Obesity and asthma: lessons from animal models

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Shore SA. Obesity and asthma: lessons from animal models. J Appl Physiol 102: 516–528, 2007; doi:10.1152/japplphysiol.00847.2006.— Epidemiological data indicate that obesity is a risk factor for asthma. These data are supported by observations in several murine models of obesity. Ob/ob, db/db, and Cpefat mice each exhibit innate airway hyperresponsiveness, a characteristic feature of asthma. These mice also respond more vigorously to common asthma triggers, including ozone. Here we discuss the implications of these data with respect to several mechanisms proposed to explain the relationship between obesity and asthma: 1) common etiologies; 2) comorbidities; 3) mechanical factors; and 4) adipokines. We focus on the role of adipokines, especially TNF-α, IL-6, leptin, and adiponectin. Understanding the mechanistic basis for the relationship between obesity and asthma may lead to novel therapeutic strategies for treatment of the obese asthmatic subject.

mice; leptin; adiponectin; tumor necrosis factor-α; interleukin-6; adipokines

ACCORDING TO THE US CENTERS for Disease Control, ~65% of the US population is either overweight or obese. The prevalence has more than doubled during the last 20 years (www.cdc.gov/ncedphp/dnpa/obesity/trend/index.htm) and continues to rise. Obesity is a known risk factor for atherosclerosis, hypertension, Type 2 diabetes, and some forms of cancer (33). Obesity is also a risk factor for asthma. Data supporting a relationship between obesity and asthma include over 30 cross-sectional studies performed in adults and children of multiple ethnicities throughout the world [for a complete list, see recent reviews (34, 115)]. With relatively few exceptions, each of these studies indicates a greater prevalence of asthma in the obese. While such cross-sectional studies cannot sort out the direction of causality, data from 13 prospective studies in adults, adolescents, and children indicate that obesity antedates asthma [see Shore and Johnston (115)]. In these studies, several hundred thousand individuals initially free of asthma were followed for periods varying from 2 to 21 yr. In aggregate, the results indicate that the relative risk of incident asthma increases with body mass index (BMI) and that even overweight conveys some increased risk. Obesity may also increase disease severity in subjects who already have asthma (1). In addition, obesity appears to alter the efficacy of standard asthma medications (98).

The observations that both weight loss and weight gain impact asthma provide additional evidence of a relationship between obesity and asthma. The impact of weight gain can be marked. For example, Camargo et al. (14) reported that women who gained >25 kg since age 18 yr had a relative risk for incident asthma almost five times that of women whose weight remained stable. In contrast, surgically induced weight loss results in significant improvements in all asthma outcomes, including prevalence, severity, use of asthma medications, and hospitalizations for asthma (27, 121, 124). Studies of diet-induced weight loss in obese asthmatic subjects also report improvements in flow rates (45, 125).

The reason for the relationship between obesity and asthma has not been established, although several possibilities have been suggested (112, 115, 142, 143) (Fig. 1). It has been hypothesized that obesity and asthma share a common etiology, such as a common genetic predisposition, or common effects of in utero conditions. It may also be that obesity per se is not the culprit. Instead, comorbidities of obesity, such as gastroesophageal reflux or sleep-disordered breathing, may provoke or worsen asthma. The obese breathe with small tidal volumes at low absolute lung volume, and both of these factors could be expected to increase airway obstruction. Finally, it is now increasingly appreciated that there are alterations in the endocrine function of adipose tissue in obesity. Obesity-related changes in adipose-derived hormones, cytokines, and other factors may also play a role in the relationship between obesity and asthma.

To examine the mechanistic basis for the relationship between obesity and asthma, we set out to determine whether we could model the relationship between obesity and asthma in mice. Here we review the various murine models of obesity that have been studied and the pulmonary phenotype of these mice, a phenotype that includes both innate airway hyperresponsiveness (AHR), a defining characteristic of asthma, as well as increased responses to ozone (O₃) and allergen, two common triggers for asthma. We also review what we have learned from these mice about the likely mechanistic basis for the relationship between obesity and asthma.

ANIMAL MODELS OF OBESITY

The pulmonary phenotype of several types of obese mice has been characterized (60, 81, 92, 103, 116, 129, 130). A brief description of the cause and nature of the obesity in each of these models is presented below, and their phenotypes are summarized in Table 1. We also describe several other murine models that may prove useful in unraveling the relationship between obesity and asthma.
**Mechanisms proposed to explain the relationship between obesity and asthma**

**Common etiologies**
- In utero conditions
- Genetics

**Co-morbidities**
- Gastroesophageal reflux
- Sleep-disordered breathing
- Type 2 diabetes
- Hypertension

**Effects of obesity on lung mechanics**
- ↓ FRC
- ↓ tidal volume

**Adipokines**
- Cytokines
- Chemokines
- Energy regulating hormones
- Acute phase reactants
- Other factors

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**Table 1. Phenotypes of obese mouse**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Ob/ob</th>
<th>Db/db</th>
<th>Cpe&lt;sup&gt;fat&lt;/sup&gt;</th>
<th>DIO</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity</td>
<td>Massive</td>
<td>Massive</td>
<td>Moderate</td>
<td>Milder</td>
<td>11, 21, 60, 70, 92, 97</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Yes</td>
<td>21, 52, 70, 87, 88, 93, 110</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Yes</td>
<td>21, 52, 70, 88, 93, 110</td>
</tr>
<tr>
<td>Increased plasma cholesterol</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Yes</td>
<td>75, 88</td>
</tr>
<tr>
<td>Innate AHR</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>60, 81, 103, 116 and unpublished observations</td>
</tr>
<tr>
<td>Small lungs</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>60, 81, 116, 130</td>
</tr>
<tr>
<td>O&lt;sub&gt;3&lt;/sub&gt;-induced AHR</td>
<td>Greater than wild type</td>
<td>Greater than wild type</td>
<td>Greater than wild type</td>
<td>N/A</td>
<td>60, 81, 103, 116</td>
</tr>
<tr>
<td>O&lt;sub&gt;3&lt;/sub&gt;-induced inflammation</td>
<td>Greater than wild type</td>
<td>Greater than wild type</td>
<td>Greater than wild type</td>
<td>60, 81, 116</td>
<td></td>
</tr>
</tbody>
</table>

AHR, airway hyperresponsiveness; O<sub>3</sub>, ozone; DIO, diet-induced obesity; N/A, not assessed. *Available data are from Cpe<sup>fat</sup> mice on a C57BL/Ks background. Otherwise, all data are from mice on a C57BL/6 background. †Most of the insulin is proinsulin.
Diet-Induced Obesity

Weanling C57BL/6 mice fed a diet in which 45 or 60% of calories are derived from fat (predominantly in the form of lard) develop obesity (11, 92, 137). After 20 wk on a 60% fat diet, body weight is ∼25% greater than for age-matched mice fed a diet in which only 10% of calories derive from fat. After 32 wk on the diet, weight is increased by ∼45% (unpublished observations). Most of the increase in body weight is the result of an increase in fat mass (11), similar to ob/ob mice. Mice rendered obese by high-fat diet, like ob/ob and db/db mice, are also hyperglycemic and insulin resistant (52, 93, 110, 126). Plasma cholesterol is also elevated in this model (75).

Other Murine Models of Obesity

Mice with other forms of genetic obesity exist (15, 21, 53), but their pulmonary phenotype has not been characterized. Tubby mice (21) and G protein-coupled receptor-7-deficient mice (53) display mature-onset obesity that may be useful in separating effects of obesity on lung development from other effects that lead to altered airway function (see below). G protein-coupled receptor-7 is the receptor for neuropeptides B and W that are involved in eating behavior (128), while tub was expressed at high levels in regions of the hypothalamus involved in eating behavior (107), whose precise function is still not defined. Since hyperinsulinemia is milder in tubby mice than in ob/ob and db/db mice, and hyperglycemia is not observed (21), tubby mice may also prove useful in determining whether insulin resistance or hyperglycemia contribute to the lung phenotype observed in obese mice. Jackson Laboratories has also developed some congenic strains of mice that differ in their susceptibility to Type 2 diabetes and obesity that may be helpful in understanding the role of hyperglycemia for asthma (72, 73, 101). In addition to genetically obese mice, methods also exist for inducing obesity in otherwise normal mice. For example, injecting mice with gold-thioglucose selectively ablates glucose-responsive neurons in the ventromedial hypothalamic nucleus, causing hyperphagia and obesity (53). Such methods may prove useful for inducing obesity in genetically altered mice.

OBESE MICE EXHIBIT INNATE AHR

Airway responsiveness to intravenous methacholine is increased in ob/ob, db/db, and Cpefat mice (60, 81, 103, 116), indicating that it is a common feature of murine obesity. In these obese mice, AHR is nonspecific, since increased responses to serotonin are also observed (81) (Fig. 2). Nonspecific AHR to multiple bronchoconstricting agonists is also a feature of human asthma. We have also examined airway responsiveness in mice with diet-induced obesity (unpublished observations). By 23 wk of age, when weight gain is still moderate (25% increase), no AHR is observed in mice on diets in which 60% of calories derive from fat, but by 35 wk, when more substantial weight gain has occurred (45% increase), airway responsiveness is increased, suggesting that weight gain must be fairly substantial before AHR is manifest. By comparison, ob/ob, db/db, and Cpefat mice weighed 175, 150, and 85% more than wild-type controls, respectively, when AHR was observed (60, 81, 116). AHR has also been associated with obesity in three large epidemiological studies (16, 19, 77), although this association is not consistently observed (34, 115). Most of the studies in which AHR was not observed to correlate with BMI involved smaller numbers of subjects and may have been less powered to detect differences, especially if substantial increases in body weight are required for the development of AHR.

We used changes in pulmonary resistance (Rt) to assess responses to methacholine in obese mice. While Rt includes contributions from both the airways and the lung tissue, measurements made in both ob/ob (103) and Cpefat mice (60) indicate that the increased responses to methacholine observed in obese mice are the result of differences in the airways. Changes in lung tissue resistance contribute very little to changes in Rt induced by intravenous methacholine in either obese or lean mice (60, 103).
While we do not currently know the mechanistic basis for the AHR observed in obese mice, we have ruled out several possible explanations. We have not observed any overt inflammation in the lungs of unchallenged obese mice, nor are there differences in the number of macrophages harvested from obese vs. lean mice by bronchoalveolar lavage (BAL) (60, 81). As discussed in more detail in MECHANISTIC BASIS FOR THE RELATIONSHIP BETWEEN OBESITY AND ASTHMA below, obesity-related alterations in functional residual capacity (FRC) and tidal volume may play a role in the relationship between obesity and asthma in spontaneously breathing humans, but additional factors must be important for the innate AHR observed in mice, since all measurements of pulmonary mechanics were made in open-chested mice, mechanically ventilation at a fixed positive end-expiratory pressure (PEEP) and a fixed tidal volume. Since AHR was observed both in ob/ob and db/db mice with leptin or leptin receptor deficiency, and in Cpefat mice and mice with diet-induced obesity that have marked increases in serum leptin (60, 92, 137), it is unlikely that leptin is involved, even though leptin has the potential to augment airway responsiveness (118). As discussed in more detail in MECHANISTIC BASIS FOR THE RELATIONSHIP BETWEEN OBESITY AND ASTHMA below, other adipokines may be involved in the innate AHR observed in obese mice. It is conceivable that hyperinsulinemia, which is marked in ob/ob and db/db mice, may contribute to the innate AHR observed in these strains. However, Cpefat mice also exhibit innate AHR. In these mice, hyperinsulinemia, although present, is the result of increased proinsulin, which is largely inactive, as described above. We also cannot rule out the possibility that hyperglycemia contributes to AHR, since all strains studied to date exhibit this phenotype to some extent (see above). There is currently increased interest in the hypothesis that increased oxidative stress, which can be a consequence of hyperglycemia, contributes to many aspects of the obese phenotype (50, 109), and oxidative stress has also been linked to asthma (64).

OBESE MICE HAVE INCREASED RESPONSES TO ACUTE O3 EXPOSURE

Exposure to O3, a common air pollutant, is a trigger for asthma. Hospital admissions for asthma are higher on days of high ambient O3 concentrations (32, 134), and, in children, O3 increases asthmatic symptoms even at concentrations below the US Environmental Protection Agency standard (39). O3 causes lung injury and an inflammatory response that includes the generation of prostanoids, cytokines, and chemokines, as well as an influx of neutrophils into the lungs (25, 56–60, 65, 81, 113). O3 also causes AHR (60, 81, 90, 156). Both O3-induced AHR and O3-induced inflammation are likely to contribute to the ability of O3 to exacerbate asthma.

Obesity impacts the effects of O3 in the lung. Pulmonary mechanics and airway responsiveness were measured 24 h after the cessation of acute O3 exposure (2 parts/million for 3 h) in ob/ob mice (103, 116), db/db mice (81), Cpefat mice (60), and their lean age- and sex-matched C57BL/6J wild-type controls. O3 exposure increased Rt. in obese mice, regardless of the modality of obesity, whereas O3 had no effect on Rt. in lean mice. In addition, O3 exposure caused much more robust changes in airway responsiveness in obese than in lean mice. In ob/ob and Cpefat mice, we only measured responses to methacholine, but in db/db mice, greater O3-induced AHR was observed, regardless of whether methacholine and serotonin was used as the bronchoconstricting agonist (Fig. 2), confirming the nonspecific nature of the AHR. A recent preliminary report also indicates greater effects of acute O3 exposure on pulmonary mechanics with increasing BMI in human subjects, especially women (6).

O3-induced injury and inflammation were also greater in ob/ob and db/db mice than in lean controls (60, 81, 116). Some investigators have reported that O3-induced AHR is mechanistically linked to certain aspects of O3-induced inflammation (20, 58, 117), although this is not always the case (74, 156). Hence it is possible that the augmented O3-induced AHR observed in obese mice is the result of their greater inflammatory response. To examine changes in the pulmonary inflammatory response to O3 over time with the development of obesity, we also exposed 7-, 10-, and 14-wk-old Cpefat mice and their age-matched wild-type controls to O3 (60, 62). Body weight averaged 20, 50, and 75% more, respectively, in these Cpefat mice than in age-matched wild-type mice. O3-induced airway inflammation was greater in Cpefat than wild-type mice, regardless of age (see Fig. 3 for data from 14-wk-old mice). Thus even a relatively moderate 20% increase in body weight is sufficient to increase the effects of O3 in mice, whereas more substantive changes appear to be required for effects on AHR (see above). Notably, serum leptin was substantially elevated at as early as 7 wk of age in Cpefat mice compared with age-matched controls, whereas changes in other measured indexes of systemic inflammation were not changed until 10 wk of age. Leptin can augment some aspects of the pulmonary response to O3 (116), but there must be important effects of obesity in addition to changes in serum leptin, since most of the inflammatory moieties induced by O3 are increased in ob/ob and db/db mice (81, 116), just as they are in Cpefat mice (60).

We initially considered the possibility that an increased inhaled dose of O3 was responsible for the increased response to O3 observed in obese mice. The inhaled dose of O3 is the product of O3 concentration, exposure time, and minute ventilation (144). O3 concentration and exposure time were identical in obese and lean mice. While there was greater minute ventilation in db/db mice than wild-type mice during O3 exposure (81), this was not true in ob/ob mice or in Cpefat mice (60, 81, 116). An additional issue related to dose is the small lung size of the ob/ob and db/db mice (see MECHANISTIC BASIS FOR THE RELATIONSHIP BETWEEN OBESITY AND ASTHMA below). Even if the inhaled dose of O3 was the same in obese and lean mice, the dose per gram of lung tissue was higher in the ob/ob and db/db mice than in wild-type controls, because their lung mass was smaller (116). We do not think this accounts for the greater inflammatory responses to O3 observed in obese mice, because similar increased responses were observed in Cpefat mice (60) and in mice with diet-induced obesity (unpublished observations). Both of these types of obese mice have lungs of normal mass.

OBESITY AND ALLERGIC RESPONSES

Atopy is an important risk factor for asthma, especially in children. What have animal models taught us about the effects of obesity on pulmonary responses to allergen challenge? Mito et al. (85) sensitized and challenged mice with diet-induced
obesity using ovalbumin (OVA). In the absence of sensitization, splenocytes from the obese mice had impaired proliferative responses compared with that from lean controls. However, following OVA sensitization and challenge, splenocyte proliferation, IL-2 production, and mast cell numbers were increased, while OVA-specific IgG1 and IgE were decreased in obese vs. lean mice. These authors did not measure airway responsiveness, but preliminary data from our laboratory indicate increased OVA-induced AHR in ob/ob mice vs. wild-type controls, even though there are no differences in T helper type 2 (Th2) airway inflammation (104). In contrast to the data from Mito et al. (85), we also observed increased effects of OVA challenge on IgE in ob/ob mice (104). The reason for this difference between our results and those of Mito et al. is not apparent, but it may be related to the modality of obesity. Although they did not examine obesity per se, Yeh and Huang (151) examined the impact of cholesterol on airway responses to OVA sensitization and challenge. They observed increased numbers of BAL fluid eosinophils and lymphocytes and increased levels of IL-5 in mice fed diets supplemented with cholesterol vs. chow-fed controls. Together, these results indicate that obesity and/or dietary constituents elevated in obesity can augment some but not all responses to allergen challenge.

Interestingly, results of studies of the effect of obesity on atopy have also been inconsistent [see Shore and Johnston for review (115)]. For example, some investigators have reported important effects of obesity on asthma in nonatopic, but not in atopic, subjects, and others have reported significant effects of BMI on asthma but not on other atopic diseases. In contrast, there have been some reports of increases in atopy with obesity, mostly in children. Since the onset of atopy usually occurs early in life, weight gain in early life may be more important for the development of atopy than weight gain as an adult. In this respect, mice in which obesity develops very early in life, such as ob/ob and db/db mice, may turn out to be more suitable as models for the interaction of obesity with atopy than other types of obese mice that become obese much later in life.

MECHANISTIC BASIS FOR THE RELATIONSHIP BETWEEN OBESITY AND ASTHMA

As described above, several mechanisms have been proposed to account for the relationship between obesity and asthma (Fig. 1). Below we summarize these mechanisms and describe, where available, evidence from animal models that addresses each one.

Epiphenomenon

When the epidemiological data demonstrating a relationship between obesity and asthma were first reported, one consideration was that this relationship was an epiphenomenon (14). It was suggested that the true relationship was between exercise and asthma or between diet and asthma. Indeed, increases in certain dietary constituents and lack of exercise can both lead to obesity. Several prospective studies controlling for exercise have since discounted the likelihood that lack of exercise is responsible for the relationship between obesity and asthma (5, 14, 91). Data from mice indicate that a relationship between obesity and an asthma-like phenotype in the lung can also exist in the absence of differences in diet. In the studies described above, mice with genetic obesity (ob/ob and db/db, and Cpeαα mice) ate exactly the same diet as their lean wild-type controls. They just ate more of it.

Common Etiologies

As described above, it has been proposed that obesity and asthma share a common etiology, for example, a common genetic predisposition, or common effects of in utero cond-
tions, and that the observed increases in the prevalence and incidence of asthma in the obese arise from this common predisposition (142). If so, there must be different factors that contribute to the relationship between obesity and an asthma-like pulmonary phenotype in mice, since the genetically obese mice in these studies differed from their wild-type controls at a single gene, a gene that was different in each model. In addition, ob/ob, db/db, and Cpefat mice are all infertile. Consequently, their mothers are all heterozygous and lean, and their in utero conditions are unlikely to differ from wild-type mice.

Comorbidities

It is also possible that comorbidities of obesity, such as gastroesophageal reflux or sleep-disordered breathing, may provoke or worsen asthma. Each of these conditions is known to affect asthma (44). To our knowledge, the presence of gastroesophageal reflux has not been assessed in any of the murine models of obesity described above. However, obese mice do have disordered sleep. Ob/ob mice have disrupted sleep architecture characterized by increased numbers of arousals and increased stage shifts compared with wild-type mice (69), and both ob/ob mice and mice with diet-induced obesity have increased amounts of 24-h non-rapid eye movement sleep (55, 69). Control of breathing during sleep is also altered in ob/ob mice, but is restored by exogenous leptin administration (92), indicating that it is the result of leptin deficiency, not some other aspect of obesity. In contrast, exogenous leptin does not reverse the increased O3-induced inflammation observed in ob/ob mice (116), suggesting that the latter phenotype is not the result of effects of sleep-disordered breathing. Similarly, control of breathing during sleep is not affected in mice with diet-induced obesity (92), even though these mice do have innate AHR and increased responses to O3 (unpublished observations). Hypercholesterolemia is a risk factor for asthma, but it appears to act independently of obesity (2). Hypertension and Type 2 diabetes are also important comorbid conditions with obesity, but little is known about the impact of these conditions on asthma.

Mechanical Factors

Both static and dynamic mechanical factors extant in the lungs of obese subjects have the capacity to aggravate airway obstruction (114, 115). The act of breathing stretches airway smooth muscle, causing actin-myosin cross bridges to detach, leading to bronchodilation (35, 43). Obese humans (108) and obese mice (115) breathe spontaneously with lower than normal tidal volumes, which would compromise this potent bronchodilating mechanism. Because of the load imposed by the increased abdominal and chest wall mass, FRC is also reduced in obesity (150). Absolute lung volume is a major determinant of airway diameter (26), and a lower FRC may unload the airway smooth muscle, allowing it to shorten excessively when activated. These mechanical factors may play a role in spontaneously breathing obese subjects. They may also be compounded by obesity-related changes in the compliance of the airways, which would further reduce the ability of tidal stretching to dilate the airways. Bergeron et al. (9) and Komakula et al. (66) each reported a decreased ability of a deep inspiration to dilate the airways of obese vs. lean asthmatic subjects. However, differences in tidal strain are unlikely to account for the AHR observed in obese mice, because measurements of pulmonary mechanics were made with the mice mechanically ventilated using a tidal volume that was the same for obese and lean mice. Nevertheless, we cannot rule out the possibility that breathing at low tidal volume for extended periods of time causes adaptations in the airway smooth muscle that are not easily reversed. We also removed the influence of the chest wall mass on absolute lung volume by measuring Rt. with the mice open chested and at a fixed PEEP. However, it is important to note that breathing at low absolute lung volume for several hours increases airway resistance, even after lung volume has been restored (24). Another important caveat must be considered with respect to lung volume. In ob/ob and db/db mice, end-expiratory lung volume is reduced, even when measured in open-chested animals with a fixed PEEP (81, 116, 130). The reduction in lung volume appears to be the result of decreased lung growth, since lung mass is also reduced (116). The small lungs of ob/ob and db/db mice may result from lack of the effects of leptin, which can act as a growth factor in the lung (8, 135). Alternatively, since obesity develops very early in these mice, it may be that the increased fat mass restricts lung growth during development. Large amounts of intrathoracic fat are observed even in 8-wk-old ob/ob and db/db mice. Alterations in lung or airway anatomy resulting from effects of obesity/leptin deficiency on lung development may contribute to the AHR observed in ob/ob and db/db mice, but the observation that lung size is normal in Cpefat mice (60) and in mice with diet-induced obesity (unpublished observations), which also exhibit AHR, does not support this hypothesis.

Adipokines

In humans, even in the absence of any overt inflammatory stimulus, the obese state is characterized by increases in the serum levels of multiple cytokines, chemokines, and soluble cytokine receptors, all of which decline with weight loss (Fig. 4). These include, but are not limited to, TNF-α, IL-1, IL-6,
These cells are the source of the bulk of the TNF-alpha expression observed in adipose tissue (141). Indeed, with the exception of adiponectin and leptin, nonadipocyte cells are the source of most of the adipokines released from human adipose tissue (31). Other serum factors that are elevated in obesity may derive from effects of these cytokines on the vasculature (122). Importantly, these cytokines and chemokines correlate with the presence of diseases common to obesity, including Type 2 diabetes, hypertension, and atherosclerosis (4, 99, 132, 140), suggesting that the inflammation is functionally important.

There are also obesity-related changes in other adipokines (proteins synthesized and released from adipose tissue), including hormones involved in energy regulation, such as leptin and resistin, as well as other factors, such as angiotensinogen and VEGF (Fig. 4) (7, 23, 37, 109, 120). In contrast, serum levels of the adipokine, adiponectin, an anti-inflammatory hormone, are decreased in obesity (29, 111, 149). Even though the liver is considered the main source of acute-phase reactants such as serum amyloid A and IL-6, the source of the increases in some of these reactants appears to be the adipose tissue (76). The observation that mice rendered adipocyte deficient have a marked decrease in their ability to increase serum amyloid A and IL-6 following injection of endotoxin also indicates that adipose tissue is an important source of acute-phase proteins (96).

Many of the adipokines listed in Fig. 4 have been associated with asthma, and it is conceivable that increases in their expression in the obese state could exacerbate airway inflammation or airway obstruction in asthma. For example, serum ectoFAC is increased in obese humans and in mice with diet-induced obesity (138). Similarly, serum levels of ectoFAC are elevated in ob/ob and db/db mice (unpublished observations). EctoFAC deficiency in mice demonstrates that ectoFAC contributes to the eosinophilic inflammation observed following allergen sensitization and airway challenge (106). VEGF, a potent angiogenesis factor, is expressed in adipose tissue (31), and VEGF is elevated in the serum of overweight and obese individuals (120). In asthmatic subjects, the number of airway cells expressing VEGF correlates with airway vascularity and correlates inversely with airway caliber (47). Obesity-related increases in serum plasminogen activator inhibitor-1, an important endogenous inhibitor of fibrinolysis and plasmin activation, could predispose toward AHR through effects on extracellular matrix turnover, as previously discussed (114, 115). Viscatin, previously described as pre-B-cell colony-enhancing factor, is an insulin mimetic that predisposes vascular smooth muscle toward a contractile phenotype (136). A similar effect of visfatin on airway smooth muscle, in conjunction with increased serum concentrations of visfatin in obesity (37), could lead to AHR.

TNF-alpha

As previously described in detail (115), there is reason to believe that obesity-related increases in circulating TNF-alpha may contribute to the relationship between obesity and asthma. A recent report indicates that peripheral blood monocytes from patients with refractory asthma have increased expression of membrane-bound TNF-alpha, TNFR1, and TNF-alpha-converting enzyme (10). Compared with placebo, asthmatic subjects in whom TNF-alpha was blocked by treatment with etanercept, a soluble TNF-alpha receptor, also showed reductions in airway responsiveness, increases in forced expiratory volume in 1 s, and improvements in asthma quality of life scores (10). Anti-TNF-alpha antibodies also reduces allergen-induced increases in methacholine responsiveness and pulmonary eosinophilia in a murine model of allergen-induced asthma (63), and the AHR induced by O3 is also attenuated in TNF-receptor-deficient mice (20, 117). Takeda together with the observation that exogenous TNF-alpha can induce AHR (133), the data suggest that the increase in circulating TNF-alpha observed in obese mice (49) may contribute to both their innate AHR and their increased responsiveness to O3. If so, it may be useful to examine the therapeutic potential of etanercept in obese asthmatic subjects.

IL-6

Obesity-related increases in IL-6 may also contribute to the relationship between obesity and asthma (Table 2). The source of much of the IL-6 in the serum of obese subjects appears to be the adipose tissue: measurements of arteriovenous differences across abdominal adipose tissue in obese subjects demonstrate higher levels of TNF-alpha and IL-6 in venous than arterial blood (86), and basal levels of IL-6 are substantially reduced in adipocyte-deficient mice (96). Serum IL-6 correlates with the development of other obesity-related syndromes, including atherosclerosis, hypertension, and diabetes mellitus (17, 100, 102), and polymorphisms of the IL-6 receptor are associated with BMI (30, 145). Treatment of mice with recombinant IL-6 also promotes the development of fatty streaks in the aortic sinus, suggesting that IL-6 is more than just a marker of atherosclerosis, but is required for early lesion development (51). We and others have previously reported that IL-6 is required for some aspects of O3-induced pulmonary inflamma-

Table 2. Evidence supporting a role for IL-6 in the relationship between obesity and asthma

<table>
<thead>
<tr>
<th>IL-6 and obesity</th>
<th>Exogenous administration of IL-6 promotes atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased IL-6 and sIL-6R in obese humans</td>
<td>Polymorphisms of the IL-6R are associated with obesity</td>
</tr>
<tr>
<td>and/or obese mice</td>
<td>Serum IL-6 correlates with the development of obesity-related syndromes</td>
</tr>
<tr>
<td>Anti-IL-6 antibodies attenuate airway responsiveness in obese mice</td>
<td>IL-6 and IL-6R are increased in asthma</td>
</tr>
<tr>
<td>Anti-IL-6 antibodies partially ablate obesity-related differences in the response to O3</td>
<td>Blockade of sIL-6R suppresses Th2 cells in a murine model of allergic airways disease</td>
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</table>

sIL-6R, soluble IL-6 receptor; Th2, T helper 2.
tion (59, 154), suggesting that obesity-related increases in IL-6 may also contribute to the exaggerated responses to O₃ observed in obese mice. Indeed, preliminary data from our laboratory demonstrate that anti-IL-6 antibody attenuates some, but not all, aspects of the increased O₃-induced inflammation observed in ob/ob mice (68). Surprisingly, we also observed reductions in innate airway responsiveness in obese, but not lean, mice treated with anti-IL-6 antibodies. Serum concentrations of the soluble IL-6 receptor are elevated, both in obesity (153) and in asthma (28, 105). IL-6 expression is also increased in the airways of asthmatic subjects (84). Increased soluble IL-6 receptor can combine with IL-6 and with membrane-bound gp130 to induce IL-6 responsiveness in cells that do not bear the IL-6 receptor (28, 105), suggesting that IL-6 may have effects in the obese state not normally observed in lean individuals.

**Leptin**

Leptin has profound effects on satiety and metabolism, as described above. However, leptin is also proinflammatory. Leptin is induced by infectious and inflammatory stimuli (41) and, in turn, stimulates proinflammatory cytokine production from monocytes and macrophages (38, 78, 83). Leptin also causes chemotaxis and release of reactive oxygen species in neutrophils (13). Since serum leptin concentrations are markedly increased in obesity (12, 23, 60, 81, 92), it has been hypothesized that these proinflammatory effects of leptin may be relevant to the increased prevalence and incidence of asthma observed in the obese. Two studies have reported an association between serum leptin and asthma (42, 123). Guler et al. (42) noted that serum leptin was predictive of asthma in boys, even after adjusting for BMI. Sood et al. (123) observed higher leptin levels and higher BMI in asthmatic vs. nonasthmatic women. Interestingly, adjusting for serum leptin levels did not affect the association between BMI and asthma in this population (42), suggesting that the relationship between obesity and asthma is not mediated via leptin. Instead, leptin appears to be a predictor of asthma, independent of obesity. Inflammatory cytokines such as TNF-α and IL-1β have been shown to induce the release of leptin from adipocytes (41), and it is conceivable that systemic manifestations of ongoing airway inflammation in asthmatic subjects lead to increased release of leptin, explaining the association between asthma and leptin. Indeed, we have reported that allergen challenge to the airways of sensitized mice increases serum leptin (118).

To address the potential role of leptin in the relationship between obesity and asthma, we implanted microosmotic pumps subcutaneously in lean OVA-sensitized mice, providing a constant infusion of leptin (118). This treatment resulted in an approximate twofold increase in serum leptin compared with mice with pumps delivering saline. The mice were then challenged with aerosolized OVA for several days. When the mice were treated with saline in the pumps, OVA challenge resulted in AHR, an increase in BAL eosinophils, and increases in BAL and lung Th2 cytokine expression. Leptin treatment augmented OVA-induced AHR, even though it did not affect OVA-induced eosinophil influx or Th2 cytokine expression. Taken together, the results suggest that leptin is capable of augmenting allergen-induced AHR through a mechanism that does not involve changes in Th2 cytokines (118). Other data from the literature support the hypothesis that any role for leptin in the relationship between obesity and asthma is unlikely to be mediated through amplification of typical Th2 mechanisms. Leptin has been shown to increase proliferative responses of CD4⁺ T cells to mitogens and to alter T-lympho-
cytokine production (80), but the effect is different for T helper type 1 (Th1) and Th2 cells. Leptin increases Th1 cytokine production. Indeed, a recent report indicates a higher percentage of CD4+ T cells secreting IFN-γ in the blood of obese vs. lean children and an association of these cells with serum leptin (95). In contrast, there was no effect of obesity on IL-4 secreting (i.e., Th2) cells. Indeed, leptin has been shown to decrease Th2 cytokine expression (80).

If leptin does play a role in the relationship between obesity and asthma, it is more likely to be mediated through effects on the innate rather than the adaptive immune system. For example, exogenous administration of leptin to lean mice before acute O3 exposure increases some aspects of their subsequent inflammation response (116). As described above, acute exposure to O3 primarily induces release of acute-phase cytokines and chemokines. To determine whether even endogenous levels of leptin can impact responses to O3, we fasted mice overnight before O3 exposure. Fasting caused a marked reduction in serum leptin, but did not reduce the ensuing inflammatory response. Four hours after acute O3 exposure (2 ppm for 3 h), BAL protein, IL-6, eotaxin, MIP-2, KC, sTNFR1, sTNFR2, and neutrophils were all increased compared with air-exposed mice, but, with the sole exception of MIP-2, levels of each of these inflammatory markers were the same in fed and fasted mice (61). These results suggest that leptin-related changes in the inflammatory response to O3 require increases in leptin above those normally observed in lean mice. Such increases are observed in obesity.

Adiponectin

In contrast to other adipokines, plasma adiponectin and adipose tissue adiponectin expression decline in obesity and rise again following weight loss (29, 111, 149). Obesity-related changes in adiponectin are likely to be functionally important, since exogenous administration of adiponectin protects obese mice against obesity-related diseases, including Type 2 diabetes and atherosclerosis (148). Like leptin, adiponectin has profound effects on energy metabolism. Adiponectin acts primarily in the liver and in skeletal muscle to increase glucose uptake, to inhibit gluconeogenesis, and to augment fatty acid oxidation (22, 148, 149). Like leptin, adiponectin also has effects on hematopoietic cells, indicating a role in immunity, but, unlike leptin and other adipokines, adiponectin is anti-inflammatory. For example, adiponectin reduces TNF-α-induced NF-kB activation in endothelial cells (94) and decreases LPS-induced TNF-α production in macrophages (152). Adiponectin has also been shown to increase expression of certain anti-inflammatory moieties, including IL-10 and the endogenous IL-1 receptor antagonist (146).

To test the hypothesis that loss of the anti-inflammatory effects of adiponectin in obesity may play a role in the relationship between obesity and asthma, we implanted mini-Alzet pumps subcutaneously in lean OVA-sensitized mice. The pumps provided a continuous infusion of full-length murine recombinant adiponectin that resulted in an ~50% increase in adiponectin vs. mice implanted with pumps delivering buffer. When the mice were subsequently challenged with aerosolized OVA, there were increases in airway responsiveness, in BAL eosinophils, and in BAL and lung Th2 cytokines in the buffer-treated mice, but these changes were either markedly attenuated or completely absent in mice treated with adiponectin (119). We also observed a decrease in serum adiponectin resulting from reduced adipose tissue adiponectin mRNA expression in mice challenged with OVA (Fig. 5), indicating that allergen challenge in the lung negatively impacts the release of adiponectin from adipocytes. Coupled with declines in mRNA expression of the three currently identified adiponectin binding proteins, adipor1, adipor2, and T-cadherin, that were observed in lungs of OVA-challenged mice, the results indicate multiple interactions between the obese state and allergic airways disease involving adiponectin (Fig. 6). Importantly, adiponectin has the potential to negatively regulate allergic inflammation in the lungs. However, allergen challenge also impacts both the levels of circulating adiponectin and the ability of the lung to respond to this adipokine. Coupled with obesity-related declines in serum adiponectin, the obese asthmatic subject is likely to have defects in this important immunomodulatory pathway that augment the effects of allergen challenge.

In conclusion, epidemiological data and studies of the effects of weight gain or weight reduction suggest a causal link between obesity and asthma. These data are supported by reports of innate AHR and increased responses to common asthma triggers in obese mice. There are several biologically plausible mechanisms that could explain a relationship between obesity and asthma. While data from animal models do not allow us to refute the possibility that common etiologies, comorbidities, or mechanical factors play a role in this relationship in humans, they suggest that other factors, possibly adipokines, are also likely to be important. Further understanding of the mechanistic basis for the relationship between obesity and asthma may lead to new therapeutic strategies for treatment in this population.

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