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Maximum volumes in excised human lungs: effects of age, emphysema, and formalin inflation

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ABSTRACT The volume of air at a transpulmonary pressure (PL) of 25 cmH_2O was measured in 28 emphysema-free and 39 emphysematous excised adult human lungs and in the lungs of 53 infants and children. In the adult emphysema-free lungs, this volume (V_{25}) was significantly correlated with body length in males but, corrected for body length, not significantly correlated with age in either males or females. V_{25} was on the average 20 per cent larger than predicted TLC in non-emphysematous lungs in vivo. The lungs were also inflated and fixed with formalin at a constant PL of 25 cm H_2O and their volume measured (VL). Marked and variable underinflation compared to V_{25} occurred in the adult lungs and VL minus lung weight averaged 75% of V_{25} and 91% of predicted TLC. In infants and children, the ratio of VL minus lung weight to V_{25} averaged 1·08 with a range of 0·58 to 1·84. The larger the lungs, the smaller the ratio, suggesting that fixation played a role in producing the small VL. In the emphysematous lungs, a significant correlation between the degree of emphysema and V_{25} was found. However, a statistically significant increase in V_{25} only occurred when the emphysema grade was greater than 5.

Many studies have demonstrated a relationship between total lung capacity (TLC) and height, age and height, or height and weight in normal subjects.[1-6] Since TLC is governed by the interaction of lung elastic properties, chest wall mechanics, and inspiratory muscle strength, it is of interest to document maximum lung volumes in excised lungs in which only lung elastic properties are a factor.

In patients with emphysema, TLC has been shown to be increased in some studies,[7-9] but not in others,[10-14] and there is uncertainty as to what degree of emphysema is necessary to produce such a change.

In morphometric studies of lung structure, it is common practice to inflate the lungs with a liquid fixative at a constant transpulmonary pressure (PL) of 25 cmH_2O (2·5 kPa).[15-17] It has been shown that the lung volume attained by this method correlates with predicted TLC in life[16 17] but that, in individual lungs, marked under and overinflation occurs.

In this study, we measured the volume of air in excised lungs distended at a PL of 25 cm H_2O (V_{25}) and the volume of the lungs fixed in formalin at a constant PL of 25 cmH_2O (VL) of 28 non-emphysematous and 39 emphysematous excised human lungs in order to define: (1) the relationship of V_{25} to age and height in non-emphysematous lungs, (2) the relationship of V_{25} to the degree of emphysema, and (3) the relationship of VL to V_{25}.

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Methods

Sixty-seven lungs (eight right, 59 left, 23 female, 44 male) were obtained at necropsy from subjects who died out of hospital from non-respiratory causes. Lungs with acute or chronic lung disease other than chronic bronchitis or emphysema were excluded. The pulmonary vasculature was ligated, the lungs weighed, the main bronchi cannulated, and the lungs tested for leaks. When small leaks were present, these were repaired by ligature or Krazy Glue[R] (Krazy Glue Inc, Chicago, Illinois). The lungs were degassed and placed on a moist tray inside a volume displacement plethysmograph and inflated to a PL of 30 cmH_2O (3 kPa), PL being measured with a Validyne DP15 pressure transducer. Volume change was measured with a Krogh spirometer. After the third inflation, the volume was measured first at a PL of 30 cmH_2O (V_{30}) and then at a PL of 25 cmH_2O to make sure that the lungs were on the flat part of the expiratory pressure-volume curve. The manoeuvre was then repeated to check for reproducibility. In a separate experiment, three lungs were inflated to a PL of 20 cmH_2O (2 kPa) and then to a PL of 30 cmH_2O in order to study the effects of varying the maximum inspiratory PL on lung volume. The average increase in lung volume at the high pressure was only 3%. Increasing the PL would, therefore, not lead to appreciably higher lung volumes and would also be impracticable as the incidence of leaks and interstitial emphysema increases substantially. V_{25} of the lungs was calculated by assuming that the left lung contributed 47% and the right lung 53% of TLC.[18]

The lungs were removed from the plethysmograph and inflated with 10% buffered formalin at a constant PL of 25 cmH_2O using a modification of the apparatus described by Heard.[15] The lungs were observed when first inflated with formalin from a PL of zero and apparent complete inflation occurred within a few minutes in all cases. Constant inflating presure of 25 cmH_2O was then maintained for two to three days. The bronchus was clamped and the lung volume measured by water displacement. Total lung volume (VL) was estimated by assuming the same proportions for left and right lungs as stated above. The lungs were sliced in a parasagittal plane and paper mounted whole lung sections produced from midsagittal slice. These were used to score the degree of emphysema on an arbitrary scale of 0-100.[19] An adjacent to midsagittal slice from each lung was impregnated with barium sulphate and examined under the dissecting microscope to facilitate detection of minor degrees of emphysema.

In a separate study, the disease-free lungs from 53 neonates, infants, and children were similarly inflated with air to a PL of 25 cmH_2O and subsequently formalin fixed at the same pressure. The ratio of (VL minus lung weight)/V_{25} was calculated for all the lungs. Lung weight was subtracted from VL since VL includes the volume of lung tissue and this was assumed to have a density of 1·0.

The relationship of V_{25} to age, postmortem body length (cm), and emphysema grade was determined using regression analysis. Predicted TLC during life for height and age were calculated using the data of Knudson *et al*[1] (TLC related to height only) and Goldman and Becklake[2] (TLC related to age and height).

Table 1 *Sex, age, and lung volumes of 28 emphysema-free lungs*

Lung	*Sex*	*Age (yr)*	*Ratio of V_{25} to predicted TLC (Knudson et al[1])*	*Ratio of V_{25} to predicted TLC (Goldman and Becklake[2])*
1	F	47	1·17	1·08
2	M	65	1·18	1·20
3	M	22	1·16	1·11
4	M	39	1·17	1·15
5	M	35	1·08	1·01
6	M	56	1·01	0·99
7	M	22	1·08	1·02
8	F	77	1·09	1·05
9	M	44	0·99	0·99
10	F	74	1·41	1·37
11	F	76	0·95	0·91
12	M	14	0·96	0·84
13	M	55	1·31	1·32
14	M	48	0·91	0·87
15	M	16	1·19	1·05
16	M	54	1·26	1·26
17	M	55	1·38	1·37
18	M	48	1·20	1·16
19	F	52	1·44	1·33
20	F	64	1·43	1·35
21	M	17	1·18	1·03
22	M	25	1·11	1·03
23	M	41	1·51	1·37
24	F	27	1·04	0·91
25	F	86	1·24	1·23
26	M	71	1·02	1·04
27	F	58	1·64	1·57
28	F	63	1·09	1·02
		Mean	1·20	1·14
		± SE	± 0·03	± 0·04

Results

Twenty-eight lungs including those of 10 females were found to be free from emphysema. The ratio ± SD of V_{25} to V_{30} in these lungs was 0·997 ± 0·005. The age, sex, observed V_{25}, and ratios of V_{25} to predicted TLC in life using the data of Knudson *et al*[1] and Goldman and Becklake[2] are shown in the table 2. It can be seen that V_{25} in excised lungs was nearly always higher than TLC expected in life and averaged 20% greater than predicted by Knudson *et al*[1] and 14% greater than predicted by Goldman and Becklake.[2] For male lungs, a significant correlation with height was found ($V_{25} = 0·089 \cdot$ height $- 8·05$, $r = 0·57$, $p < 0·05$) but not for females ($r = 0·47$). However, there was a trend for the females and the lack of significance may have been the result of the narrow height range (156–168 cm) compared with the males (160–187 cm) and the small number of cases. The regression line for males is compared with that obtained by Knudson *et al*[1] in fig 1. There was no difference in slope but a significant difference in elevation ($p < 0·025$). Since the volumes in this study were measured at ATP and the volumes in vivo are corrected to BTPS, the

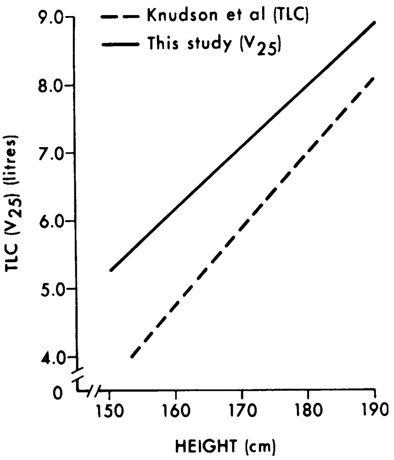

Fig 1 *Relationship between the regression lines of TLC versus height in vivo and V_{25} versus height in excised lungs. The slopes are not significantly different, but the elevation of V_{25} versus height is significantly higher.*

difference in elevation is even greater. There was no significant correlation of V_{25} with age independent of height in males or females (age versus V_{25}/height^3, $r = 0{\cdot}32$, $r = 0{\cdot}09$ for males and females, respectively). For the latter calculation, the data of the three males under the age of 20 years were excluded since height increases up to adulthood and then declines with age. There was also no significant correlation between (V_L minus lung weight)/height^3 and age ($r = 0{\cdot}07$, $r = 0{\cdot}25$, for males and females, respectively).

The ratio of V_L minus lung weight to V_{25} in the normal lungs was $0{\cdot}75 \pm 0{\cdot}02$ with no significant difference between males and females. There was also no correlation between the lung size (V_{25}) and this ratio ($r = -0{\cdot}11$) or age and this ratio ($r = 0{\cdot}06$). The range of ratios was 0·48 to 0·94. The ratio of V_L minus lung weight to predicted TLC was $0{\cdot}91 \pm 0{\cdot}03$ and was not related to age ($r = 0{\cdot}28$). The V_L minus lung weight was significantly lower than predicted TLC ($p < 0{\cdot}01$, paired Student's *t* test). Therefore, the correction factor which would need to be applied to any linear measurement in these lungs to correct back to V_{25} ranged from 1·28 to 1·02 (mean 1·10), being given by $(V_{25}/V_L \text{ minus lung weight})^{1/3}$ However, to correct back to TLC in life, the mean factor is only 1·03 $(\text{predicted TLC}/V_L \text{ minus lung weight})^{1/3}$

In the children's lungs, V_{25} ranged from 4·4 to 1905 ml and the V_L minus lung weight/V_{25} ratios ranged from 0·58 to 1·84 (mean ± SD = 1·08 ± 0·38). However, a significant relationship between V_{25} and this ratio was found (V_L minus lung weight/V_{25} ratios ranged from 0·58 to 1·84 (mean ± SD = 1·08 ± 0·38). However, a significant relationship between V_{25} and this ratio was found (V_L minus lung weight/V_{25} = 1·14 − 0·00026 V_{25}, $r = -0{\cdot}41$, $p < 0{\cdot}01$).

In 39 lungs (age range 37–77 yr), varying degrees of emphysema were noted ranged from trace to grade 60. The ratio of V_{25} to V_{30} in the emphysematous lungs was $0{\cdot}999 \pm 0{\cdot}002$. A significant relationship between the ratio of V_{25} to predicted TLC during life (Knudson *et al*[1]) and the emphysema grade was found (V_{25}/predicted TLC = 0·0075 emphysema grade ± 1·23, $r = 0{\cdot}45$, $p < 0{\cdot}01$). The emphysematous lungs were divided into three groups on the basis of the degree of emphysema. The ratios of V_{25}/predicted TLC in the three groups can be seen in fig 2. Only in groups 2 and 3 was this ratio significantly higher than in the emphysema-free lungs. However, group 1 was not significantly different from groups 2 and 3. A significant relationship between the ratio of V_{25}/predicted V_{25} was also obtained (V_{25}/predicted V_{25} = 0·0051 emphysema grade + 1·06, $r = 0{\cdot}40$, $p < 0{\cdot}05$). The ratios of V_{25}/predicted V_{25} in the three groups (fig 3) yielded similar results to those in fig 2 with only groups 2 and 3 being significantly different from the emphysema-free lungs, but no difference between groups 1, 2, and 3.

The mean ratio ± SD of V_L minus lung weight/V_{25} was $0{\cdot}77 \pm 0{\cdot}12$ with a range of

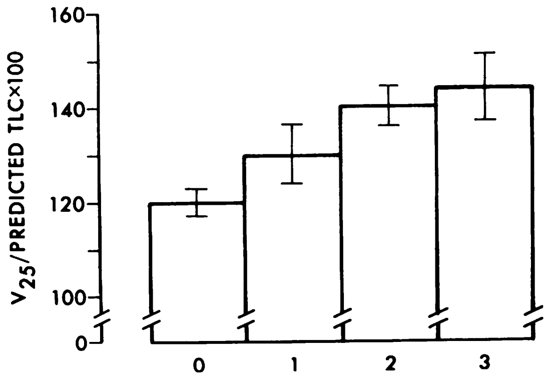

Fig 2 *V_{25} expressed as a percentage of predicted TLC in vivo in the four groups of lungs. Groups 2 and 3 are significantly different from group 0 but there is no significant difference between groups 1, 2, and 3. Group 0 = 28 emphysema-free lungs, group 1 = 11 lungs with emphysema grade 0-5, group 2 = 20 lungs with emphysema grade 5-20, group 3 = eight lungs with emphysema > 20.*

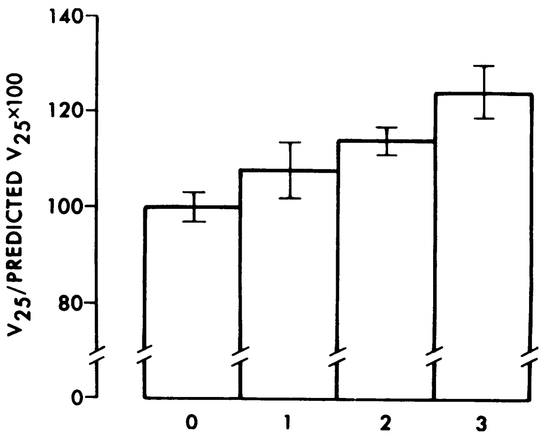

Fig 3 *V_{25} expressed as a percentage of predicted V_{25} in the four groups of lungs. (Predicted V_{25} obtained from the relationship of V_{25} to body length in emphysema-free lungs.) Groups 2 and 3 are significantly different from group 0 but there is no significant difference between groups 1, 2, and 3.*

0·43 to 0·98 and this was very similar to the normal lungs. This ratio was not related to the emphysema grade ($r = 0\cdot10$).

The emphysema-free lungs were then combined with those showing minimal emphysema—that is, group 1, in order to recalculate the age regression of V_{25}/height^3. Again, the three male lungs under age 20 years were excluded. Now, the males demonstrated a significant relationship between age and V_{25}/height^3 ($V_{25} = 0\cdot0072$ age $+ 1\cdot12, r = 0\cdot48, p < 0\cdot05$), but not the females.

Discussion

Many studies have shown that TLC in life in normal individuals is positively correlated with height.[1-6] In some studies, TLC has also been shown to have a significant negative correlation with age independent of height.[2 4] Since it has been well documented that lung elastic recoil decreases with age,[1 20 21] it is thought that weaker inspiratory muscles in conjunction with a stiffer chest wall[22] in older individuals either exactly counterbalances the loss of elastic recoil of the lungs or slightly overcompensates for it. In this study, V_{25} was measured in excised lungs free of the effects of age-related changes in chest wall and respiratory muscle mechanics. It is, therefore, somewhat surprising that in the emphysema-free lungs, V_{25} was only significantly related to height and not to age, although this is in keeping with the results of a previous study[18] which showed no increase with age of V_L corrected for height. There was a trend for the males to have a greater V_{25} with increasing age but not for females, and this may be related to the observation that loss of elastic recoil with age is greater in males than in females.[21] The V_{25} was shown to be close to maximum lung volume by the demonstration that V_{25} was on the plateaux of both the inspiratory and expiratory pressure-volume curves. The V_{25} was consistently larger than predicted from data obtained in living subjects, averaging 20% increase compared with the best prediction data. This suggests that the chest wall at all ages constrains the lungs and prevents them reaching their maximum volume or, alternatively, that some basic alteration in elastic properties of the lungs occurs after death. In addition, despite loss of elastic recoil with age, the maximum lung volume remains fixed throughout life, governed by body size. Astrand *et al*,[23] in a longitudinal study, demonstrated an increase in TLC with age in a group of physically fit subjects. In the light of the present findings, this would be difficult to explain unless the chest wall became more compliant and respiratory muscles stronger with age.

There are a number of possible factors in this study which may affect these conclusions. If height had been systematically underestimated by the measurement of postmortem body length, then the predicted values for TLC would be falsely low. In addition, little was known about the medical histories of these subjects. It is possible that some of them may not have been included in a series of normal, healthy volunteers during life. On the other hand, it is also quite possible, indeed likely, that the minimal or equivocal degreees of emphysema of the lungs in group 1 would not have led to their exclusion from such a series. When they, with their inherent age bias and larger V_{25}, were combined with the emphysema-free lungs, a significant age-related change in V_{25} resulted in males.

If lungs are distended with formalin, the bronchi tied and left overnight, they lose about 25% of lung volume.[24] It is in order to overcome this shrinkage that a constant P_L is used during the time of fixation. However, in the present study, despite application of constant pressure, underinflation of varying degree occurred compared to V_{25}. Because of this underinflation, the linear measurement of a structure in the fixed lung would need to be increased by an average of 10% but in some individual lungs, by up to 30% to correct back to V_{25}, whereas much lower factors correct back to TLC during life.

The question arises as to which is the "correct" lung volume for morphometric purposes. VL minus lung weight is close to predicted TLC in this series, averaging 91%. (VL minus predicted ideal lung weight would be closer to TLC since many of the lungs were heavy.) In another study,[18] we have shown that VL is, on the average, 8% greater than radiologically determined TLC and when allowance is made for volume of tissue, the results of the two studies are similar. It thus appears that fixation proceeds rapidly enough to make the lung stiffer so that VL never reaches V_{25} and fortuitously, approaches TLC. However, since the relationship between V_{25} and VL was not related in the adult lungs to sex, age, or lung size, it may be better to measure V_{25} first so that the correction factors in individual lungs can be calculated.

It is interesting, however, that in the children's lungs which had a much greater range of lung volumes, a significant relationship between the degree of formalin inflation and V_{25} was found. This suggests that since formalin inflation of the larger lungs takes longer, perhaps some fixation takes place during inflation thus limiting the maximum volume obtainable.

The lungs with emphysema demonstrated a V_{25} significantly greater than the normal lungs. However, when divided into groups according to the severity of emphysema, it became apparent that significant increases in V_{25} occurred only when the emphysema grade was greater than 5. However, since groups 1, 2, and 3 were not significantly different from each other, it may also be argued that the presence of emphysema is more important than the grade in determining increases in lung volume.

Underinflation with formalin of the emphysematous lungs occurred to an almost equal degree to that observed in the emphysema-free lungs. By inflating the lungs with air first and meticulously sealing all leaks when present, we can be certain that leakage was not a factor. However, emphysematous lungs tend to be leaky and when this occurs, VL may be expected to be even smaller. Therefore, once again, this suggests the need for preliminary air inflation. It should be noted that overinflation (VL minus lung weight/V_{25} ratio of $> 1\cdot0$) was not observed in any lung in the present series.

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References

1 Knudson RJ, Clark DF, Kennedy TC, Knudson DE. Effect of aging alone on mechanical properties of the normal adult human lung. *J Appl Physiol* 1977; **43**:1054–62.

2 Goldman HI, Becklake MR. Respiratory function tests. Normal values at median altitudes and the prediction of normal results. *Am Rev Respir Dis* 1959; **79**:457–67.

3 Kaltreider NL, Fray WW, Van Zile Hyde H. The effect of age on the total pulmonary capacity and its subdivisions. *Am Rev Tuberc* 1938; **37**:662–89.

4 Needham CD, Rogan MC, McDonald I. Normal standards for lung volumes, intrapulmonary gas-mixing, and maximum breathing capacity. *Thorax* 1954; **9**:313–25.

5 Grimby G, Söderholm B. Spirometric studies in normal subjects. III. Static lung volumes and maximum voluntary ventilation in adults with a note on physical fitness. *Acta Med Scand* 1963; **173**:199–206.

6 Marshall R. The physical properties of the lungs in relation to the subdivisions of lung volume. *Clin Sci* 1957; **16**:507–15.

7 Finucane KE, Colebatch HJH. Elastic behavior of the lung in patients with airway obstruction. *J Appl Physiol* 1969; **26**:330–8.

8 Boushy SF, Aboumrad MH, North LB, Helgason AH. Lung recoil pressure, airway resistance and forced flows related to morphologic emphysema. *Am Rev Respir Dis* 1971; **104**:551–61.

9 Pratt PC, Jutabha O, Klugh GA. Quantitative relationship between structural extent of centrilobular emphysema and post mortem volume and flow characteristics of lungs. *Med Thorac* 1965; **22**:197–208.

10 Gelb AF, Gold WM, Wright RR, Bruch HR, Nadel JA. Physiologic diagnosis of subclinical emphysema. *Am Rev Respir Dis* 1973; **107**:50–63.

11 Zamel N, Hogg J, Gelb A. Mechanisms of maximal expiratory flow limitation in clinically unsuspected emphysema and obstruction of the peripheral airways. *Am Rev Respir Dis* 1976; **113**:337–45.

12 Watanabe S, Mitchell M, Renzetti AD. Correlation of structure and function in chronic pulmonary emphysema. *Am Rev Respir Dis* 1965; **92**:221–7.

13 Thurlbeck WM, Henderson JA, Fraser RG, Bates DV. Chronic obstructive lung disease. A comparison between clinical, roentgenologic, functional and morphologic criteria in chronic bronchitis, emphysema, asthma and bronchiectasis. *Medicine* 1970; **49**:81–145.

14 Jenkins DE, Greenberg SD, Boushy SF, Schweppe HI, O'Neal RM. Correlation of morphologic emphysema with pulmonary function parameters. *Trans Assoc Am Physicians* 1965; **78**:218–30.

15 Heard BE. A pathological study of emphysema

of the lungs with chronic bronchitis. *Thorax* 1958; **13**:136–49.
16 Matsuba K, Thurlbeck WM. The number and dimensions of small airways in non-emphysematous lungs. *Am Rev Respir Dis* 1971; **104**: 516–24.
17 Matsuba K, Thurlbeck WM. The number and dimensions of small airways in emphysematous lungs. *Am J Pathol* 1972; **67**:265–75.
18 Thurlbeck WM. Post mortem lung volumes. *Thorax* 1980; in press.
19 Thurlbeck WM, Dunnill MS, Hartung WS, Heard BE, Heppleston AG, Ryder RC. A comparison of three methods of measuring emphysema. *Hum Pathol* 1970; **1**:215–26.
20 Turner JM, Mead J, Wohl ME. Elasticity of human lungs in relation to age. *J Appl Physiol* 1968; **25**:664–71.
21 Bode FR, Dosman J, Martin RR, Ghezzo H, Macklem PT. Age and sex differences in lung elasticity, and in closing capacity in non-smokers. *J Appl Physiol* 1976; **41**:129–35.
22 Mittman C, Edelman NH, Norris AH, Shock NW. Relationship between chest wall and pulmonary compliance and age. *J Appl Physiol* 1965; **20**:1211–6.
23 Astrand I, Astrand PO, Hallbäck I, Kilbom A. Reduction in maximal oxygen uptake with age. *J Appl Physiol* 1973; **35**:649–54.
24 Thurlbeck WM. The incidence of pulmonary emphysema: with observations on the relative incidence and spatial distribution of various types of emphysema. *Am Rev Respir Dis* 1963; **87**: 206–15.