

Effect of leptin on allergic airway responses in mice

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Background: Epidemiologic data indicate that the incidence of asthma is increased in obese patients.

Objective: Because the serum levels of the satiety hormone and proinflammatory cytokine leptin are increased in obese individuals, we sought to determine whether leptin can augment allergic airway responses.

Methods: We sensitized and challenged BALB/cJ mice with ovalbumin. Alzet® micro-osmotic pumps were implanted in the mice to deliver a continuous infusion of either saline or leptin (1.75 µg/g/d). Two days later, the mice were challenged with either aerosolized saline or ovalbumin once per day for 3 days. We measured airway responsiveness, performed bronchoalveolar lavage, and obtained blood to measure serum leptin and IgE 24 or 48 hours after the last challenge.

Results: Leptin infusion increased serum leptin concentrations, which were increased further after ovalbumin sensitization and challenge. Ovalbumin challenge increased bronchoalveolar lavage fluid cells and cytokines, serum IgE, lung cytokine mRNA expression, and responses to inhaled, aerosolized methacholine. It is important to note that the changes in methacholine responsiveness and IgE were augmented in leptin- versus saline-infused mice.

Conclusions: These results indicate that serum leptin is increased during allergic reactions in the airways and may play a role in the relationship between obesity and asthma. (*J Allergy Clin Immunol* 2005;115:103-9.)

Key words: Airway responsiveness, bronchoalveolar lavage, cytokines, IgE, leptin, ovalbumin

Both asthma and obesity are prevalent and growing problems, and epidemiologic data indicate an increased incidence of asthma in overweight or obese adults and children.¹⁻³ The importance of understanding this relationship is underscored by the observation that the prevalence of obesity is extremely high among inner-city children, who are at the greatest risk of developing asthma.³

Abbreviations used

AHR:	Airway hyperresponsiveness
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
GAPDH:	Glyceraldehyde phosphate dehydrogenase
LTD ₄ :	Leukotriene D ₄
MCh:	Methacholine
OVA:	Ovalbumin

It is likely that the relationship between obesity and asthma is causal. Longitudinal studies indicate that obesity antedates asthma and that the relative risk of incident asthma increases with increasing obesity.⁴⁻⁶ Moreover, weight reduction in obese patients with asthma results in a decline in asthma severity and symptoms.^{7,8} In addition, airway responsiveness is increased in genetically obese mice, and exposure to ozone, an asthma trigger, enhances ozone-induced airway hyperresponsiveness (AHR) and inflammation to a greater extent in obese versus lean mice.⁹

The mechanistic basis for the relationship between obesity and asthma is unknown; however, it is possible that leptin plays a role. Leptin is a satiety hormone expressed in adipocytes in proportion to their mass,¹⁰ and serum leptin concentrations are 4 to 6 times greater in severely obese humans compared with their lean counterparts.¹¹ Leptin and/or leptin mRNA levels are also increased in obese mice.^{12,13} There is evidence demonstrating that leptin, besides suppressing satiety and increasing metabolism, is also proinflammatory. Leptin is a member of the IL-6 family of cytokines. Hematopoietic cells express leptin receptors, and bone marrow cells cultured in the presence of leptin demonstrate growth of granulocyte-macrophage colonies.^{14,15} CD4⁺ T cells, monocytes, and macrophages can each respond to leptin with increased proliferative responses, increased production of some cytokines, or both.^{14,16-18} Leptin also causes activation of the transcription factors activating protein 1 and nuclear factor-κB in endothelial cells.¹⁹ It is important to note our recent report that in wild-type C57BL/6J mice, exogenous leptin administration augments ozone-induced cytokine release in the lung.⁹

The purpose of this study was to determine whether leptin can augment allergic responses in the lung. To that end, we sensitized mice with ovalbumin (OVA). Alzet® micro-osmotic pumps were implanted subcutaneously to provide a continuous infusion of leptin or saline.

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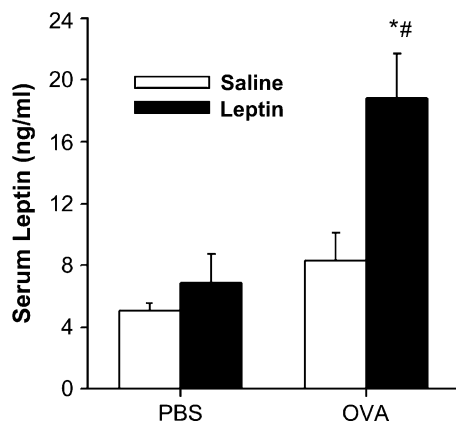


FIG 1. Effect of infusing either saline (0.5 μ L/h) or leptin (1.75 μ g/g/d) on serum leptin concentrations determined by an ELISA. Blood was collected from the animals 48 hours after the last challenge with either PBS or ovalbumin (OVA) in OVA-sensitized mice; $n = 10$ to 17 for each group. $^*P < .001$ versus leptin/PBS; $^{\#}P < .001$ versus saline/OVA.

Beginning 2 days later, the mice were challenged with aerosols of either PBS or OVA 30 min/d for 3 days. Responses to inhaled methacholine (MCh) were measured 24 hours after the last challenge. Blood was collected, bronchoalveolar lavage (BAL) was performed, and the lungs were harvested for preparation of RNA 48 hours after the last challenge.

METHODS

Allergen sensitization and challenge

This study was approved by the Harvard Medical Area Standing Committee on Animals. Male and female 4-week-old BALB/cJ mice were purchased from The Jackson Laboratory (Bar Harbor, Me). Mice were sensitized to chicken egg albumin (OVA; Grade V; Sigma-Aldrich, St Louis, Mo) on day 0 by an intraperitoneal injection of 20 μ g of OVA and adjuvant—2 mg of aluminum hydroxide (J. T. Baker, Phillipsburg, NJ) dispersed in 0.2 mL of PBS. The mice were given a second injection of identical reagents on day 14. On days 28, 29, and 30, animals were challenged with an aerosol of either PBS containing 6% OVA (wt/vol) or PBS. The aerosol was generated by driving compressed air into an LC StarTM nebulizer (Pari Respiratory Equipment, Inc, Monterey, Calif) containing 8 mL of solution to be nebulized. During aerosol delivery, mice were placed individually into 1 of 12 chambers of a specially modified mouse pie cage (Braintree Scientific, Braintree, Mass).

Implantation of Alzet micro-osmotic pumps

On day 26, 2 days before the initiation of OVA or PBS aerosol challenges, Alzet micro-osmotic pumps (model 1007D; Durect Corporation, Cupertino, Calif) were implanted subcutaneously in the intrascapular region of each mouse. The pumps infused solutions at 0.5 μ L/h for 7 days. The reservoir of each pump was preloaded with 96 μ L of either sterile PBS or recombinant mouse leptin (3.6 μ g/ μ L; R&D Systems, Minneapolis, Minn), resulting in a leptin infusion rate of approximately 1.75 μ g/g/d. During this procedure, mice were anesthetized with ketamine (100 mg/kg) and xylazine (15 mg/kg). After implantation, mice were administered an analgesic: buprenorphine hydrochloride (0.1 mg/kg; Sigma-Aldrich).

Whole-body plethysmography

Responses to inhaled, aerosolized MCh were assessed in awake, unrestrained animals by whole-body plethysmography (Buxco Electronics, Inc, Sharon, Conn), as previously described.²⁰ Measurements were performed 24 h after the last OVA or PBS aerosol challenge. The outcome indicator used, enhanced pause, has been described by others and has been demonstrated empirically to correlate with pulmonary resistance.^{21,22} The validity of whole-body plethysmography for the measurement of allergen-induced AHR in BALB/cJ mice has been verified by others.²¹

Bronchoalveolar lavage

Forty-eight hours after the last aerosol challenge, mice were killed with an overdose of sodium pentobarbital. Blood was drawn, and the serum was stored at -20°C for subsequent assay of total serum leptin and corticosterone (both R&D Systems) and IgE (BD Biosciences, San Diego, Calif) by ELISA. BAL was performed, and BAL fluid (BALF) cells and differentials were counted as previously described.⁹ BAL supernatant was stored at -80°C and subsequently analyzed by ELISA for IL-4, IL-13 (R&D Systems), and leukotriene D₄ (LTD₄) (Cayman Chemical Co, Ann Arbor, Mich) and by a Cytometric Bead Array kit (BD Biosciences) for IL-5 and IFN- γ . The limits of detection were 1.5 and 2 pg/mL for the IL-4 and IL-13 ELISAs, respectively, and 15 pg/mL for the LTD₄ ELISA. The limit of detection for the IL-5 and IFN- γ bead arrays were each 2.5 pg/mL.

RNA extraction and real-time PCR

Lungs were homogenized with the PowerGen 125 (Fisher Scientific, Springfield, NJ) at full speed in 3 mL of TRIzol reagent (Invitrogen, Carlsbad, Calif) for 5 minutes, and total RNA was then extracted in accordance with the manufacturer's instructions and stored at -80°C . Reverse transcription (RT) was performed, and the RT reactions were stored at -80°C . Quantitative real-time RT-PCR was performed with an iCycler iQ Real Time Detection System and iQ SYBR Green Supermix in accordance with the manufacturer's instructions (Bio-Rad, Hercules, Calif). Primer sets and product sizes were as follows: IL-4 forward, 5'-ACA GGA GAA GGG ACG CCA T-3'; IL-4 reverse, 5'-GAA GCC CTA CAG ACG AGC TCA-3' (95 bp); IL-13 forward, 5'-AGA CCA GAC TCC CCT GTG CA-3'; IL-13 reverse, 5'-TGG GTC CTG TAG ATG GCA TTG-3' (123 bp); IL-5 forward, 5'-AGC ACA GTG GTG AAA GAG ACC TT-3'; IL-5 reverse, 5'-TCC AAT GCA TAG CTG GTG ATT T-3' (116 bp); leptin forward, TCT CCG AGA CCT CCT CCA TCT; leptin reverse, TTC CAG GAC GCC ATC CAG (100 bp); glyceraldehyde phosphate dehydrogenase (GAPDH) forward, 5'-TTC ACC ACC ATG GAG AAG GC-3'; and GAPDH reverse, 5'-GGC ATG GAC TGT GGT CAT GA-3' (236 bp). Except in the case of leptin, the PCR products of cDNA from OVA-challenged mouse lung were gel-purified and cloned into TOPO-TA vectors (Invitrogen) and used as positive controls to construct standard curves for real-time PCR. In the case of leptin, we used cDNA generated from murine adipose tissue to clone the leptin control used for the standard curve. For each set of primers, melting curve analysis yielded a single peak consistent with 1 PCR product. Changes in lung cytokine mRNA transcript copy number were assessed relative to changes in GAPDH mRNA transcript copy number.

Statistical analysis

Data were analyzed by repeated-measures ANOVA (airway responsiveness and BALF cells) or factorial ANOVA (BALF cytokines, leptin, IgE, corticosterone, and cytokine mRNA expression) with Statistica software (StatSoft, Tulsa, Okla). For IgE, BALF cell counts, and cytokine mRNA expression, data were logarithmically

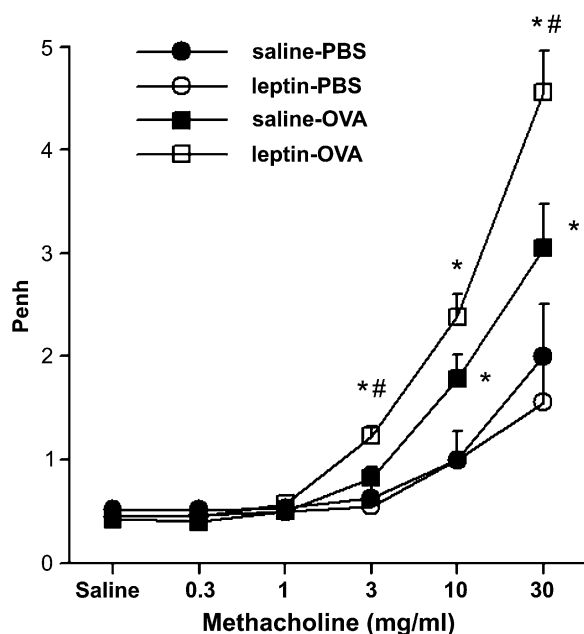


FIG 2. Effect of infusing either saline or leptin on airway responsiveness to inhaled methacholine, measured by whole-body plethysmography. Results were obtained 24 hours after the last challenge with either PBS or ovalbumin (OVA) in OVA-sensitized mice; $n = 10$ to 17 for each group. * $P < .05$ versus the PBS group with the same treatment; # $P < .05$ versus the saline OVA group. *Penh*, Enhanced pause.

transformed before analysis because untransformed data were not normally distributed. A P value $< .05$ was considered significant.

RESULTS

Serum leptin

The continuous infusion of leptin from subcutaneous Alzet micro-osmotic pumps at $1.75 \mu\text{g/g/d}$ resulted in a significant increase in serum leptin. When both PBS and OVA-challenged mice were considered together, leptin increased from $6.9 \pm 1.1 \text{ ng/mL}$ in saline-infused mice to $15.1 \pm 2.2 \text{ ng/mL}$ in leptin-infused mice ($P < .01$). When the mice were further categorized according to the type of aerosol challenge, factorial ANOVA analysis also indicated a significant effect of leptin versus saline infusion ($P < .01$). In addition, we observed a significant effect of OVA versus PBS challenge ($P < .005$) on serum leptin and found a significant interaction between leptin infusion and OVA challenge ($P < .05$) such that animals continuously infused with leptin and OVA challenged had substantially higher serum leptin than animals in any other group (Fig 1). Although adipose tissue is the primary source of leptin, it has been reported that lymphocytes in inflamed lung tissue are capable of expressing this gene.²³ However, this is unlikely to be the source of the increases in serum leptin induced by OVA, because real-time PCR indicated no change in lung leptin mRNA expression either with leptin infusion or with OVA challenge (data not shown). Changes in serum leptin were sufficient to

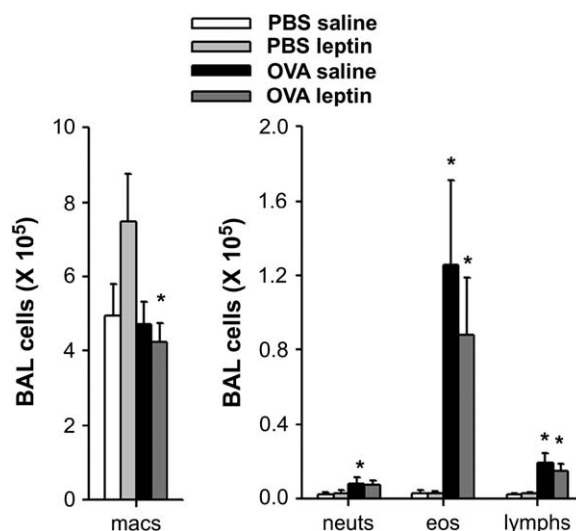


FIG 3. Effect of infusing either saline or leptin on bronchoalveolar lavage (BAL) fluid leukocytes. BAL was performed 48 hours after the last challenge with either PBS or ovalbumin (OVA) in OVA-sensitized mice; $n = 10$ to 17 for each group. * $P < .05$ or ** $P < .001$ versus the PBS group with the same treatment. *macs*, Macrophages; *neuts*, neutrophils; *eos*, eosinophils; *lymphs*, lymphocytes.

induce a physiological response: mice treated with saline increased their body weight by $0.7 \pm 0.2 \text{ g}$ in the 6 days after pump implantation ($P < .01$), whereas in leptin-infused mice, there was no significant change in body weight ($0.1 \pm 0.3 \text{ g}$; $P < .05$ compared with saline-infused mice). Because no effect of sex was observed on any of the outcome indicators used in our analysis, data from both sexes were combined and are presented together.

Airway responsiveness

Repeated-measures ANOVA indicated that there was a significant effect of OVA compared with PBS aerosol challenge on airway responsiveness ($P < .001$). We also observed a significant interaction between OVA aerosol and the type of infusion (PBS versus leptin; $P < .05$), such that airway responsiveness was greater in leptin-infused than saline-infused mice after OVA but not after PBS aerosol challenge (Fig 2). *Post hoc* analysis indicated that the interaction was significant at MCh doses of 3 and 30 mg/mL ($P < .05$ in each instance). There was no effect of leptin on airway responsiveness in PBS-challenged mice.

BALF cells and cytokines

Repeated-measures ANOVA indicated a significant effect of OVA on BALF macrophages ($P < .05$), neutrophils ($P < .01$), eosinophils ($P < .001$), and lymphocytes ($P < .001$) (Fig 3), but no effect of leptin infusion and no interaction between leptin infusion and OVA aerosol challenge, except for a significant increase in BALF macrophages in the leptin-infused, PBS aerosol-challenged group ($P < .05$). Factorial ANOVA also indicated a significant effect of OVA exposure on BALF IL-4, IL-5, and IL-13 (Fig 4), but there was no effect of leptin infusion and no interaction between leptin infusion

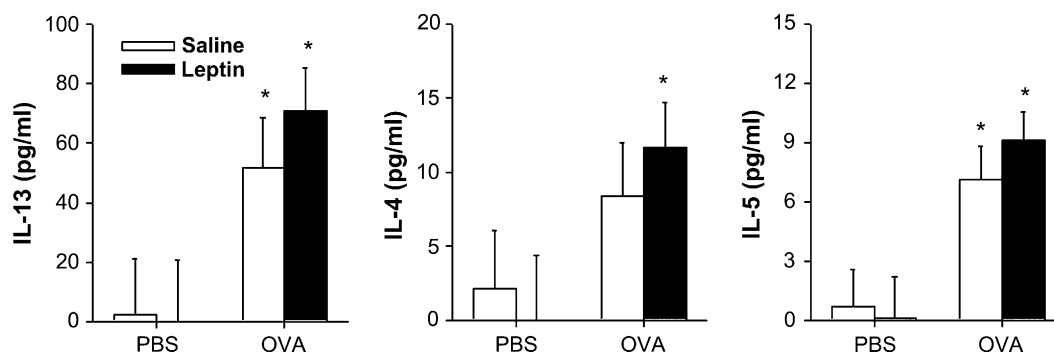


FIG 4. Effect of infusing either saline or leptin on BAL fluid Th2 cytokines measured by ELISAs or a cytometric bead array kit. BAL was performed 48 hours after the last challenge with either PBS or ovalbumin (OVA) in OVA-sensitized mice; $n = 10$ to 17 for each group. * $P < .05$ versus the PBS group with the same treatment.

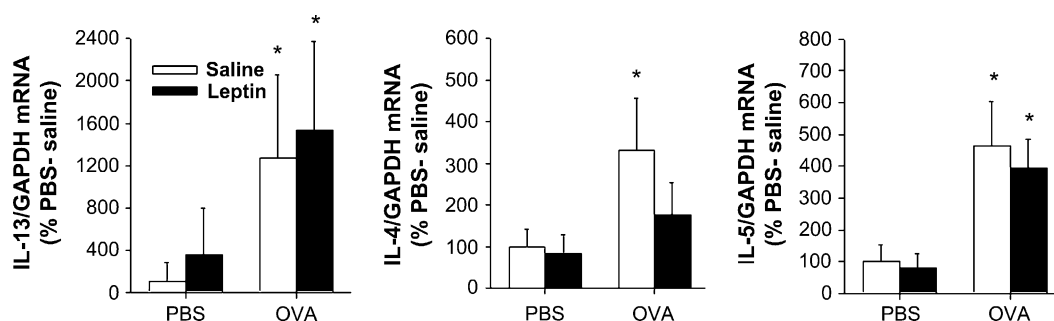


FIG 5. Effect of infusing either saline or leptin on lung cytokine mRNA expression. Lungs were harvested 48 hours after the last challenge with PBS or ovalbumin (OVA) in OVA-sensitized mice. Results are geometric means $\pm$ SEs, are normalized to glyceraldehyde phosphate dehydrogenase (GAPDH) expression, and are expressed relative to saline-infused, PBS-challenged mice; $n = 9$ to 14 for each group. * $P < .05$ versus the PBS group with the same treatment.

and OVA aerosol challenge. IFN- γ and LTD $_4$ were undetectable in the BALF of all treatment groups.

Cytokine mRNA expression

To examine cytokine expression in lung tissue, we measured lung IL-4, IL-13, and IL-5 mRNA expression by real-time PCR (Fig 5). Factorial ANOVA indicated a significant effect of OVA versus PBS exposure for all 3 Th2 cytokines ($P < .05$ in each case). However, there was no effect of leptin infusion on cytokine mRNA expression and no interaction between leptin infusion and OVA exposure.

Total serum IgE

Factorial ANOVA indicated a significant effect of OVA versus PBS aerosol challenge on serum IgE ($P < .01$) and a significant interaction between OVA and leptin ($P < .05$), such that mice infused with leptin had a greater increase in serum IgE after OVA exposure than PBS-infused mice (Fig 6).

Serum corticosterone

Because leptin can affect the adrenal-pituitary axis^{24,25} and because corticosteroids have the potential to substantially affect allergic airway responses,²⁶ we also measured serum corticosterone levels in these mice.

Serum corticosterone levels were on the order of 50 ng/mL; this is consistent with other reports in this species.²⁷ Factorial ANOVA indicated no significant effect of leptin versus saline and no significant effect of OVA versus PBS aerosol (data not shown).

DISCUSSION

Our results indicate that in BALB/cJ mice, sensitization and repeated airway challenge with OVA increases serum leptin. Our results also indicate that leptin augments OVA-induced responses to MCh and increases in serum IgE. Taken together, the results suggest that the increases in serum leptin that are observed in obese patients may exacerbate symptoms of asthma in this population.

Measurements of serum leptin were performed to confirm the efficacy of our pharmacologic intervention. Continuous infusion of leptin at approximately 1.75 μ g/g/d resulted in an approximately 40% increase in total serum leptin in mice challenged with PBS and a greater (approximately 2.5-fold) increase in serum leptin in OVA-challenged mice. The changes were significant and leptin was clearly capable of inducing physiological responses, because body weight increased during the treatment period in saline-infused, but not leptin-infused, mice.

Although the relative reductions in body weight induced by leptin were modest, it should be noted that leptin was administered for only 6 days. Furthermore, the changes in body weight are consistent with other reports indicating that leptin, at similar concentrations, causes only modest reductions in body weight in normal mice but causes much greater reductions in body weight in *ob/ob* mice, which are genetically deficient in leptin.^{12,28} The changes in serum leptin that were induced by our treatment protocol were of the magnitude that would be induced by modest rather than extreme obesity¹¹ and are likely to be pathophysiologically relevant. Male C57BL/6 mice maintained on high-fat diets from the time of weaning until 20 weeks of age weighed approximately 33% more than their low-fat-diet controls and had approximately 2-fold increased serum leptin, similar to the magnitude of serum leptin change achieved here (unpublished data).

Compared with PBS aerosol challenge, OVA aerosol challenge increased serum leptin, particularly in leptin-infused mice. Increased serum leptin is also observed in boys with asthma versus those without.²⁹ Because adipocytes are the primary source of leptin, the data suggest that allergic responses in the airways are capable of altering adipocyte function. It has been well established that a variety of inflammatory mediators, including IL-1 β and TNF- α , are capable of inducing the release of leptin from adipocytes.³⁰ Furthermore, both IL-1 β and TNF- α have been shown to contribute to allergic airway responses in mice.^{31,32} Serum leptin is also increased in a number of other inflammatory conditions, including intravenous injection of endotoxin or ileal injection of toxin A from *Clostridium difficile*,^{30,33} as well as during acute exacerbations of chronic obstructive pulmonary disease.³⁴ Such exacerbations are often triggered by infections.

Leptin is expressed in the skin at sites of wound healing,³⁵ and leptin is also produced by inflamed colonic epithelial cells.³⁶ Hence, it is theoretically possible that that leptin is also produced in the airways after allergic inflammation and might be the source of the changes in serum leptin observed in the OVA-challenged mice. Indeed, a preliminary report by Bruno et al²³ indicates immunoreactive leptin associated with lymphocytes in airways of subjects with chronic obstructive pulmonary disease. However, our data do not support this hypothesis. Leptin mRNA was detectable in the lung, although its expression in this organ was much less than that observed in adipose tissue. Moreover, lung leptin mRNA was not altered by OVA either in the presence or absence of exogenous leptin.

We were surprised to find that the effect of OVA aerosol challenge on serum leptin was particularly important in mice given continuous infusions of leptin. It is theoretically possible that this is the result of a greater release of exogenous leptin from the Alzet micro-osmotic pumps or decreased degradation of leptin in the OVA- versus PBS-challenged mice, but we know of no reason why this should have occurred. It is more likely that the increased serum leptin observed in the leptin-treated and OVA-challenged mice is the result of more endogenous leptin

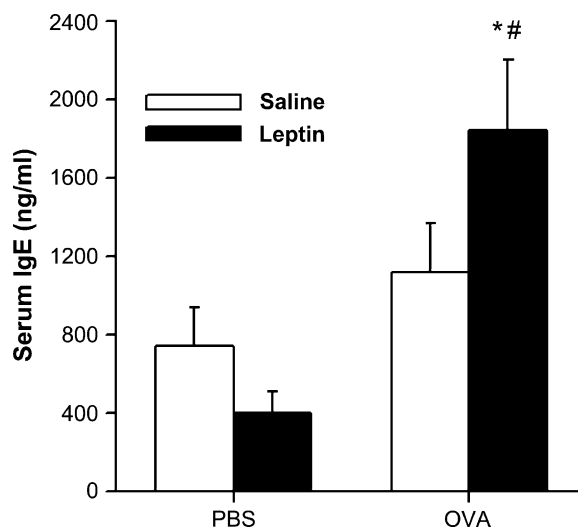


FIG 6. Effect of infusing saline or leptin on total serum IgE concentrations determined by an ELISA. Blood was collected from the animals 48 hours after the last challenge with either PBS or ovalbumin (OVA) in OVA-sensitized mice; n = 9 to 17 for each group. *P < .05 versus leptin/PBS; #P < .05 versus saline/OVA.

release. It may be that in the presence of leptin, there is a greater systemic inflammatory response resulting from OVA challenge that leads to a greater release of leptin from adipocytes.

Compared with PBS aerosol challenge, challenge with aerosolized OVA resulted in increased responses to MCh (Fig 2); increases in BALF neutrophils, eosinophils, and lymphocytes (Fig 3); increased release of cytokines into BALF (Fig 4); increased lung T_H2 cytokine expression (Fig 5); and increases in serum IgE (Fig 6), consistent with other reports in the literature.^{26,37,38} Our results indicate that increases in serum leptin lead to increased effects of OVA on responses to MCh (Fig 2) and on serum IgE (Fig 6), without any apparent effect on the inflammatory response in the airways, at least as indicated by BALF cells (Fig 3) and BALF or lung cytokines (Figs 4 and 5). We were somewhat surprised by these results. Although we had hypothesized that leptin might augment responses to MCh in animals with allergy, we had anticipated that this would occur as a result of an increased inflammatory response in the airways.^{14,15,17,19} The changes in serum leptin induced by our intervention (2.5-fold increase) were small by comparison with the 4- to 6-fold increases that are observed in severely obese individuals. Thus, it is possible that more substantial increases in leptin might induce effects on allergic inflammatory responses. It is also possible that there were increases in some unmeasured aspect of allergic airway inflammation—for example, events downstream of IL-4 or IL-13 receptor activation—or that systemic, as opposed to pulmonary, inflammatory or immune responses were augmented. A weak but significant correlation between serum leptin and serum IgE is also observed in asthmatic children.²⁹

The ability of leptin to increase airway responsiveness and IgE was observed only in mice that had been both sensitized and challenged. In the absence of allergic airway challenge, no significant effect of leptin was observed on either outcome indicator (Figs 2 and 6). These results indicate that leptin is interacting with allergen challenge rather than inducing effects on its own. Similarly, effects of leptin on macrophages and lymphocytes are observed only when these cells are activated by other moieties; leptin alone is without effect.^{14,16,17}

The observation that OVA-induced increases in total IgE were greater in leptin-infused mice argues that some alterations in the immune system occurred in response to leptin administration. In most mouse strains, including the BALB/cJ mice we used, OVA challenge in sensitized animals results in an increase in IgE compared with OVA sensitization alone, thus indicating that allergen challenge is an important stimulus for IgE production.³⁹ Thus, it is certainly reasonable that leptin administered only during the challenge phase of the study could have exerted effects on IgE. T_H2 cytokines affect B-cell class-switching to IgE, and leptin does influence T-cell function.^{16,18} However, the effect of leptin is to shift $CD4^+$ T cells toward T_H1 cytokine production,^{16,18} an event that would have been expected to decrease OVA-induced IgE production and not increase it, as we observed (Fig 6; right columns). In fact, we found no evidence for the effects of leptin on either T_H1 - or T_H2 -derived cytokines in the BALF or lung tissue. Instead, it is possible that there were direct effects of leptin on B lymphocytes that contributed to the production of IgE, consistent with the expression of leptin receptors by these cells.⁴⁰

We do not know the mechanistic basis for the ability of leptin to augment OVA-induced AHR. It is theoretically possible that this effect is the result of the technique used for the measurements. Enhanced pause correlates empirically with airway resistance after MCh challenge, but it can also be affected by other factors, such as changes in ventilation. We think such an explanation unlikely, because this technique has been validated in the setting of OVA sensitization and challenge in BALB/cJ mice.²¹ Furthermore, we did not observe any differences in baseline ventilation between saline- and leptin-infused mice (data not shown).

It is also possible that the effects of leptin on airway responsiveness were related to effects on the adrenal-pituitary axis. For example, leptin has been reported to induce release of corticotrophin-releasing hormone from the hypothalamus, thus leading to increased corticosteroid release.^{24,41} Leptin has also been shown to activate sympathetic nerves to some, but not all, organs.²⁵ However, corticosterone levels were not different in saline- versus leptin-treated mice. Furthermore, increases in corticosterone or increases in sympathetic activation would be expected to decrease, not increase, OVA-induced AHR,²⁶ as was observed. The lack of effect of leptin on corticosterone levels also argues that the small relative reductions in body weight induced by leptin were not sufficient to impose any significant stress on the animals.

It is also possible that the changes in airway responsiveness were related to the effects of leptin on IgE (Fig 5). Cross-linking FcεRI receptors on mast cells upon binding of allergen to IgE results in the secretion of mediators, many of which have the capacity to elicit AHR. Although the relationship between allergen-induced AHR and allergen-induced IgE production in mice is controversial (for review, see Shore³⁷), there are reports of reduced OVA-induced AHR in mast cell-deficient mice,³⁸ and cross-linking FcεRI receptors on mast cells can induce AHR.⁴² Leptin deficiency has been shown to reduce the secretion of cysteinyl leukotrienes from alveolar macrophages,⁴³ although whether the same is true for mast cells is not known. Because leukotrienes are capable of inducing AHR, it is possible that the effects of leptin on OVA-induced AHR were the result of a greater release of leukotrienes from mast cells. We cannot rule out this possibility, because although we could not detect LTD₄ in BALF from these mice, it is possible that the eicosanoid was released but that this occurred earlier than when BAL was performed. Others have reported increased mast cell numbers in the tracheal mucosa of obese mice upon sensitization by OVA.⁴⁴

Finally, it is possible that the enhancement of OVA-induced AHR in leptin-infused mice occurs through a direct effect of leptin on the airway smooth muscle. For example, after OVA aerosol challenge, leptin may potentiate the actions of other inflammatory mediators that lead to AHR. There are also leptin receptors on epithelial cells,⁴⁵ and it is also possible that the effect of leptin on airway responses to MCh occurs through some epithelial-dependent process, for example, effects on mucus hypersecretion.

In summary, our data indicate that in BALB/cJ mice, OVA sensitization and challenge increases serum leptin and that even relatively modest increases in serum leptin can increase some aspects of the allergic airway response. The results suggest that leptin, which is markedly increased in severe obesity, has the potential to exacerbate asthma in these individuals.

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