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Effect of Specific Allergen Inhalation on Serum Adiponectin in Human Asthma*

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Background: Adiponectin is associated with asthma. The direction of this association is not known in humans. In mice, this association is bidirectional: allergen inhalation affects serum adiponectin, and exogenous adiponectin administration affects asthma. We sought to evaluate whether allergen inhalation affects serum adiponectin in human asthma.

Methods: This study included eight sensitized subjects with mild asthma and six healthy control subjects. Asthmatic subjects were challenged with inhaled specific allergen (positive allergen skin test), methacholine, and irrelevant allergen (negative allergen skin test). Control subjects were challenged with irrelevant allergen. Sequential serum samples were obtained before and nine times after each challenge. Serum adiponectin- (primary outcome), leptin-, adiponectin-to-leptin ratio-, eotaxin-, and tumor necrosis factor- α -response curves, area under the curves, and baseline and peak concentrations were evaluated. Statistical analysis used repeated-measures analysis of variance and paired *t* tests.

Results: There were no significant differences in outcome measures among the challenges in asthmatic subjects or when compared to control subjects. Type II error is an unlikely explanation for these findings because the study was adequately powered to detect changes in serum adiponectin, as reported in the literature. Further, pooled data showed that serum adiponectin diurnal variation curves were lower in asthmatic subjects than in control subjects.

Conclusions: Serum adiponectin concentrations are lower in asthmatic subjects than in control subjects. Specific allergen inhalation in asthmatic subjects does not acutely affect serum adiponectin concentrations. The reverse association (*ie*, effect of adiponectin on asthma) needs further study. If future studies prove adiponectin to be a protective factor for asthma, modulating adiponectin may open a new approach toward managing asthma.

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Key words: adipokine; adiponectin; allergen inhalation challenge; asthma; leptin

Abbreviations: DEXA = dual energy X-ray absorptiometry; RM-ANOVA = repeated-measures analysis of variance; TNF = tumor necrosis factor

Adipokines, a diverse group of proteins secreted from adipose tissue, include various hormones, cytokines, chemokines, and acute-phase proteins. These may be antiinflammatory, such as adiponectin, or proinflammatory, such as leptin, eotaxin, and tumor necrosis factor (TNF)- α . Some investigators^{1–3} have hypothesized that adipokines may cause asthma. If this hypothesis is correct, modulating adipokines may open a new approach toward managing asthma.

Studies^{4,5} have confirmed that serum adiponectin is associated with asthma. Exogenous adiponectin administration in sensitized mice attenuates allergen-

induced increase in airway hyperresponsiveness.⁵ In addition, serum adiponectin concentrations decrease following allergen challenge in sensitized mice.⁵ The adiponectin-asthma relationship is therefore bidirectional in mice, whereby allergen inhalation affects serum adiponectin and exogenous adiponectin administration affects asthma.

For editorial comment see page 255

Our recent cross-sectional study⁴ shows that high serum adiponectin concentrations are associated

Table 1—List of Inhalational Challenges Performed in Random Order

Sensitized Subjects With Asthma	Nonatopic Healthy Control Subjects
Irrelevant allergen inhalation (allergen tested negative on skin-prick test on screening evaluation)	Irrelevant allergen inhalation (allergen tested negative on skin-prick test on screening evaluation)
Methacholine inhalation (nonimmunologic stimulus)	
Specific allergen inhalation (allergen tested positive on skin-prick test on screening evaluation and starting challenge dose subsequently evaluated on titration skin allergen prick test)	

with decreased risk for asthma in women. However, cross-sectional studies are unable to determine the direction of association and hence the need for longitudinal and interventional studies in this field. Therefore, we determined whether specific allergen inhalation affects serum adipokines in humans with asthma, similar to mice. The primary aim was to determine whether serum concentrations of adiponectin decrease following specific allergen inhalation in asthma. The secondary aim was to determine whether serum concentrations of leptin, eotaxin, and TNF- α increase, and whether the ratio of serum adiponectin-to-leptin decreases following specific allergen inhalation.

MATERIALS AND METHODS

Study Design

This was an interventional study including eight sensitized subjects with asthma and six healthy control subjects. Asthma was

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defined by the presence of all of the following criteria: physician diagnosis of asthma, confirmed skin test "atopy," presence of nonspecific airway hyperreactivity (provocative concentration of methacholine causing a 20% fall in FEV₁ $\leq$ 16 mg/mL), and presence of specific airway reactivity (FEV₁ decline $\geq$ 20%) to either inhaled Juniper-Mountain cedar or Bermuda grass allergen on screening evaluation. Control subjects were defined as those who met none of the first three criteria. All subjects with asthma had mild disease (intermittent or mild persistent in severity). This phenotype was chosen to minimize serious adverse reactions to allergen inhalation and to minimize the confounding effect of asthma medications. The study inclusion and exclusion criteria are outlined in the online supplement. The study protocol was approved by the local institutional review board. Informed consent was obtained from all study participants.

Exposures

All subjects underwent one to three inhalational challenges in random order on separate days (Table 1). All subjects inhaled

Table 2—Baseline Characteristics of Study Subjects*

Characteristics	Asthma (n = 8)	Control Subjects (n = 6)
Women†	5	4
Premenopausal†	4/5	4/4
Age, yr†	31.4 $\pm$ 8.5	39.9 $\pm$ 9.7
White race†	7	4
Ex-smokers†	2	3
Age of onset of asthma, yr	12.4 $\pm$ 5.4	N/A
Use of inhaled corticosteroids	1	0
Confirmed skin test atopy to common aeroallergens	8	0
Body composition measurements		
Body mass index, kg/m ² †	26.4 $\pm$ 4.9	29.4 $\pm$ 6.6
Percentage of body fat (DEXA)†	35.1 $\pm$ 6.3	38.8 $\pm$ 12.3
Percentage of truncal fat (DEXA)†	37.9 $\pm$ 7.2	42.3 $\pm$ 11.4
Insulin resistance (HOMA units)†	2.4 $\pm$ 1.2	2.5 $\pm$ 1.7
Baseline FEV ₁ , L/s prior to irrelevant challenge†	3.1 $\pm$ 0.5	3.3 $\pm$ 0.8
Inhalational challenge results		
Provocative concentration of methacholine causing a 20% fall in FEV ₁	10.5 $\pm$ 9.9	N/A
Percentage drop in FEV ₁ following methacholine challenge	26.8 $\pm$ 8.9	N/A
Percentage drop in FEV ₁ following specific challenge	28.8 $\pm$ 5.3	N/A
Percentage drop in FEV ₁ following irrelevant challenge†	− 3.0 $\pm$ 5.2	− 2.8 $\pm$ 5.7

*Data are presented as No. or mean $\pm$ SD. HOMA = homeostasis model assessment; N/A = not applicable.

†There was no significant difference between the two groups with respect to these variables ($p > 0.05$ for all analyses).

incremental concentrations of an irrelevant allergen to which they were not sensitive (*ie*, associated with a negative skin-prick test result). Two additional inhalational challenges were similarly performed on all subjects with asthma, with a nonimmunologic stimulus (methacholine),^{6,7} and with specific allergen to which the subjects were sensitive (associated with a positive skin-prick test result).⁸ The specific allergens used were Bermuda grass ($n = 3$) and Juniper-Mountain Cedar ($n = 5$). Additional details of these procedures are in the online supplement.

Outcome Measures

The primary outcome measure was the postchallenge change in concentration of serum adiponectin from baseline. Secondary outcome measures included similar changes in serum concentrations of leptin, adiponectin-to-leptin ratio, eotaxin, and TNF- α . Multiple venous blood samples were obtained in a fasting state at 15 min and just before the start of the challenge, just after the completion of the challenge (0 h), and at 1, 2, 3, 4, 5, 6, 9, and 23 h after the challenge. Baseline (or pretest) levels were derived from the mean of the first two levels obtained. Additional details are provided in the online data supplement.

Statistical Analysis

All outcome measures other than serum adiponectin-to-leptin ratio were logarithmically transformed due to their nonnormal distribution. Statistical analysis used repeated-measures analysis of variance (RM-ANOVA), with both the three challenges and time as repeated factors in subjects with asthma (SAS PROC-MIXED, SAS 9.1.3; SAS Institute; Cary, NC). Outcomes between subjects with asthma and control subjects were similarly

compared using case status as the grouping factor and time as the repeated factor. If there were significant differences, *post hoc* testing with paired *t* tests was performed. Challenge response curves were plotted as geometric means for logarithmically transformed outcome measures, and as arithmetic means for serum adiponectin-to-leptin ratio. Additionally, area under the curves and baseline and peak concentrations of outcome measures were compared. The covariates studied included measures of obesity and insulin resistance that may affect serum adiponectin concentrations in humans.^{9,10} These covariates are further described in the online supplement. Statistical significance was accepted as $p < 0.05$.

Sample Size and Power Estimates

Based on a previous study¹¹ and assuming a modest correlation of 0.7 between baseline and follow-up values, the SD of the difference was estimated at 4.4 $\mu\text{g/mL}$ for serum adiponectin (Table E4 in online supplement). Therefore, our sample size of eight subjects with asthma was adequate to detect a difference of 5.1 $\mu\text{g/mL}$ for serum adiponectin with $\alpha = 0.05$ and $\beta = 0.2$. This study was therefore adequately powered because the published absolute difference in serum adiponectin in a human intervention study was 6.7 $\mu\text{g/mL}$.¹¹ Further, the power estimate based on mice allergen challenge studies was also adequate.⁵ The actual power of the study was in fact greater than the above estimation because data were logarithmically transformed.

RESULTS

Fourteen subjects, 8 subjects with asthma and 6 control subjects, were studied, mostly premenopausal,

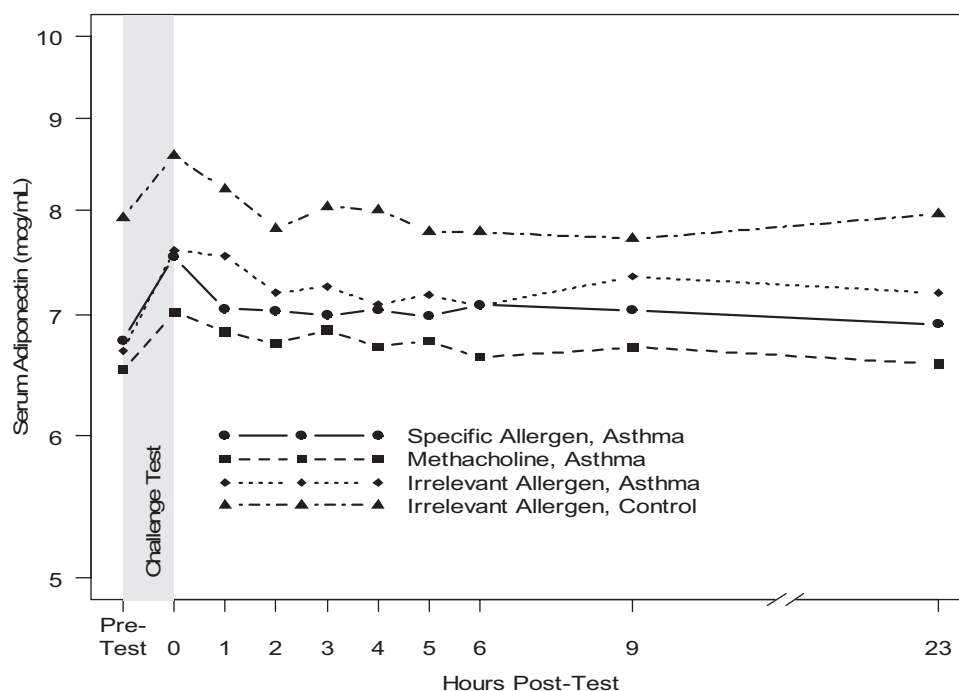


FIGURE 1. Serum adiponectin (geometric) mean response curves to inhalational challenge. Following any challenge, there was an immediate transient rise in serum adiponectin concentration. Comparison of the three response curves among the eight subjects with asthma using RM-ANOVA showed no significant differences. Similar results were noted when specific allergen challenge in asthma and irrelevant allergen challenge in control subjects were compared. Logarithmically transformed data shown in Table E2 in the online supplement.

overweight women (Table 2). Among all challenges, the decline in FEV₁ following specific allergen inhalation was greatest in magnitude ($28.8 \pm 5.3\%$) and duration (Table 2; Table E3 in online supplement).

Following any inhalational challenge (in both asthma subjects and control subjects), there was an immediate rise in serum adiponectin concentration above baseline, lasting about an hour (Fig 1; Table E2 in online supplement). In addition, there was an immediate decline in serum leptin concentration below baseline, lasting several hours, returning to baseline by the next morning (Fig 2; Table E2 in online supplement). Despite these changes, there was no significant change in serum adiponectin-to-leptin ratio as compared to baseline (Fig 3). This lack of significant change was attributed to large SDs associated with this measure (Table E2 in online supplement). While serum eotaxin concentration (Fig 4) was unaffected by any inhalational challenge, a small transient decline in serum TNF- α concentration 2 to 3 h after the specific allergen and methacholine challenges in subjects with asthma was suggested (Fig 5).

Repeated-Measures Analysis of Outcome Measures Among Subjects With Asthma

Comparison of the three challenges among subjects with asthma showed no significant differences

with respect to the response curves, area under the curves, baseline and peak concentrations of serum adiponectin, leptin, adiponectin-to-leptin ratio, and TNF- α (Figs 1-3, 5; Table E1 in online supplement). A difference in the main effect of the eotaxin response curves between specific allergen and methacholine and irrelevant allergen challenges was noted. However, no significant difference at individual time points in (geometric) mean serum eotaxin level was seen among the various curves (all $p > 0.30$). Furthermore, there were no significant differences among the three challenges with respect to area under the curve and baseline and peak concentrations of serum eotaxin (Fig 4, Table E1 in online supplement).

Repeated-Measures Analysis of Outcome Measures Between Subjects With Asthma and Control Subjects

Comparison of the specific allergen challenge in subjects with asthma and irrelevant allergen challenge in control subjects again showed no significant differences with respect to the above outcome measures, except for a difference in main effects between the two eotaxin response curves (Figs 1-5; Table E1 in online supplement). Again, no significant differences in (geometric) mean serum eotaxin concentrations at individ-

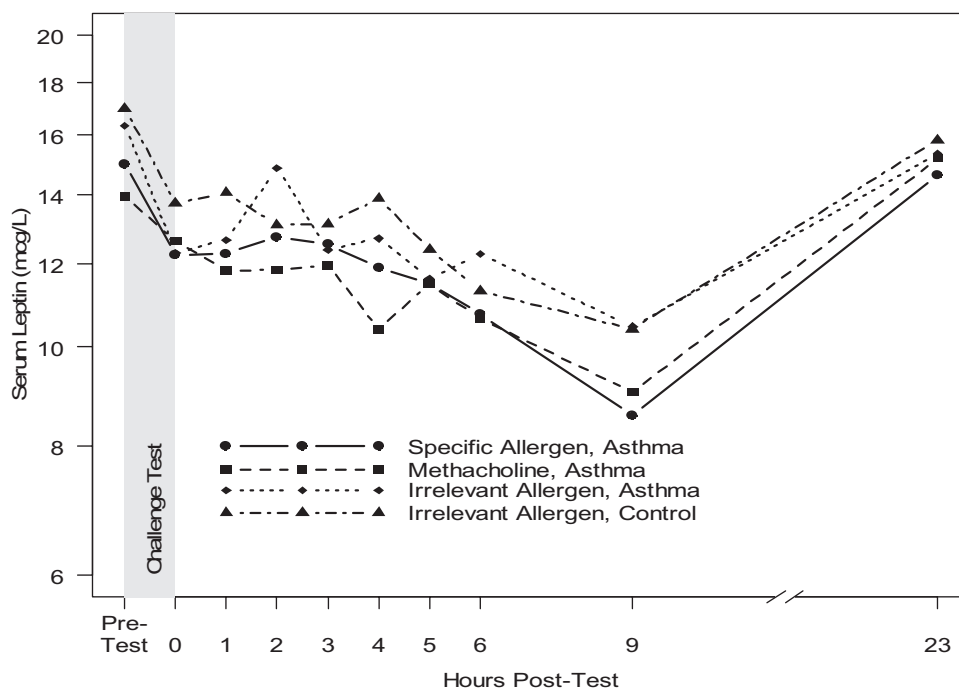


FIGURE 2. Serum leptin (geometric) mean response curves to inhalational challenge. Following any challenge, there was an immediate transient decline in serum leptin concentration. Comparison of the three response curves among the eight subjects with asthma using RM-ANOVA showed no significant differences. Similar results were noted when specific allergen challenge in asthma and irrelevant allergen challenge in controls were compared. Logarithmically transformed data shown in Table E2 in the online supplement.

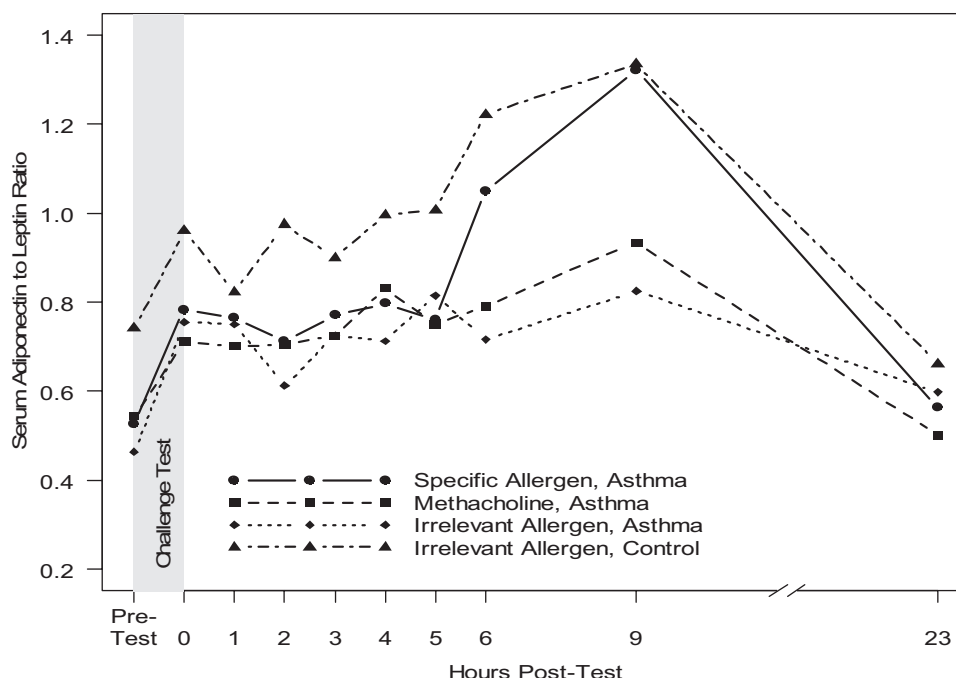


FIGURE 3. Mean response curves of serum adiponectin to leptin ratio to inhalational challenge. Comparison of the three response curves among eight subjects with asthma using RM-ANOVA showed no significant differences. Similar results were noted when specific allergen challenge in asthma and irrelevant allergen challenge in control subjects were compared. Raw data shown in Table E2 in the online supplement.

ual time points, including the baseline, between the two curves were noted (all $p > 0.18$).

Further, when data from the three challenges in subjects with asthma were pooled, serum adiponectin curves during the 24-h period were significantly lower in subjects with asthma, compared to control subjects ($p = 0.02$). Adjustment for covariates, *ie*, obesity (body mass index, dual energy X-ray absorptiometry [DEXA]-assessed percentage of body fat, or percentage of truncal fat separately) and insulin resistance did not explain this observation, and in fact further strengthened the association of adiponectin diurnal variation curves with asthma status ($p \leq 0.01$ for all analyses). Similar results were seen when serum adiponectin-to-leptin ratio was used instead of adiponectin.

DISCUSSION

This study of human subjects with mild asthma does not support the hypothesis established in mice that specific allergen inhalation affects serum adiponectin concentrations. We postulate the following explanations for this discrepancy. First, mice are not the same as humans, and mouse asthma is not the same as human asthma.¹² Quite like the current study, the effects of adipokines (such as adipisin, resistin, and interleukin-6) on insulin resistance vary between humans and mice.¹³ Second, our results

may be negative because of subject characteristics. Our subjects were predominantly overweight premenopausal and postmenopausal women and men with a mild asthma phenotype. The choice of subjects was partly based on safety of challenge testing. Further, the relevant animal studies were done on lean (BALB/cJ) mice of both sexes.^{5,14} Third, the severity of intervention in this study could not match that of the mice allergen challenge studies. Fourth, the results may be negative because of a small sample size (type II statistical error). Despite the small sample size, our power analysis was adequate to detect changes in serum adiponectin, as reported in the referenced human interventional study as well as in the mice allergen challenge experiments.^{5,11} A careful *post hoc* evaluation of the power analysis showed that our assumptions for SDs used to calculate effect size were in fact correct (Table E4 in online supplement). Furthermore, logarithmic transformation of data resulted in a greater effect size than was postulated in the prior power analysis.

Serum concentrations of adiponectin, an anti-inflammatory adipokine, are reduced among obese subjects.^{15,16} High serum adiponectin concentrations are associated with reduced risk of asthma in women after adjusting for obesity.⁴ In sensitized mice, exogenous adiponectin administration attenuates allergen-induced airway hyperresponsiveness.⁵ Further, adi-

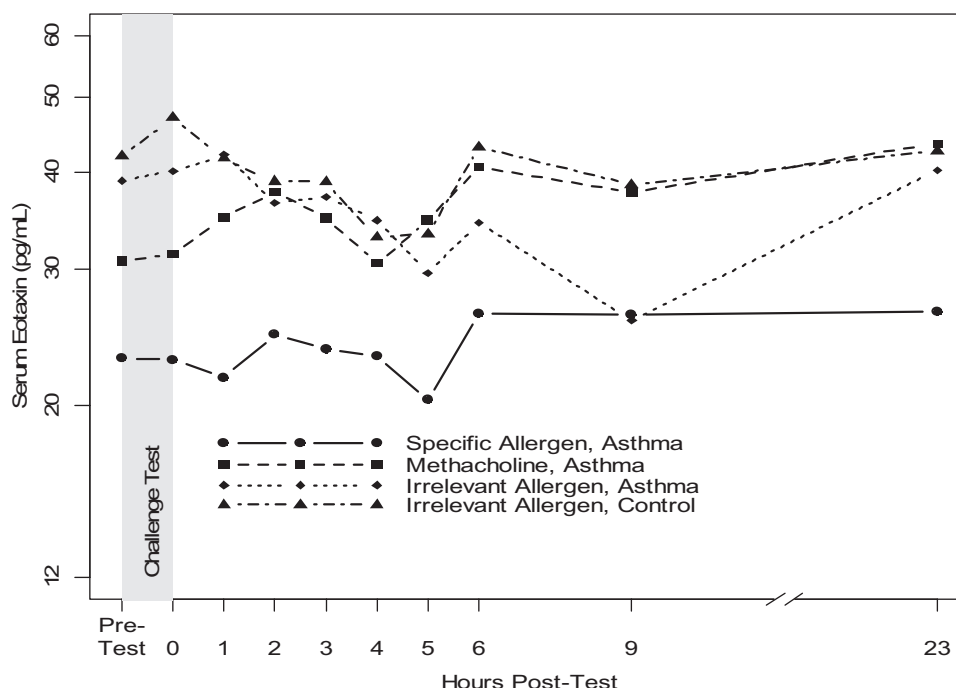


FIGURE 4. Serum eotaxin (geometric) mean response curves to inhalational challenge. A main effect difference in eotaxin response curves was seen in the eight subjects with asthma using RM-ANOVA, between specific allergen challenge and methacholine and irrelevant allergen challenges. However, no significant differences at individual time points were seen between the various curves. Similar results were noted when specific allergen challenge in asthma and irrelevant allergen challenge in control subjects were compared. Logarithmically transformed data are shown in Table E2 in the online supplement.

ponectin messenger RNA production in mice decreases following allergen challenge.⁵ The adiponectin-asthma relationship in mice is therefore bidirectional. However, the direction of the adiponectin-asthma association is not known in humans.

This study demonstrates transient changes in serum adiponectin and leptin concentrations (Fig 1, 2) following any inhalational challenge in both asthma and control subjects. There are two possible explanations for these findings. First, multiple spirometry maneuvers during the challenge test may itself affect the release of adipokines through a mechanism that is independent of the nature of the inhalant. Second and more likely, the change is due to the superimposed diurnal variations in serum adiponectin and leptin concentrations.^{17,18} The diurnal variation in serum adiponectin concentration is characterized by a nocturnal decline with a nadir in the early morning and a subsequent peak in the late morning. After peak levels are reached, there is minimum daytime variation.¹⁷ Since all challenges were performed between 7:00 AM and 8:00 AM in this study, the subsequent increase in adiponectin in Figure 1 may represent the described diurnal peak. Similarly, leptin shows diurnal variation with a peak between 10:00 PM and 3:00 AM (median, 1:20 AM) and a nadir between 8:00

AM and 5:40 PM (median, 10:33 AM) in one study,¹⁸ with an additional delay if subjects were fasting. The decline in serum leptin in Figure 2 may therefore also be explained by normal diurnal variation.¹⁸

Although we found a difference in main effects between the eotaxin response curves (Fig 4; Table E1 in online supplement), no significant differences at individual time points in (geometric) mean serum eotaxin concentration were seen among the various curves on *post hoc* analysis. We therefore postulate that the statistical difference between the eotaxin curves, as detected by the powerful RM-ANOVA analysis, does not have any clinical significance.

If specific allergen inhalation does not affect serum adiponectin concentrations in human asthma, how can we explain our previous cross-sectional observation⁴ that high concentrations of serum adiponectin are associated with decreased risk for asthma? One explanation is that this association is determined by the long-term effects of asthma on adipose tissue. If correct, the short-term experimental study conditions may not reflect the long-term effects of asthma on adipose tissue. However, we believe that the more plausible explanation is that serum adiponectin affects asthma status in humans (*ie*, a reverse direction of the adiponectin-asthma

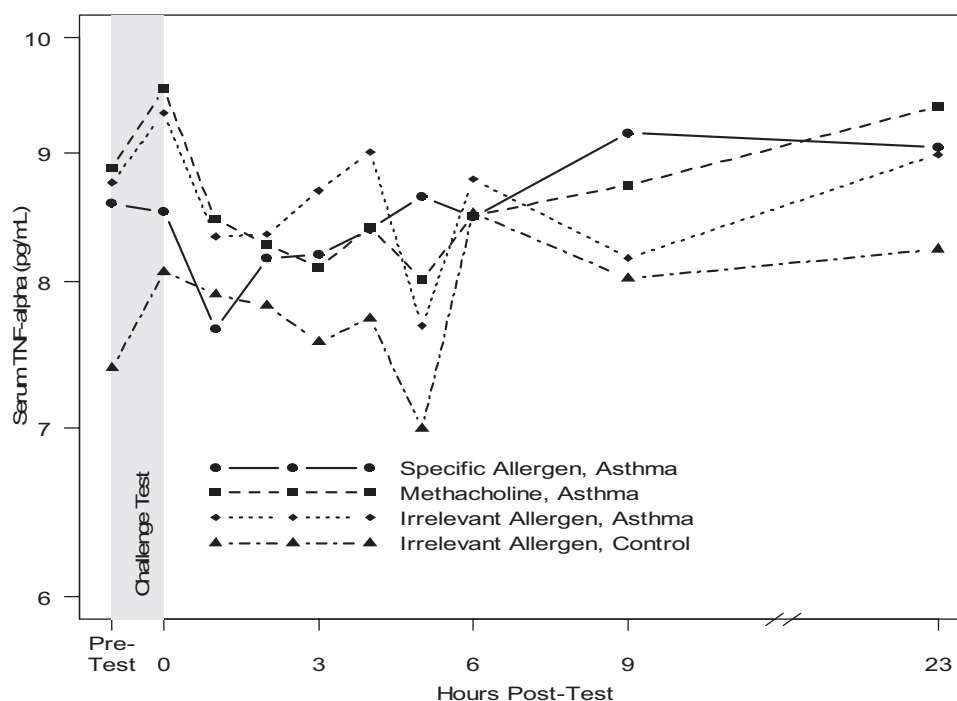


FIGURE 5. Serum TNF- α (geometric) mean response curves to inhalational challenge. Comparison of the three response curves among eight subjects with asthma using RM-ANOVA showed no significant differences. Similar results were noted when specific allergen challenge in asthma and irrelevant allergen challenge in control subjects were compared. Logarithmically transformed data are shown in Table E2 in the online supplement.

association than was hypothesized in this study). Thus, high serum concentration of adiponectin may be a protective factor for human asthma.^{4,19} However, this still remains to further investigated.

Additionally, we found that the diurnal variation curves of serum adiponectin concentrations in asthma subjects were lower than control subjects. This finding is consistent with the result of our previous large cross-sectional study⁴ in which serum adiponectin measure at a single time point was studied. Adiponectin inhibits mitogen-induced proliferation and migration of cultured murine vascular smooth-muscle cells,^{16,20} and may have similar effects on airway smooth muscle.⁵ Given that adiponectin receptors are expressed in human airway smooth cells,²¹ it is possible that continually decreased serum adiponectin may contribute toward increased airway smooth-muscle mass in asthma.¹

The strengths of this study include careful characterization of subjects, multiple (within-group and between-group) comparison of specific allergen challenge, randomized and blinded administration of challenges, and extensive time-sensitive sampling of outcome measures in a fasting state. Further, this is the first human study translated from the novel theory formulated on mice experiments.^{5,14,22}

Potential limitations include possibly lower level of severity of allergen intervention and truncated tim-

ing of the last serum sample drawn after challenge in this human study, as compared to the relevant mice study. Our abbreviated time strategy (23 h compared to 48 h in the mice study⁵) was influenced by the limits we could safely impose on fasting human volunteers to be interned in controlled conditions. It is also possible that airway adipokines may have been more relevant than serum adipokines in this study. However, the study objective was to replicate the mouse experiment that measured serum adipokines.⁵ Further, measurement of airway adipokines is technically difficult, and multiple measurements would not have been possible. Another potential weakness may be the inclusion of men and postmenopausal women in this study. However, *post hoc* analysis of the four premenopausal women in each group revealed similar results. It should be noted that this study was not designed to evaluate the interaction of sex, menopausal status, or obesity on adiponectin response to allergen challenge. The relevant animal study (that this human study was translated from) was done on lean mice of both sexes.⁵

To summarize, this study is the first to report lower diurnal variation curves of serum adiponectin in subjects with asthma as compared to healthy control subjects. Further, unlike mice, specific

allergen inhalation in humans with mild asthma does not acutely affect serum adiponectin concentrations. We instead hypothesize the reverse association (*ie*, serum adiponectin may affect asthma status in humans).⁴ If future studies prove that serum adiponectin is a protective factor for human asthma, modulating adiponectin may open a unique and innovative approach toward managing asthma.

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REFERENCES

- 1 Shore SA. Obesity and asthma: lessons from animal models. *J Appl Physiol* 2007; 102:516–528
- 2 Beutner DA, Weiss ST, Sutherland ER. Obesity and asthma. *Am J Respir Crit Care Med* 2006; 174:112–119
- 3 Shore SA. Obesity and asthma: implications for treatment. *Curr Opin Pulm Med* 2007; 13:56–62
- 4 Sood A, Cui X, Qualls C, et al. Association between asthma and serum adiponectin concentration in women. *Thorax* 2008; 63:877–882
- 5 Shore SA, Terry RD, Flynt L, et al. Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. *J Allergy Clin Immunol* 2006; 118:389–395
- 6 Chai H, Farr RS, Froehlich LA, et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56:323–327
- 7 Allen ND, Davis BE, Hurst TS, et al. Difference between dosimeter and tidal breathing methacholine challenge: contributions of dose and deep inspiration bronchoprotection. *Chest* 2005; 128:4018–4023
- 8 Gerblich AA, Campbell AE, Schuyler MR. Changes in T-lymphocyte subpopulations after antigenic bronchial provocation in asthmatics. *N Engl J Med* 1984; 310:1349–1352
- 9 Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and β -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28:412–419
- 10 Mather KJ, Hunt AE, Steinberg HO, et al. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab* 2001; 86:5457–5464
- 11 Fallo F, Scarda A, Sonino N, et al. Effect of glucocorticoids on adiponectin: a study in healthy subjects and in Cushing's syndrome. *Eur J Endocrinol* 2004; 150:339–344
- 12 Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol* 2004; 172:2731–2738
- 13 Arner P. Resistin: yet another adipokine tells us that men are not mice. *Diabetologia* 2005; 48:2203–2205
- 14 Shore SA, Schwartzman IN, Mellema MS, et al. Effect of leptin on allergic airway responses in mice. *J Allergy Clin Immunol* 2005; 115:103–109
- 15 Wulster-Radcliffe MC, Ajuwon KM, Wang J, et al. Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun* 2004; 316:924–929
- 16 Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999; 100:2473–2476
- 17 Gavrilu A, Peng CK, Chan JL, et al. Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. *J Clin Endocrinol Metab* 2003; 88:2838–2843
- 18 Saad MF, Riad-Gabriel MG, Khan A, et al. Diurnal and ultradian rhythmicity of plasma leptin: effects of gender and adiposity. *J Clin Endocrinol Metab* 1998; 83:453–459
- 19 Sood A, Camargo CA Jr, Ford ES. Association between leptin and asthma in adults. *Thorax* 2006; 61:300–305
- 20 Okamoto Y, Kihara S, Ouchi N, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2002; 106:2767–2770
- 21 Shore SA, Johnston RA. Obesity and asthma. *Pharmacol Ther* 2006; 110:83–102
- 22 Yeatts K, Sly P, Shore S, et al. A brief targeted review of susceptibility factors, environmental exposures, asthma incidence, and recommendations for future asthma incidence research. *Environ Health Perspect* 2006; 114:634–640

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Title

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Online Supplemental File

METHODS

Inclusion and exclusion criteria: The inclusion criteria were English speaking adult subjects who met the study definitions. The exclusion criteria were history of diabetes, abnormal fasting glucose, or use of anti-diabetes medications; history of atherosclerotic cardiovascular disease or abnormal electrocardiogram suggestive of the same or use of statins; history of chronic infectious, active inflammatory, or active neoplastic conditions; use of systemic corticosteroids, leukotriene receptor antagonists, or cromolyn in the previous three months or allergen immunotherapy within the previous 5 years; presence of HIV risk factors; history of abnormal chest radiograph; elevated serum creatinine on screening visit (> 1.8 mg/dL in men and > 1.5 mg/dL in women [1]); current history of smoking confirmed by urinary cotinine measurement ; and use of supplemental reproductive hormones. In addition, the challenge tests were delayed in the event of an acute infection or surgery within prior 4 weeks and respiratory tract infections within prior 8 weeks.

Definition of atopy: Absence of atopy was defined as a lack of immediate type skin test reactivity (< 3 mm. wheal diameter) to all of the aeroallergens common in the Albuquerque region of southwestern United States. The allergens tested in duplicate by a skin prick test included Cottonwood, Juniper-Mountain cedar, Bermuda grass, Kentucky bluegrass, Meadow fescue grass, Russian thistle, Kochia, Western ragweed, cat hair, and dust mites

Dermatophagoides farinae and *Dermatophagoides pteronyssinus* (Greer Labs, Lenoir, NC). On the other hand, presence of atopy was defined as immediate type skin test reactivity (≥ 3 mm. wheal diameter) to Juniper-Mountain cedar and/or Bermuda grass. We focused on these two

allergens since they were approved for inhalational testing by the United States Federal Drug Administration in this study (Investigational new drug application number BP-IND-13063) .

Exposures: All subjects were studied with one to three inhalational challenge/s in random order (as outlined in Table 1). A titration skin allergen prick test was additionally performed on subjects with asthma, a week prior to the challenges. All tests were performed at the Clinical Translational Science Center at the University of New Mexico, Albuquerque, NM.

Titration skin allergen prick test –This test was performed with half-logarithmic dilutions of Juniper-Mountain cedar or Bermuda grass allergen. The concentration of the most dilute allergen solution that produced a positive skin test (as defined by a ≥ 3 mm wheal) was used to determine the starting dose of the specific allergen inhalational challenge [2].

Inhalational Challenge Tests (Table 1) - These tests were performed at 0700 hours and lasted approximately an hour. Medications were held prior to the test, as outlined in the ATS guidelines [3]. Post-challenge, 2.5 mg. of nebulized albuterol was given for either clinical distress or $\geq 40\%$ decrease in FEV₁.

All subjects inhaled incremental concentrations of an irrelevant allergen (to which they were not sensitive *i.e.* associated with a negative skin prick test). In the event of an irrelevant allergen not being available (as some atopic subjects had multiple positive skin tests), saline inhalation was performed with an equal number of steps. Non-immunological stimulus (methacholine) inhalational challenge was performed on subjects with asthma using the recent Cockcroft

modification of the methods of Chai *et al.* [4, 5]. Briefly, subjects serially inhaled the following increasing concentrations (0.15, 0.31, 0.62, 1.25, 2.5, 5.0, 10.0, 16 and 25 mg/ml) of methacholine by tidal breathing via a nebulizer through a Rosenthal-French dose-metering device until the FEV₁ decreased by $\geq 20\%$ from baseline values. Additionally, all subjects with asthma inhaled a specific allergen to which they were sensitive *i.e.* associated with a positive skin prick test. Using the protocol by Gerblich *et al* [6], allergen was inhaled in incremental concentrations via a similar nebulizer and dose-metering device until the FEV₁ decreased by $\geq 20\%$ from baseline, starting at three-to-four half-logarithmic concentrations below that caused skin test positivity in the titration prick test [2]. The specific allergen used was Bermuda grass (n = 3) and Juniper-Mountain Cedar (n = 5) since these aeroallergens are common in our region and were approved for inhalational testing by the United States Federal Drug Administration in this study.

The inhalational challenges for the subjects with asthma were in random order, each on a separate visit, with a window of at least 72 hour duration following irrelevant allergen and methacholine challenges and of at least 7-day duration following specific allergen challenge, in order to minimize the ‘carry-over’ effect on subsequent test results. For actively menstruating women, the testing was done within 3-14 days following the cessation of menstrual flow to minimize the effect of luteal menstrual phase on serum leptin concentrations [7]. The subjects were kept fasting overnight prior to the challenge test. Wherever possible, the subjects, phlebotomists, technicians, and nurses were blinded to the exposure status of the subjects with asthma.

Outcome Measures: The primary outcome measure was the post-challenge change in concentration of serum adiponectin from baseline. Secondary outcome measures included similar changes in serum concentrations of leptin, adiponectin-to-leptin ratio, eotaxin, and TNF- α . Multiple venous blood samples were obtained in a fasting state at 15 minutes and just before the start of the challenge, just after the completion of the challenge (0 hours), and at 1, 2, 3, 4, 5, 6, 9, and 23 hours after the challenge. Baseline or 'pre-test' levels were derived from the mean of the first two levels obtained. The described *in vivo* half-lives of adiponectin, leptin, and TNF- α are approximately 2.5 hours [8], 25 minutes [9], and < 10 minutes respectively [10]. The described *in vitro* half life of eotaxin is about 2 hours [11]. Therefore, the timing of the sampling of outcome measures was appropriate for the study design.

Venous blood samples were centrifuged and frozen at -70° C within 90 minutes of collection. Serum adiponectin and leptin concentrations were measured using ELISA and radioimmunoassay techniques respectively (Linco Research, Inc., St. Charles, MO). Serum eotaxin concentrations were measured by Luminex technology using singleplex reagents (Invitrogen/BioSource, Camarillo, CA). Serum TNF- α concentrations were measured using a solid-phase chemiluminescent immunometric assay (Siemens, Los Angeles, CA). The sensitivity for the various assays was 0.78 ng/mL for adiponectin; 0.5 ng/mL for leptin; 9.7 pg/mL for eotaxin; and 1.7 pg/mL for TNF- α . The intra-assay and inter-assay test coefficients of variation were < 8.4% for adiponectin; < 5% for leptin; $\leq$ 9% for eotaxin; and < 3% for TNF- α .

Covariates – Serum adiponectin levels may be affected by obesity and insulin resistance.

Therefore, data were obtained regarding measures of total body fat (body mass index or BMI and

percent body fat by Dual energy X-ray absorptiometry or DEXA), and central fat (percent truncal fat by DEXA). DEXA was assessed using a well-calibrated GE Lunar DPX-NT pencil beam densitometer (General Electric Medical Systems, USA). Insulin resistance was calculated using the homeostasis model assessment from fasting glucose and insulin levels [12, 13].

REFERENCES

1. Steffes MW, Gross MD, Schreiner PJ, Yu X, Hilner JE, Gingerich R, Jacobs DR, Jr. Serum adiponectin in young adults--interactions with central adiposity, circulating levels of glucose, and insulin resistance: the CARDIA study. *Ann Epidemiol* 2004; 14(7): 492-498.
2. American Academy of Allergy and Immunology. Allergen Challenge Protocol, AAAI recommendation, http://www.aaaai.org/members/only/teaching_slides/. 2004.
3. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, MacIntyre NR, McKay RT, Wanger JS, Anderson SD, Cockcroft DW, Fish JE, Sterk PJ. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med* 2000; 161(1): 309-329.
4. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL, Spector SL, Townley RG. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975; 56(4): 323-327.
5. Allen ND, Davis BE, Hurst TS, Cockcroft DW. Difference between dosimeter and tidal breathing methacholine challenge: contributions of dose and deep inspiration bronchoprotection. *Chest* 2005; 128(6): 4018-4023.
6. Gerblich AA, Campbell AE, Schuyler MR. Changes in T-lymphocyte subpopulations after antigenic bronchial provocation in asthmatics. *N Engl J Med* 1984; 310(21): 1349-1352.
7. Ludwig M, Klein HH, Diedrich K, Ortmann O. Serum leptin concentrations throughout the menstrual cycle. *Arch Gynecol Obstet* 2000; 263(3): 99-101.

8. Hoffstedt J, Arvidsson E, Sjolin E, Wahlen K, Arner P. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. *J Clin Endocrinol Metab* 2004; 89(3): 1391-1396.
9. Lankarani KB, Moghadami M, Masoumpoor M, Geramizadeh B, Omrani GR. Serum leptin level in patients with functional dyspepsia. *Dig Liver Dis* 2004; 36(11): 717-721.
10. Barlan IB, Basaran MM, Bakir M, Tukenmez F, Bekiroglu N. Serum TNF-alpha, sCD8 and sIL-2R levels in childhood tuberculosis. *Infection* 1995; 23(4): 237-239.
11. Atasoy U, Curry SL, Lopez de Silanes I, Shyu AB, Casolaro V, Gorospe M, Stellato C. Regulation of eotaxin gene expression by TNF-alpha and IL-4 through mRNA stabilization: involvement of the RNA-binding protein HuR. *J Immunol* 2003; 171(8): 4369-4378.
12. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28(7): 412-419.
13. Mather KJ, Hunt AE, Steinberg HO, Paradisi G, Hook G, Katz A, Quon MJ, Baron AD. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab* 2001; 86(11): 5457-5464.
14. Fallo F, Scarda A, Sonino N, Paoletta A, Boscaro M, Pagano C, Federspil G, Vettor R. Effect of glucocorticoids on adiponectin: a study in healthy subjects and in Cushing's syndrome. *Eur J Endocrinol* 2004; 150(3): 339-344.
15. Shore SA, Terry RD, Flynt L, Xu A, Hug C. Adiponectin attenuates allergen-induced airway inflammation and hyperresponsiveness in mice. *J Allergy Clin Immunol* 2006; 118(2): 389-395.

Table E1: Response of outcome measures to various inhalational challenges among subjects with asthma and controls

Outcome measure		Specific Allergen, Asthma (S-A)	Methacholine, Asthma (M-A)	Irrelevant Allergen, Asthma (I-A)	Irrelevant Allergen, Controls (I-C)	p value - comparison of S-A, M-A & I-A	p value – comparison of S-A & I-C
Serum Adiponectin	Challenge response curve (log concentration)					0.45	0.06
	AUC	3.43 ± 4.48	2.29 ± 3.04	6.77 ± 8.05	0.66 ± 1.83	0.28	0.15
	Log Baseline	0.83 ± 0.14	0.81 ± 0.17	0.83 ± 0.13	0.90 ± 0.17	0.97	0.44
	Log Peak	0.05 ± 0.03	0.04 ± 0.02	0.07 ± 0.05	0.04 ± 0.02	0.21	0.41
Serum Leptin	Challenge response curves (log concentration)					0.64	0.53
	AUC	-30.94 ± 10.32	-29.65 ± 25.36	-30.83 ± 25.07	-67.59 ± 63.94	0.99	0.22
	Log Baseline	1.18 ± 0.25	1.15 ± 0.28	1.21 ± 0.21	1.23 ± 0.50	0.86	0.82
	Log Peak	0.02 ± 0.02	0.07 ± 0.07	0.05 ± 0.06	0.04 ± 0.06	0.14	0.49
Serum adiponectin- to-leptin ratio	Challenge response curves					0.59	0.37
	AUC	3.66 ± 6.73	2.23 ± 3.11	2.63 ± 3.06	3.05 ± 3.77	0.82	0.83
	Baseline	0.53 ± 0.35	0.54 ± 0.35	0.46 ± 0.24	0.74 ± 0.76	0.87	0.54
	Peak	0.57 ± 1.05	0.34 ± 0.38	0.39 ± 0.42	0.49 ± 0.68	0.80	0.87
Serum Eotaxin	Challenge response curves (log concentration)					0.02	0.02
	AUC	-28.43 ± 127.52	129.41 ± 394.68	-21.06 ± 184.39	-39.81 ± 79.38	0.42	0.85
	Log Baseline	1.36 ± 0.54	1.49 ± 0.34	1.59 ± 0.32	1.62 ± 0.36	0.55	0.30
	Log Peak	0.25 ± 0.26	0.28 ± 0.32	0.16 ± 0.12	0.14 ± 0.05	0.63	0.32
Serum TNF- α	Challenge response curves (log concentration)					0.93	0.11
	AUC	-1.41 ± 7.13	-3.57 ± 8.43	-3.21 ± 15.26	3.07 ± 9.27	0.92	0.36
	Log Baseline	0.83 ± 0.12	0.95 ± 0.14	0.94 ± 0.14	0.87 ± 0.17	0.98	0.44
	Log Peak	0.07 ± 0.06	0.06 ± 0.04	0.08 ± 0.07	0.08 ± 0.08	0.65	0.67

Note 1: For challenge response curves, main effects due to test are reported from a RM-ANOVA analysis with time and challenge as the repeat factors.

Note 2: AUC or Area under the curve for serum leptin, adiponectin, adiponectin-to-leptin ratio, and eotaxin was obtained from nine observations at -1 to +9 hours after challenge;

Negative AUC suggests that the curve is overall below the baseline value.

Note 3: Mean ± S.D. of logarithmic values to the base 10 are reported for all outcome measures except for AUC and for serum adiponectin-to-leptin ratio.

Note 4: Basic unit for measurement of serum adiponectin is µg/mL, serum leptin is µg/L, serum eotaxin and TNF-α is pg/mL.

Table E2: Mean adipokine concentrations at various time points of inhalational challenges among subjects with asthma (n = 8) and controls (n = 6).

Adipokine	Inhalational Challenge Test	Pre-test	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour	9 hour	23 hour
Serum Adiponectin	Specific Allergen, Asthma	0.83 ± 0.14	0.88 ± 0.15	0.85 ± 0.14	0.85 ± 0.14	0.85 ± 0.14	0.85 ± 0.13	0.84 ± 0.14	0.85 ± 0.15	0.85 ± 0.13	0.84 ± 0.14
	Methacholine, Asthma	0.81 ± 0.17	0.85 ± 0.18	0.84 ± 0.17	0.83 ± 0.18	0.84 ± 0.16	0.83 ± 0.17	0.83 ± 0.17	0.82 ± 0.16	0.83 ± 0.15	0.82 ± 0.17
	Irrelevant Allergen, Asthma	0.83 ± 0.13	0.88 ± 0.16	0.88 ± 0.15	0.86 ± 0.16	0.86 ± 0.14	0.85 ± 0.15	0.86 ± 0.15	0.85 ± 0.14	0.87 ± 0.15	0.86 ± 0.15
	Irrelevant Allergen, Control	0.90 ± 0.17	0.93 ± 0.18	0.92 ± 0.16	0.89 ± 0.18	0.91 ± 0.17	0.90 ± 0.17	0.89 ± 0.18	0.89 ± 0.18	0.89 ± 0.17	0.90 ± 0.18
Serum Leptin	Specific Allergen, Asthma	1.18 ± 0.25	1.09 ± 0.26	1.09 ± 0.29	1.11 ± 0.29	1.10 ± 0.32	1.08 ± 0.32	1.06 ± 0.29	1.03 ± 0.36	0.93 ± 0.38	1.17 ± 0.28
	Methacholine, Asthma	1.15 ± 0.28	1.10 ± 0.30	1.07 ± 0.29	1.07 ± 0.31	1.08 ± 0.32	1.02 ± 0.34	1.06 ± 0.33	1.03 ± 0.29	0.96 ± 0.33	1.18 ± 0.26
	Irrelevant Allergen, Asthma	1.21 ± 0.21	1.09 ± 0.27	1.10 ± 0.28	1.17 ± 0.28	1.09 ± 0.28	1.10 ± 0.30	1.07 ± 0.31	1.09 ± 0.30	1.02 ± 0.26	1.19 ± 0.29
	Irrelevant Allergen, Control	1.23 ± 0.50	1.14 ± 0.46	1.15 ± 0.43	1.12 ± 0.50	1.12 ± 0.44	1.14 ± 0.52	1.09 ± 0.50	1.05 ± 0.54	1.02 ± 0.54	1.20 ± 0.38
Serum Adiponectin-to-Leptin Ratio	Specific Allergen, Asthma	0.53 ± 0.35	0.78 ± 0.71	0.76 ± 0.78	0.71 ± 0.69	0.77 ± 0.85	0.80 ± 0.81	0.76 ± 0.64	1.05 ± 1.40	1.32 ± 1.78	0.57 ± 0.41
	Methacholine, Asthma	0.54 ± 0.35	0.71 ± 0.63	0.70 ± 0.54	0.70 ± 0.56	0.72 ± 0.61	0.83 ± 0.71	0.75 ± 0.64	0.79 ± 0.71	0.93 ± 0.77	0.50 ± 0.30
	Irrelevant Allergen, Asthma	0.46 ± 0.24	0.75 ± 0.57	0.75 ± 0.58	0.61 ± 0.48	0.73 ± 0.54	0.71 ± 0.61	0.81 ± 0.67	0.72 ± 0.51	0.82 ± 0.51	0.60 ± 0.47
	Irrelevant Allergen, Control	0.74 ± 0.76	0.96 ± 0.95	0.82 ± 0.74	0.97 ± 1.07	0.90 ± 0.81	0.99 ± 1.11	1.01 ± 1.05	1.22 ± 1.44	1.33 ± 1.58	0.66 ± 0.50
Serum Eotaxin	Specific Allergen, Asthma	1.36 ± 0.54	1.36 ± 0.48	1.34 ± 0.46	1.39 ± 0.44	1.37 ± 0.42	1.36 ± 0.47	1.31 ± 0.51	1.42 ± 0.42	1.42 ± 0.54	1.42 ± 0.59
	Methacholine, Asthma	1.49 ± 0.34	1.50 ± 0.36	1.54 ± 0.32	1.58 ± 0.49	1.54 ± 0.40	1.48 ± 0.42	1.54 ± 0.41	1.61 ± 0.42	1.58 ± 0.40	1.64 ± 0.32
	Irrelevant Allergen, Asthma	1.59 ± 0.31	1.60 ± 0.36	1.62 ± 0.30	1.56 ± 0.31	1.57 ± 0.35	1.54 ± 0.34	1.47 ± 0.36	1.54 ± 0.42	1.41 ± 0.35	1.60 ± 0.42
	Irrelevant Allergen, Control	1.62 ± 0.36	1.67 ± 0.34	1.62 ± 0.33	1.59 ± 0.37	1.59 ± 0.40	1.52 ± 0.37	1.52 ± 0.35	1.63 ± 0.28	1.59 ± 0.38	1.63 ± 0.44
Serum TNF- α	Specific Allergen, Asthma	0.93 ± 0.12	0.93 ± 0.09	0.88 ± 0.12	0.91 ± 0.15	0.91 ± 0.15	0.92 ± 0.14	0.94 ± 0.13	0.93 ± 0.13	0.96 ± 0.12	0.96 ± 0.10

	Methacholine, Asthma	0.95 ± 0.14	0.98 ± 0.15	0.93 ± 0.12	0.92 ± 0.15	0.91 ± 0.13	0.92 ± 0.12	0.90 ± 0.14	0.93 ± 0.14	0.94 ± 0.18	0.97 ± 0.10
	Irrelevant Allergen, Asthma	0.94 ± 0.14	0.97 ± 0.10	0.92 ± 0.09	0.92 ± 0.14	0.94 ± 0.12	0.95 ± 0.14	0.89 ± 0.11	0.94 ± 0.13	0.91 ± 0.12	0.95 ± 0.12
	Irrelevant Allergen, Control	0.87 ± 0.17	0.91 ± 0.12	0.90 ± 0.12	0.89 ± 0.12	0.88 ± 0.10	0.89 ± 0.15	0.84 ± 0.12	0.93 ± 0.09	0.90 ± 0.10	0.92 ± 0.11

Note 1: This data, after antilogarithmic transformation, were the bases for Graphs 1-5 in the text.

Note 2: Basic unit for measurement of serum adiponectin is $\mu\text{g/mL}$, serum leptin is $\mu\text{g/L}$, serum eotaxin and $\text{TNF-}\alpha$ is pg/mL .

Note 3: Mean $\pm$ S.D. of logarithmic values to the base 10 are reported for all outcome measures except for serum adiponectin-to-leptin ratio.

Table E3: Mean pulmonary function test values at various time points of inhalational challenges among subjects with asthma (n = 8) and controls (n = 6).

Pulmonary Function Measure	Inhalational Challenge Test	Pre-test	0 hour	1 hour	2 hour	3 hour	4 hour	5 hour	6 hour	9 hour	23 hour
FEV ₁ (liters)	Specific Allergen, Asthma	3.44 ± 0.67	2.22 ± 0.53	2.71 ± 0.51	3.01 ± 0.43	3.17 ± 0.52	3.16 ± 0.49	3.17 ± 0.51	3.10 ± 0.39	2.98 ± 0.36	3.12 ± 0.55
	Methacholine, Asthma	3.35 ± 0.65	2.52 ± 0.57	3.15 ± 0.55	3.29 ± 0.57	3.33 ± 0.56	3.24 ± 0.52	3.26 ± 0.58	3.34 ± 0.65	3.32 ± 0.59	3.24 ± 0.54
	Irrelevant Allergen, Asthma	3.10 ± 0.47	3.18 ± 0.56	3.18 ± 0.60	3.14 ± 0.61	3.20 ± 0.62	3.15 ± 0.60	3.15 ± 0.64	3.23 ± 0.59	3.16 ± 0.61	3.14 ± 0.53
	Irrelevant Allergen, Control	3.30 ± 0.79	3.41 ± 0.81	3.30 ± 0.86	3.29 ± 0.83	3.31 ± 0.82	3.33 ± 0.85	3.33 ± 0.84	3.27 ± 0.79	3.34 ± 0.85	3.32 ± 0.84
Percent predicted FEV ₁	Specific Allergen, Asthma	93.09 ± 12.50	60.82 ± 14.22	73.41 ± 8.92	81.91 ± 9.92	86.16 ± 11.55	86.06 ± 12.68	85.91 ± 9.94	84.53 ± 11.29	81.89 ± 14.39	85.17 ± 17.19
	Methacholine, Asthma	90.82 ± 13.58	68.75 ± 14.98	85.39 ± 12.79	89.23 ± 12.46	90.09 ± 10.36	87.81 ± 10.44	88.38 ± 11.79	90.20 ± 12.99	89.93 ± 12.24	87.93 ± 11.89
	Irrelevant Allergen, Asthma	84.35 ± 11.56	86.58 ± 15.79	86.40 ± 15.35	85.29 ± 14.92	86.95 ± 15.20	85.56 ± 14.41	85.49 ± 16.25	87.93 ± 15.09	85.90 ± 15.05	85.72 ± 14.87
	Irrelevant Allergen, Control	97.56 ± 19.03	100.83 ± 18.53	97.18 ± 19.18	97.31 ± 19.02	97.61 ± 17.15	98.44 ± 19.51	98.21 ± 17.20	96.47 ± 16.22	98.71 ± 19.55	98.06 ± 19.94

Note 1: Mean ± S.D. of values are reported

Table E4: Minimum absolute detectable differences in serum adiponectin concentration (primary outcome measure) in this study - using two-sided paired t-test with significance level α of 0.05, power of 80%, sample size of 8 subjects with asthma, and correlation between time points ρ of 0.7.

Outcome measure	S.D. at one time point	S.D. of difference	Minimum absolute detectable difference	Absolute difference published in the literature	Population description
Serum adiponectin	5.7 [14]	4.4	5.1 $\mu\text{g/mL}$	6.7 $\mu\text{g/mL}$ [14]	Men and women
Serum adiponectin	2.8 [15]	2.2	2.5 $\mu\text{g/mL}$	5.0 $\mu\text{g/mL}$	BALB\cJ male and female mice

Note 1: Estimates of standard deviations (S.D.) at one time point were obtained from the referenced human and mice interventional studies.