understand the link between asthma and obesity. However, indicate that obese mice may be a useful tool to better responsiveness in obese vs. lean mice (38). These studies (41), also causes greater inflammation and greater hyperre-

an important pollutant that exacerbates asthma (6, 10, 36, known.

are reduced with weight loss (5, 39). The mechanism(s) by which obesity increases the incidence of asthma is un-

a causal one, because longitudinal studies indicate that asthma symptoms antedates asthma (2, 3) and that asthma symptoms are most often thought of as being an airway phenomenon, there is good reason to believe that lung parenchymal tissues might contribute. For example, in many species, lung tissue resistance (Rtis), a measure of the pressure losses across the lung tissue that are in phase with flow, represents a substan-

tial part of baseline Rt. These pressure changes have been ascribed to frictional losses within connective tissues and contractile cells and to losses associated with hysteresis of the alveolar air-liquid interface (8, 21, 24). Furthermore, changes in Rtis contribute substantially to changes in Rt induced by bronchoconstricting agonists or allergens (15, 22, 28, 29, 42), although there are no data regarding the locus of changes in responsiveness that are induced by O3. In addition, despite the usual classification of asthma as an airway disease, the lung parenchyma can be substantially impacted by this disease, as emphasized by observations of lung parenchymal inflammation in human asthma (18). Moreover, the lung periphery also appears to contribute to mechanical dysfunction even in asthmatic individuals with normal spirometry (17, 47).

Understanding the locus (airways vs. parenchyma) of changes in pulmonary mechanics may contribute to our understanding of the mechanistic basis for these changes. For example, there are contractile elements in the alveolar walls that may behave differently from airway smooth muscle. In addition, changes in surfactant can impact parenchymal mechanics without any effect on the airways. Determining the locus of pulmonary mechanical responses may also have important implications for targeting drug delivery. Thus, to better understand how obesity and ozone exposure impact the lung and its responsiveness to nonspecific contractile agonists, we partitioned lung responses between the airways and lung parenchymal tissue. In particular, we sought to establish in wild-type mice whether O3-induced hyperresponsiveness is the result of changes in the airways or the lung tissue or both. Similarly, we sought to determine the locus (airways vs. parenchyma) of the enhanced O3-induced hyperresponsiveness observed in obese mice (38).

Rivera-Sanchez, Y. M., R. A. Johnston, I. N. Schwartzman, J. Valone, E. S. Silverman, J. J. Fredberg, and S. A. Shore. Differential effects of ozone on airway and tissue mechanics in obese mice. J Appl Physiol 96: 2200–2206, 2004. First published February 13, 2004; 10.1152/japplphysiol.00960.2003.—Obesity is an important risk factor for asthma. We recently reported increased ozone (O3)-induced hyperresponsiveness to methacholine in obese mice (Shore SA, Rivera-Sanchez YM, Schwartzman IN, and Johnston RA. J Appl Physiol 95: 938–945, 2003). The purpose of this study was to determine whether this increased hyperresponsiveness is the result of changes in the airways, the lung tissue, or both. To that end, we examined the effect of O3 (2 parts/million for 3 h) on methacholine-induced changes in lung mechanics with the use of a forced oscillation technique in wild-type C57BL/6J mice and mice obese because of a genetic deficiency in leptin (ob/ob mice). In ob/ob mice, O3 increased baseline values for all parameters measured in the study: airway resistance (Raw), lung tissue resistance (Rtis), lung tissue damping (G) and elastance (H), and lung hysteresivity (η). In contrast, no effect of O3 on baseline mechanics was observed in wild-type mice. O3 exposure significantly increased Raw, Rtis, lung resistance (Rl), G, H, and η responses to methacholine in both groups of mice. For G, Rtis, and Rl there was a significant effect of obesity on the response to O3. Our results demonstrate that both airways and lung tissue contribute to the hyperresponsiveness that occurs after O3 exposure in wild-type mice. Our results also demonstrate that changes in the lung tissue rather than the airways account for the amplification of O3-induced hyperresponsiveness observed in obese mice.

airway responsiveness; leptin; tissue damping; elastance; hysteresivity; methacholine

THE INCIDENCE OF ASTHMA IS increased in the obese (9, 20). It is likely that the relationship between obesity and asthma is a causal one, because longitudinal studies indicate that obesity antedates asthma (2, 3) and that asthma symptoms are reduced with weight loss (5, 39). The mechanism(s) by which obesity increases the incidence of asthma is unknown.

Using murine models of obesity and measurements of lung resistance (Rl), we have recently reported that obese mice exhibit increased responsiveness to intravenous methacholine compared with wild-type controls (38). Ozone (O3), an important pollutant that exacerbates asthma (6, 10, 36, 41), also causes greater inflammation and greater hyperresponsiveness in obese vs. lean mice (38). These studies indicate that obese mice may be a useful tool to better understand the link between asthma and obesity. However, these studies in ob/ob mice fall short of identifying target tissues, cells, and effector pathways that might account for the observed inflammatory and mechanical responses.

The locus of pulmonary mechanical responses can be either the airways or the lung parenchyma or both, but measurements of Rt do not allow discrimination between these two sites. Although changes in lung responsiveness, such as those we observed in obese mice exposed to O3 (38), are most often thought of as being an airway phenomenon, there is good reason to believe that lung parenchymal tissues might contribute. For example, in many species, lung tissue resistance (Rtis), a measure of the pressure losses across the lung tissue that are in phase with flow, represents a substan-

tial part of baseline Rt. These pressure changes have been ascribed to frictional losses within connective tissues and contractile cells and to losses associated with hysteresis of the alveolar air-liquid interface (8, 21, 24). Furthermore, changes in Rtis contribute substantially to changes in Rt induced by bronchoconstricting agonists or allergens (15, 22, 28, 29, 42), although there are no data regarding the locus of changes in responsiveness that are induced by O3. In addition, despite the usual classification of asthma as an airway disease, the lung parenchyma can be substantially impacted by this disease, as emphasized by observations of lung parenchymal inflammation in human asthma (18). Moreover, the lung periphery also appears to contribute to mechanical dysfunction even in asthmatic individuals with normal spirometry (17, 47).

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METHODS

Animals

This study was approved by the Harvard Medical Area Standing Committee on Animals. Male and female mice aged 8–12 wk at the time of study were purchased from Jackson Laboratory (Bar Harbor, ME). Ob/ob mice have a single base pair mutation in codon 105 of the leptin gene that results in a premature stop codon (49). In the absence of leptin, the mice eat excessively and are obese as early as 4 wk of age. Because the ob/ob mice are on a C57BL/6j background, wild-type C57BL/6j mice were used as controls.

O3 Exposure

Exposure to O3 [2 parts/million (ppm) for 3 h] or filtered room air was conducted in a stainless steel chamber with a Plexiglas door (~145 liters in volume). O3 was generated by passing dry 100% oxygen through ultraviolet light and mixing it with filtered room air in the chamber. Chamber atmosphere was drawn continuously via a calibrated by an ultraviolet photometric O 3 exiVent system was used both for ventilating animals (OK). A descriptive by 10.220.33.5 on November 11, 2017 http://jap.physiology.org/ Downloaded from
RESULTS

Baseline Values of Pulmonary Mechanics

Table 1 shows baseline values of Raw, Rtis, G, H, and \( \eta \) in \( ob/ob \) and wild-type mice 24 h after they were exposed to either \( O_3 \) (2 ppm for 3 h) or air. The \( ob/ob \) mice exposed to \( O_3 \) had significantly higher baseline values than mice under any of the other conditions for all the parameters measured in the study: Raw, Rtis, G, H, and \( \eta \). However, there was no statistically significant difference among any of the other groups studied. Table 1 also shows the weights of \( ob/ob \) and wild-type mice at the time of the study. The \( ob/ob \) mice weighed approximately twice as much as the wild-type mice.

Pulmonary Responses to Methacholine

Air-exposed mice. After air exposure, methacholine caused a significant increase in Raw (Fig. 2A) in both the \( ob/ob \) and the wild-type mice. In contrast, there was no significant effect of methacholine on G, H, or \( \eta \) and no effect on Rtis in either group of mice after air exposure. ED\(_{200}\)Raw was significantly lower in air-exposed \( ob/ob \) compared with wild-type mice (Table 2). In contrast, in most air-exposed mice regardless of genotype, ED\(_{200}\)Rtis could not be assessed because Rtis did not achieve an increase to 200% of baseline.

\( O_3 \)-exposed mice. ANOVA analysis indicated a significant increase in responses to methacholine after \( O_3 \) exposure whether Raw (\( P < 0.05 \); Fig. 2A), Rtis (\( P < 0.005 \); Fig. 2B), Rtis (\( P < 0.005 \); Fig. 2C), G (\( P < 0.005 \); Fig. 2D), H (\( P < 0.05 \); Fig. 2E), or \( \eta \) (\( P < 0.005 \) Fig. 1F) was the outcome indicator. Note that for Rtis and Rtis, which are frequency dependent, we used a frequency of 2.5 Hz to compute the responses. In wild-type mice, comparison of Fig. 2, A and B, indicates that, although \( O_3 \) increased both Raw and Rtis responses to methacholine, changes in Raw continued to dominate changes in Rtis. For example, in air-exposed wild-type mice at the highest dose of methacholine, changes in Raw averaged 600% of baseline, whereas changes in Rtis averaged only 300% of baseline, and the dose of methacholine required to double Raw was about a half log lower than that required to double Rtis (Table 2).

After \( O_3 \) exposure, methacholine-induced changes in G, Rtis, and Rtis were significantly greater in obese than in wild-type mice (\( P < 0.05 \) in each case; Fig. 2, B–D). A similar trend was observed for \( \eta \) and for H (Fig. 2, E and F), but the effects were not statistically significant. In contrast to the other mechanical indexes, methacholine-induced changes in Raw were not significantly different in obese and lean mice after \( O_3 \) exposure (Fig. 2A). Indeed, \( O_3 \) did not cause a significant decrease in log ED\(_{200}\)Raw in obese mice (Table 2), even though it induced a marked decrease in log ED\(_{200}\)Rtis. Thus the increased pulmonary responses to methacholine observed in obese mice exposed to \( O_3 \) (Fig. 2C and Ref. 38) were the result of obesity-related effects on the lung tissue rather than the airways.

DISCUSSION

Our results indicate that 1) under air-exposed conditions, most of the increase in Rtis induced in mice by intravenous methacholine is the result of a change in Raw, whereas Rtis is unaffected; 2) under \( O_3 \)-exposed conditions in wild-type mice, increases in Rtis induced by intravenous methacholine are the result of increases in both Raw and Rtis, although increases in Raw dominate; and 3) the exaggerated pulmonary responses to methacholine observed after \( O_3 \) exposure in obese compared with wild-type mice are the result of more robust changes in Rtis, whereas Rtis exposure does not cause a significant increase in Raw responses to methacholine in obese mice.

To separate changes in Rtis into changes in Raw vs. Rtis, a parameter estimation model (12) was used to partition \( Z_L \) derived from multiple forcing frequencies into components representing the airway and parenchymal mechanical properties. Examination of the real and imaginary parts of \( Z_L \) as a function of frequency (Fig. 1) indicated that \( Z_L \) conformed to the model used. Use of this system for the measurement of Raw, Rtis, G, H, and \( \eta \) in mice has been verified by Tomioka et al. (42), who compared it with the alveolar capsule technique. The latter has been regarded as the most direct way to assess parenchymal mechanics in animals (7). Lung mechanics obtained in mice after methacholine challenge yielded equivalent results when these two techniques were compared (42). Thus, in mice, the parameter estimation model of Hantos et al. (12) can be used to partition Rtis into Raw and Rtis.

In air-exposed mice, we did not observe any effect of intravenous methacholine on Rtis (Fig. 2B), whereas Raw did increase (Fig. 2A). In contrast, studies performed in several other species, including dogs (22), guinea pigs (15), and rats (29), indicate that changes in Rtis represent a substantial fraction of the changes in Rtis that are induced by methacholine. Contraction of parenchymal interstitial cells, contraction of smooth muscle within alveolar ducts, alveolar shape change and its effects on the air-liquid interface, and inhomogeneities in airway narrowing have all been proposed to account for these changes in Rtis (8, 15, 21, 22, 24, 29). It is possible that the mode of delivery of methacholine (aerosol vs. intravenous)

<table>
<thead>
<tr>
<th></th>
<th>( \text{Raw, cmH}_2\text{O/mL/s} )</th>
<th>( \text{Rtis, cmH}_2\text{O/mL/s} )</th>
<th>( G, \text{cmH}_2\text{O/ml} )</th>
<th>( H, \text{cmH}_2\text{O/ml} )</th>
<th>( \eta )</th>
<th>Body Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-air</td>
<td>0.31 ± 0.015</td>
<td>0.33 ± 0.02</td>
<td>3.93 ± 0.24</td>
<td>25.8 ± 1.82</td>
<td>0.15 ± 0.01</td>
<td>19.6 ± 2.6</td>
</tr>
<tr>
<td>WT-( O_3 )</td>
<td>0.36 ± 0.057</td>
<td>0.38 ± 0.03</td>
<td>4.41 ± 0.31</td>
<td>23.4 ± 4.42</td>
<td>0.14 ± 0.67</td>
<td>17.7 ± 1.9</td>
</tr>
<tr>
<td>( ob/ob )-air</td>
<td>0.36 ± 0.031</td>
<td>0.34 ± 0.03</td>
<td>4.09 ± 1.67</td>
<td>27.6 ± 2.3</td>
<td>0.15 ± 0.01</td>
<td>50.8 ± 8.3†</td>
</tr>
<tr>
<td>( ob/ob )-( O_3 )</td>
<td>0.61 ± 0.09*</td>
<td>0.79 ± 0.18*</td>
<td>8.67 ± 1.49*</td>
<td>43.4 ± 6.54*</td>
<td>0.19 ± 0.01*</td>
<td>52.4 ± 7.9†</td>
</tr>
</tbody>
</table>

Values are means ± SE. Measurements were made 24 h after ozone (\( O_3 \)) exposure. Raw, airway resistance; Rtis, lung tissue resistance; G, lung tissue damping; H, lung tissue elastance; \( \eta \), lung tissue hysteresivity; ppm, parts/million; WT, wild type. Rtis was calculated from G and H at a frequency of 2.5 Hz. *\( P < 0.05 \) compared with all other groups. †\( P < 0.05 \) compared with wild-type mice with same exposure. Significant differences among the groups were assessed by factorial ANOVA using Fisher’s least significant difference test for post hoc comparisons.
accounts for the lack of an Rtis response to methacholine in normal mice vs. other species. Changes in Rtis are greater when methacholine is delivered by aerosol rather than intravenously, perhaps as a result of more heterogeneous constriction of airways (29). Nonetheless, our findings are in agreement with other studies performed in normal mice that indicate that methacholine does not affect Rtis even when the methacholine is delivered by aerosol (42). Thus it is more likely that mice have inherently different lung mechanical properties than other animal species. In this respect, it is interesting to note that changes in Raw also dominate during methacholine-induced bronchoconstriction in normal humans (16).

In contrast to the condition of air exposure, both Raw and Rtis increased with methacholine in mice exposed to O₃ (Fig. 2), although in wild-type mice Raw continued to dominate. Furthermore, responses to methacholine were greater in O₃-exposed than in air-exposed mice for both Raw and Rtis. Inhomogeneous airway constriction can substantially impact the measurement of G without any actual change in the properties of the lung tissue (23, 24), resulting in changes in Rtis that do not really reflect the parenchyma. Hence, it possible that at least part of the increase in Rtis after methacholine challenge represents heterogeneous effects of O₃ resulting in altered and more inhomogenous distribution of airway narrowing during bronchoconstriction. Such inhomogeneities could also account for the increased impact of methacholine on \( \eta \) (23, 24). However, this is unlikely to be the only reason for the increases in Rtis. The observation that there were also substantive
Table 2. Log ED_{200}Raw and ED_{200}Rtis values for obese and wild-type mice exposed to O3 (2 ppm for 3 h)

<table>
<thead>
<tr>
<th></th>
<th>Log ED_{200}Raw</th>
<th>Log ED_{200}Rtis</th>
</tr>
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<tbody>
<tr>
<td>WT-air</td>
<td>−0.07±0.13†</td>
<td>0.5*</td>
</tr>
<tr>
<td>WT-O3</td>
<td>−0.59±0.13†‡</td>
<td>−12.12±0.13†‡</td>
</tr>
<tr>
<td>Ob/ob-air</td>
<td>−0.55±0.14‡</td>
<td>0.29±0.16</td>
</tr>
<tr>
<td>Ob/ob-O3</td>
<td>−0.45±0.15</td>
<td>−0.57±0.14‡</td>
</tr>
</tbody>
</table>

Values are means ± SE given in mg/ml. Measurements were made 24 h after exposure. ED_{200}Raw and ED_{200}Rtis, doses of methacholine required to double Raw and Rtis, respectively. *In cases where methacholine-induced changes in Rtis did not reach 200% of baseline, an ED_{200} value of 3 mg/ml was assigned. †P < 0.05 compared with air-exposed mice in the same group. ‡P < 0.05 compared with lean mice in the same exposure group. Significant differences among the groups were assessed by factorial ANOVA using Fisher’s least significant difference test for post hoc comparisons.

Changes in H, a measure of lung elastance, during methacholine challenge after O3 exposure, particularly in obese mice (Fig. 2), in conjunction with data from Lutchen et al. (23, 24) indicating that H is not substantially affected by inhomogeneities of airway constriction, suggest that, in O3-exposed mice, changes in the mechanical properties of the lung tissue were indeed occurring during methacholine challenge. Thus our data suggest that both airways and lung tissue contribute to the hyperresponsiveness that occurs after O3 exposure, although in wild-type mice Raw dominates. To our knowledge, this is the first report in any species indicating the partitioning of O3-induced hyperresponsiveness between airways and tissue.

O3 causes lung inflammation characterized by release of prostanoids, cytokines, and chemokines and an influx of neutrophils. It is possible that, after O3 exposure, some of these inflammatory mediators acting on the airway smooth muscle or its innervation account for the increased effects of methacholine on Raw because increased airway contractile responses can be observed in airways of O3-exposed animals even in vitro (26). In contrast, we do not know what accounts for the effects of O3 on Rtis. Effects of contractile on contractile elements in the lung parenchyma could play a role. It is also possible that O3-induced effects on surfactant contribute to the observed lung tissue effects because the surface tension of bronchoalveolar lavage samples from rats exposed to O3 is increased likely as a result of oxidation of surfactant phospholipids (25, 27, 34). Finally, it is possible that airway closure contributes to changes in Rtis in O3-exposed animals. By closing off some parts of the lung, a reduced parenchymal mass would receive relatively greater volume changes during oscillations, leading to greater frictional losses. The observation that, in O3-exposed mice, methacholine caused changes in H (Fig. 2E), a measure of lung elastance, supports the idea that airway closure was occurring. Note that these explanations are not mutually exclusive because changes in lung surfactant could contribute to airway closure.

The baseline values of Raw, Rtis, G, and H after O3 exposure, but before methacholine exposure, were increased substantially in the ob/ob but not in the wild-type mice (Table 1). η was also increased but to a lesser extent. Because η is not sensitive to airway closure, whereas Raw, Rtis, G, and H are, airway closure induced by O3 exposure in the ob/ob but not wild-type mice could account for these changes. Airway closure has been invoked as an explanation for similar findings in a murine model of airway inflammation (46). However, a significant degree of airway closure would need to take place to explain the magnitude of the increase in H in the ob/ob O3-exposed mice (nearly 60% over air-exposed mice).

Previously published data from these same mice indicate that responsiveness is increased even in air-exposed obese compared with wild-type mice (38). The data presented here suggest that this is primarily the result of differences in the airways (Table 2). Indeed in air-exposed mice, there were no significant changes in Rtis induced by methacholine in either obese or wild-type mice (Fig. 2B). Breathing at low lung volume has been shown to augment airway responsiveness in both animals and humans (4). Because of changes in the chest wall, obese humans breathe at a lower than normal functional residual capacity (FRC) (35, 37), and it is possible that a similar phenomenon occurs in awake, spontaneously breathing obese mice. However, changes in lung volume resulting from alterations in the chest wall cannot explain the differences in baseline airway responsiveness reported in our study, as these mice were all studied with an open chest and a fixed PEEP.

Even in open-chest ob/ob mice, FRC (defined as the volume at a fixed PEEP) is reduced compared with wild-type mice (40), but the change in FRC is the result of a smaller lung mass (38) and the FRC-to-total lung capacity ratio is actually the same in ob/ob and wild-type mice (40). It is not clear that small lungs per se should lead to airway hyperresponsiveness, although immature animals usually have airway hyperresponsiveness compared with adults. Instead, we have previously hypothesized (38) that the airway hyperresponsiveness observed in the air-exposed ob/ob mice may be the result of low grade systemic inflammation, which is a consistent feature of both human and rodent obesity (14, 19, 30, 44, 45). Nevertheless, we cannot rule out the possibility that issues of methacholine dose also contributed to differences in baseline responsiveness between wild-type and ob/ob mice. We elected to deliver methacholine intravenously on a milligram per kilogram basis. The bolus delivery time was slow in comparison with the normal cardiac cycle of a mouse, so it is likely the methacholine was relatively well mixed in the total blood volume. In the absence of information concerning the relative blood volumes of wild-type and ob/ob mice, we cannot refute the possibility that the concentration of methacholine delivered to the lungs was actually higher in the ob/ob compared with the wild-type mouse, accounting for the increase in responsiveness. However, given that the ED_{200}Raw of air-exposed ob/ob mice was about threefold lower than that of wild-type mice (Table 2), the blood volume of the ob/ob mouse would have to be ~30% less in the ob/ob mouse despite a twofold increase in body mass to account for these findings on the basis of dilution alone.

We have also reported that responsiveness, as indicated by changes in Rtis, is increased after O3 exposure and that this effect of O3 is amplified in obese compared with normal mice (38). Our present results indicate that these O3-induced changes are primarily the result of effects on the lung tissue (Fig. 2B), whereas responsiveness is similar in O3-exposed obese and wild-type mice when only the airways are considered (Fig. 2A). Indeed, O3 did not induce a significant increase in Raw responses to methacholine in ob/ob mice, at least not with respect to changes in ED_{200}Raw (Table 2). We had previously hypothesized that an increased dose of O3 in the ob/ob mice might have accounted for the increased effects of
O₃ on both airway responsiveness and airway inflammation: because minute ventilation during O₃ exposure is similar in wild-type and ob/ob mice, but ob/ob mice have smaller lungs, the inhaled dose of O₃ per gram of lung tissue is higher in ob/ob than wild-type mice despite the same inhaled concentration (38). Our present results do not support this interpretation because an increased dose of O₃ would be as likely to affect the airways as the lung tissues, whereas in fact, Rats but not Raw was impacted by obesity during methacholine challenge.

It is also possible that lack of leptin in the ob/ob mice rather than obesity per se contributes to the enhanced effects of O₃ on the lung tissue. Leptin causes release of surfactant in cultured lung epithelial cells (1, 43), and we have observed increases in alveolar lavage cytokines (38). We do not know which cells in the lung tissue contribute to the hyperresponsiveness that occurs after O₃ exposure in wild-type mice, although changes in the lung tissues dominate the response. In contrast, changes in the lung tissue rather than the airways account for the amplification of O₃-induced hyperresponsiveness observed in obese mice.

ACKNOWLEDGMENTS

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REFERENCES

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