Apo{superscript}tm1Unc{subscript} mice have impaired alveologenesis, low lung function, and rapid loss of lung function

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Massaro D, Massaro GD. Apo{superscript}tm1Unc{subscript} mice have impaired alveologenesis, low lung function, and rapid loss of lung function. Am J Physiol Lung Cell Mol Physiol 294: L991–L997, 2008. First published March 14, 2008; doi:10.1152/ajplung.00013.2008.—Diminished lung function, indicated by a low forced expiratory volume in one second (FEV{subscript}1), and short physical stature, predict early mortality from all causes, including cardiovascular, among smokers and never smokers. The basis for these associations is unclear, and, it is not known if there is a pulmonary morphological component to the relationship between low FEV{subscript}1 and early death in a general population. Some apolipoprotein E genotypes also predict atherosclerosis and early mortality. These considerations led us to examine the Apo{superscript}tm1Unc{subscript} (Apo) mouse, in which the apolipoprotein E gene is deleted, and that develops dyslipidemia, atherosclerosis at an early age, and has a shorter life span than the founder wild-type (wt) strain.

We asked if Apoe mice have a morphological or functional pulmonary phenotype. We measured the size, number, and surface area of pulmonary gas-exchange units (alveoli) and mechanical properties of the lung. Compared with wt mice, Apoe mice had: 1) diminished developmental alveologenesis, 2) increased airway resistance in early adulthood, 3) high lung volume and high dynamic and static compliance in later adulthood, 4) more rapid loss of lung recoil with age, and 5) were less long than wt mice. These findings in mice indicate the association of a low FEV{subscript}1 with early death in humans may have developmental, and accelerated ageing, related pulmonary components, and that dietary, genetic, or dietary and genetic influences, on lipid metabolism may be an upstream cause of inflammation and oxidative stress, currently considered to be major risk factors for COPD.

FEV{subscript}1; predict early death; atherosclerosis; short stature; diet; genetics; cholesterol

Epidemiological studies indicate that in a general population of never smokers or smokers, a low forced expiratory volume in one second (FEV{subscript}1), compared with its expected value, is a predictor of early mortality from all causes, including cardiovascular (3, 33). Why FEV{subscript}1 has this predictive capacity in a general population, especially among never smokers, is unclear. A putative factor linking a low for expected FEV{subscript}1 to cardiovascular disease is evidence of the presence of chronic systemic inflammation, based on finding elevated concentrations of markers of systemic inflammation, in individuals with a low FEV{subscript}1 (31), and in those with cardiovascular disease (34). Oxidative stress is also considered a risk factor for cardiovascular disease (34), for a low FEV{subscript}1 in a general population (26), and for chronic obstructive pulmonary disease (COPD), which is a cause of a low FEV{subscript}1 (27). However, in COPD, there are morphological abnormalities associated with, and indicative of, a low FEV{subscript}1 (10). In contrast, it is not known if in a general population without COPD, or without other identified pulmonary diseases, a pulmonary morphological abnormality accompanies a low FEV{subscript}1. It is also unknown if impaired pulmonary development contributes to a low FEV{subscript}1 in a general population. A related, equally unclear, association is that of short stature, a low for predicted FEV{subscript}1, and early death (2).

Apolipoprotein E has an important role in triglyceride and cholesterol metabolism, but substantial evidence links it to biological processes other than lipid transport (18–20), and certain apolipoprotein genotypes are associated with early death (15). The Apo{superscript}tm1Unc{subscript} (Apo) mouse, which lacks a functional apolipoprotein E gene (38), develops atherosclerosis at an early age and has a shorter life span than its founder wild-type (wt) mouse (25). These considerations led us to test the hypothesis that Apoe mice have a pulmonary morphological and functional phenotype consistent with the presence of a low FEV{subscript}1 in humans, and that lung function diminishes with age at a more rapid rate in Apoe mice than in wt mice. We found Apoe mice 1) were shorter than wt mice, 2) had diminished formation of pulmonary gas-exchange units (diminished developmental alveologenesis), 3) exhibited high airways resistance as young adults consistent with a low FEV{subscript}1 in humans, and 4) had a more rapid loss of mechanical lung function with age compared with wt mice. Thus, this model of atherosclerosis and early death has a pulmonary phenotype of impaired developmental alveologenesis, low lung mechanical function, a rapid decline of lung function with age, and low body length.

MATERIALS AND METHODS

Animals. We purchased wt C57BL/6J and B6.129P2-Apo{superscript}tm1Unc male mice from Jackson Laboratory. The mutant mice were originally generated by inactivation of the apoliprotein E gene (38). The C57BL/6J was produced by Jackson Laboratory by back crossing the Apo{superscript}tm1Unc mutation 10 times to C57BL/6J mice (Jax Mice Data Sheet, The Jackson Laboratory). All mice were housed in the Department of Comparative Medicine at Georgetown University School of Medicine, maintained on a 12:12-h light-dark cycle, and allowed Rodent Chow 5001 and tap water ad libitum. Mice were killed by anesthesia (indicated by failure to withdraw from a toe pinch) with xylazine (10 mg/kg) plus ketamine (75 mg/kg). All procedures were approved by the Georgetown University Animal Care and Use Committee and comply with the National Institutes of Health guidelines.

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Morphometry. Lungs were fixed at a transpulmonary pressure of 20 cmH₂O. This was achieved by inserting a cannula into the trachea and instilling 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4. The trachea was then ligated, the lungs excised from the chest, and fixation was continued for 2 h at 0–4°C. Lung volume was measured by volume displacement (29). Lungs were then cut into blocks, and blocks were selected for further processing by a systematic sampling technique (7). Fixation was continued as previously described (22, 24). Serial sections of lung were cut, and alveolar air spaces were identified by following gas-exchange structures through a complete set of prints (22, 24). The selector method was used to select alveoli for analysis; the volume of an average alveolus was determined by the point-sample intercepts method (4) as previously described in detail (22, 24); and the number of alveoli was calculated from the average volume of an alveolus and the total volume of alveolar air. Alveolar surface area was estimated by point and intersection counting (35).

Measurement of lung mechanical properties. We achieved a surgical level of anesthesia as described above, then shaved the ventral side of the neck, made a skin incision, identified the trachea by blunt dissection, made a small incision between two tracheal rings, and inserted and tied an 18-gauge cannula into the trachea. The mouse was placed in a supine position and connected via the tracheal cannula to a computer-controlled small animal ventilator (flexiVent, Scireq Scientific Respiratory Equipment). Mice were ventilated at 150 breaths/min with room air at a volume of 10 ml/kg body mass. After 3 min of ventilation, we initiated the computer-generated program to measure lung compliance (C) and the impedance of the respiratory system. The latter was measured 2 min after an inflation of 30 cmH₂O and was then fitted by flexiVent software to a constant-phase model to provide measures of airway resistance (Rn), tissue damping (G), tissue elastance (H), and the ratio of G to H (tissue hysteresivity). For measurement of impedance, the flexiVent software provides a computer-generated volume signal made up of 19 mutually primed sinusoids ranging from 0.25 to 19.5 Hz that was applied to the airway opening.

Statistical methods. For each parameter measured or calculated from measurements, the values for individual animals were averaged.
per experimental group, and the standard deviation of the group mean was calculated. A one-way analysis of variance was used for multiple group comparisons, and the statistical significance of the difference between two groups was obtained by an unpaired two-tailed t-test (StatMost3).

RESULTS

Body mass, nose to rump length, and lung volume. Apoe mice weighed less than wt mice at ages 1 and 3 mo; both groups weighed the same at age 8 mo (Fig. 1A). The nose-rump distance was less in Apoe than in wt mice at 1 and 3 mo of age (not measured at 8 mo) (Fig. 1B).

Alveolar dimensions. One-month- and 3-mo-old Apoe mice had gas-exchange units with a smaller surface-to-volume ratio (Fig. 1C) and lower surface area (Fig. 1D) than wt mice. This indicates their gas-exchange units are larger than those of wt mice. To further document the differences in alveolar dimensions between wt and Apoe mice, we measured alveolar dimensions from serial sections of lung of 3-mo-old mice. Three-month-old Apoe mice had larger and fewer alveoli than same-age wt mice (Table 1), confirming the surface-to-volume measurements. Thus, alveoli in Apoe mice were larger, fewer, and had a smaller surface area than alveoli in wt mice. Some of these differences can be appreciated by examination of histological sections (Fig. 2).

Table 1. Volume of an average alveolus (v̄a), alveolar number (Na), and body mass-specific number of alveoli (Na/kg)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>v̄a × 10⁻⁴</th>
<th>Na × 10⁻⁶</th>
<th>Na × 10⁻⁵/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6J</td>
<td>4.3±0.3</td>
<td>9.2±0.4</td>
<td>331±39</td>
</tr>
<tr>
<td>Apoe tm1Unc (4)</td>
<td>7.7±0.3</td>
<td>4.6±0.7</td>
<td>183±24</td>
</tr>
<tr>
<td>P value</td>
<td>0.0002</td>
<td>0.0006</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Means ± SD are given. Values in parentheses indicate the number of mice.

Comparison of ageing-related changes in lung function. Between ages 3 and 8 mo, there was a 2.5-fold greater percentage increase of lung volume in Apoe than in wt mice (Fig. 5A). Over the same period, lung compliance increased about twice as much in Apoe mice as in wt mice (Fig. 5B), and resistance in the conducting airways fell 25% in wt mice and 42% in Apoe mice (Fig. 5C). An age-related fall of airways resistance in wt mice, as we found, was reported earlier by others (11). The basis for the decline is

Fig. 2. Light microscopic view of the lung of 1-mo-old wt (A) and Apoe (B) mice and 3-mo-old wt (C) and Apoe (D) mice. Scale bars, 100 μm.
not clear. However, airway resistance measured with the flexiVent is measured in inspiration and expiration. Therefore, it is possible bronchial smooth muscle, as other muscles, atrophies with age resulting in loss of tone of the conducting airways, with consequent greater widening of the conducting airways during inspiration in older mice.

Tissue damping declined in both groups of mice, but the decline did not differ to a statistically significant degree between wt and Apoe mice (Fig. 6A). Tissue elastance fell twice as much in Apoe as in wt mice, between ages 3 and 8 mo (Fig. 6B). Although the absolute values for hysteresivity did not differ among groups, when calculated as a percentage change with age, the percent increase of hysteresivity between age 3 mo and age 8 mo was greater in Apoe compared with wt mice (Fig. 6C). An increase of hysteresivity is thought to signify alteration in the properties of tissues, increased heterogeneity of tissue, or a combination of the two events (12, 16).


DISCUSSION

Apolipoprotein E has a major role in triglyceride and cholesterol metabolism, and differences in Apoe genotype frequencies are associated with increased mortality (19). Compared with wt, Apoe mice die sooner (25), have earlier onset and more severe atherosclerosis (25), and have hyperlipidemia (9), increased circulating levels of LDL cholesterol (9), very low density lipoprotein cholesterol (9), triglycerides (25), and total cholesterol (20).

The lung is not widely considered an organ that suffers the ill effects of dyslipidemia, including hypercholesterolemia. However, in animals, a diet high in cholesterol produces atheromatous changes in the pulmonary vasculature (5, 6, 8, 13, 30). Providing rabbits with a diet high in cholesterol results in “senile emphysema” and has led to the suggestion that disordered lipid metabolism results in abnormal pulmonary alveoli (14). This latter notion is supported by our present findings, and, on a more mechanistic level, by the demonstration that a high cholesterol diet in rabbits induces in the lung a several hundred-fold elevation of 15-lipoxygenase activity (1); the latter has a pro- as well as an anti-inflammatory effect, and may have a proatherogenic effect (for review, see Ref. 36).

The biological basis of the epidemiological link between atherosclerosis and COPD, among never smokers and smokers (32), is not understood, but it is thought that systemic inflammation present in COPD may foster atherosclerosis (17, 33). However, our study with Apoe knockout mice, which have disordered lipid metabolism including hypercholesterolemia (38), earlier work by others indicating hypercholesterolemia in rabbits results in senile emphysema (14), and the marked increase of 15-lipoxygenase in lung and heart in mice fed a

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Fig. 4. Tissue damping (A), elastance (B), and hysteresivity (C). Means ± SD are given. Values within the bars give the number of mice. A: ANOVA = <0.009; *P < 0.02 vs. 8-mo-old wt; †P < 0.003 vs. 8-mo-old Apoe, and because this is the second comparison, the Bonferroni method indicates the intergroup difference is not statistically significant. B: ANOVA = 4.9E-007; *P = 0.0004 vs. 8-mo-old wt; †P < 0.0001 vs. 8-mo-old Apoe. C: there were no differences among the group means.

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Fig. 5. Change of lung volume (A), compliance (B), and airway resistance (C) with age. Means ± SD are given.
high cholesterol diet (1), suggest the influence of diet, genetics, or both on lipid metabolism, is the more upstream mediator of the concurrence of COPD and cardiovascular disease than is inflammation and oxidative stress (17, 33).

The formation of mature pulmonary gas-exchange units (alveoli) is, in part, by subdivision (septation) of gas-exchange sacculles present at birth. This subdivision results in gas-exchange structures (alveoli) that are smaller, more numerous, and with a greater surface area than the gas-exchange sacculles present at birth (23). Thus, subdivision of the sacculles present at birth produces gas-exchange units with a higher surface-to-volume ratio than those present at birth. The lung’s gas-exchange surface area per lung volume is also larger at age 14 days than at birth. Compared with 1-mo-old wt mice, septation is impaired in Apoe mice, and by age 3 mo, there is not “catch-up” septation, i.e., the impairment of septation persists into adulthood. The presence of fewer alveoli in Apoe mice would result in fewer alveolar attachments to, and hence less exchange surface area per lung volume. Thus, subdivision of the sacculles present at birth produces gas-exchange units with a higher surface-to-volume ratio than those present at birth. The lung’s gas-exchange surface area per lung volume is also larger at age 14 days than at birth. Compared with 1-mo-old wt mice, septation is impaired in Apoe mice, and by age 3 mo, there is not “catch-up” septation, i.e., the impairment of septation persists into adulthood. The presence of fewer alveoli in Apoe mice would result in fewer alveolar attachments to, and hence less exchange surface area per lung volume. Thus, subdivision of the sacculles present at birth produces gas-exchange units with a higher surface-to-volume ratio than those present at birth. The lung’s gas-exchange surface area per lung volume is also larger at age 14 days than at birth.

Compared with its predicted value, a low FEV₁ is a marker for increased all-cause mortality (3, 33). Short stature is also associated with a low for predicted FEV₁ and early death (2). Thus, finding Apoe mice are not as long as wt mice indicates that age-related alveolar loss is, in part, a pathogenic alveolar developmental component.

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GRANTS

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REFERENCES


