559	481 ±79
4	35	10	0	3611 ±537	735 ±133
5	35	40	0	3716 ±632	461 ±105
6	35	25	100	3812 ±672	471 ±70
7	35	25	200	3883 ±605	497 ±93

Vreb: rebreathing volume; RR: respiratory rate; AV_D: additional deadspace.

Results

Mean and standard deviation of V_A and Vt under the various experimental conditions are indicated in table 1, and statistical significance from analyses of variance of the effects of Vreb, RR and AV_D on V_A and Vt are shown in table 2; the effects of Vreb, RR and AV_D on Vt always had height as a significant covariant (p=0.029, 0.026, and 0.019, respectively). Means and standard deviations of individual measurements of Vt for each subject with respect to the seven protocols are shown in table 3.

Because RR had a significant effect on Vt, we determined the regression lines of Vt in relation to height for various RR when Vreb was 35% of VC and AV_D was 0 ml. Figure 1 shows individual values and regression lines of Vt *versus* height at various RR (r=0.718 for 10 br·min^{-1}, r=0.741 for 25 br·min^{-1}, r=0.745 for 40 br·min^{-1}; df=8; p<0.02 under all conditions).

Significance of correlations between Vt, V_A, and Vreb when AV_D was maintained at 0 ml and RR

Table 2. – Significance of the effects of changes in rebreathing conditions on alveolar volume and lung tissue volume

Rebreathing condition	Effect on Alveolar volume (V_A)	Effect on Lung tissue volume (Vt)
Tidal volume (TV)	p<0.0001	NS
Respiratory rate (RR)	NS	p<0.0001
Additional deadspace (AV_D)	NS	NS

NS: not significant.

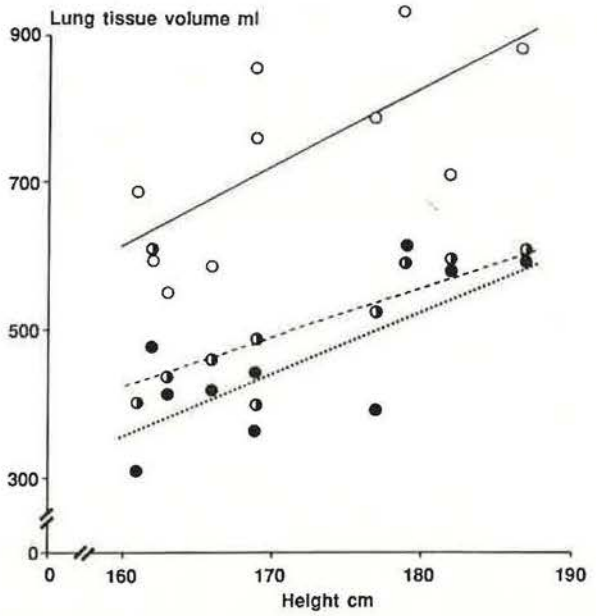

Fig. 1. – Individual values and regression lines of lung tissue volume *versus* height in ten normal subjects rebreathing at three respiratory rates (—○— 10 br·min^{-1}; --◑-- 25 br·min^{-1};··· ●··· 40 br·min^{-1}) with 35% vital capacity rebreathing volume and 0 ml additional deadspace.

Table 4. – Significance of correlations between lung tissue volume (Vt) alveolar volume (V_A) and rebreathing volume (Vreb), at various Vreb without the height covariant.

	Vreb 20% VC	Vreb 35% VC	Vreb 50% VC
Vreb – Vt	p<0.05	NS	NS
V_A – Vt	NS	p<0.05	p<0.05
V_A – Vreb	p<0.02	p<0.01	p<0.05

VC: vital capacity; NS: not significant.

Table 3. – Mean±SD of lung tissue volume (ml) for each subject with respect to rebreathing conditions and height (cm)

Rebreathing condition	JP 169	GH 179	DD 187	GL 162	MM 162	TC 169	CD 162	TK 182	OP 177	AG 166
	Subjects and their heights									
1	487	592	601	434	557	396	402	587	521	458
	±51	±84	±79	±22	±57	±51	±70	±43	±51	±69
2	540	615	599	452	327	459	346	464	449	408
	±73	±69	±92	±23	±29	±22	±51	±56	±50	±47
3	600	598	486	415	443	463	422	537	355	495
	±66	±60	±47	±47	±48	±41	±45	±82	±42	±71
4	856	933	886	549	595	759	687	709	790	585
	±83	±147	±154	±84	±87	±82	±89	±39	±136	±106
5	361	615	596	413	477	442	313	584	392	418
	±45	±81	±88	±51	±38	±47	±40	±75	±58	±54
6	417	527	551	464	507	396	390	591	411	461
	±53	±37	±82	±63	±71	±45	±51	±76	±78	±45
7	432	480	656	437	436	484	439	684	470	457
	±32	±60	±82	±57	±61	±69	±28	±90	±74	±70

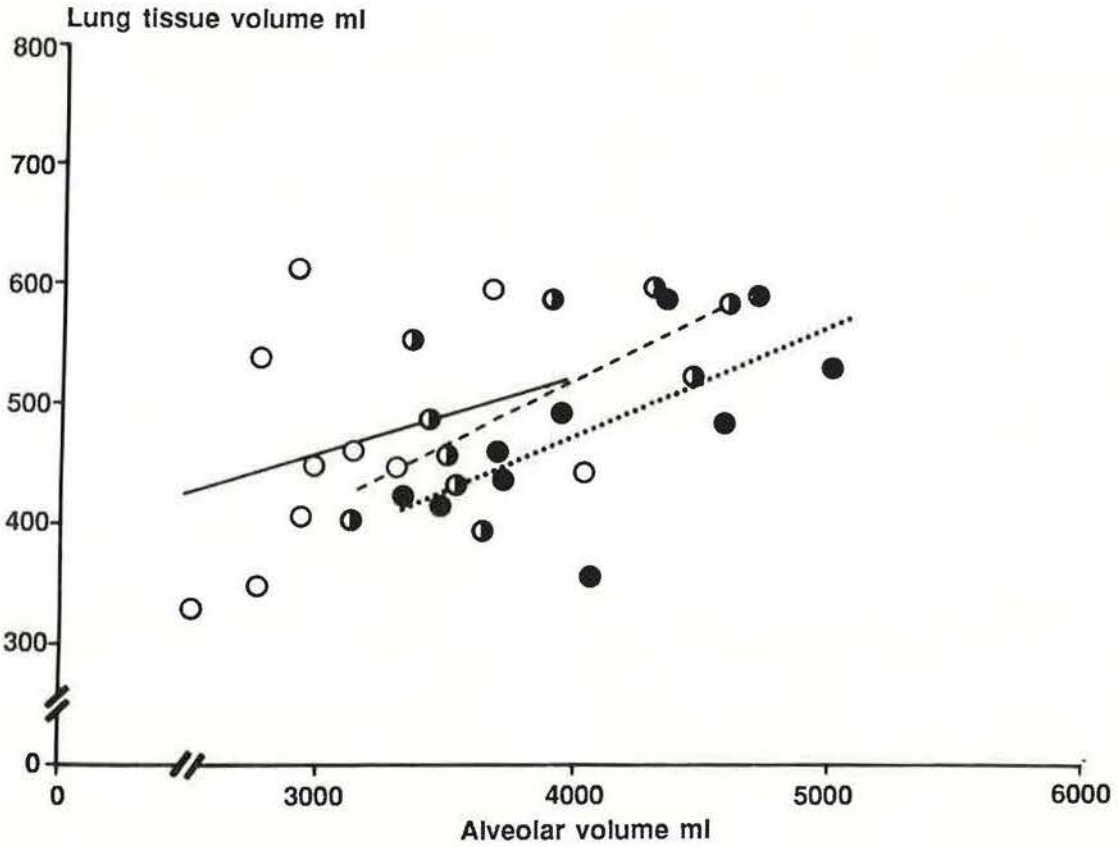

Fig. 2. – Individual values and regression lines of lung tissue volume *versus* alveolar volume in ten normal subjects rebreathing at three rebreathing volumes (—○— 20% VC, r=0.344; --◑-- 35% VC, r=0.659; ··●··· 50% VC, r=0.655) with 25 br·min^{-1} respiratory rate and 0 ml additional deadspace.

at 25 br·min^{-1} is shown in table 4. Figure 2 shows individual values and regression lines of Vt *versus* VA.

Discussion

Our study of normal subjects showed that measurements of Vt were influenced neither by AVD nor by changes in Vreb. However, Vt increased both with height and when RR was low (10 br·min^{-1}).

Additional deadspace

AVD up to 200 ml did not cause any change in Vt. This result is no different from that of PETRINI *et al.* [15]; using a mathematical model of the human lung, they found only a 22 ml (4%) increase in Vt for a 200 ml deadspace, and an 83 ml (14%) increase in Vt for a 600 ml deadspace. Since, in our study, the total deadspace volume (anatomical, apparatus and additional deadspaces) never exceeded one third of Vreb, we may have precluded any demonstrable effect on Vt of increasing AVD. Furthermore, in our study, the effect of increasing AVD is quite different from that of an increase in physiological deadspace, since the tracer gas cannot diffuse across the inorganic material of AVD as it does across the conducting airways.

Rate of rebreathing

Slow RR was associated with a rise in Vt. Vt in the standard condition of rebreathing (Vreb=35% VC, AVD=0 ml) increased from 503 ml for a 25 br·min^{-1} RR to 735 ml for a 10 br·min^{-1} RR. KALLAY *et al.* [13] also found an inverse relationship between Vt and RR and concluded that the time allowed for alveolar mixing is an important determinant of Vt. Part of this increase might be explained by technical artifacts. Average Vt decreased to 627 ml when the fifth and sixth breaths were excluded from the Vt calculation. At a 10 br·min^{-1} RR these two breaths are obviously affected by C_2H_2 recirculation, as already observed [21]. Unfortunately, using only the second, third, and fourth end-expiratory fractional concentrations leads to a larger variability in the results.

Rebreathing volume

Vreb did not have any effect on Vt in our normal human subjects. Various cofactors may influence the effect of Vreb on Vt: the species, the lung conditions (normal or oedematous), and the end-expiratory lung volume during the rebreathing manoeuvre. Changes in Vreb did not have any effect on Vt in normal dogs, but did have an effect in dogs with pulmonary oedema [5].

In contrast, Vt increases with Vreb in both normal sheep and sheep with pulmonary oedema [6]; this discrepancy may be explained by better access of the tracer gas to the terminal respiratory units of the animal with "low" inspiratory capacity at the functional residual capacity, either under normal conditions (sheep) or under the effects of pulmonary oedema (dog) [6]. KALLAY *et al.* [13] studied the effect of Vreb on Vt in six normal human subjects keeping the inspired volume and V_A constant but with various end-expiratory volumes during the rebreathing manoeuvre. They found an increase in Vt when Vreb increased and the end-expiratory lung volume decreased. By contrast, in our study the end-expiratory lung volume (residual functional capacity) was constant, a difference in experimental conditions which may explain the difference in the effects of Vreb on Vt.

Height

Vt was strongly correlated with the height of the subjects – the taller the subjects, the higher the Vt. OVERLAND *et al.* [10] related Vt to predicted total lung capacity (TLC) in order to normalize for differences in body size. CRAPO *et al.* [12] normalized TLC by expressing it as TLC/FRC (functional residual capacity). They reasoned that height is the major factor in predicted TLC or FRC and that expressing Vt as fraction of predicted TLC or FRC is a simple way of allowing for height, as lung size depends on the subject's height. This reasoning agrees with the positive correlation also found between Vt and V_A in most studies [1, 10–13, 22].

V_A depends on both height and Vreb. Therefore, the relationship between V_A and Vt may interfere with height. We have shown that Vt was positively correlated with V_A when Vreb was maintained at 35 and 50% of VC, and pooling all the data, that Vt was also correlated with V_A. Similar results have been observed by OVERLAND *et al.* [10] in man and by PETERSON *et al.* [1] in dogs. KALLAY *et al.* [13] found that when V_A was increased from residual volume (RV) by progressively larger inspiratory volume and Vreb, Vt increased 50 $ml \cdot l^{-1}$ V_A. The usual explanation for this effect is that an increase in Vt caused by an increase in V_A is related to deeper penetration of the tracer gas in the peripheral pulmonary units where the ratio between tissue volume and gas volume is maximal. Another explanation is that because V_A depends on both height and Vreb, the relationship between Vt and V_A might be explained by an effect of height on V_A and on Vt. Our data in normal human subjects show that Vreb has no effect on Vt and that Vt has height as a covariant in all statistical analyses that we performed. However, KALLAY *et al.* [13] showed that increasing V_A by increasing the end-expiratory volume whilst keeping the inspiratory volume and Vreb constant did not significantly alter Vt. This might be explained by the much narrower range of height of Kallay's subjects compared to ours, which might preclude any detection of the effects of height on Vt. This strongly suggests that the link between Vt and V_A is due to a common dependence of V_A and Vt on height in normal human subjects when starting to rebreathe from FRC.

In conclusion, our study in normal human subjects shows that Vt is not influenced by an increase in AV_D and that Vt increases when RR is low. Whilst changes in Vreb influence V_A, they do not affect Vt, suggesting that in normal human subjects and over a wide range of Vreb and RR, the relationship between V_A and Vt is probably due to height.

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Effets des conditions de "rebreathing" et de la taille corporelle sur le volume tissulaire pulmonaire chez l'homme normal.
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RÉSUMÉ: Pour évaluer les conséquences des variations du type respiratoire inhérent à la maladie pulmonaire sur la mesure du volume du tissu pulmonaire par "rebreathing" (Vt), nous étudié 10 sujets humains normaux, chez qui nous avons apprécié les effets des modifications du volume de "rebreathing" (Vreb), du volume de l'espace mort additionnel (AV_D), de la fréquence respiratoire (RR), et de la taille des sujets. Vt et le volume alvéolaire (V_A) ont été déterminés à partir des concentrations d'acétylène et d'hélium, à la fin du volume courant. Nous avons exécuté des mesures de Vt en utilisant différentes combinaisons de Vreb (20, 35 et 50% de la capacité vitale prédite), de AV_D (0, 100 et 200 ml) et de RR (10, 25 et 40 resp·min^{-1}). Seuls les RR bas (10 resp·min^{-1}) entraînaient une augmentation du Vt ($p<0.001$). Une augmentation de Vreb entraîne une augmentation de V_A, mais pas de Vt. V_A et Vt sont en corrélation positive avec la taille des sujets. Nous concluons que, chez les sujets normaux, Vt augmente: 1) avec la taille des sujets; et 2) en cas de rythme respiratoire lent. L'interprétation des résultats de Vt doit donc prendre ces deux variables en considération.
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