

## Dietary vitamin E supplement does not inhibit changes in lung pressure-volume characteristics produced by bleomycin in hamsters

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*Dietary vitamin E supplement does not inhibit changes in lung pressure-volume characteristics produced by bleomycin in hamsters. S. Sato, H. Nakamura, K. Takada, K. Takahashi.*

**ABSTRACT:** We tested the hypothesis that a dietary supplement of vitamin E (VE) may lessen changes in pulmonary pressure-volume characteristics induced by intratracheal instillation of bleomycin. Ninety-nine male hamsters were separated into a control group (C), a VE supplement group (E), a control plus bleomycin group (CB) and a VE supplement plus bleomycin group (EB). Animals were killed at 30, 40, 55, 70 and 90 days and the pressure-volume curves of their lungs, both air-filled and saline-filled, were determined. Bleomycin was instilled on the thirtieth day. Lung volumes, compliance and curve-fitting data were compared. The mean serum VE concentration was  $17.5 \mu\text{g} \cdot \text{ml}^{-1}$  in groups E and EB as compared to  $5.7 \mu\text{g} \cdot \text{ml}^{-1}$  in groups C and CB. Despite the remarkably high VE content, no significant difference was found between groups CB and EB for the parameters compared.

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Bleomycin is an effective agent in the control of a number of human cancers but produces diffuse pulmonary fibrosis [1, 2]. This adverse effect is used experimentally to induce a lesion that resembles human idiopathic pulmonary fibrosis [3, 4]. The ability of bleomycin to induce pulmonary fibrosis may be partially attributable to its metabolism into free radicals [5, 6], since it acts as a potent generator of hydroxyl radicals [7, 8] and enhances lipid peroxidation [9]. Vitamin E is a potent biological antioxidant, that inhibits lipid peroxidation by scavenging free radicals generated during the univalent reduction of molecular oxygen [10, 11, 12]. This evidence led us to hypothesize that supplemental administration of vitamin E may lessen the toxic effects of bleomycin on pulmonary mechanics.

The present work was designed to see if bleomycin-induced changes in pulmonary mechanics might be prevented by supplemental vitamin E in hamsters. The vitamin E supplement was administered in the diet. Feeding of the test diet began thirty days before the first estimation of the effect of increased serum level in order to observe the effect of a steady-state elevation of vitamin E. We obtained air- and saline-filled lung pressure-volume curves and analysed lung volumes, compliances and curve-fitting data.

### Methods

Ninety-nine male golden hamsters at postnatal week 3, weighing 30-44 g, were separated into the following four groups; control diet (C), vitamin E

supplemented diet (E), control diet plus bleomycin (CB), and vitamin E supplemented diet plus bleomycin (EB). The vitamin E content of the control diet was 2 mg/100 g. Vitamin E supplemented animals were fed on the control diet supplemented with 58.5 mg/100 g. After 30, 40, 55, 70 and 90 days of feeding the test diets, air- and saline-filled lung pressure-volume (P-V) curves were obtained. On day 30, a single dose of bleomycin (0.5 mg/100 g body weight in 0.4 ml of saline) was administered intratracheally to groups CB and EB. To groups C and E, 0.4 ml of normal saline was instilled.

Intraperitoneal pentobarbital sodium anaesthesia (7 mg/100 g body weight) was followed by tracheal cannulation. A suture was placed around the trachea to prevent air or saline from leaking. The anterior side of thorax was removed and the animal was exsanguinated by ventricular puncture. The blood was centrifuged and the serum was frozen for the measurement of vitamin E concentration by a modified Rindi's method [13]. The lung was not removed from the thorax in order to avoid pleural injury. The vertebral column was severed at the lumbar-thoracic junction. The lung-thorax preparation was degassed in a vacuum chamber for 10 min until the lung appeared liver-like. The evacuation pressure was controlled through a needle valve to avoid boiling of the water. Immediately after degassing, P-V curves with air were determined in a semi-closed plastic box containing water. The air was kept at  $37 \pm 0.5^\circ\text{C}$ , and saturated with water vapour. The lungs were inflated by a series of 5 sec infusions of



0.33 ml, each followed by 5 sec for stress relaxation. When the transpulmonary pressure (Ptp) reached 25 cmH<sub>2</sub>O, the lungs were deflated in similar steps, until Ptp reached approximately 1.0 cmH<sub>2</sub>O. Three infusion-withdrawal cycles were obtained, and the last curve was subjected to analysis. The air volume at a Ptp of 25 cmH<sub>2</sub>O was defined as total lung capacity (TLC). The tracheal pressure was monitored with a pressure transducer (NIHON KOHDEN, TP-101T), amplified (NIHON KOHDEN, DC amplifier), and recorded on a direct writing recorder (SAN - EI SOKKI RECTI - HORIZ 8S).

The second degassing was followed by measurement of three infusion-withdrawal cycles with isotonic saline in a 0.9% saline bath heated to 37°C. The open end of the tracheal catheter was placed under degassed saline with the animal inclined at an angle of about 20° head down. Saline at 37°C was injected and withdrawn in the same way as for the air P-V curves. The total saline volume injected on the first inflation was equal to the total air volume injected on the first inflation with air. The third curve was analysed.

The volume was monitored with a potentiometer (MIDORI SOKKI CPP - 35) coupled to the movement of the glass syringe mounted in the infusion-withdrawal pump, and recorded on the recorder described above. Compliance of the lung was measured as the slope of the steepest portion of the P-V curve. The lung compliances were expressed as the

ratio to the predicted total lung capacity (P-TLC) which was obtained using an allometric equation obtained from nose-to-tail length and TLC of normal hamsters in our laboratory (TLC in ml =  $3.929 \times \text{length in cm} - 1.051$ ). Another analysis of the pressure-volume curves was performed using the method reported by PENGELLY [14]. Pressure-volume points above 50% of TLC on a deflation limb were used to determine a theoretical curve for each lung.

Data were subjected to the Student's unpaired t-test for all possible comparisons between groups. A probability value  $p < 0.05$  was considered statistically significant.

### Results

Two hamsters died immediately after bleomycin instillation. On every experimental day, animals fed on the vitamin E supplemented diet had significantly higher serum vitamin E levels than those fed on the normal diet (fig. 1). The average vitamin E level of the supplemented groups ( $17.5 \mu\text{g} \cdot \text{ml}^{-1}$ ) was approximately three times the average value of the control diet groups ( $5.7 \mu\text{g} \cdot \text{ml}^{-1}$ ).

Average air-filled P-V curves are shown in figure 2. Animals treated with bleomycin had significantly smaller absolute values of TLC than those of groups C and E on experimental day 55, i.e. 25 days after

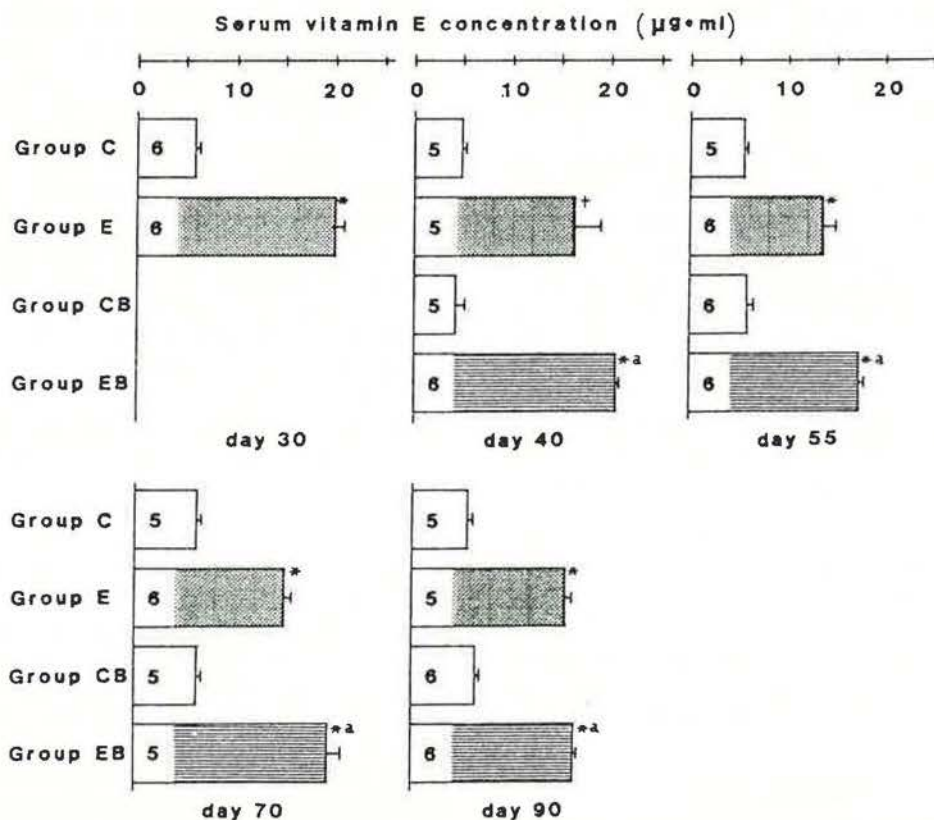


Fig. 1. Serum vitamin E concentration in  $\mu\text{g} \cdot \text{ml}^{-1}$  (mean  $\pm$  SE). A statistically significant difference compared with the animals fed on the control diet is denoted by \* $p < 0.0001$ ,  $\dagger p < 0.005$ . A statistically significant difference compared with the animals in group CB is denoted by (a)  $p < 0.0001$ . The number of animals is given in the histograms.

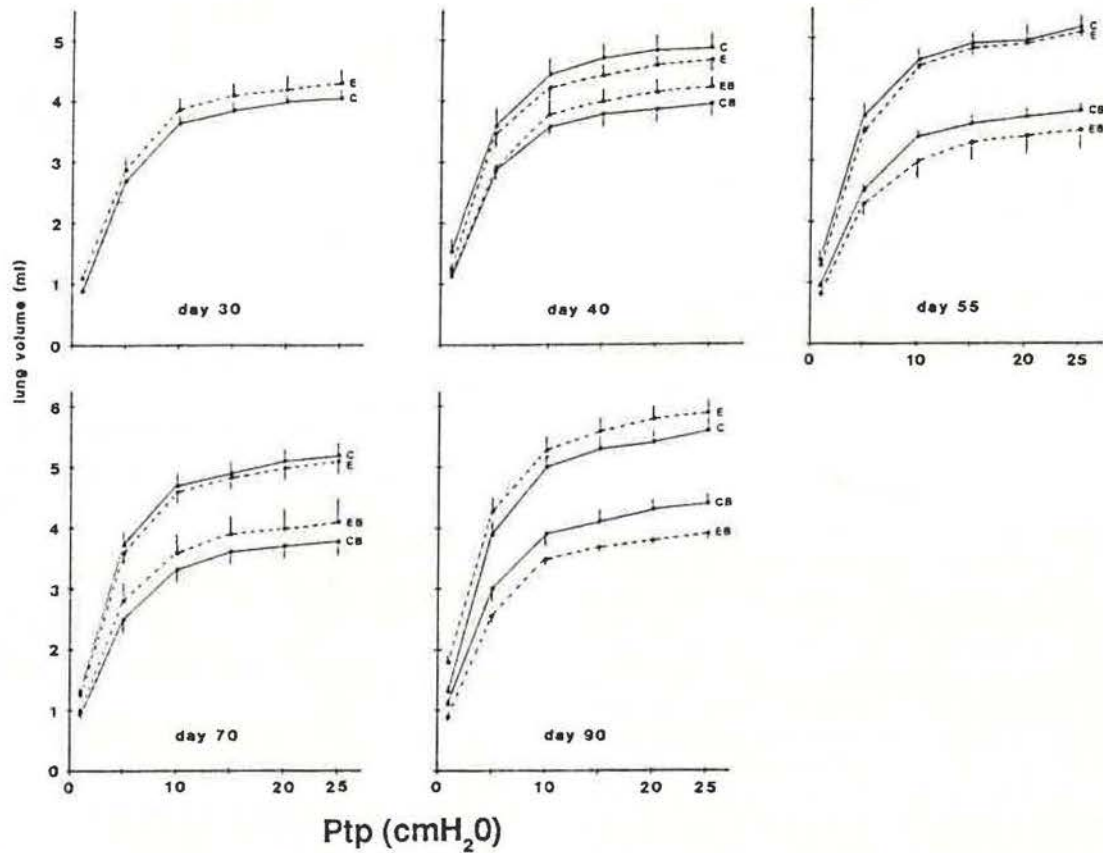


Fig. 2. Average air-filled pressure-volume curves. The mean volumes and SE at transpulmonary pressures of 1, 5, 10, 15, 20 and 25 cmH<sub>2</sub>O are shown. The capital letters C, E, CB and EB refer to the groups. The number of animals is as in figure 1.

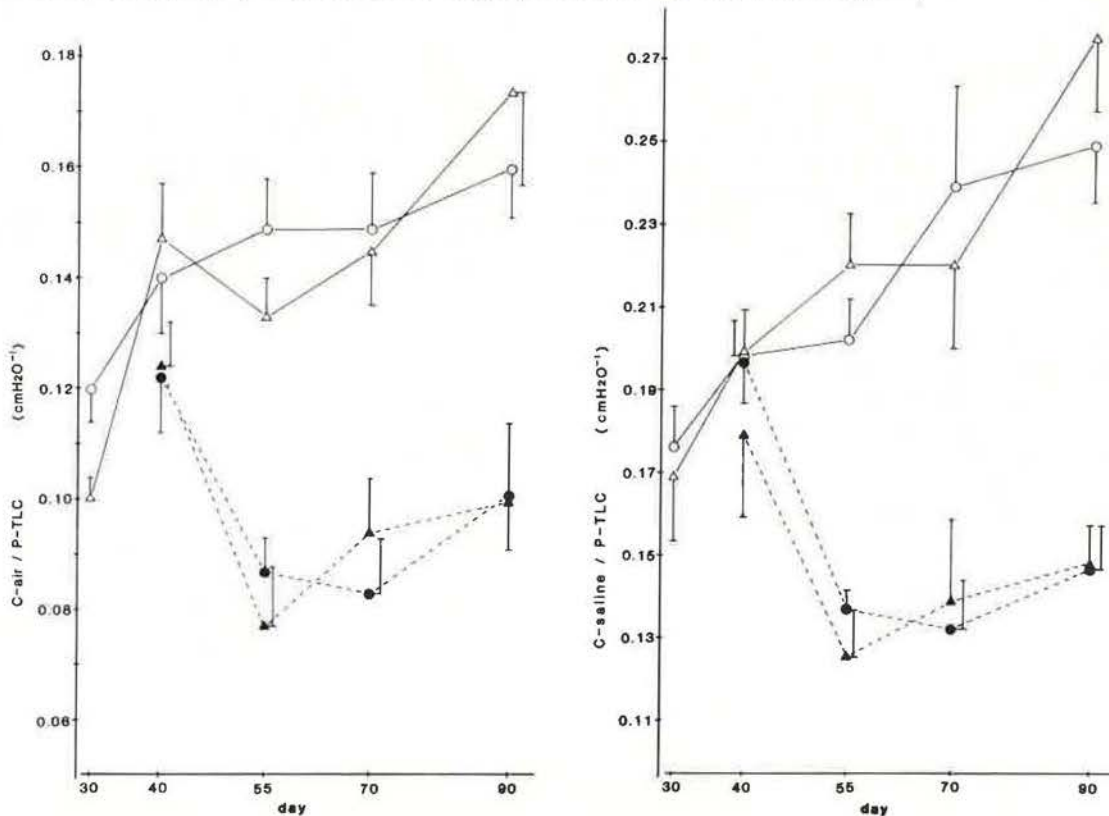


Fig. 3. Means and SE of lung compliance on air-filled (C-air, left) and saline-filled (C-saline, right) P-V curves expressed as a fraction of predicted TLC. Open circles: group C; open triangles: group E; closed circles: group CB; closed triangles: group EB. From 55 days onwards, the two bleomycin-treated groups had less compliant lungs than the two untreated groups ( $p < 0.001-0.05$ ), and there was no difference between the two groups of bleomycin-treated animals (CB vs EB) at any stage. The number of animals is as in figure 1.



bleomycin administration and thereafter. The result was the same even when the values were expressed as a fraction of P-TLC. Vitamin E supplement did not affect the TLC values.

Means and SE of lung compliance on air and saline P-V curves expressed as a ratio to P-TLC are plotted against experimental days in figure 3. The values were significantly lower for the bleomycin groups from experimental day 55 onwards. There was no significant effect from vitamin E supplement.

The half-inflation pressure of the groups treated with bleomycin was significantly higher than that of control groups after experimental day 55. However, there was again no significant effect of vitamin E supplement.

### Discussion

In studies where body size, and consequently lung size, varies among the different groups, it is appropriate to express lung volume and compliance as a percentage of predicted TLC, as we have done here, rather than in absolute values. However, no regression equations for the prediction of TLC can ever be free from variability, which leads to some correction errors. Thus we applied a curve-fitting method [14] in the analyses of our P-V curves. Half-inflation pressure obtained using this method has been demonstrated to be independent of lung size and a good index of lung stiffness. No significant difference between half-inflation pressure of groups CB and EB was detected, supporting the results derived from the analysis of compliances.

We measured only serum vitamin E content. It is possible that serum vitamin E level does not reflect true lung tissue levels of vitamin E. However, vitamin E concentrations have been reported to increase comparably in both plasma and lung tissue after administration of a vitamin E supplemented diet in rat [15]. We therefore believe that in our hamsters the lung tissue level of vitamin E as well as that of the serum was raised.

Alternatively, bleomycin-induced pulmonary damage as well as recovery of the lung tissue may be a multistep process and the step in which vitamin E is involved may not require more vitamin E than the normal amount. The serious inflammatory stage inherent to bleomycin toxicity may be under the control of a multiplicity of mechanisms or substrates other than vitamin E which protects cells and tissues from oxidant.

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**RÉSUMÉ:** Nous avons testé l'hypothèse qu'un supplément diététique de vitamine E pourrait diminuer les modifications des caractéristiques de la courbe pression-volume pulmonaire induites par l'instillation intra-trachéale de bleomycine. Nonante-neuf hamsters mâles ont été répartis en un groupe contrôle (C), un groupe avec supplément vitaminique E (E), un groupe contrôle plus bleomycine (CB), et finalement un groupe avec supplément de vitamine E et bleomycine (EB). Les animaux ont été tués aux jours 30, 40, 55, 70 et 90, et les courbes de pression-volume de leurs poumons ont été déterminées, le poumon étant rempli d'air ou de solution saline. La bleomycine a été instillée au trentième jour. L'on a comparé les volumes pulmonaires, la compliance et les données d'ajustement des courbes. La concentration sérique de vitamine E dans les groupes E et EB fut approximativement trois fois élevée (valeur moyenne  $17.5 \mu\text{g}\cdot\text{ml}^{-1}$ ) que celle des groupes C et CB (valeur moyenne  $5.7 \mu\text{g}\cdot\text{ml}^{-1}$ ). Malgré ce contenu remarquablement élevé en vitamine E, l'on n'a trouvé aucune différence significative entre les groupes CB et EB pour les paramètres étudiés. Ces résultats suggèrent qu'une élévation du taux sérique de vitamine E est sans importance pour normaliser les altérations induites par la bleomycine dans les caractéristiques pression-volume pulmonaires.