

Article Type: Original

Obesity, Asthma Prevalence and IL-4: Roles of Inflammatory Cytokines, Adiponectin and Neuropeptide Y

Running title: Asthma, Obesity, Cytokines and NPY

Yanxia Lu,^{1,2} Hugo P.S. Van Bever,³ Tow Keang Lim,⁴ Win Sen Kuan,⁵ Daniel Yam Thiam Goh,³

Malcolm Mahadevan,⁵ Tiong Beng Sim,⁵ Roger Ho,² Anis Larbi,¹ Tze Pin Ng²

¹Singapore Immunology Network (SiGN), Agency for Science, Technology and Research (A*STAR), Singapore

²Department of Psychological Medicine, Yong Loo Lin School of Medicine, National University of Singapore

³Department of Pediatrics, Yong Loo Lin School of Medicine, National University of Singapore

⁴Division of Respiratory and Critical Care Medicine, Department of Medicine, National University Health System, Singapore

⁵Emergency Medicine Department, National University Health System, Singapore

This work was supported by the Singapore Immunology Network (A*STAR).

More information about Methods is submitted as electronic repository.

Author responsible for correspondence and request for reprints: A/P Tze Pin Ng

Yong Loo Lin School of Medicine, National University of Singapore,

Department of Psychological Medicine, National University Hospital,

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12428

This article is protected by copyright. All rights reserved.

5 Lower Kent Ridge Road, Singapore 119074;

Fax: 65-67772191, Tel: 65-67724514, Email: pcmngtp@nus.edu.sg

Pediatr Allergy Immunol

Obesity, Asthma Prevalence and IL-4: Roles of Inflammatory Cytokines, Adiponectin and Neuropeptide Y

Lu Y, Van Bever HP, Lim TK, Kuan WS, Goh DY, Mahadevan M, Sim TB, Ho R, Larbi A, Ng TP

Abstract

Background: Obesity is associated with asthma risk and severity, but the underlying biological mechanisms are poorly understood. We hypothesized that cytokine markers of systemic inflammation, and adiponectin and NPY markers of immuno-modulating and neuro-hormonal regulation are involved in the obesity-asthma association.

Methods: We explored the relationships between BMI, CRP, IL-6, and TNF- α , adiponectin and neuropeptide Y (NPY) with asthma prevalence and IL-4 levels in 70 youth with asthma and 69 age- and gender-matched healthy controls using cross-sectional and longitudinal data.

Results: Mean BMI level was higher among asthma patients than healthy controls ($p<0.001$). In logistic regression models controlling for potential confounders, independent associations with asthma prevalence were found for obesity ($p=0.001$), increasing tertiles of CRP (linear trend $p<0.001$), IL-6 (linear trend $p<0.001$) and lowest and highest tertiles of TNF- α (quadratic trend $p<0.05$), increasing adiponectin (linear $p=0.022$) and decreasing tertiles of NPY (linear trend $p=0.001$). Among patients with

asthma, NPY level was positively correlated with adiponectin ($p<0.05$) and TNF- α ($p<0.05$), and levels of NPY and IL-6 were significantly associated with IL-4 level at baseline and one-year follow-up.

Conclusions: The obesity-asthma association was not explained by systemic inflammation. Specifically, CRP, TNF- α , IL-6, NPY and adiponectin were independently associated with asthma prevalence. NPY and IL-6 were associated with IL-4 marker of allergic airway inflammation in asthma and should be further investigated as prognostic markers of asthma outcomes.

Key Words: Adiponectin; Adiposity; Asthma control; Inflammation; Neuropeptide Y.

INTRODUCTION

The association between obesity and asthma is well documented. Epidemiological and clinical studies show increased risk and severity of asthma, and less favourable response to asthma therapy in obese children and adults (1). Weight loss may improve lung function and asthma control (2, 3). Because C-reactive protein (CRP), tumour necrosis factor- α (TNF- α) and interleukin (IL)-6 concentrations are higher in obese than in non-obese individuals, it is proposed that systemic inflammation modulates airway disease such as asthma in obese subjects, skewing asthma towards a more difficult-to-control phenotype (4). However, whether systemic inflammation mediates obesity-associated asthma is debatable (5).

Adiponectin, exclusively secreted from adipose tissues, is found abundantly in the blood and modulates glucose regulation and fatty acid oxidation, and other metabolic processes. Although murine studies show that the administration of adiponectin attenuates airway

changes of asthma and promotes weight loss, possibly through inhibiting proinflammation and strengthening host immunity (9), paradoxically allergen-induced bronchoprovocation decreases adiponectin concentrations (10). Human studies are limited and have reported conflicting findings that high levels of adiponectin (11) or low levels of adiponectin (12) were associated or there were no associations with asthma (8).

Neuroendocrine hormones are involved in both obesity and asthma. Neuropeptide Y (NPY) is found abundantly in the brain and peripheral nerves and organs, and interestingly it is colocalized with adiponectin receptors in the arcuate nucleus (13). In response to stress, peripheral NPY released from sympathetic nerve terminals innervating visceral adipose tissue stimulates adipocyte growth and rapid fat storage, supplying energy to deal with the external threat (14). NPY Y1 receptor (Y1R) and Y5R knockout mice show increased food intake and greater adipose deposition (16, 17). NPY is a potent immunomodulator of the airway inflammatory response in asthma (18), although the underlying neuro-immunological mechanisms remain poorly understood.

As asthma is a highly complex disease that remains poorly understood, its relationship with obesity likely involves a complex interaction between cytokines released from adipocytes, airway endothelial cells and macrophages, possibly modulated by the central regulating influences of neuropeptide hormones. Previous studies have investigated inflammatory cytokines, adiponectin and NPY in obesity and asthma, but only individually as single pieces in a large puzzle. We hypothesized that cytokine markers of systemic inflammation, and adiponectin and NPY markers of immunomodulating and neuro-hormonal regulation are involved in both asthma and obesity. In

Accepted Article

this case control study of youth with asthma and age-and sex-matched healthy controls, we explored the associations of BMI, TNF- α , IL-6, CRP, adiponectin and NPY with asthma prevalence using cross-sectional and longitudinal data. Among patients with asthma, we examined whether plasma levels of these cytokines and NPY were associated with Th2-mediated allergic airway inflammation activity reflecting asthma severity.

METHODS

Study design and participants

This is a clinical study conducted in young asthma adults without co-existing chronic medical illnesses. Participants included 70 young adults (aged 21-35 years) with physician diagnosed asthma based on the Global Initiative for Asthma (GINA) criteria recruited from outpatient clinics of National University Hospital (NUH) of Singapore during June 2011 to April 2013. A group of 69 healthy controls were age- (+/- 2 years) and sex-matched with asthma patients, without current or previous history of asthma and agreed to provide health information, blood samples, and written informed consent. Participants were followed and 131 (87.9%) subjects were re-assessed at the one-year follow-up. A description of the sample size calculation for the study is found in the online Supplement. This study was conducted in accordance with the amended Declaration of Helsinki. All participants signed written informed consent for the study, which was approved by the National Healthcare Group Domain Specific Review Board.

The participants' demographic, asthma clinical, neuro-endocrine and immunological profiles were assessed. The degree of asthma control and asthma quality of life was determined using the Asthma Control Test (ACT) (21) and the Asthma Quality of Life Questionnaire (AQLQ) (22). Spirometric test was administered and 20ml venous blood

sample was collected into the BD Vacutainer® CPT™ Cell Preparation tube with Sodium Citrate. Within 2 h after sample collection, plasma was isolated from each blood sample and stored in -80°C freezer until the time the experiments were performed. The levels of NPY, adiponectin and CRP were measured by ELISA. Plasma concentration of IL-4, IL-6, and TNF- α was analysed utilizing a custom Milliplex. BMI was calculated as weight (kg) divided by the squared height (m^2). BMI was missing in four participants at baseline and ten participants at one-year follow-up assessment. More details of Methods are provided in the online Supplementary.

Statistical analyses

Data analyses were performed using PASW/SPSS 18.0. Differences in BMI, TNF- α , IL-6, CRP, adiponectin and NPY between the asthma and healthy control groups were compared for the whole sample, and by subgroups of male and female sex, and obese and non-obese participants, with test of statistical interactions with gender and obesity in generalized linear models (GLMs). Pearson correlation was performed to test the association of BMI, adiponectin, obesity-related cytokines, and neuropeptides at baseline and one-year follow-up. Multiple logistic regressions were used to determine the relationships of BMI, cytokines and NPY with asthma prevalence, adjusting for age, sex, ethnicity, smoking status, and perceived stress, in the whole sample and by male and female subgroups. We evaluated in hierarchical models the changes in the estimates of odds ratio of association of BMI with asthma when markers of systemic inflammation, adiponectin and NPY were sequentially introduced individually and together in the models. Finally, among asthma patients, the independent associations of BMI, TNF- α , IL-6, CRP, adiponectin and NPY (and their interactions with gender and obesity) with IL-4

levels at baseline and one-year follow-up were examined in GLMs, adjusting for potential confounders

RESULTS

Table 1 shows the demographic and clinical characteristics of asthma patients and healthy controls. Participants with asthma were more likely to be smokers ($p<0.001$) and report poorer self-rated health status ($p<0.001$). Asthma patients had mean duration of 16.0 years, and included 30 (47.6%) who reported previously taking medium to high dose of inhaled corticosteroids, and showed higher levels of IL-4 than healthy controls ($p=0.001$).

As shown in **Table 2**, there was a higher mean BMI level (25.61 vs. 22.4 kg/m², $p<0.001$) and greater prevalence of obesity (45.5% vs. 14.5%, $p<0.001$) among asthma patients than healthy controls. The asthma group had significantly higher levels of IL-6 (3.68 versus 0.78, $p=0.001$) and CRP (6.32 versus 1.70, $p<0.001$). Mean adiponectin level, as expected, was lower among obese versus non-obese participants ($p=0.008$; 4.86 versus 6.71 in the control group, and 6.37 versus 8.77 in the asthma group), and was higher in females than males ($p=0.009$; 7.36 versus 5.64 in the control group, and 8.62 versus 6.91 in the asthma group). For both obese and non-obese participants, adiponectin was higher in the asthma than control group (7.72 versus 6.44, $p<0.05$ in the whole sample). NPY level was significantly lower in the asthma group compared to healthy controls (0.45 versus 0.62, $p<0.01$).

The mean level of TNF- α was not significantly different between asthma and controls at baseline. However, among asthma patients, TNF- α , IL-6 and CRP levels were

significantly correlated with one another (r from 0.398 to 0.562, $p < 0.001$), and NPY was significantly correlated with TNF- α ($r = 0.272$, $p = 0.027$) and adiponectin ($r = 0.283$, $p = 0.021$). Similar correlation was observed in the analysis of one-year follow-up assessments. **Table 3**

In logistic regression models controlling for confounders, obesity was significantly associated with asthma prevalence (OR=5.55, $p = 0.001$). We examined in hierarchical models whether the OR was altered after adjusting for the levels of cytokines and NPY individually and together. The OR of association of BMI with asthma remained significantly elevated in all adjustments, and increased to 8.48 after adjusting for cytokines and NPY. **Table 4**

Also, positive associations with asthma that remained significantly elevated independently of each other were observed for increasing tertiles of CRP (OR=2.44 and OR=5.15, p (linear trend)=0.010) and increasing tertiles of IL-6 (OR=2.23 and OR=5.63, p (linear trend)=0.011). TNF- α showed a U-shaped association with asthma prevalence (lowest tertile OR=2.44, upper tertile OR=15.1, p (quadratic trend) < 0.001. A positive association with asthma prevalence was observed for increasing tertiles of adiponectin (OR=8.23 and OR=3.85, p (quadratic trend)=0.012). **Table 4** The associations of cytokines and NPY with asthma prevalence did not vary significantly by male and female sex. **Table E1**

Among the asthma patients, we examined whether BMI and levels of cytokine markers and NPY were independently associated with baseline (**Table 5**) and one-year (**Table 6**) IL-4, and whether the associations varied by sex or obesity subgroups. Adjusted for age,

sex, ethnicity, smoking status, and perceived stress, BMI was not associated with IL-4, and with further adjustment for cytokines and NPY, was inversely associated with IL-4 level ((baseline: standardized beta=-0.285, $p=0.019$; one-year: standardized beta=-0.277, $p=0.023$). IL-6 levels were positively associated with baseline (standardized beta=0.582, $p<0.001$) and one-year IL-4 (standardized beta=0.625, $p<0.001$) in both sexes, and particularly among females, although the interaction effect was not statistically significant. NPY ($p<0.001$) showed most strongly positive association with baseline and one-year IL-4 in the whole sample, and particularly among lean participants, and among females.

DISCUSSION

As asthma is a complex and heterogeneous disease, more than a few cytokines or neuro-hormones are likely to play critical roles in orchestrating, perpetuating and amplifying the inflammatory response in asthma. Unlike other studies of the kind, this study used several diverse biomarkers to better elucidate the complex neuro-hormonal and immune-mediated and inflammatory responses involved in both obesity and asthma. The results in this study showed that prevalent asthma was associated with high BMI. Furthermore, the obesity-asthma association was not explained by systemic inflammation. CRP, TNF- α , IL-6, NPY and adiponectin were independently associated with asthma prevalence. NPY and IL-6 were associated with baseline and one-year IL-4 which is an index of allergic airway inflammation in asthma.

Obesity and inflammation The association between obesity and asthma reported in prior studies is amply supported in this study. Obese individuals were shown to be eight times as likely to have asthma compared to non-obese individuals after adjusting for

potential confounders. Importantly, we found that the association of obesity and asthma was not explained by systemic inflammation (CRP, IL-6, TNF- α), adiponectin and NPY. This suggests that the effect of systemic inflammation associated with obesity is unlikely to fully explain the asthma-obesity association observed in humans (5, 7). Studies have also failed to observe an association between BMI and airway inflammation measured by sputum cell counts, IL-1 β , IL-5, IL-6 and IL-8 in sputum supernatants or nitric oxide (1, 4). The obesity-asthma association is thus more likely to be mediated by other factors, which include lung compression mechanics, co-morbidities such as gastro-oesophageal reflux, sleep apnea, type 2 diabetes, the modulating effects of estrogen, or shared genetic and prenatal and early life nutritional influences, which contribute to asthma pathogenesis, modulate asthma expression, or trigger or aggravate asthma (18).

Adiponectin contributes to the development or expression of asthma in general or among obese individuals (6). In this study, asthma patients showed higher mean levels of adiponectin than healthy controls. Mid and high tertile levels of adiponectin compared to low tertile adiponectin were associated with higher prevalence of asthma, but the effect estimate did not differ significantly between mid and high adiponectin. Because adiponectin has known sexual dimorphism, and previous studies have indicated significant asthma association only for females, we examined the associations for males and females, but found no clear indications of sex-specific associations.

In asthma, the anti-inflammatory effect of adiponectin is evidenced by, for example, the attenuation of airway hyperreactivity and neutrophilic inflammation mediated by acute phase cytokines and chemokines in adiponectin-deficient mice (26). However, studies also report that adiponectin possesses apparently contradictory pro-inflammatory

activities (6, 11), and modulates immune responses. Thus, it both activates dendritic cells and enhances Th1 and Th17 responses, and also down-regulates expression of co-stimulatory molecules and increases expression of inhibitory molecules on dendritic cells, leading to upregulation of T regulatory cell activity (27). Adiponectin activity in inflammatory and immune-mediated diseases, in turn, may be down-regulated by hormonal milieu (such as insulin, androgen) and sympathetic tone (activation of the beta-adrenergic pathway). This may possibly explain the attenuated OR of association with asthma in the high adiponectin level group.

Neuropeptide Y In this study, asthma patients showed significantly lower levels of NPY than healthy controls. We have earlier reported that NPY level was depressed in association with high level of perceived stress observed in this group of asthma patients (28). Studies show that chronic stress, combined with a high-fat/high-sugar diet, shifts sympathetic signaling toward NPY, leading to obesity and the metabolic syndrome (29). Although NPY is produced by adipose tissue macrophages and regulates obesity-induced inflammation (30), we found no evidence of significant interactive effects of circulating NPY and obesity with increased asthma prevalence in this study.

However, among asthmatics, increasing NPY level was associated with baseline and one-year IL-4 levels. NPY appears to have a bimodal role in APCs and T cells, as NPY-Y1 paradoxically modulates hyper-responsiveness in T cells (32). We observed that NPY was significantly correlated with IL-4 in lean and not obese asthmatics. This suggests that the APC-activating and negative T cell regulating activity of NPY in the autocrine and/or paracrine signalling pathway in asthma may be blunted by obesity. As the Th1-type inflammatory response is associated with obesity and cardio-metabolic diseases,

the asthma-associated responses directed towards the Th2-type of inflammatory response may be thwarted by the presence of obesity among asthma subjects.

The results are robust in showing a strong positive association of NPY with IL-4 overall, and particularly among lean participants, and among females. Increasing IL-6 level was also associated with IL-4, but the results for TNF- α could be spurious. Taken together, IL-6 and NPY may possibly be useful prognostic markers of IgE-mediated allergic airway inflammation and asthma severity. However, because of the cross-sectional design of the study, only prevalent cases of asthma were studied, and it is not known if chronic allergic airway disease may have reverse causative effects on the level of cytokines and hormones observed. The expression of adiponectin and NPY may vary across different populations; for example, the adiponectin levels were relatively lower (lowest tertile 6.0 versus 7.0 $\mu\text{g/ml}$ in women) than in a US study sample (33). Because of sample size limitations, tests of statistical interaction may be under-powered and modelling of trends of association with sparse data may not be robust and possibly yield spurious results. Our results therefore require replication in further studies.

In conclusion, our study shows that while obesity was evidently associated with asthma, systemic inflammation did not mediate this association. However, TNF- α , CRP and IL-6 cytokines, together with adiponectin and NPY are evidently involved in the complex neuro-hormonal, immune-mediated and inflammatory responses underlying individual predisposition and perpetuation of asthma disorder. Further research in understanding the modulating roles played by adiponectin and NPY in the development and progression of asthma may help in better phenotypic characterization of asthma, and to

identify important new targets for therapy and pave the way for more effective treatment.

Acknowledgements

TPN had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. All listed authors meet the criteria for authorship set forth by the International Committee for Medical Journal Editors. Conception and design: TPN, AL, RH, HVB; Analysis and interpretation: YL, TKL, DG, MM, TBS; Drafting the manuscript for important intellectual content: YL, TPN, WSK. We gratefully acknowledge the assistance and support of asthma nurses in the Division of Respiratory and Critical Care Medicine (RCCM) and staff in the Emergency Medicine Department (EMD) of National University Hospital (NUH) in patient recruitment and sample collection.

References

- 1 McLachlan CR, Poulton R, Car G, *et al.* Adiposity, asthma, and airway inflammation. *J Allergy Clin Immunol.* 2007; **119**: 634-9.
- 2 Stenius-Aarniala B, Poussa T, Kvarnstrom J, Gronlund EL, Ylikahri M, Mustajoki P. Immediate and long term effects of weight reduction in obese people with asthma: randomised controlled study. *Bmj.* 2000; **320**: 827-32.
- 3 Maniscalco M, Zedda A, Faraone S, *et al.* Weight loss and asthma control in severely obese asthmatic females. *Respir Med.* 2008; **102**: 102-8.
- 4 Lessard A, Turcotte H, Cormier Y, Boulet LP. Obesity and asthma: a specific phenotype? *Chest.* 2008; **134**: 317-23.
- 5 Hancox RJ, Poulton R, Greene JM, *et al.* Systemic inflammation and lung function in young adults. *Thorax.* 2007; **62**: 1064-8.
- 6 Sood A, Shore SA. Adiponectin, Leptin, and Resistin in Asthma: Basic Mechanisms through Population Studies. *J Allergy (Cairo).* 2013; **2013**: 785835.
- 7 Yuksel H, Sogut A, Yilmaz O, Onur E, Dinc G. Role of adipokines and hormones of obesity in childhood asthma. *Allergy Asthma Immunol Res.* 2012; **4**: 98-103.
- 8 Jartti T, Saarikoski L, Jartti L, *et al.* Obesity, adipokines and asthma. *Allergy.* 2009; **64**: 770-7.
- 9 Kim KW, Shin YH, Lee KE, Kim ES, Sohn MH, Kim KE. Relationship between adipokines and manifestations of childhood asthma. *Pediatr Allergy Immunol.* 2008; **19**: 535-40.
- 10 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**: 389-95.
- 11 Sood A, Dominic E, Qualls C, *et al.* Serum Adiponectin is Associated with Adverse Outcomes

- of Asthma in Men but Not in Women. *Front Pharmacol*. 2011; **2**: 55.
- 12 Sood A, Qualls C, Schuyler M. Leptin, adiponectin, and asthma: findings from a population-based cohort study. *Ann Allergy Asthma Immunol*. 2010; **104**: 355; author reply 55-6.
- 13 Guillod-Maximin E, Roy AF, Vacher CM, *et al*. Adiponectin receptors are expressed in hypothalamus and colocalized with proopiomelanocortin and neuropeptide Y in rodent arcuate neurons. *J Endocrinol*. 2009; **200**: 93-105.
- 14 Kuo LE, Kitlinska JB, Tilan JU, *et al*. Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med*. 2007; **13**: 803-11.
- 15 Segal-Lieberman G, Trombly DJ, Juthani V, Wang X, Maratos-Flier E. NPY ablation in C57BL/6 mice leads to mild obesity and to an impaired refeeding response to fasting. *Am J Physiol Endocrinol Metab*. 2003; **284**: E1131-9.
- 16 Kushi A, Sasai H, Koizumi H, Takeda N, Yokoyama M, Nakamura M. Obesity and mild hyperinsulinemia found in neuropeptide Y-Y1 receptor-deficient mice. *Proc Natl Acad Sci U S A*. 1998; **95**: 15659-64.
- 17 Higuchi H, Niki T, Shiiya T. Feeding behavior and gene expression of appetite-related neuropeptides in mice lacking for neuropeptide Y Y5 receptor subclass. *World J Gastroenterol*. 2008; **14**: 6312-7.
- 18 Sood A, Ford ES, Camargo CA, Jr. Association between leptin and asthma in adults. *Thorax*. 2006; **61**: 300-5.
- 19 Doniec Z, Pierzchala-Koziec K, Tomalak W, Kurzawa R. [Serum level of leptin and neuropeptide Y in children with mild asthma]. *Pneumonol Alergol Pol*. 2004; **72**: 9-13.
- 20 Dahlof C, Dahlof P, Lundberg JM, Strombom U. Elevated plasma concentration of neuropeptide Y and low level of circulating adrenaline in elderly asthmatics during rest and acute severe asthma. *Pulm Pharmacol*. 1988; **1**: 3-6.
- 21 Nathan RA, Sorkness CA, Kosinski M, *et al*. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol*. 2004; **113**: 59-65.
- 22 Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. *Thorax*. 1992; **47**: 76-83.
- 23 Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. *International journal of biological sciences*. 2012; **8**: 1281-90.
- 24 Bradding P, Roberts JA, Britten KM, *et al*. Interleukin-4, -5, and -6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *American journal of respiratory cell and molecular biology*. 1994; **10**: 471-80.
- 25 Kattan M, Kumar R, Bloomberg GR, *et al*. Asthma control, adiposity, and adipokines among inner-city adolescents. *J Allergy Clin Immunol*. 2010; **125**: 584-92.
- 26 Zhu M, Hug C, Kasahara DI, *et al*. Impact of adiponectin deficiency on pulmonary responses to acute ozone exposure in mice. *American journal of respiratory cell and molecular biology*. 2010; **43**: 487-97.
- 27 Jung MY, Kim HS, Hong HJ, Youn BS, Kim TS. Adiponectin induces dendritic cell activation via PLC γ /JNK/NF- κ B pathways, leading to Th1 and Th17 polarization. *J Immunol*. 2012; **188**: 2592-601.
- 28 Lu Y, Ho R, Lim TK, *et al*. Neuropeptide Y may mediate psychological stress and enhance T2 inflammatory response in asthma. *J Allergy Clin Immunol*. 2014.
- 29 Kuo LE, Czarnecka M, Kitlinska JB, Tilan JU, Kvetnansky R, Zukowska Z. Chronic stress, combined with a high-fat/high-sugar diet, shifts sympathetic signaling toward neuropeptide Y and leads to obesity and the metabolic syndrome. *Ann N Y Acad Sci*. 2008; **1148**: 232-7.
- 30 Singer K, Morris DL, Oatmen KE, *et al*. Neuropeptide Y Is Produced by Adipose Tissue Macrophages and Regulates Obesity-Induced Inflammation. *Plos One*. 2013; **8**.
- 31 Jaakkola U, Kakko T, Juonala M, *et al*. Neuropeptide Y polymorphism increases the risk for asthma in overweight subjects; protection from atherosclerosis in asthmatic subjects - The

cardiovascular risk in young Finns study. *Neuropeptides*. 2012; **46**: 321-28.

32 Macia L, Rao PT, Wheway J, Sierro F, Mackay F, Herzog H. Y1 signalling has a critical role in allergic airway inflammation. *Immunol Cell Biol*. 2011; **89**: 882-8.

33 Sood A, Cui X, Qualls C, et al. Association between asthma and serum adiponectin concentration in women. *Thorax*. 2008; **63**: 877-82.

Table 1. Baseline socio-demographic and clinical characteristics of participants by asthma and healthy control (n=139)

	Asthma (n = 70)	Healthy Control (n = 69)	Significance Tests	
			χ^2/t	p
Sex				
Male	36 (51.4)	37 (53.6)	0.067	0.796
Female	34 (48.6)	32 (46.4)		
Age	27.00 $\pm$ 5.06	25.48 $\pm$ 3.38	1.450	0.150
Housing type				
1-3 room public housing	20 (30.3)	23 (33.3)	0.143	0.706
4-5 room public housing or private house	46 (69.7)	46 (66.7)		
Smoking status				
Non-smoker	41 (62.1)	65 (94.2)	20.584	<0.001
Past or current smoker	25 (37.9)	4 (5.8)		
Self-rated health				
Very poor to fair to good	59 (88.1)	27 (39.1)	35.005	<0.001
Very good to excellent	8 (11.9)	42 (60.9)		
IL-4 (pg/ml)	4.59 $\pm$ 7.00	1.57 $\pm$ 2.27	3.337	0.001
FEV1 Pred (%)	80.51 $\pm$ 24.56	87.21 $\pm$ 28.88	-1.374	0.172
FVC Pred (%)	86.21 $\pm$ 23.13	95.81 $\pm$ 31.03	-1.918	0.057
Number of asthma attack in past 1 year	3.99 $\pm$ 5.23	NA		
Number of asthma-related hospitalization in past year	0.84 $\pm$ 1.24	NA		
History of asthma (years)	15.98 $\pm$ 10.30	NA		
Dose of inhaled corticosteroids (ICS)				
Medium to high dose ($\geq$ 250 μ g/day)	30 (47.6)	NA		
Low dose (<250 μ g/day)	17 (27.0)	NA		
No use	16 (25.4)	NA		
Asthma control	17.70 $\pm$ 5.31	NA		
Asthma quality of life	4.85 $\pm$ 1.45	NA		
Activity limitation	2.33 $\pm$ 0.70	NA		
Symptom	4.75 $\pm$ 1.56	NA		
Emotional function	4.76 $\pm$ 1.69	NA		
Exposure	4.78 $\pm$ 1.60	NA		

Figures in table denote M $\pm$ SD or number and %. IL-4 = interleukin 4, FEV1 Pred = forced expiratory volume in 1 second predicted, FVC Pred = forced vital capacity predicted.

Table 2. Case-control analysis of association of BMI, NPY and adipokine levels with asthma (n=139)

	Asthma (n = 70)	Healthy Control (n = 69)	Significance Tests	
			t/ χ^2	p
Total Samples, N=135				
BMI	25.61 ± 6.04	22.41 ± 3.53	3.739	<0.001
Obese (BMI ≥ 25 kg/m², n (%))	30 (45.5)	10 (14.5)	15.509	<0.001
TNF-α (pg/ml)	11.08 ± 8.50	10.58 ± 2.35	0.458	0.649
IL-6 (pg/ml)	3.68 ± 6.90	0.78 ± 1.44	3.341	0.001
CRP (μg/ml)	6.32 ± 8.39	1.70 ± 2.68	4.270	<0.001
Adiponectin (μg/ml)	7.72 ± 3.17	6.44 ± 4.25	2.001	0.048
NPY (ng/ml)	0.45 ± 0.22	0.62 ± 0.40	-3.087	0.003
IL-4 (pg/ml)	4.59 ± 7.00	1.57 ± 2.27	3.337	0.001
Males, N=70				
	N = 33	N = 37		
BMI	25.89 ± 6.45	23.49 ± 3.10	1.943	0.058
TNF-α (pg/ml)	10.89 ± 7.20	11.21 ± 2.38	-0.247	0.806
IL-6 (pg/ml)	2.08 ± 1.90	0.70 ± 1.09	3.701	0.001
CRP (μg/ml)	4.91 ± 7.88	2.01 ± 2.91	2.021	0.050
Adiponectin (μg/ml)	6.91 ± 2.29	5.64 ± 3.43	1.855	0.068
NPY (ng/ml)	0.41 ± 0.15	0.57 ± 0.26	-3.138	0.003
IL-4 (pg/ml)	3.39 ± 2.57	1.88 ± 2.76	2.379	0.020
Females, N=65				
	N = 33	N = 32		
BMI	25.34 ± 5.68	21.16 ± 3.63	3.540	0.001
TNF-α (pg/ml)	11.28 ± 9.82	9.86 ± 2.12	0.801	0.429
IL-6 (pg/ml)	5.37 ± 9.50	0.87 ± 1.77	2.635	0.013
CRP (μg/ml)	7.82 ± 8.79	1.34 ± 2.36	4.031	<0.001
Adiponectin (μg/ml)	8.62 ± 3.76	7.36 ± 4.94	1.142	0.258
NPY (ng/ml)	0.50 ± 0.26	0.68 ± 0.52	-1.794	0.079
IL-4 (pg/ml)	5.86 ± 9.62	1.21 ± 1.50	2.702	0.011
Obese (BMI ≥ 25 kg/m²), N=40				
	N = 30	N = 10		
BMI	30.47 ± 5.58	29.08 ± 3.04	0.745	0.461
Adiponectin (μg/ml)	6.37 ± 1.60	4.86 ± 2.65	1.695	0.117
TNF-α (pg/ml)	10.15 ± 7.05	11.08 ± 1.92	-0.410	0.684
IL-6 (pg/ml)	3.78 ± 6.29	1.08 ± 1.45	1.337	0.189
CRP (μg/ml)	7.21 ± 6.22	5.27 ± 5.17	0.882	0.383
NPY (ng/ml)	0.44 ± 0.15	0.68 ± 0.25	-2.818	0.016
IL-4 (pg/ml)	4.08 ± 4.51	2.88 ± 3.33	0.767	0.448
Non-Obese (BMI<25 kg/m²), N=95				
	N = 36	N = 59		
BMI	21.56 ± 2.19	21.28 ± 2.05	0.638	0.525
Adiponectin (μg/ml)	8.77 ± 3.86	6.71 ± 4.43	2.269	0.026
TNF-α (pg/ml)	11.99 ± 9.96	10.50 ± 2.42	0.844	0.405
IL-6 (pg/ml)	2.36 ± 3.21	0.73 ± 1.45	2.762	0.009
CRP (μg/ml)	4.98 ± 9.30	1.09 ± 1.29	2.389	0.023
NPY (ng/ml)	0.46 ± 0.27	0.61 ± 0.42	-1.896	0.061
IL-4 (pg/ml)	4.98 ± 8.51	1.34 ± 2.00	2.558	0.015

Figures in table denote M $\pm$ SD or number and %. BMI = body mass index, IL-6 = interleukin 6, CRP = C-reactive protein, NPY = neuropeptide Y, TNF- α = tumor necrosis factor alpha.

Tests for interactions (asthma*sex, asthma*obesity) were not statistically significant.

Table 3. Correlation ρ (p) of BMI, obesity-related cytokines and neuropeptides at baseline (n=139) and one-year follow-up (n=131)

	BMI	Adiponectin	TNF- α	IL-6	CRP	NPY	IL-4
Baseline							
BMI	1						
Adiponectin	-0.191 (0.028)	1					
TNF- α	-0.062 (0.481)	0.106 (0.221)	1				
IL-6	0.251 (0.004)	0.019 (0.828)	0.416 (<0.001)	1			
CRP	0.350 (<0.001)	0.069 (0.424)	0.497 (<0.001)	0.449 (<0.001)			
NPY	-0.148 (0.092)	0.173 (0.045)	0.086 (0.321)	-0.026 (0.763)	-0.028 (0.743)	1	
IL-4	0.038 (0.665)	0.005 (0.957)	0.010 (0.909)	0.275 (0.001)	0.115 (0.183)	0.176 (0.041)	1
Asthma Patients							
BMI	1						
Adiponectin	-0.294 (0.020)	1					
TNF- α	-0.120 (0.352)	0.192 (0.122)	1				
IL-6	0.189 (0.141)	0.007 (0.958)	0.447 (<0.001)	1			
CRP	0.187 (0.146)	0.095 (0.449)	0.562 (<0.001)	0.398 (0.001)	1		
NPY	-0.042 (0.745)	0.283 (0.021)	0.272 (0.027)	0.160 (0.198)	0.214 (0.085)	1	
IL-4	-0.096 (0.456)	-0.027 (0.828)	-0.001 (0.993)	0.226 (0.068)	0.015 (0.903)	0.453 (<0.001)	1
Healthy Controls							
BMI	1						
Adiponectin	-0.271 (0.025)	1					
TNF- α	0.117 (0.340)	-0.039 (0.750)	1				
IL-6	0.073 (0.550)	-0.217 (0.074)	0.038 (0.757)	1			
CRP	0.650 (<0.001)	-0.180 (0.140)	0.034 (0.781)	0.290 (0.015)	1		
NPY	-0.111 (0.362)	0.211 (0.082)	-0.095 (0.438)	-0.127 (0.298)	-0.098 (0.424)	1	
IL-4	0.122 (0.318)	-0.127 (0.299)	-0.006 (0.959)	0.038 (0.758)	0.036 (0.767)	0.239 (0.048)	1
One-year follow-up							
BMI	1						
Adiponectin	-0.361 (<0.001)	1					
TNF- α	-0.044 (0.635)	-0.023 (0.2803)	1				
IL-6	0.189 (0.039)	-0.013 (0.891)	0.075 (0.420)	1			
CRP	0.526 (<0.001)	-0.273 (0.003)	-0.022 (0.809)	0.254 (0.005)			
NPY	0.084 (0.365)	0.186 (0.043)	0.039 (0.676)	-0.008 (0.935)	0.000 (1.000)	1	
IL-4	-0.029 (0.758)	0.052 (0.574)	0.047 (0.609)	0.147 (0.111)	0.115 (0.214)	0.188 (0.041)	1
Asthma Patients							
BMI	1						
Adiponectin	-0.353 (0.011)	1					
TNF- α	-0.128 (0.369)	0.169 (0.236)	1				
IL-6	0.539 (<0.001)	-0.020 (0.888)	-0.065 (0.648)	1			
CRP	0.456 (0.001)	-0.257 (0.069)	-0.119 (0.408)	0.476 (<0.001)	1		
NPY	0.334 (0.017)	-0.063 (0.660)	-0.087 (0.543)	0.374 (0.007)	0.183 (0.198)	1	
IL-4	-0.105 (0.465)	0.095 (0.509)	0.034 (0.815)	0.125 (0.383)	0.043 (0.762)	0.226 (0.111)	1
Healthy Controls							
BMI	1						
Adiponectin	-0.313 (0.009)	1					
TNF- α	0.082 (0.506)	-0.253 (0.037)	1				
IL-6	0.159 (0.196)	-0.035 (0.780)	0.294 (0.015)	1			
CRP	0.482 (<0.001)	-0.203 (0.097)	0.268 (0.027)	0.539 (<0.001)	1		
NPY	0.015 (0.905)	0.240 (0.048)	0.231 (0.058)	-0.066 (0.591)	-0.119 (0.332)	1	
IL-4	-0.041 (0.742)	0.154 (0.211)	0.003 (0.980)	0.305 (0.011)	0.148 (0.228)	0.321 (0.008)	1

NPY = Neuropeptide Y, BMI = body mass index, TNF- α = tumor necrosis factor alpha, IL-6 = interleukin 6, CRP = C-reactive protein.

Table 4. Logistic and quadratic regression models of association of BMI, adiponectin, NPY, TNF- α , CRP, and IL-6 levels with asthma (n=139)

	BMI	Asthma	Healthy Control	<i>p</i> for Wald χ^2	Adjusted*	Adjusted* + BMI	Adjusted* + TNF- α , CRP, IL-6	Adjusted* + Adiponectin	Adjusted* + NPY	Adjusted* + BMI, Adiponectin, NPY, TNF- α , CRP, IL-6
BMI	Obese	30 (45.5)	10 (14.5)	<0.001	5.55 (2.10-14.7)	NA	4.63 (1.37-15.7)	8.20 (2.83-23.7)	8.32 (2.72-25.4)	8.48 (2.05-35.14)
	Non-obese	36 (54.5)	59 (84.5)		1	NA	1	1	1	1
	<i>P</i>				0.001	NA	0.014	<0.001	<0.001	0.003
Adiponectin	Low tertile	11 (16.4)	34 (49.3)	0.022	1	1	1	NA	1	1
	Mid-tertile	34 (50.7)	12 (17.4)		7.18 (2.50-20.6)	5.82 (1.93-17.5)	8.23 (2.37-28.6)	NA	8.53 (2.79-26.1)	8.01 (2.16-29.6)
	High tertile	22 (32.8)	23 (33.3)		2.04 (0.74-5.61)	3.07 (1.01-9.36)	3.85 (1.11-13.3)	NA	2.57 (0.88-7.52)	4.78 (1.26-18.1)
	Linear Trend, <i>p</i>				0.127	0.027	0.004	NA	<0.001	0.012
NPY	Low tertile	32 (48.5)	13 (18.8)	0.001	4.46 (1.84-10.84)	8.63 (0.26-28.4)	7.75 (2.12-28.3)	10.9 (3.07-39.1)	NA	9.71 (2.42-38.9)
	Mid-tertile	18 (27.3)	27 (39.1)		1.21 (0.52-2.84)	3.24 (0.99-10.6)	3.52 (0.94-13.2)	3.64 (1.05-12.6)	NA	3.92 (0.98-5.7)
	High tertile	16 (24.2)	29 (42.0)		1	1	1	1	NA	1
	Linear Trend, <i>p</i>				0.001	<0.001	0.002	<0.001	NA	0.001
IL-6	Low tertile	16 (24.2)	44 (63.8)	<0.001	1	1	1	1	1	1
	Mid-tertile	13 (19.7)	17 (24.6)		2.10 (0.84-5.28)	2.56 (0.86-7.63)	2.23 (0.71-6.98)	4.12 (1.22-13.9)	2.65 (0.89-7.88)	3.79 (1.04-13.9)
	High tertile	37 (56.1)	8 (11.6)		12.7 (4.90-33.0)	10.0 (3.05-33.1)	5.63 (1.45-21.9)	11.1 (3.20-38.8)	14.3 (4.32-47.4)	5.98 (1.44-24.80)
	Linear Trend, <i>p</i>				<0.001	<0.001	0.011	<0.001	<0.001	0.008
CRP	Low tertile	14 (21.2)	31 (44.9)	<0.001	1	1	1	1	1	1
	Mid-tertile	20 (30.3)	25 (36.2)		2.22 (0.78-6.27)	1.54 (0.51-4.65)	2.44 (0.82-7.28)	2.53 (0.86-7.43)	2.22 (0.77-6.42)	1.80 (0.51-6.37)
	High tertile	32 (48.5)	13 (18.8)		7.70 (2.49-23.8)	4.35 (1.28-14.8)	5.15 (1.47-18.0)	9.59 (2.94-31.2)	7.81 (2.36-25.9)	3.63 (0.80-16.44)
	Linear Trend, <i>p</i>				<0.001	0.018	0.010	<0.001	0.001	0.096
TNF- α	Low tertile	30 (45.5)	15 (21.7)	0.400	8.22 (2.55-26.6)	9.27 (2.55-33.7)	8.38 (1.67-42.0)	9.04 (2.71-30.1)	10.7 (3.12-36.7)	25.5 (4.47-145.2)
	Mid-tertile	10 (15.2)	35 (50.7)		1	1	1	1	1	1
	High tertile	26 (39.4)	19 (27.5)		7.59 (2.33-24.7)	8.83 (2.50-31.2)	15.1 (2.55-89.3)	7.98 (2.40-26.6)	6.90 (2.10-22.7)	11.0 (2.25-53.8)
	Quadratic Trend, <i>p</i>				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* Adjusted for: age, sex, race, smoking status, and perceived stress. BMI = body mass index, IL-6 = interleukin 6, CRP = C-reactive protein, NPY = neuropeptide Y, TNF- α = tumor necrosis factor alpha. BMI: obese: BMI $\geq$ 25kg/m²; non-obese: BMI<25kg/m². Adiponectin: low tertile: $\leq$ 4.80 μ g/ml in male, $\leq$ 6.04 μ g/ml in female; mid-tertile: $>$ 4.80 μ g/ml in male, $>$ 6.04 μ g/ml & $\leq$ 9.24 μ g/ml in female; high tertile: $>$ 7.44 μ g/ml in male, $>$ 9.24 μ g/ml in female. NPY: low tertile: $\leq$ 0.40 ng/ml; mid-tertile: $>$ 0.40 ng/ml & $\leq$ 0.51 ng/ml; high tertile: $>$ 0.51 ng/ml IL-6: low tertile: $\leq$ 0.06 pg/ml; mid-tertile: $>$ 0.06 pg/ml & $\leq$ 1.98 pg/ml; high tertile: $>$ 1.98 pg/ml. CRP: low tertile: $\leq$ 0.53 μ g/ml; mid-tertile: $>$ 0.53 μ g/ml & $\leq$ 2.37 μ g/ml; high tertile: $>$ 2.37 μ g/ml. TNF- α : low tertile: $\leq$ 8.77 pg/ml; mid-tertile: $>$ 8.77 pg/ml & $\leq$ 11.41 pg/ml; high tertile: $>$ 11.41 pg/ml

Table 5. Independent associations of BMI, adiponectin, NPY, TNF- α , CRP and IL-6 levels with baseline IL-4 concentration among patients with asthma (n=139)

	Dependent variable: IL-4							
	Adjusted (1)				Adjusted (2)			
	B	SE	β	<i>p</i>	B	SE	β	<i>p</i>
Whole sample								
BMI	-0.037	0.158	-0.032	0.813	-0.333	0.138	-0.285	0.019
IL-6	0.491	0.191	0.339	0.013	0.708	0.206	0.489	0.001
CRP	0.069	0.124	0.078	0.580	-0.061	0.127	-0.069	0.634
TNF- α	0.066	0.110	0.080	0.551	-0.246	0.130	-0.299	0.065
Adiponectin	-0.210	0.303	-0.096	0.490	-0.343	0.252	-0.157	0.179
NPY	16.508	3.715	0.508	<0.001	18.924	3.547	0.582	<0.001
NPY*Gender	21.478	8.081	1.453	0.010	29.471	7.069	1.994	<0.001
NPY*Obesity	7.634	1.740	0.505	<0.001	11.368	3.120	0.752	0.001
Females								
BMI	-0.203	0.329	-0.119	0.544	-0.421	0.226	-0.247	0.078
IL-6	0.624	0.303	0.421	0.051	1.158	0.277	0.781	0.001
CRP	0.322	0.244	0.293	0.200	-0.148	0.177	-0.134	0.415
TNF- α	0.202	0.216	0.205	0.360	-0.808	0.215	-0.819	0.001
Adiponectin	-0.497	0.593	-0.194	0.411	-0.762	0.321	-0.298	0.029
NPY	24.267	5.552	0.667	<0.001	34.895	4.589	0.960	<0.001
Males								
BMI	-0.037	0.071	-0.119	0.606	-0.040	0.080	-0.128	0.625
IL-6	0.151	0.226	0.140	0.510	0.562	0.243	0.521	0.032
CRP	-0.080	0.060	-0.271	0.193	-0.070	0.077	-0.237	0.376
TNF- α	-0.113	0.053	-0.412	0.046	-0.137	0.071	-0.503	0.068
Adiponectin	-0.087	0.197	-0.096	0.664	-0.267	0.186	-0.294	0.168
NPY	-3.017	3.204	-0.223	0.356	-2.887	3.089	-0.214	0.362
Obese								
BMI	0.202	0.165	0.268	0.236	-0.057	0.084	-0.076	0.506
IL-6	0.597	0.081	0.894	<0.001	0.810	0.093	1.214	<0.001
CRP	0.046	0.133	0.068	0.731	-0.117	0.066	-0.172	0.096
TNF- α	0.188	0.126	0.311	0.150	-0.221	0.075	-0.364	0.009
Adiponectin	0.451	0.481	0.163	0.360	0.179	0.212	0.064	0.412
NPY	3.773	5.801	0.126	0.523	-3.488	2.831	-0.116	0.236
Non-obese								
BMI	-0.665	0.849	-0.163	0.440	-0.997	0.666	-0.244	0.149
IL-6	0.633	0.523	0.227	0.237	0.725	0.541	0.260	0.194
CRP	0.078	0.201	0.082	0.700	-0.139	0.248	-0.144	0.582
TNF- α	-0.008	0.179	-0.009	0.963	-0.330	0.217	-0.367	0.143
Adiponectin	-0.374	0.509	-0.161	0.468	-0.695	0.378	-0.299	0.079
NPY	21.772	5.254	0.659	<0.001	27.512	5.445	0.833	<0.001

Adjusted (1): base model variables: age, gender, ethnicity, smoking status, perceived stress, and BMI (for sex).

Adjusted (2): base model variables: age, gender, ethnicity, smoking status, perceived stress, and BMI (for sex) and circulating biomarkers.

BMI = body mass index, IL-6 = interleukin 6, CRP = C-reactive protein, NPY = neuropeptide Y, TNF- α = tumor necrosis factor alpha.

Table 6. Independent associations of BMI, adiponectin, NPY, TNF- α , CRP and IL-6 levels with IL-4 level at 1-year follow-up among patients with asthma (n=131)

	Dependent variable: IL-4							
	Adjusted (1)				Adjusted (2)			
	B	SE	β	<i>p</i>	B	SE	β	<i>p</i>
Whole sample								
BMI	-0.044	0.158	-0.039	0.580	-0.312	0.131	-0.277	0.023
IL-6	0.495	0.212	0.337	0.025	0.550	0.195	0.374	0.008
CRP	0.113	0.147	0.108	0.447	0.040	0.128	0.039	0.755
TNF- α	0.200	0.140	0.201	0.160	0.065	0.131	0.066	0.620
Adiponectin	-0.218	0.395	-0.081	0.584	-0.008	0.296	-0.003	0.978
NPY	20.815	4.272	0.562	<0.001	23.183	3.844	0.625	<0.001
NPY*Gender	13.740	2.245	0.792	<0.001	30.132	6.484	1.738	<0.001
NPY*Obesity	9.651	2.159	0.544	<0.001	8.915	3.220	0.502	0.009
Females								
BMI	-0.034	0.332	-0.021	0.920	-0.051	0.271	-0.032	0.853
IL-6	0.577	0.383	0.389	0.149	0.569	0.357	0.383	0.133
CRP	0.457	0.367	0.308	0.230	0.141	0.245	0.095	0.575
TNF- α	0.406	0.434	0.278	0.362	0.015	0.382	0.010	0.970
Adiponectin	-0.829	0.809	-0.237	0.319	-0.142	0.538	-0.041	0.796
NPY	33.007	6.283	0.762	<0.001	33.303	5.656	0.769	<0.001
Males								
BMI	-0.106	0.077	-0.295	0.183	-0.089	0.108	-0.254	0.422
IL-6	0.060	0.319	0.041	0.854	-0.101	0.402	-0.070	0.806
CRP	-0.049	0.072	-0.145	0.511	-0.137	0.118	-0.408	0.266
TNF- α	0.044	0.066	0.142	0.515	0.157	0.105	0.508	0.160
Adiponectin	0.176	0.221	0.164	0.436	0.214	0.245	0.205	0.399
NPY	6.860	3.534	0.448	0.069	4.467	4.098	0.291	0.295
Obese								
BMI	0.008	0.226	0.008	0.972	-0.255	0.162	-0.254	0.143
IL-6	0.788	0.119	0.896	<0.001	0.743	0.215	0.844	0.005
CRP	0.078	0.199	0.083	0.700	-0.090	0.134	-0.096	0.512
TNF- α	0.524	0.158	0.624	0.004	0.083	0.190	0.099	0.671
Adiponectin	0.440	0.762	0.108	0.571	-0.031	0.482	-0.008	0.950
NPY	6.542	8.550	0.164	0.455	2.164	5.686	0.054	0.710
Non-obese								
BMI	-1.097	0.854	-0.287	0.214	-0.691	0.530	-0.180	0.214
IL-6	-0.361	0.876	-0.087	0.684	-0.360	0.520	-0.086	0.499
CRP	0.117	0.252	0.101	0.647	0.135	0.191	0.117	0.491
TNF- α	-0.015	0.242	-0.014	0.950	-0.020	0.163	-0.018	0.904
Adiponectin	-0.308	0.646	-0.118	0.639	-0.026	0.336	-0.010	0.940
NPY	35.893	4.106	0.998	<0.001	35.309	4.574	0.982	<0.001

Adjusted (1): base model variables: age, gender, ethnicity, smoking status, perceived stress, and BMI (for sex).

Adjusted (2): base model variables: age, gender, ethnicity, smoking status, perceived stress, and BMI (for sex) and circulating biomarkers.

BMI = body mass index, IL-6 = interleukin 6, CRP = C-reactive protein, NPY = neuropeptide Y, TNF- α = tumor necrosis factor alpha.

Online Data Supplement

Obesity, Asthma Prevalence and IL-4: Roles of Inflammatory Cytokines, Adiponectin and Neuropeptide Y

Yanxia Lu, Hugo P.S. Van Bever, Tow Keang Lim, Win Sen Kuan, Daniel Yam Thiam Goh,
Malcolm Mahadevan, Tiong Beng Sim, Roger Ho, Anis Larbi, Tze Pin Ng

METHODS

Study design and participants

The inclusion criteria were: asthma patients a) with doctor-diagnosed asthma based on GINA diagnostic criteria; b) aged 21-35 years; c) able to understand and respond to questions in English; healthy controls a) aged 21-35 years; b) able to understand and respond to questions in English. The exclusion criteria were individuals a) with other pulmonary disease (cystic fibrosis, etc.); b) having neurological or psychiatric illness (depression, schizophrenia, bipolar disorder, post-traumatic stress disorder, attention deficit hyperactivity disorder, autistic disorder, etc.); c) having gastro-intestinal (e.g. irritable bowel syndromes), autoimmune disorders (arthritis, lupus, etc), pituitary syndromes, Cushing's syndrome, involving disturbances in the hypothalamic-pituitary-adrenal (HPA) axis; d) on oral steroid treatment within two weeks before visiting; e) having mental incapacitation or learning disability with difficulty understanding and responding to questions in English. At one-year follow-up, four asthma patients and one healthy control declined to be re-assessed; two patients were lost contact; and one patient had to quit from this study due to pregnancy.

Interested volunteer patients and healthy controls were invited to attend a screening interview to assess their eligibility and provide informed consent before enrolment. After this, trained nurses would bring the participant to a designated room within the department that is free from distraction and where the conversation between the investigator and the participant could not be overheard. Spirometric test and questionnaires were administered and blood samples were collected from participants.

Blood samples were centrifuged at room temperature in a horizontal rotor for 20 minutes at 1650 rpm with the brake off.

Spirometric test

Calibration for spirometer (Easy-One; Model 2001 Diagnostic Spirometer, NDD Medical Technologies, Zurich, Switzerland) was checked daily with a 3-L syringe. Forced expiratory maneuvers were performed with the respondent seated according to American Thoracic Society (ATS) recommended guidelines, and standardization of procedures: at least three acceptable maneuvers, with forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) reproducible within 200 mL (1).

Asthma control and asthma quality of life

The degree of asthma control was determined using the Asthma Control Test (ACT) (2), a widely-used questionnaire in clinical and research settings for the assessment of asthma. ACT has shown strong evaluative and discriminative properties in general (2) and among local patients (3). The questions in ACT verified occurrences of episodes of dyspnea, sleep impairment due to asthma symptoms, limitations in daily activities, the use of rescue inhalatory medications, and self-rated degree of asthma control. Participants responded to 5 questions pertaining to a recent 4-week period, with each question scored from 1 to 5 points. The higher the ACT score (25 points is the maximum achievable), the better controlled is asthma. A score of 20 points or higher corresponds to well-controlled asthma, 19 or less indicates less than optimal control.

Asthma-related functional impairment was assessed using the Asthma Quality of Life Questionnaire (AQLQ) (4), a well-validated questionnaire with excellent psychometric properties. AQLQ consists of 32 items relating to the day-to-day problems that patients with asthma find most troublesome. Consequences of asthma are evaluated in four life domains: activity limitations (ability to carry out daily activities), symptoms (frequency and nature of asthma symptoms such as coughing and chest tightness), emotional distress (intensity of the discomfort, fear, or distress associated with asthma), and exposure (to environmental stimuli) (capacity to manage environmental triggers of asthma such as smoke, dust, and pollution). Patients were asked to recall their experience over the previous 2 weeks and respond to each question on a 7-point scale. Average scores on each domain and a total AQLQ score were generated for each patient. The internal consistency reliability of AQLQ ranged from 0.90 to 0.95, and the reproducibility was as high as 0.81 to 0.93 (5).

Enzyme-linked immunosorbent assay (ELISA) and Multiplex analysis

Plasma specimens were prepared for ELISA and Multiplex analysis in 96-well plates. Neuropeptide Y-like immunoreactivity was measured using a competitive enzyme immunoassay (Bachem/Peninsula Laboratories Inc., Bachem, San Carlos, California). The quantitative measurement of adiponectin was performed using a human adiponectin ELISA (competitive) kit (Biovendor Laboratory Medicine Inc., Brno, Czech Republic). The levels of CRP were measured by a human CRP ELISA kit (Millipore Corp., Billerica, Massachusetts). Plates were run according to the manufacturers' instructions.

Plasma concentration of IL-4, IL-6, and TNF- α was analysed utilizing a custom Milliplex MAP Human Cytokine Magnetic Bead Panel (Millipore Corp., Billerica, Massachusetts) following the kit-specific protocols provided by Millipore. Results were acquired using the Luminex 200 platform (Millipore Corp., Billerica, Massachusetts), with Bioplex Manager 6.0 software, based on standard curves plotted through a 5-parameter logistic curve setting.

Body mass index (BMI)

Height and weight were measured wearing light clothing only. BMI was calculated as weight (kg) divided by the squared height (m^2). Obesity was defined according to WHO recommended cut-off for Asians ($\text{BMI} \geq 25 \text{ kg/m}^2$).

Psycho-social and clinical profile

Socio-demographic variables included gender, age, family housing type as a proxy for socioeconomic status (1-3 room public housing, 4-5 room public housing or private house), and self-reported smoking status (non-smoker, past or current smoker). A potential psychological confounding variable perceived stress was measured using Perceived Stress Scale (PSS) (6). The items in this questionnaire are of a general nature of measurements of subjectively perceived stress and hence are not directed at any particular sub-population group. Asthma-related clinical assessments consisted of frequency of asthma attack within the past one year, frequency of hospitalization due to asthma within the past one year, duration of history of asthma, and dose of inhaled corticosteroids (ICS). The self-rated health (SRH) status was assessed by a single question that was well validated in previous studies (7): "Would you say that in general

your health is...?” Participants were asked to answer with a 5-category Likert response scale (excellent, very good, good or fair, poor, or very poor).

Sample size estimation

This study enlisted 70 asthma patients and 69 non-asthma controls. The levels of NPY measured from the method described by Allen et al in a previous study (8) vary from 150 to 600 ng/dl (mean 350, SD 100), demonstrating between group differences of 100 ng/dl. Sample size requirement for 80% power of the present study to demonstrate statistical significance at $p < 0.05$ is estimated to be $n=17$ for per group. Similar sample size estimates were obtained based on other measurements methods (9). Previous investigations with NPY have effectively used sample sizes ranging between 25 to 50. Allowing for lower sensitivity with single adiponectin measurements and confounding variables, a larger sample size of 69-70 per group is deemed to be adequately powered for the purpose of this study.

References

- E1 Ng TP, Niti M, Fones C, Yap KB, Tan WC. Co-morbid association of depression and COPD: a population-based study. *Respir Med*. 2009; **103**: 895-901.
- E2 Nathan RA, Sorkness CA, Kosinski M, *et al*. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol*. 2004; **113**: 59-65.
- E3 Lai CK, Ko FW, Bhome A, *et al*. Relationship between asthma control status, the Asthma Control Test and urgent health-care utilization in Asia. *Respirology*. 2011; **16**: 688-97.
- E4 Juniper EF, Guyatt GH, Epstein RS, Ferrie PJ, Jaeschke R, Hiller TK. Evaluation of impairment of health related quality of life in asthma: development of a questionnaire for use in clinical trials. *Thorax*. 1992; **47**: 76-83.
- E5 Barzi F, Woodward M. Imputations of missing values in practice: results from imputations of serum cholesterol in 28 cohort studies. *Am J Epidemiol*. 2004; **160**: 34-45.
- E6 Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983; **24**: 385-96.
- E7 DeSalvo KB, Bloser N, Reynolds K, He J, Muntner P. Mortality prediction with a single general self-rated health question. A meta-analysis. *J Gen Intern Med*. 2006; **21**: 267-75.
- E8 Morgan CA, 3rd, Wang S, Mason J, *et al*. Hormone profiles in humans experiencing military survival training. *Biol Psychiatry*. 2000; **47**: 891-901.
- E9 Cardell LO, Uddman R, Edvinsson L. Low plasma concentrations of VIP and elevated levels of other neuropeptides during exacerbations of asthma. *Eur Respir J*. 1994; **7**: 2169-73.

Table S1. Logistic and quadratic regression models of association of BMI, adiponectin, NPY, TNF-a, CRP, and IL-6 levels with asthma by sex (n=139)

		Males					Females				
		Asthma	Healthy Control	<i>p</i> for Wald χ^2	Adjusted*	Adjusted* for BMI, Adiponectin, TNF-a, NPY, CRP, IL-6	Asthma	Healthy Control	<i>P</i> for Wald χ^2	Adjusted*	Adjusted* for BMI, Adiponectin, TNF-a, NPY, CRP, IL-6
BMI	Obesity	15 (65.2)	8 (34.8)	0.037	5.20 (1.30-20.8)	29.20 (2.23-382.5)	15 (88.2)	2 (11.8)	<0.001	15.6 (2.42-100.2)	10.6 (0.72-157.2)
	Non-obesity	18 (38.3)	29 (61.7)		1	1	18 (37.5)	30 (62.5)		1	1
	<i>P</i>				0.020	0.010				0.004	0.086
	Interaction (Wald, <i>p</i>)									(1.915), 0.166	(0.278), 0.598
Adiponectin	Low tertile	5 (14.3)	19 (51.4)	0.011	1	1	6 (18.8)	15 (46.9)	0.538	1	1
	Mid-tertile	16 (45.7)	8 (21.6)		9.16 (1.83-45.9)	14.46 (1.43-146.8)	18 (56.3)	4 (12.5)		11.4 (2.18-59.9)	17.5 (1.86-165.6)
	High tertile	14 (40.0)	10 (27.0)		2.92 (0.62-13.6)	4.05 (0.49-33.3)	8 (25.0)	13 (40.6)		0.90 (0.17-4.67)	3.99 (0.34-46.4)
	Linear Trend, <i>p</i>				0.148	0.100				0.685	0.100
	Interaction (Wald), <i>p</i>									1	1
NPY	Low tertile	19 (55.9)	7 (18.9)	0.004	6.20 (1.7-21.5)	29.1 (2.67-317.6)	13 (40.6)	6 (18.8)	0.089	3.13 (0.86-11.3)	5.40 (0.49-59.2)
	Mid-tertile	8 (23.5)	14 (37.8)		1.31 (0.38-4.52)	3.09 (0.29-33.2)	10 (31.3)	13 (40.6)		1.11 (0.34-3.63)	8.29 (0.80-86.1)
	High tertile	7 (20.6)	16 (43.2)		1	1	9 (28.1)	13 (40.6)		1	1
	Linear Trend, <i>p</i>				0.004	0.004				0.089	0.158
	Interaction (Wald), <i>p</i>									(0.686), 0.407	(2.202), 0.138
IL-6	Low tertile	9 (26.5)	25 (67.6)	0.001	1	1	7 (21.9)	19 (59.4)	<0.001	1	1
	Mid-tertile	8 (23.5)	6 (16.2)		3.70 (1.01-13.6)	5.61 (0.70-44.9)	5 (15.6)	11 (34.4)		1.23 (0.31-4.84)	1.37 (0.17-11.2)
	High tertile	17 (50.0)	6 (16.2)		7.87 (2.36-26.0)	6.97 (0.91-53.4)	20 (62.5)	2 (6.3)		27.1 (5.00-147.4)	18.9 (1.16-307.3)
	Linear Trend, <i>p</i>				0.001	0.048				<0.001	0.068
	Interaction (Wald), <i>p</i>									(0.804), 0.370	(0.215), 0.643
CRP	Low tertile	9 (26.5)	15 (40.5)	0.188	1	1	5 (15.6)	16 (50.0)	<0.001	1	1
	Mid-tertile	13 (38.2)	13 (35.1)		2.69 (0.63-11.5)	1.86 (0.23-15.20)	7 (21.9)	12 (37.5)		1.76 (0.36-8.61)	1.32 (0.19-9.22)
	High tertile	12 (35.3)	9 (24.3)		3.58 (0.72-17.8)	0.65 (0.05-8.57)	20 (62.5)	4 (12.5)		15.2 (2.70-85.1)	8.26 (0.87-78.1)
	Linear Trend, <i>p</i>				0.113	0.753				0.002	0.075
	Interaction (Wald), <i>p</i>									(2.802), 0.094	(2.769), 0.096
TNF-a	Low tertile	15 (4.1)	4 (10.8)	0.153	76.6 (6.00-977.1)	27.6 (4.73-3690.0)	15 (46.9)	11 (34.4)	0.760	8.74 (1.42-53.78)	11.5 (0.94-142.4)
	Mid-tertile	5 (14.7)	20 (54.1)		1	1	5 (15.6)	15 (46.9)		1	1
	High tertile	14 (41.2)	13 (35.1)		19.4 (2.55-148.3)	63.8 (1.13-3612.1)	12 (37.5)	6 (18.8)		8.74 (1.48-51.7)	9.66 (0.94-99.8)
	Quadratic Trend, <i>p</i>				0.001	0.005				0.009	0.034
	Interaction (Wald), <i>p</i>									(0.457), 0.499	(0.204), 0.651

* Adjusted for: age, sex, race, smoking status, and perceived stress. BMI = body mass index, IL-6 = interleukin 6, CRP = C-reactive protein, NPY = neuropeptide Y, TNF- α = tumor necrosis factor alpha. BMI: obesity: BMI $\geq$ 25kg/m²; non-obesity: BMI<25kg/m². Adiponectin: low tertile: $\leq$ 4.80 μ g/ml in male, $\leq$ 6.04 μ g/ml in female; mid-tertile: > 4.80 μ g/ml & $\leq$ 7.44 μ g/ml in male, > 6.04 μ g/ml & $\leq$ 9.24 μ g/ml in female; high tertile: > 7.44 μ g/ml in male, > 9.24 μ g/ml in female. NPY: low tertile: $\leq$ 0.40 ng/ml; mid-tertile: > 0.40 ng/ml & $\leq$ 0.51 ng/ml; high tertile: >0.51 ng/ml. IL-6: low tertile: $\leq$ 0.06 pg/ml; mid-tertile: > 0.06 pg/ml & $\leq$ 1.98 pg/ml; high tertile: >1.98 pg/ml. CRP: low tertile: $\leq$ 0.53 μ g/ml; mid-tertile: > 0.53 μ g/ml & $\leq$ 2.37 μ g/ml; high tertile: >2.37 μ g/ml. TNF- α : low tertile: $\leq$ 8.77 pg/ml; mid-tertile: > 8.77 pg/ml & $\leq$ 11.41 pg/ml; high tertile: >11.41 pg/ml.