HIGHLIGHTED TOPIC | Pulmonary Physiology and Pathophysiology in Obesity

Onset of obesity in carboxypeptidase E-deficient mice and effect on airway responsiveness and pulmonary responses to ozone

Richard A. Johnston,1,2 Ming Zhu,1 Christopher B. Hernandez,2 Erin S. Williams,1 and Stephanie A. Shore1

1Molecular and Integrative Physiological Sciences Program, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts; and 2Division of Pulmonary, Allergy, and Critical Care Medicine, Department of Internal Medicine, The University of Texas Medical Branch, Galveston, Texas

Submitted 17 July 2009; accepted in final form 16 March 2010

Johnston RA, Zhu M, Hernandez CB, Williams ES, Shore SA. Onset of obesity in carboxypeptidase E-deficient mice and effect on airway responsiveness and pulmonary responses to ozone. J Appl Physiol 108: 1812–1819, 2010. First published March 18, 2010; doi:10.1152/japplphysiol.00784.2009.— When compared with lean, wild-type mice, obese Cpefat mice, 14 wk of age and older, manifest innate airway hyperresponsiveness (AHR) to intravenous methacholine and enhanced pulmonary inflammation following acute exposure to ozone (O3). The purpose of this study was to examine the onset of these augmented pulmonary responses during the onset of obesity. Thus airway responsiveness and O3-induced pulmonary inflammation and injury were examined in 7- and 10-wk-old Cpefat and age-matched, wild-type, C57BL/6 mice. Compared with age-matched controls, 7- and 10-wk-old Cpefat mice were approximately 25 and 61% heavier, respectively. Airway responsiveness to intravenous methacholine was assessed via forced oscillation in unexposed Cpefat and wild-type mice. The 10- but not 7-wk-old Cpefat mice exhibited innate AHR. O3 exposure (2 ppm for 3 h) increased markers of pulmonary inflammation and injury in the bronchoalveolar lavage fluid of all mice. However, most markers were greater in Cpefat vs. wild-type mice, regardless of age. Serum levels of leptin, a satiety hormone and proinflammatory cytokine, were increased in Cpefat vs. wild-type mice of both age groups, but the serum levels of other systemic inflammatory markers were greater only in 10-wk-old Cpefat vs. wild-type mice. These results demonstrate that a 25% increase in body weight is sufficient to augment pulmonary responses to O3, but innate AHR is not manifest until the mice become much heavier. These results suggest that the mechanistic bases for these responses are different and may develop according to the nature and degree of the chronic systemic inflammation that is present.

leptin; neutrophil; pulmonary resistance; sTNFR1; sTNFR2

Obesity is a major public health problem in both the developed and developing world (34, 44). Obesity is also a risk factor for the development of certain respiratory diseases, including asthma (14, 47). Epidemiological studies indicate an increased prevalence and incidence of asthma in overweight or obese children, adolescents, and adults (reviewed in Refs. 14, 47). Prospective studies controlling for a number of potential confounders, including physical activity, total energy intake, and smoking status, indicate that the relative risk of incident asthma progressively increases with increasing body mass index (BMI) and that obesity precedes the development of asthma (7, 10). In addition, obese asthmatic individuals examined after diet-induced or surgically induced weight loss report a decrease in both the severity and symptoms of asthma (37, 53). Obese humans also have greater decrements in pulmonary function following exposure to ozone (O3) (1, 3), a common environmental pollutant and an asthma trigger (13). Taken together, these observations indicate that obesity either causes and/or worsens asthma, but the mechanistic basis underlying this relationship is not well understood.

Our laboratory has been utilizing mouse models of obesity to understand the relationship between obesity and asthma. We have reported that in the absence of any inciting stimulus, airflow hyperresponsiveness (AHR) to intravenously administered bronchoconstrictors is observed in mice obese as a result of a genetic deficiency in leptin, a satiety hormone (ob/ob mice) (27, 45, 50), or the long form of the leptin receptor (db/db mice) (36, 52); in mice obese due to a genetic deficiency in carboxypeptidase E (Cpe) (25), an enzyme involved in processing prohormones and proneuropeptides involved in satiety and energy expenditure (Cpefat mice); and in mice obese due to the consumption of a high-fat diet (24). We have also shown that acute exposure to O3 augments airway responsiveness, pulmonary injury, and pulmonary inflammation to a greater extent in obese compared with lean mice (24, 25, 32, 36, 45, 50, 52). Thus obese mice manifest many of the characteristic features of asthma, including AHR to nonspecific bronchoconstrictors and enhanced pulmonary responses to O3, and may serve as useful tools to enhance our understanding of the relationship between obesity and asthma.

Besides being a major storage depot for lipids, adipose tissue is now recognized as an important endocrine organ that synthesizes many hormones, including leptin, adiponectin, and resistin (39). In obesity, adipose tissue becomes infiltrated with macrophages and also synthesizes acute-phase proteins, cytokines, chemokines, and soluble cytokine receptors, including IL-6, monocyte chemotactic protein (MCP)-1, plasminogen activator inhibitor (PAI)-1, and TNF-α (6, 39, 46, 59, 60). These substances, collectively termed adipokines, are largely proinflammatory in nature and once released into the systemic circulation result in a condition commonly referred to as chronic systemic inflammation. Importantly, the levels of some of these adipokines correlate directly with the severity of obesity-related diseases, such as atherosclerosis and insulin
were exposed to O3 (2 ppm) for 3 h. Separate but identical chambers (145-liter stainless steel and Plexiglas exposure chamber where they individual wire-mesh cages, which were subsequently placed inside a...tion between the innate AHR and the increased responses to O3 C57BL/6 control mice. Our data indicate a temporal disso- ciation from The Jackson Laboratory. Since the...marked across obesity groups. The percentage of male mice was 45% and 40% among the 7-wk-old wild-type and Cpefat mice, respectively, and 32% and 27% for the 10-wk-old wild-type and Cpefat mice, respectively. Protocol. Two cohorts each of 7- and 10-wk-old wild-type, C57BL/6 and Cpefat mice were used in this study. In the first cohort of mice, the animals were anesthetized and instrumented for the measurement of baseline pulmonary mechanics and airway responsiveness to methacholine (Sigma-Aldrich; St. Louis, MO). In the second cohort of 7- and 10-wk-old mice, the animals were euthanized, blood was collected, and a bronchoalveolar lavage (BAL) was performed 4 h following the cessation of a 3-h exposure to either O3 (2 ppm) or room air. We chose to examine the animals 4 h following the cessation of exposure because we have previously reported that this O3 exposure regimen significantly elevates the BAL fluid (BALF) concentrations of many inflammatory cytokines and chemokines of interest, and because O3-induced inflammation is increased in Cpefat mice as well as other types of obese mice compared with lean, wild-type control mice with this exposure regimen (24, 25, 32, 36, 45, 50). After PBS (1 μl/g) or increasing concentrations of methacholine, dissolved in PBS (1 μl/g), were injected, we measured pulmonary resistance (Rt), using a sinusoidal forcing function at a frequency of 2.5 Hz every eighth breath until resistance peaked. At that point, measurements of total lung imped- ance (Zl) were obtained using an 8-s optimized pseudorandom signal containing frequencies ranging from 0.25 to 19.63 Hz. A parameter estimation model (16) was used to partition Zl into components representing airway resistance (Raw) and the coefficients of lung tissue damping (G) and lung tissue elastance (H).

Blood collection and BAL. The animals were euthanized with an intraperitoneal injection of pentobarbital sodium, and blood was subsequently collected from the heart via cardiac puncture. The red blood cells in a 10-μl aliquot of blood were lysed in a cell lysis solution, and the total number of blood leukocytes was then enumerated with a hemacytometer. In addition, serum was isolated from the blood and stored at −20°C until analyzed.

After blood was collected from each animal, a BAL was performed and the BALF cells and differentials were counted as previously described (23). The BALF was stored at −80°C until needed. Subsequently, the total BALF protein concentration was determined spectrophotometrically according to the Bradford protein assay procedure (Bio-Rad Laboratories; Hercules, CA). The concentrations of BALF and/or serum adiponectin, etoxacin, KC, monocyte chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-2, and soluble tumor necrosis factor receptor (sTNFR) 1 and 2 were deter- mined by Quantikine ELISA kits or DuoSet ELISA development systems (R&D Systems; Minneapolis, MN), according to the manufac- turer’s instructions.

Statistical analysis of data. Comparisons of body weight and serum markers of inflammation were assessed by factorial ANOVA with genotype and age as the main effects. In the case of serum IL-6, the data were logarithmically transformed to conform to a normal distribution. Within each age group, comparisons of BALF parameters were assessed by factorial ANOVA with genotype, age, and exposure as the main effects. Fisher’s least significance difference test was used as a follow-up to determine the significance of differences between the individual groups. For the analyses of BALF cells, values were logarithmically transformed to conform to a normal distribution. Within each age group, comparisons of methacholine-induced changes in pulmonary mechanics were assessed by repeated-measures ANOVA using the dose of methacholine as the repeated variable and genotype and age as the main effect. STATISTICA software (StatSoft; Tulsa, OK) was used to perform all statistical analyses. A P value < 0.05 was considered significant. All values are expressed as means ± standard error of the mean (SE), unless otherwise noted.

RESULTS

Body mass. Factorial ANOVA indicated a significant effect of age and genotype on body mass in both male and female mice (P < 0.001 in each case). Regardless of age, Cpefat mice weighed significantly more than their age- and sex-matched, wild-type controls (Fig. 1), but the magnitude of obesity increased with age. At 7 and 10 wk of age, Cpefat mice weighed approximately 25 and 61% more, respectively, than age-matched controls.
Serum markers of inflammation. Factorial ANOVA indicated a significant ($P < 0.05$) effect of genotype on the levels of all systemic markers of inflammation (sTNFR1, sTNFR2, IL-6, leptin, adiponectin, and blood leukocytes) examined in the serum or blood, with the exception of MCP-1 ($P = 0.084$). In each case, the effect lay in the 10-wk-old mice, in which levels were significantly greater in $Cpe^{fat}$ mice compared with age-matched controls (Fig. 2). In 7-wk-old mice, the levels of each systemic marker of inflammation were slightly greater in $Cpe^{fat}$ compared with wild-type mice. However, in the 7-wk-old mice, the only outcome indicator that was significantly increased in $Cpe^{fat}$ vs. wild-type mice was serum leptin (Fig. 2E).

Airway responsiveness to intravenous methacholine. We have previously reported that by 14 wk of age, $Cpe^{fat}$ mice exhibit innate AHR (25). To examine the onset of this phenomenon with increasing obesity, we measured changes in $R_l$ induced by intravenous methacholine in unexposed 7- and 10-wk-old $Cpe^{fat}$ and age-matched controls (Fig. 3, A and B). Repeated-measures ANOVA indicated a significant ($P < 0.001$) effect of genotype in 10-wk-old mice, but not in 7-wk-old mice. In the 10-wk-old mice, responses to methacholine were significantly greater in the $Cpe^{fat}$ than the wild-type mice (Fig. 3B). We also observed a significant ($P < 0.001$) interaction between methacholine dose and age in the $Cpe^{fat}$ mice, with greater changes in $R_l$ at 0.3 mg/ml methacholine in 10- vs. 7-wk-old $Cpe^{fat}$ mice. In contrast, in wild-type mice, there was no significant interaction between dose and age, and the trend was in the opposite direction (i.e., lower responses to methacholine in the older mice). To determine if changes in the airways or the lung tissues accounted for the AHR observed in 10-wk-old $Cpe^{fat}$ mice, we also examined methacholine-induced changes in $R_{aw}$, $G$, and $H$ in these animals (Fig. 4). Methacholine caused a dose-related increase in $R_{aw}$ in 10-wk-old mice (Fig. 4A), and repeated-measures ANOVA indicated that changes in $R_{aw}$ were significantly greater ($P < 0.001$) in $Cpe^{fat}$ compared with wild-type mice. There was no significant difference in baseline $G$ and $H$ between 10-wk-old $Cpe^{fat}$ and their age-matched controls, although there was a trend for both to be increased in the $Cpe^{fat}$ mice (Fig. 4, B and C). Further-

Fig. 1. Total body masses of male and female wild-type and $Cpe^{fat}$ mice at 7 or 10 wk of age. Values are means ± standard error of the means (SE); $n = 6–16$ mice for each group. *$P < 0.05$ compared with age- and sex-matched, wild-type mice.

Fig. 2. Serum concentrations of soluble tumor necrosis factor receptor 1 (sTNFR1) (A), sTNFR2 (B), IL-6 (C), monocyte chemotactic protein (MCP)-1 (D), leptin (E), and adiponectin (F), as well as the number of blood leukocytes (G) from room air-exposed wild-type and $Cpe^{fat}$ mice, which are 7 or 10 wk of age. Values are means ± SE; $n = 6–9$ mice for each group. *$P < 0.05$ compared with age-matched, wild-type mice.
more, methacholine did not significantly increase G or H, in either 10-wk-old Cpefat or wild-type mice (Fig. 4, B and C).

O₃-induced lung injury and inflammation. We have previously reported that the lung injury and inflammation induced by acute O₃ exposure are significantly greater in Cpefat vs. wild-type mice that are at least 14 wk of age (25). To examine the onset of this phenomenon with increasing obesity, we measured the concentrations of BALF protein, IL-6, KC, MIP-2, and eotaxin as well as the number of macrophages, neutrophils, and epithelial cells in the lavage fluid of 7- and 10-wk-old wild-type and Cpefat mice (Fig. 5). Exposure to O₃ increased BALF protein, IL-6, KC, MIP-2, eotaxin, neutrophils, and epithelial cells in 7- and 10-wk-old wild-type and Cpefat mice (Fig. 5). Factorial ANOVA indicated a significant interaction between genotype and exposure on BALF protein, IL-6, KC, MIP-2, neutrophils, and epithelial cells (p ≤ 0.05 in each case): when considered without regard to age, the response to O₃ was greater in Cpefat vs. wild-type mice for each of these outcome indicators, consistent with previously reported data from mice at least 14 wk of age (25). Follow-up analysis using Fisher’s least significance difference yielded essentially similar results when each genotype was considered separately (Fig. 5). Note that the number of BALF macrophages was reduced rather than increased by ozone (Fig. 5), which likely reflects their increased...
activation and adhesion, making them less easy to wash out of the lungs. Furthermore, lymphocytes and eosinophils were virtually absent in the BALF of both wild-type and Cpefat mice, regardless of exposure (data not shown).

DISCUSSION

Our results demonstrate that small increases in body weight (i.e., 25%) were sufficient to augment O₃-induced pulmonary injury and inflammation in 7-wk-old Cpefat compared with age-matched, wild-type mice, whereas greater increases in body mass (i.e., 61%) were necessary before innate AHR was manifest in Cpefat mice at 10 wk of age. Augmented responses to O₃ were observed in Cpefat mice when only the serum levels of leptin, a satiety hormone and proinflammatory cytokine, were elevated, whereas innate AHR was not observed until numerous markers of systemic inflammation (i.e., sTNFR1, sTNFR2, IL-6, leptin, and blood leukocytes) were increased. Consequently, our data suggest that the mechanistic basis for these phenomena are not the same and may develop according to the nature and degree of the chronic systemic inflammation that is present.

In this study, we monitored the onset of obesity in Cpefat mice from 7 until 10 wk of age (Fig. 1). Cpefat mice become obese due to a missense mutation in the gene encoding carboxypeptidase E, an enzyme important in processing prohormones and pronuropeptides involved in satiety and energy expenditure (33). Obesity develops more slowly in Cpefat mice than in ob/ob and db/db mice, which are obese due to a genetic deficiency in either leptin or the long isoform of the leptin receptor, respectively (33). At 8–12 wk of age, ob/ob and db/db mice weigh ~150% more than their lean, wild-type controls (36, 50), suggesting that leptin is of greater importance in regulating body mass than carboxypeptidase E. Nevertheless, in Cpefat mice, obesity can be quite marked: mice 14 wk of age and older weighed 88% more than age-matched controls (25). However, the less rapidly developing obesity observed in Cpefat mice make this strain a better model of obesity for examining pulmonary changes during the onset of obesity.

Other investigators have examined age-related differences in airway responsiveness to intravenous methacholine in naive mice and report that airway responsiveness is greatest in young (3–4 wk of age) mice and then declines in mice 6 and 8 wk of age (4). Our data indicate that at 7 wk of age, wild-type and Cpefat mice have similar responsiveness (Fig. 3A), but at 10 wk of age, airway responsiveness increases in the Cpefat mice (Fig. 3B). This innate AHR is also observed in 14- to 16-wk-old Cpefat mice (25) and in other types of obese mice (24, 36, 50). Anesthetics can either increase or decrease responsiveness to bronchoconstrictors (9, 29), so we cannot rule out the possibility that effects of excess adipose tissue on the absorption, distribution, and metabolism of the anesthetics might be impacting responsiveness in the obese mice. However, the data
from 10-wk-old mice indicate that the locus of this innate AHR is the airways rather than the lung tissues, consistent with our previous findings in obese mice (24, 25, 45). It would be surprising if alterations in the pharmacokinetic profile of the anesthetics differentially impacted the airways and the lung tissues.

Small increases in body mass, such as those observed in the 7-wk-old mice, were not sufficient to evoke AHR (Fig. 3A). Aside from increases in serum leptin, we also did not observe any significant increases in measures of systemic inflammation in the 7-wk-old Cpefat mice, whereas most of those outcomes were increased by 10 wk of age (Fig. 2), when AHR was observed (Fig. 3). Taken together, the results indicate that the onset of innate AHR correlates temporally with the onset of systemic inflammation in these obese mice, suggesting that this inflammation may contribute to obesity-related AHR. We do not think that changes in serum leptin play a role in this hyperresponsiveness: leptin was already elevated at 7 wk of age in Cpefat mice, but these mice did not exhibit AHR. Furthermore, exogenous administration of leptin does not augment airway responsiveness to methacholine in unexposed C57BL/6 mice (26), nor does leptin augment contraction of isolated, airway smooth muscle in tissue baths (41). The soluble forms of the two TNF-α receptors, TNFR1 and TNFR2, were also elevated in the serum of 10-wk-old Cpefat mice (Fig. 2, A and B). These receptors are cleaved from the cell surface by TNF-α converting enzyme, an enzyme that has been shown to be activated under conditions of oxidative stress (17). Thus it is conceivable that obesity-related elevations in serum sTNFR1 and sTNFR2 are markers of the systemic oxidative stress that is characteristic of obesity (15). The relationship between oxidative stress and AHR in obesity remains to be established, but it is important to note that obese asthmatics have elevated levels of 8-isoprostane, a marker of oxidative stress, in exhaled breath condensate (31). We also observed increased levels of the pleiotropic cytokine, IL-6, in the serum of 10- but not 7-wk-old mice (Fig. 2C). We previously reported increased serum IL-6 in 24-wk-old Cpefat vs. wild-type mice (49). While it is conceivable that this increased serum IL-6 contributes to the innate AHR observed in obese Cpefat mice, others have reported that transgenic overexpression of IL-6 in the lungs of mice decreases rather than increases responsiveness to methacholine (12). We were surprised to find elevated rather than reduced levels of adiponectin in the blood of 10-wk-old Cpefat vs. wild-type mice (Fig. 2F), since we and others have previously observed reduced adiponectin expression in obese mice (19, 36). Similarly, we have observed reduced adiponectin in Cpefat mice, which are 25 wk of age and older (unpublished observations). Others have reported similar findings in obese ob/ob mice: younger (8 wk old) ob/ob mice, which are still substantially obese, have increased adiponectin vs. wild-type controls, whereas older (12 wk old) ob/ob mice have reduced adiponectin (61). One possible explanation is that during initial expansion of adipose tissue, serum adiponectin increases as a result of an increase in adipose tissue mass, whereas with continuing obesity, endoplasmic reticulum stress and other factors act to decrease the ability of individual adipocytes to express adiponectin. When increased by exogenous administration, adiponectin reduces airway responsiveness in allergen-sensitized and -challenged mice, but not in allergen-sensitized, PBS-challenged mice (51). Taken together, the results suggest that changes in serum adiponectin are unlikely to account for the obesity-related increases in airway responsiveness. There were also substantial, albeit not quite significant increases in serum MCP-1 in the 10-wk-old Cpefat mice (Fig. 2D). The relationship between serum MCP-1 and AHR has not been established, but others have reported that the intratracheal administration of recombinant murine MCP-1 to CBA/J mice increases responsiveness to methacholine (8). Furthermore, dramatic increases in adipose tissue MCP-1 mRNA expression in mice with diet-induced obesity also correspond with the onset of insulin resistance (62). Finally, there may be other unmeasured aspects of this systemic inflammation that contribute to AHR in obese mice. For example, TNF-α is increased in the serum of obese mice (18), and exogenous TNF-α can augment airway responsiveness (55).

We do not know exactly how systemic inflammation might contribute to the induction of AHR in obese mice. It is conceivable that inflammatory moieties in the serum act on airway smooth muscle cells to increase their contractility. However, it is also possible that neurons that innervate the airways or blood vessels that pass close to the airway walls are the target of these inflammatory moieties and that alterations in these cells impact their modulatory effects on airway narrowing.

Compared with age-matched controls, both 7- and 10-wk-old Cpefat mice had increased O3-induced pulmonary injury and inflammation compared with wild-type mice, as indicated by increased levels of BALF protein, IL-6, KC, MIP-2, eotaxin, neutrophils, and epithelial cells (Fig. 5). Similar results were obtained in Cpefat mice that were 14 wk of age and older (25), and in other types of obese mice (24, 32, 36, 50, 52). Since the 7-wk-old obese mice weighed only 25% more than wild-type controls, these results indicate that even small increases in body mass are sufficient to augment the effects of acute O3 exposure. For comparison, depending on exactly what BMI one uses as a reference “normal”, a 25% increase in body weight in a human subject would place that subject just into the “overweight” range. The results also reveal discordance between the onset of innate AHR and enhanced responses to O3 exposure in obese mice: whereas 7-wk-old Cpefat mice had not yet developed innate AHR (Fig. 3A), increased responses to O3 were already manifest in these mice (Fig. 5). Of the various markers of systemic inflammation measured, only serum leptin was significantly elevated in 7-wk-old Cpefat mice (Fig. 2E). It is certainly possible that there were other unmeasured aspects of this systemic inflammation that were also increased in the 7-wk-old Cpefat mice that account for the increased responses to O3 in these mice. However, elevations in leptin could also account for these observations. In addition to being a satiety hormone, leptin is also proinflammatory (35). Importantly, we have reported that an intraperitoneal bolus administration of exogenous leptin to wild-type mice before and again just after exposure to 2 ppm O3 increased BALF IL-6, KC, and protein compared with mice that received intraperitoneal PBS (50). Interestingly, in that study, the magnitude of the change in serum leptin we induced with leptin treatment was very similar to the magnitude of the increase in leptin observed in 7-wk-old Cpefat vs. wild-type mice (Fig. 2E). Both macrophages and airway epithelial cells express leptin receptors (35, 56) and are targets of O3 (2, 20). If leptin accounts for the enhancement of O3-induced pulmonary injury and inflammation in Cpefat mice,
there must be an additional factor or factors involved since similar results are observed in obese ob/ob and db/db mice (32, 36, 50, 52), which have impaired leptin signaling.

Although the goal of this study was not to examine the impact of age on responses to O₃, we did observe age-related differences in the cellular inflammation associated with O₃ exposure: BALF macrophages, neutrophils, and epithelial cells were lower in 10- than in 7-wk-old mice, whereas, with the exception of BALF IL-6, O₃-induced changes in other aspects of the inflammatory response to O₃ were largely unaffected by age (Fig. 5). Others, including ourselves, have reported greater responses to O₃ in adult mice than in neonates (21, 48, 58), although the impact of age can be modified by mouse strain (58). However, none of these studies compared 7- and 10-wk-old mice. Importantly, in this study, the effect of age was similar in wild-type and in Cpefat/ mice.

In addition to obesity, Cpefat/ mice display many common obesity-related comorbidities, including hyperglycemia, hyper-triglycerideremia, and hypercholesterolemia (11, 40, 42, 43). These conditions could contribute to the onset of obesity-induced asthma. For example, statins, which reduce the levels of cholesterol in the blood, have been shown to reduce allergen-induced airway inflammation in mice (30, 38). In contrast, we have established that reducing fasting blood glucose by metformin treatment fails to ameliorate the innate AHR or increased responses to O₃ observed in db/db mice (52), suggesting that hyperglycemia is not involved.

In conclusion, our results indicate that an approximate 25% increase in body mass was sufficient to augment O₃-induced pulmonary injury and inflammation in Cpefat/ mice. These results suggest that even small increases in body weight in human subjects may increase their susceptibility to the toxic effects of O₃. In contrast, greater increases in body mass (i.e., 61%) were necessary before the innate AHR characteristic of obese mice was manifest. The results suggest that the mechanistic bases for the innate AHR and the increased pulmonary responses to O₃ common to murine obesity are different.

GRANTS
This study was supported by National Heart, Lung, and Blood Institute Grant HL-084044 (to S. A. Shore), National Institute of Environmental Health Sciences Grants ES-013307 (to S. A. Shore) and ES-00002 (to D. W. Dockery), and American Lung Association Research Training Fellowship RT-41-N (to R. A. Johnston).

DISCLOSURES
No conflicts of interest (financial or otherwise) are declared by the authors.

REFERENCES


