Responses to ozone are increased in obese mice

S. A. Shore, Y. M. Rivera-Sanchez, I. N. Schwartzman, and R. A. Johnston

Physiology Program, Harvard School of Public Health, Boston, Massachusetts 02115
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Shore, S. A., Y. M. Rivera-Sanchez, I. N. Schwartzman, and R. A. Johnston. Responses to ozone are increased in obese mice. J Appl Physiol 95: 938–945, 2003. First published June 6, 2003; 10.1152/japplphysiol.00336.2003.—Epidemiological data indicate an increased incidence of asthma in overweight adults and children. Ozone (O₃) is a common trigger for asthma. Accordingly, the purpose of this study was to compare O₃-induced airway hyperresponsiveness and airway inflammation in lean, wild-type (C57BL/6J) mice and mice that are obese as a consequence of a genetic defect in the gene encoding the satiety hormone leptin (ob/ob mice). The ob/ob mice eat excessively and weighed more than twice as much as age- and gender-matched wild-type mice. Airway responsiveness to intravenous methacholine was measured by forced oscillation. In air-exposed controls, baseline pulmonary resistance was greater, and the dose of methacholine required to double pulmonary resistance was lower in ob/ob than wild-type mice. Exposure to O₃ (2 parts/million for 3 h) caused AHR and airway inflammation in both groups of mice, but responses to O₃ were enhanced in ob/ob compared with wild-type mice. Admiration of exogenous leptin did not reverse the enhanced inflammatory response observed in ob/ob mice, but augmented airway inflammation in wild-type mice. The inhaled dose of O₃ per gram of lung tissue was greater in ob/ob than wild-type mice. Our results indicate that O₃-induced airway responses are enhanced in ob/ob mice and suggest that inhaled O₃ dose may be one factor contributing to this difference, but other aspects of the obese phenotype may also contribute. Our results also indicate that the hormone leptin, which is increased in the obese, has the capacity to increase airway inflammation.

Ozone (O₃) is a trigger for asthmatic episodes. The number of hospital admissions for asthma increases on days of high-ambient O₃ concentrations (14, 51). O₃ exposure causes airway hyperresponsiveness (AHR) and airway inflammation in both humans and animals (11, 18, 23, 34, 44, 46, 60), and these effects are likely to worsen asthmatic episodes. The purpose of this study was to determine whether obesity enhances the AHR and/or airway inflammation induced by O₃. To that end, we used mice with a genetic defect in the gene encoding leptin, a satiety hormone produced in adipocytes (ob/ob mice). Because of their inability to produce leptin, ob/ob mice eat excessively and are markedly obese (25). Our results indicated that O₃-induced AHR and airway inflammation are increased in ob/ob compared with lean wild-type mice. To determine whether differences between ob/ob and wild-type mice were the direct result of their leptin deficiency, rather than some other aspect of the ob/ob mouse phenotype, we also acutely administered exogenous leptin to wild-type and ob/ob mice before air or O₃ exposure and examined effects of this hormone on bronchoalveolar lavage (BAL) cells and cytokines.

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). The ob/ob mice have a single base pair mutation in codon 105 of the leptin gene that results in a premature stop codon (61). In the absence of leptin, the mice eat excessively and are severely obese as early as at 4 wk of age. The increased body weight is entirely the result of an increase in fat mass. Because the mutation is expressed 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 on the front (~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 sampling port, and O₃ concentration was measured by an O₃ chemiluminescent analyzer (model 49, ThermoElectron Instruments, Hopkinton, MA), which was calibrated by an ultraviolet photometric O₃ calibrator (model 49PS, ThermoElectron Instruments). During air or O₃ exposures, mice were placed in individual wire mesh cages within the chamber and were awake during the exposure.

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Protocol. The ob/ob and wild-type mice were exposed to O₃ (2 ppm for 3 h) or to filtered air. Twenty-four hours later, mice were anesthetized and instrumented for the measurement of pulmonary mechanics by the forced oscillation technique, and airway responsiveness to intravenous methacholine was measured. We chose to examine airway responsiveness 24 h after the cessation of O₃ exposure based on previous reports using this species (56, 60). BAL was performed either 4 or 24 h after the cessation of O₃ exposure in a separate cohort of mice. We used the 24-h time point to correspond to that used for measurements of pulmonary mechanics and because BAL polymorphonuclear leukocytes (PMN) and protein peak at this time. We used the 4-h time point to capture O₃-induced changes in BAL cytokines, which peak earlier than PMN and protein and then decline (see below).

We also examined the effect of exogenous saline or leptin administration on O₃-induced airway inflammation to determine whether exogenous leptin could reverse the effects observed in leptin-deficient ob/ob mice. For these experiments, C57BL/6J and ob/ob mice were divided into two groups. One group received intraperitoneal injections of saline (2.5 μl/g) on day 1, spaced 3.5 h apart. On day 2, this group again received two injections of saline. The first was administered 0.5 h before initiation of O₃ (2 ppm for 3 h) or air exposure. The second injection was administered just after cessation of the exposure. Mice were killed, and BAL was performed 4 h after cessation of O₃. The second group was treated identically, except that the mice were given injections of saline rather than leptin. The dose of leptin was chosen based on published data indicating its efficacy in reducing food consumption (5, 37, 38). To confirm that leptin levels were still elevated at the time the BAL was performed, blood was drawn at the end of the experiment by cardiac puncture, and the serum was stored at −20°C until assayed for leptin by radioimmunoassay (Lincor, St. Louis, MO).

A final series of experiments was performed to determine whether differences in O₃-induced AHR or airway inflammation between wild-type and ob/ob mice could be the result of differences in the inhaled dose of O₃ resulting from differences in minute ventilation (V̇E) during exposure (47). For these experiments, V̇E, tidal volume (VT), breathing frequency (f), and end-expiratory pause (EEP) were measured under baseline conditions and/or during O₃ exposure in mice placed in nose-only-exposure plethysmographs, as previously described (46, 47). Some of these mice were killed at least 1 wk after O₃ exposure, and the lungs and the heart were excised and weighed.

Measurement of pulmonary mechanics. Twenty-four hours after exposure to air or O₃, mice were anesthetized with xylazine (7 mg/kg) and pentobarbital sodium (50 mg/kg). A tracheostomy was performed, and a tubing adaptor (18 gauge) (Becton-Dickinson, Franklin Lakes, NJ) was used to cannulate the trachea. The mice were then ventilated at 150 and 180 Hz in wild-type and ob/ob mice, respectively. Both groups were ventilated at a VT of 0.3 by using a specialized ventilator (flexiVent, SCIREQ, Montreal, Quebec). The slightly higher frequency used for the ob/ob was chosen to conform to their spontaneous breathing frequencies (see below), whereas, during spontaneous breathing, VT did not differ between the strains. The ventilator that we used featured a computer-controlled piston capable of producing any desired waveform and accurately measuring delivered volume (and hence flow) by tracking piston movement, as described by others (40). Pressure at the airway opening was also measured by the flexiVent system. Once ventilation was established, a wide incision was made in the chest wall bilaterally to expose the lungs to atmospheric pressure, and a positive end-expiratory pressure of 3 cmH₂O was applied by placing the expiratory line under water. The tail vein was cannulated for the delivery of methacholine.

Measurements of dynamic pulmonary resistance (RL) were made by the forced oscillation technique by using a 2.5-Hz sinusoidal forcing function. The flexiVent system was used both for ventilating the lungs and for delivering the oscillations. Calibration of the system with the tracheostomy tube in place removes the mechanical properties of the tubing, so that the data reported by the system represent the mechanical properties of the lungs only. Dose-response curves to intravenously administered methacholine were obtained as follows. Mice were given an inflation to three times VT. One minute later, a bolus of PBS was administered (1 μl/g), and VT was measured every eighth breath for the next 1–2 min, until VT peaked and began to decline. The mouse was then given another inflation to three times VT. The procedure was repeated by using doses of methacholine chloride dissolved in PBS that increased in half-log intervals from 0.03 to 3.0 mg/ml at a dose of 1 μl/g. Five minutes were allowed to elapse between doses of methacholine. The three highest values of VT obtained after each dose were averaged to obtain the final values for each dose. The dose of methacholine required to double RT (ED₂₀₀RT) was obtained by log linear interpolation between the doses bounding the point at which RT was exactly 200% of the PBS value.

BAL. Mice were killed with an overdose of pentobarbital sodium. The trachea was cannulated with a tubing adaptor, and the lungs were lavaged twice with PBS (1 ml), which was instilled and then slowly withdrawn over 30 s. The recovered BAL fluid (~80–90% of that delivered) was placed on ice until it was centrifuged at 1,200 rpm at 4°C for 10 min. Cell pellets were resuspended in PBS, and the total number of cells was counted with a hemocytometer. Aliquots of cells were also centrifuged onto glass slides at 800 rpm for 10 min (Cytospin 3, Shandon, Sewickley, PA), air-dried, and stained with Wright-Giemsa (LeukoStat, Fisher Scientific, Pittsburgh, PA). Cell differentiation was determined by counting 300 cells under ×400 magnification. Cells were assigned as macrophages, neutrophils, eosinophils, lymphocytes, or epithelial cells based on standard morphological characteristics. The supernatant was frozen at −70°C and subsequently analyzed for protein concentration by using the Bio-Rad dye reagent (Bio-Rad, Richmond, CA), according to the manufacturer's instructions. An aliquot of the supernatant was re-centrifuged at 40,000 g at 4°C for 30 min and subsequently analyzed for eotaxin, IL-6, macrophage inflammatory protein-2 (MIP-2), and KC by using enzyme immunoassay kits (Endogen, Woburn, MA, for IL-6; and R&D Systems, Minneapolis, MN, for eotaxin, MIP-2, and KC).

Measurement of V̇E. To measure ventilation during O₃ exposure, mice were placed in a Plexiglas restraining tube that served as a head-out flow plethysmograph, as previously described (46, 47). Different sized tubes were used for lean and obese mice. The tube was fitted with a rubber gasket designed to fit snugly around the animal’s neck. Once the animal was in the tube, a large piston fitted with a rubber O-ring was moved into place behind it, thus restraining the animal’s movement. Air displaced at the body surface as the animal breathed passed across a pneumotach attached to a differential pressure transducer. The front end of the tube was inserted into a port in the O₃ exposure chamber, and the animals were exposed nose only. The flow signal from the pneumotach was fed to a personal computer and analyzed by using software (BUXCO Electronics, Sharon, CT) that...
Table 1. Baseline $R_t$, $ED_{200}R_t$, and body weight of wild-type and $ob/ob$ mice exposed to air or ozone (2 ppm for 3 h)

<table>
<thead>
<tr>
<th></th>
<th>$R_t$, cm$^3$H$_2$O·ml$^{-1}$·s$^{-1}$</th>
<th>Log $ED_{200}R_t$</th>
<th>Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type - Air</td>
<td>0.89 ± 0.02</td>
<td>−0.24 ± 0.05</td>
<td>19.6 ± 2.6</td>
</tr>
<tr>
<td>Wild type - O$_3$</td>
<td>0.88 ± 0.15</td>
<td>−0.42 ± 0.11*</td>
<td>17.7 ± 1.9</td>
</tr>
<tr>
<td>$ob/ob$ - air</td>
<td>0.76 ± 0.04†</td>
<td>−0.69 ± 0.07†</td>
<td>50.8 ± 8.3†</td>
</tr>
<tr>
<td>$ob/ob$ - O$_3$</td>
<td>1.59 ± 0.29†</td>
<td>−0.88 ± 0.15†</td>
<td>52.4 ± 7.9†</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 6$–7/group. Measurements were made 24 h after exposure. $R_t$, pulmonary resistance; $ED_{200}R_t$, dose of methacholine (in mg/ml) required to double $R_t$; O$_3$, ozone. *$P < 0.05$ compared with mice in same group exposed to air; †$P < 0.05$ compared with wild-type mice with same exposure.

allowed for breath-by-breath measurements of $V_e$, $V_r$, $f$, and EEP.

Statistics. Differences in ventilatory parameters during O$_3$ exposure were assessed by repeated-measures ANOVA. Differences in BAL cells and cytokines, baseline $R_t$, and $ED_{200}R_t$ were assessed by ANOVA. In each case, follow-up $t$-tests were used to determine the significance of differences between individual groups. Differences in serum leptin were assessed by unpaired $t$-tests. Statistical analyses were carried out by using SAS software (SAS Institute, Cary, NC).

RESULTS

Table 1 shows baseline $R_t$, $ED_{200}R_t$, and body weight in wild-type and $ob/ob$ mice 24 h after exposure to air or O$_3$. ANOVA indicated statistically significant differences among the four groups for weight ($P < 0.001$), baseline $R_t$ ($P < 0.005$), and $ED_{200}R_t$ ($P < 0.001$). When the air- and O$_3$-exposed mice were grouped together, $ob/ob$ mice weighed 51.7 ± 2.2 g, more than twice as much as wild-type mice (18.7 ± 0.7 g). After air exposure, baseline $R_t$ was slightly, but significantly, greater ($P < 0.05$) in $ob/ob$ than wild-type mice. The $ob/ob$ air-exposed mice were also significantly more responsive to methacholine than wild-type mice after air exposure (Figure 1, Table 1).

There was no effect of O$_3$ on baseline $R_t$ in wild-type mice. However, in $ob/ob$ mice, O$_3$ caused a significant increase in baseline $R_t$ ($P < 0.02$). O$_3$ exposure increased airway responsiveness in both wild-type and $ob/ob$ mice (Figure 1, Table 1). The effect of O$_3$ was to decrease $ED_{200}R_t$ (Table 1) and to increase the maximal response to methacholine (Figure 1). O$_3$ exposure resulted in a significantly greater increase in airway responsiveness in the $ob/ob$ mice than in the wild-type mice (Figure 1).

BAL protein, eotaxin, MIP-2, KC, and IL-6 concentrations were not significantly different in $ob/ob$ mice compared with wild types after air exposure (Figure 2, B–E). O$_3$ exposure caused a significant increase in BAL eotaxin, MIP-2, IL-6, and KC measured 4 h after cessation of O$_3$ exposure that declined toward baseline by 24 h. In contrast, BAL protein was increased at 4 h and increased further at 24 h (Figure 2A). There was a significantly greater increase in BAL protein, eotaxin, KC, and IL-6 in the $ob/ob$ mice compared with the wild-type mice 4 h after cessation of O$_3$ exposure. A similar trend was observed for MIP-2. There was no statistically significant effect of O$_3$ exposure or obesity on the total number of cells recovered from BAL. However, O$_3$ exposure influenced the percentage of BAL cells that were PMN (Figure 2F). In both wild-type and $ob/ob$, O$_3$ exposure resulted in a significant increase in the percentage of BAL cells that were PMN measured at 4 h, and PMN remained elevated at 24 h. However, O$_3$-induced changes in BAL PMN were not different between wild-type and $ob/ob$ mice.

To determine whether differences in responses to O$_3$ observed in $ob/ob$ mice were the direct result of these animals’ leptin deficiency or due to some other aspect of the phenotype of these mice, we examined the ability of leptin to reverse changes in O$_3$-induced inflammation that were observed in $ob/ob$ mice (Figure 3). As described above, exposure to O$_3$ (2 ppm for 3 h) induced greater increases in BAL protein, eotaxin, KC, MIP-2, and IL-6 in $ob/ob$ mice treated with saline than in wild-type mice treated with saline, whereas PMN were not affected. However, no significant differences were observed between leptin-treated and saline-treated $ob/ob$ mice exposed to either air or O$_3$. In contrast, O$_3$-induced changes in BAL IL-6, KC, and protein were greater in leptin-treated than in saline-treated wild-type mice (Figure 3). A similar trend was observed for eotaxin and MIP-2, although the effects were not statistically significant. After the experiments were terminated, blood was drawn. Serum leptin levels averaged 27.9 ± 4.3 ng/ml in leptin-treated wild-type mice, but only 5.1 ± 1.7 ng/ml in saline-treated wild-type mice ($P < 0.01$).

The dose of O$_3$ delivered to the lungs is the product of O$_3$ concentration, exposure time, and $V_e$ (57). $V_e$ depends on metabolism, and, in rodents, there are changes in metabolism during O$_3$ exposure (33, 46, 47) and with obesity (25). To determine whether differences in O$_3$ dose resulting from differences in $V_e$ might
account for observed differences between wild-type and ob/ob mice in the response to O₃, we measured Vₑ and the pattern of breathing in ob/ob and wild-type mice during O₃ exposure (Fig. 4). We also measured Vₑ and the pattern of breathing during O₃ exposure in wild-type mice treated with exogenous saline or leptin.

We did not observe any statistically significant differences in Vₑ between wild-type and ob/ob mice before O₃ exposure (Fig. 4A, time 0), although there were differences in the pattern of breathing. In particular, f was higher in ob/ob (282 ± 19 breaths/min) than in wild-type mice (246 ± 13 breaths/min) (P < 0.05), but Vₚ was not different (0.25 ± 0.01 and 0.26 ± 0.02 ml in wild-type and ob/ob mice, respectively). As previously reported (46, 47), there was a marked decrease in Vₑ during O₃ exposure in wild-type mice (Fig. 4A). The ob/ob showed a similar pattern of response, and, although Vₑ tended to be higher in ob/ob than in wild-type mice at all times during O₃ exposure, the differences were not statistically significant. When the total volume of air inhaled during the 3-h O₃ exposure was computed for each group, the difference was also not statistically significant (7.77 ± 0.92 and 9.70 ± 0.84 liters for wild-type and ob/ob mice, respectively). These results indicate that the total inhaled dose of O₃ was not different between wild-type and ob/ob mice. However, ob/ob mice have been reported to have smaller lungs than wild-type controls (49), suggesting that the inhaled dose of O₃ per gram of lung tissue might, in fact, be different in the obese mice. To examine this possibility, we excised the lungs of a separate cohort of ob/ob and wild-type mice at least 1 wk after O₃ exposure and weighed them. The weight of the lungs of ob/ob mice (0.18 ± 0.02 g) was less than that of age-

Fig. 2. Bronchoalveolar lavage (BAL) protein, cytokine concentrations, and polymorphonuclear leukocytes in wild-type (open bars) and ob/ob (solid bars) mice. A: protein; B: eotaxin; C: macrophage inflammatory protein-2 (MIP-2); D: KC; E: IL-6; and F: neutrophils. Mice were exposed to air or O₃ (2 ppm for 3 h), and BAL was performed 4 or 24 h later. Values are means ± SE of data from 6–7 mice in each group. *P < 0.05, compared with O₃-exposed wild-type mice.

Fig. 3. The ob/ob and wild-type mice were treated with saline or leptin (2.5 μg/g) 2 times/day for 2 days and exposed to air or O₃ (2 ppm for 3 h). BAL was performed 4 h later. A: protein; B: eotaxin; C: MIP-2; D: KC; E: IL-6; and F: neutrophils. Values are means ± SE of data from 6–7 mice in each group. *P < 0.05, leptin-treated compared with saline-treated wild-type mice. *P < 0.05, saline-treated ob/ob compared with saline-treated wild-type mice.
DISCUSSION

Our results indicate that $O_3$-induced AHR and $O_3$-induced airway inflammation are enhanced in ob/ob mice (Figs. 1 and 2). Moreover, the difference in the inflammatory response to $O_3$ is not corrected by acute exogenous administration of leptin (Fig. 3), suggesting that obesity per se, or some other aspect of the chronic phenotype of these mice that is not corrected by acute leptin treatment, accounts for the enhanced response to $O_3$. For example, the difference in response to $O_3$ may be a consequence of differences in $O_3$ dose, resulting from the reduced lung size in the ob/ob mice. Surprisingly, acute exogenous administration of leptin did result in an increase in $O_3$-induced airway inflammation in wild-type mice, even though it did not affect responses to $O_3$ in ob/ob mice (Fig. 3).

We observed a small but significant increase in $R_L$ in ob/ob compared with wild-type mice (Table 1). Lung volumes are reduced in ob/ob mice (49), and it is likely that the increase in baseline $R_L$ is the result of this reduction in lung volume, because airway diameter is strongly influenced by lung volume. Indeed, in obese humans, there is a linear correlation between changes in airway conductance and functional residual capacity (59). In obese humans, the reductions in lung volume associated with obesity are thought to result from gravitational effects, resulting from the increased abdominal load (58). However, we observed a decrease in the actual mass of the lung in leptin-deficient ob/ob mice, despite their marked increase in body weight, suggesting that simple gravitational effects likely do not explain all of the reduction in lung volume observed in these mice. Rather, it may be that leptin is required for lung growth (3), because leptin receptors are present in developing prealveolar acini of fetal lungs (2) and because leptin stimulates surfactant synthesis in fetal lung cells (2, 52), as well as proliferation of tracheal epithelial cells (53). Alternatively, reductions of the volume of the thorax as a result of the increased abdominal mass and even the accumulation of fat between the lungs and the rib cage may limit lung growth and development in ob/ob mice, because obesity is present very early on.

Airway responsiveness was increased in ob/ob compared with wild-type mice, even in the absence of $O_3$ exposure (Fig. 1). An association between increased body mass index and onset of AHR has also been observed in a longitudinal study of aging in humans (26). Breathing at low lung volume has been shown to augment airway responsiveness both in animals and in humans (12). The ob/ob mice do breathe at lower lung volumes than wild-type mice (49), but, in our study,
lungs per se should lead to AHR, although immature animals usually have AHR compared with adults. In both obese humans (24, 35, 56) and obese mice (19, 55), there is chronic systemic inflammation, even in the absence of any overt inflammatory insult. Although we did not note any differences in BAL cytokines in the ob/ob compared with wild-type air-exposed mice (Fig. 2), it is possible that differences existed but were below the limit of detection of the assays used. The relationship between airway inflammation and AHR is quite complex, and it is clear that AHR can occur in the absence of airway inflammation (6), particularly under conditions that promote airway remodeling (6, 10). However, multiple stimuli that promote airway inflammation also promote AHR (6), and it is possible that the AHR observed in the air-exposed ob/ob mice may be the result of low-grade inflammation.

It is unlikely that differences in tidal stretching of airway smooth muscle account for the difference in baseline airway responsiveness between the air-exposed ob/ob and the wild-type mice. Fredberg et al. (15) have shown that the periodic strains on airway smooth muscle imposed by tidal lung inflations reduce the muscle’s ability to shorten. The amount of tidal stretching of airway smooth muscle depends on the ventilation in relation to the size of the lungs. We used identical ventilation values during measurements of pulmonary mechanics for the ob/ob and wild-type mice based on the observation that their spontaneous ventilation values were not different. However, the obese mice had smaller lungs. Hence, when normalized for the size of their lungs, ventilation values were actually greater in the obese mice, implying greater tidal smooth muscle stretching, which should have resulted in reduced, not increased, airway responsiveness, as observed. Nevertheless, obese humans do breath with smaller ventilation values (42), and reduced tidal stretching of airway smooth muscle could play a role in the increased airway responsiveness observed in obese humans (26).

Our results indicate that both O₃-induced AHR and O₃-induced airway inflammation are increased in ob/ob compared with wild-type mice (Figs. 1 and 2). Data from many investigators suggest that the mechanistic basis for O₃-induced AHR is inflammation arising from oxidant injury to the lungs and airways, although the precise aspect(s) of the inflammatory cascade required for AHR is still not firmly established. Thus it is likely that the increased O₃-induced AHR observed in ob/ob mice (Fig. 1) is a consequence of the increase in airway inflammation (Fig. 2). It is unlikely that the enhanced O₃-induced airway inflammation observed in the ob/ob mice is the direct result of leptin deficiency, because treating ob/ob mice with leptin did not restore O₃-induced inflammation to that observed in wild-type mice (Fig. 3). Instead, our results suggest that the changes observed in ob/ob mice resulted from an effect of chronic leptin deficiency, such as obesity per se, or reduced lung size, that could not be reversed with acute leptin treatment. Although the inhaled volume of O₃ was the same in ob/ob and wild-type mice, a greater dose of O₃ per gram of lung tissue was actually delivered to the obese mice, because their ventilation was essentially the same as that of the wild-type mice, but their lungs were smaller. Such differences in dose could have accounted for at least part of the differences in O₃-induced inflammation and AHR. It will ultimately be important to examine other murine models of obesity in which lung growth is not impacted to determine whether the reduced lung size of the ob/ob mouse accounted for the differences in response to O₃ or whether other aspects of the obese phenotype also contribute. For example, chronic systemic inflammation in the obese (19, 24, 35, 55, 56) may amplify subsequent inflammatory responses.

One of the most interesting results of this study was the observation that, in wild-type mice, exogenous leptin increased O₃-induced cytokine and protein release into BAL fluid (Fig. 3). To our knowledge, this is the first report of proinflammatory effects of leptin in the lung in vivo, although leptin has also been shown to enhance inflammatory and/or immune responses in other organs. For example, treatment of mice with exogenous leptin has been shown to augment the inflammatory and fibrogenic response of the liver to hepatotoxic chemicals (21). Leptin treatment has also been shown to render mice more susceptible to the development of autoimmune encephalomyelitis after immunization with myelin-derived peptides (32). The tertiary structure of leptin resembles that of many cytokines, and the leptin receptor has close homology to class I cytokine receptors, along with JAK/STAT signaling capabilities (50). Hematopoietic cells express leptin receptors, and bone marrow cells cultured in the presence of leptin demonstrate growth of granulocyte-macrophage colonies (16, 54). The leptin receptor is also expressed in CD4⁺ T cells, and both mouse and human T cells respond to leptin with increased proliferative responses to mitogenic stimuli and increased production of some cytokines (28, 31). Peritoneal macrophages also express leptin receptors and respond to leptin administration with an increase in LPS-stimulated TNF-α, IL-6, and IL-12 production (16, 27). In human monocytes, leptin induces the expression of activation markers, causes proliferation, and increases IL-6 and TNF-α expression (43). Leptin receptors are also present on endothelial cells and cause activation of activator protein-1 and NF-κB, transcription factors important in the induction of many inflammatory proteins (4). We do not know the precise cell types that are the target of leptin’s effects on O₃-induced pulmonary inflammation. However, both macrophages and airway epithelial cells have been shown to be targets of O₃ (1, 39), both cell types express leptin receptors (27, 53), and both cell types express cytokines after O₃ exposure (22).

In summary, our results demonstrate increased airway responsiveness in ob/ob mice. Whereas the re-
duced lung size of these mice may contribute to part of this difference in responsiveness, it is possible that low-grade systemic inflammation also plays a role. Our results also demonstrate that both O₃-induced AHR and O₃-induced airway inflammation are enhanced in ob/ob mice. Differences in inhaled O₃ dose consequent to differences in lung size may account for some of these changes. As such, the effects of leptin on lung growth may confound use of this leptin-deficient model of obesity for understanding the relationship between obesity and asthma. Other models in which obesity develops without attendant effects on lung size may prove more useful. However, the results suggest the possibility that the obese may represent an “at risk” population in terms of their susceptibility to air pollutants.

Our results also indicate that leptin has the capacity to augment O₃-induced airway inflammation. Given that leptin is produced in proportion to adipocyte mass and that leptin concentrations are four to six times greater in severely obese compared with lean human subjects (30, 41), it is possible that differences in serum leptin may also promote allergic airway inflammation and asthma. In this respect, it is interesting to note that the association between obesity and asthma is stronger in females than in males (9, 20). Similarly, for equivalent body mass index, leptin levels are higher in women than in men (41).

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DISCLOSURES

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