



Allergen exposure induces adipose tissue inflammation and insulin resistance



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ABSTRACT

This study investigates whether exposure to allergen elicits insulin resistance as a result of adipose tissue inflammation. Male C57BL/6 mice were challenged with ovalbumin (OVA) allergen for 12 weeks, and blood and adipose tissue samples were collected at 24 h after the last challenge. Levels of adhesion molecules, fasting insulin, fasting glucose, and adipokines in the blood were analyzed, and fasting homeostasis model assessment was applied to determine insulin resistance (HOMA-IR). The expression of pro- and anti-inflammatory genes in dissected adipose tissues was analyzed by real-time RT-PCR. Our results showed that OVA exposure increased insulin resistance as well as resistin and E-selectin, but reduced adiponectin in the serum. Resistin level was significantly correlated with HOMA-IR. Moreover, in adipose tissues of OVA-challenged mice, the pro-inflammatory M1 genes were more abundant while the anti-inflammatory M2 genes were less than those of PBS-treated mice. The expression changes of both M1 and M2 genes were significantly associated with serum levels of adiponectin, resistin, and E-selectin. Hematoxylin and eosin (HE) and immunohistochemistry (IHC) stain also showed that there was more obvious inflammation in OVA-challenged mice. In conclusion, the current study suggests the relationship between allergen-elicited adipose tissue inflammation and circulating inflammatory molecules, which are possible mediators for the development of insulin resistance. Therefore, we propose that allergen exposure might be one risk factor for insulin resistance.

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1. Introduction

Diabetes is a group of metabolic diseases that are prevalent in all countries, and the number of adults with diabetes is estimated to increase by 20% in developed countries and 69% in developing countries from 2010 to 2030 [1]. Diabetes is generally classified into type 1 and type 2, which are characterized by insulin deficiency and insulin resistance, respectively. As opposed to insulin deficiency that is partly inherited, the development of insulin resistance is primarily due to lifestyle factors such as diet and environmental pollutant exposure [2–5]. Understanding the risk factors for insulin resistance is crucial to the prevention and development of therapeutic strategies for type 2 diabetes.

Pathophysiological studies indicated that inflammation plays a key role in the development of insulin resistance [6,7]. For example, high-fat-fed mice produced more pro-inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β) in the liver, which caused hepatic and systemic insulin resistance [8,9]. Inflammatory markers vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and endothelial-selectin (E-selectin), which are released from stimulated vascular endothelium, appear to participate in the development of insulin resistance [6,7,10,11]. Moreover, the levels of circulating VCAM-1, ICAM-1 and E-selectin were shown to significantly correlate with insulin sensitivity [12–14]. Therefore, inflammation is now known as a pathological condition that associates with the development of insulin resistance.

Adipose tissue produces a large number of bioactive mediators and has a central role in both immune responses and metabolism of lipid and glucose. Adipose tissue inflammation induced elevated plasminogen activator inhibitor-1 (PAI-1), IL-1 β , IL-6, and TNF- α while it decreased the expression of anti-inflammatory markers like macrophage galactose lectin-1 (Mgl1), macrophage galactose lectin-2 (Mgl2) and interleukin-10 (IL-10), and alteration of these inflammatory factors

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appeared to promote insulin resistance [6,7,15–17]. Members of adipokines participated in glucose production and insulin resistance; in particular, adiponectin had a protective role in insulin resistance by increasing glucose uptake [18–20], while resistin was implied to increase the propensity of insulin resistance [21,22]. More importantly, epidemiological studies found that type 2 diabetes patients had higher serum resistin levels compared to healthy subjects [23,24], while several cohort studies indicated that a lower level of adiponectin is associated with a higher incidence of diabetes [25–27]. Taken together, the inflammatory factors and adipokines that are produced by adipose tissues may play an important role in the development of insulin resistance and type 2 diabetes.

We recently discovered that acute exposure to allergen lowered adiponectin levels in serum and adipose tissues and concurrently induced adipose tissue inflammation [28]. In the current study, we hypothesize that allergen exposure promotes insulin resistance through the action of adipose tissue inflammation. Our data of a long-term exposure model found that allergen could modulate serum IL-6, adipokines and E-selectin, and alteration of these circulating factors correlated with pro-inflammatory phenotype of macrophage in adipose tissues. More importantly, allergen-induced fluctuation of serum resistin is associated with insulin sensitivity that was obtained by homeostasis model assessment insulin resistance (HOMA-IR). Collectively, our study suggests that allergen exposure may be one risk factor for the development of insulin resistance.

2. Materials and methods

2.1. Animal

Eight-week-old male C57BL/6 mice were obtained from the National Cheng-Kung University Laboratory Animal Center. Mice were grouped and kept in constant lighting cycle (12-h light/dark), temperature ($23 \pm 2^\circ\text{C}$) and relative humidity (50–60%) with free access to food and water. In this study, at least repeated experiments were performed until trend of the response curve was consistent before we started collection of study data. All study protocols were approved by the Animal Care and Use Committee of the National Cheng-Kung University.

2.2. Allergen sensitization and challenge

In the first stage, mice were sensitized with four intraperitoneal injections of 10 μg ovalbumin (OVA, Grade IV, Sigma-Aldrich, St Louis, MO, USA) bound to 1 mg aluminum hydroxide (Sigma-Aldrich, St. Louis, MO, USA) in 200 μl phosphate buffered saline (PBS, pH 7.4) on days 1, 8, 15 and 22. On day 29, for the second stage, the mice were intranasally challenged with 50 μg OVA in 25 μl PBS once a day and five days per week for 12 weeks. Equal volume of PBS was used in parallel experiments as a control. Twenty-four hours after the last intranasal challenge, mice were weighed (AE240, Mettler-Toledo, Switzerland) and sacrificed with cytosol (Kyorin Pharmaceuticals, Tokyo, Japan), and the blood, bronchoalveolar lavage fluid (BALF) and adipose tissue were collected.

2.3. Blood glucose, insulin, adiponectin, resistin and immune markers

Oral glucose tolerance test (OGTT) was performed at the eighth week of allergen challenge. Mice were deprived of food beginning at 9 AM for 4 h and then supplied with 400 mg/ml glucose (D-(+)-glucose, Sigma-Aldrich, St. Louis, MO, USA) by gavage feeding. Blood samples were collected before and at 15, 30, 60 and 120 min after the feeding, centrifuged at 1250 $\times g$ for 15 min at 4°C , and stored at -80°C . The blood glucose and insulin were determined by GLUC-PAP (RANDOX, Crumlin, United Kingdom) and insulin kit (Mercodia, Uppsala, Sweden) in accordance with the manufacturer's protocols, respectively.

Mice were sacrificed at 24 h after the last intranasal challenge with 4-hour fast. Blood samples were centrifuged at 12,500 rpm for 30 min at 4°C , and the serum was stored at -80°C . Enzyme-linked immunosorbent assays (ELISA) were performed to determine the serum levels of IL-6, TNF- α , adiponectin, resistin, VCAM-1, ICAM-1, E-selectin (R&D System, Minneapolis, MN, USA), and total IgE (BD Biosciences, San Diego, CA, USA). Fasting homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by fasting blood glucose (mg/dl) $\times$ fasting insulin ($\mu\text{U}/\text{ml}$) / 405 [29].

2.4. Cell populations in the bronchoalveolar lavage fluid (BALF)

This study has drawn BALF samples and determined the number and population of cell to understand whether allergen exposure successfully elicit airway inflammation. Two tubes of BALF were collected by order, and each included 3 repeated, 30-second-long drawings using 1 ml saline. The BALF was centrifuged at 4°C and 2000 rpm for 10 min, and the pellets were re-suspended in 1 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum. Twenty microliters of cell suspension was drawn for total cell counts by using a hemocytometer (Marienfeld, Germany) and a microscope (BH-2, Olympus, Tokyo, Japan). The rest of the suspension was cytocentrifuged (Thermo Electron Corporation, Waltham, MA, USA) to transfer cells onto a microscopic slide, which was subsequently stained with Diff-quick (Sysmex Corporation, Tokyo, Japan). Macrophages, eosinophils and neutrophils were differentiated by stain, and cell numbers of each population were counted under a microscope (CX31RBSF, Olympus, Tokyo, Japan).

2.5. Inflammatory marker expression in adipose tissues

Brown (interscapular) and white (inguinal and gonado) adipose tissues were harvested and conserved in RNAlater (Protech, Taipei, Taiwan) at 4°C for 24 h before storage in -20°C . For RNA extraction, 1 ml RNA Plus Extraction Reagent (Invitrogen, Carlsbad, CA, USA) was mixed with the adipose tissue, and the mixture was homogenized by a BeadBeater (BioSpec Products, Inc, Bartlesville, OK, USA). The extracted sample was further treated with RNA pure kit (Geneaid, New Taipei, Taiwan) to increase the purity. The mRNA expressions of PAI-1, IL-6, TNF- α , IL-1 β (pro-inflammatory M1 macrophage), IL-10, Mgl1, and Mgl2 (anti-inflammatory M2 macrophage) in adipose tissues were determined by real-time quantitative RT-PCR. Reverse transcription was performed by using SuperScript III First-Strand Synthesis Super-Mix (Invitrogen, Carlsbad, CA, USA). Quantitative real-time RT-PCR was performed with Fast SYBR Green Master Mix (Applied Biosystem, Foster, California, USA) and SYBR Green system (Applied Biosystem, StepOne Plus Real-Time PCR SYSTEM Thermal CyclInguinal Block, Model 4376592, Foster, CA, USA). The data were expressed as relative gene expression to the endogenous reference, 18S rRNA, by the $\Delta\Delta\text{Ct}$ method [30]. For comparison between the OVA- and PBS-treated groups, the level of hypoxanthine guanine phosphoribosyl transferase (HPRT) expression was used as the internal control. Primer sets were as follows: PAI-1 forward 5'-TGA TGG CTC AGA GCA ACA AG-3' and reverse 5'-GCC AGG GTT GCA CTA AAC AT-3'; IL-6 forward 5'-GAT GCT ACC AAA CTG GAT ATA ATC-3' and reverse 5'-GGT CCT TAG CCA CTC CTT CTG TG-3'; TNF- α forward 5'-ATG AGC ACA GAA AGC ATG-3' and reverse 5'-TCA CAG AGC AAT GAC TCC-3'; IL-1 β forward 5'-GCA GCT ATG GCA ACT GTT CCT-3' and reverse 5'-TCA TAT GGG TCC GAC AGC ACG-3'; IL-10 forward 5'-CAG AGC CAC ATG CTC CTA GA-3' and reverse 5'-TGT CCA GCT GGT CCT TTG TT-3'; Mgl1 forward 5'-TGA GAA AGG CTT TAA GAA CTG GG-3' and reverse 5'-GAC CAC CTG TAG TGA TGT GGG-3'; Mgl2 forward 5'-TTA GCC AAT GTG CTT AGC TGG-3' and reverse 5'-GGC CTC CAA TTC TTG AAA CCT-3'; and HPRT forward 5'-CTC ATG GAC TGA TTA TGG ACA GGA-3' and reverse 5'-GCA GGT CAG CAA AGA ACT TAT AGC-3'.

2.6. Hematoxylin and eosin (HE) and immunohistochemistry (IHC) staining

This study evaluated the inflammation of adipose tissues by hematoxylin and eosin (HE) staining method. Adipose tissues were fixed in 10% neutral formalin (Macron chemicals, Charlotte, NC, USA) for 24 h. The tissues were embedded with paraffin by paraffin embedding mechanism (LIPSHAW), and sliced to 5 μ m by paraffin slicing mechanism (LIPSHAW). The histological sections were stained with Harris hematoxylin and counterstained with eosin. In addition, this study used F4/80 expression as a marker to identify the adipose tissue macrophage (ATM). Finally, we evaluated the inflammation state by using a light microscope (CX31RBSF, Olympus, Tokyo, Japan).

2.7. Statistical analysis

Comparisons of biomarkers in BALF, serum and adipose tissue between the PBS- and OVA-treated mice were assessed by Student *t*-test. Pearson correlation was applied to analyze the relationship. SAS 9.2 was used to analyze the data. Statistical significance was set at the level $p < 0.05$.

3. Results

3.1. Cell population in BALF and IgE level in serum

The study has applied chicken egg albumin (ovalbumin, OVA), one type of mouse allergen, to sensitize the mice and investigated whether allergen exposure can induce insulin resistance. To evaluate whether OVA exposure effectively causes immune responses, we have examined airway inflammation by looking into the cell population in BALF and total IgE in the serum. Table 1 shows that repeated administration of OVA caused a marked and significant increase in the number of total cells, macrophages, neutrophils and eosinophils in BALF, as compared to the PBS-treated group ($p < 0.05$). Likewise, OVA induced a significant increase of serum IgE in the OVA-challenged over the PBS-treated mice ($p < 0.05$). These results confirmed that OVA exposure induced hyper-reactivity of the immune system in the study group of mice.

3.2. Glucose and insulin in serum

We performed OGTT to address whether allergen exposure influences glucose metabolism. The time course of blood glucose and insulin levels upon gavage feeding is displayed in Fig. 1, revealing abnormal elevation of glucose and insulin in OVA-challenged mice at 30 min after feeding, as compared with their PBS-treated counterparts ($p < 0.05$). Furthermore, Fig. 2 shows increased HOMA-IR in OVA-challenged over the PBS-treated mice at 24 h after the last challenge ($p < 0.05$). These results suggest that OVA exposure causes insulin resistance in mice.

3.3. Inflammatory markers and adipokines in serum

We hypothesized that inflammatory responses may contribute to OVA-induced insulin resistance. Therefore, we investigated the serum level of inflammation markers IL-6, TNF- α , ICAM-1, VCAM-1, and E-selectin that are expressed by vascular endothelium, as well as resistin

Table 1
Immune cell counts in BALF and serum IgE (n = 4–6/group).

Cell composition	PBS	OVA
Total cells (10^5 /ml)	0.73 $\pm$ 0.40	775.55 $\pm$ 108.26*
Macrophages (10^5 /ml)	0.58 $\pm$ 0.24	30.31 $\pm$ 3.56*
Eosinophils (10^5 /ml)	0.04 $\pm$ 0.05	7.58 $\pm$ 0.89*
Neutrophils (10^5 /ml)	0.11 $\pm$ 0.14	730.08 $\pm$ 103.28*
Total IgE (μ g/ml)	1.95 $\pm$ 0.94	4.86 $\pm$ 0.27*

* $p < 0.05$ when compared with PBS.

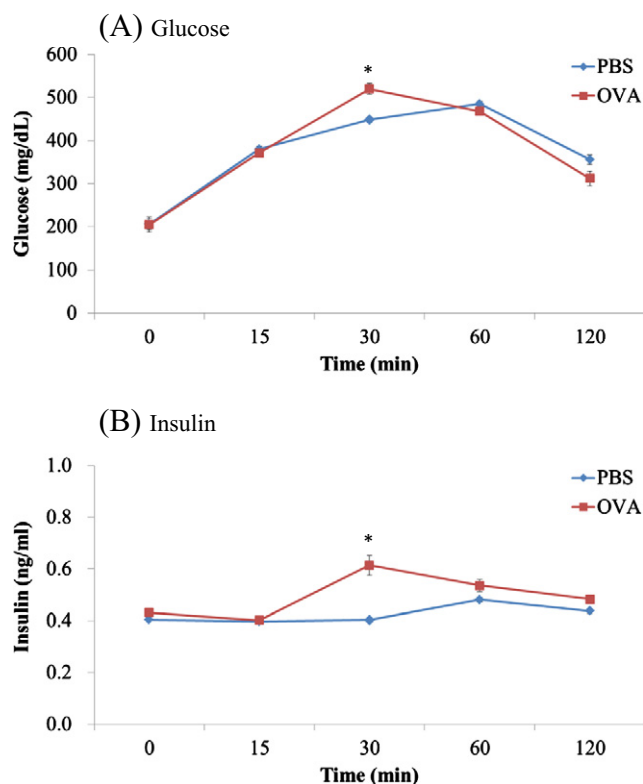


Fig. 1. Blood glucose and insulin levels by oral glucose intolerance test. (A) glucose and (B) insulin (* $p < 0.05$ between OVA and PBS treatments) (n = 4–6/group).

and adiponectin which are expressed by adipose tissues. Fig. 3A shows that there was a higher level of IL-6 in mice with OVA-challenge compared to the mice with PBS-challenge ($p < 0.05$), and OVA-challenged mice also expressed a higher level of E-selectin ($p < 0.05$), while no significant difference was found in the level of ICAM-1 and VCAM-1 (Fig. 3B). On the other hand, resistin increased by nearly two-fold ($p < 0.05$) while adiponectin decreased significantly ($p < 0.05$) in OVA-challenged mice (Fig. 3C). To further assess whether HOMA-IR is influenced by inflammation and adipokines, we have analyzed the correlation of HOMA-IR with IL-6 (Fig. 4A), E-selectin (Fig. 4B), resistin (Fig. 4C), and adiponectin (Fig. 4D) by Pearson correlation. We found a significant relationship between HOMA-IR and resistin ($p < 0.05$). On the other hand, the correlation of HOMA-IR with E-selectin or adiponectin was close to but not statistically significant ($p = 0.07$ and $p = 0.06$, respectively). Taken together, our data indicate that OVA exposure stimulates vascular endothelium and causes fluctuations in the serum level of adipokines; moreover, the level of resistin, one member of adipokines, significantly correlates with insulin resistance.

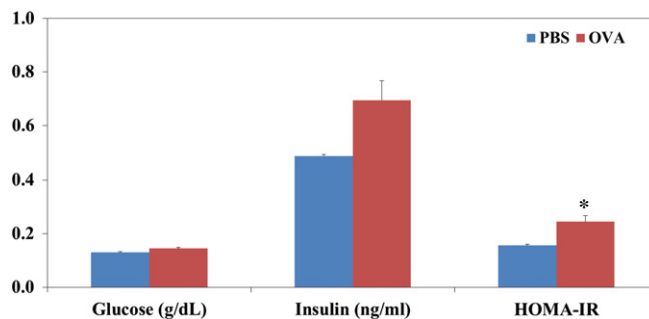


Fig. 2. Fasting glucose, fasting insulin, and HOMA-IR values (* $p < 0.05$ when compared with PBS) (n = 4–6/group).

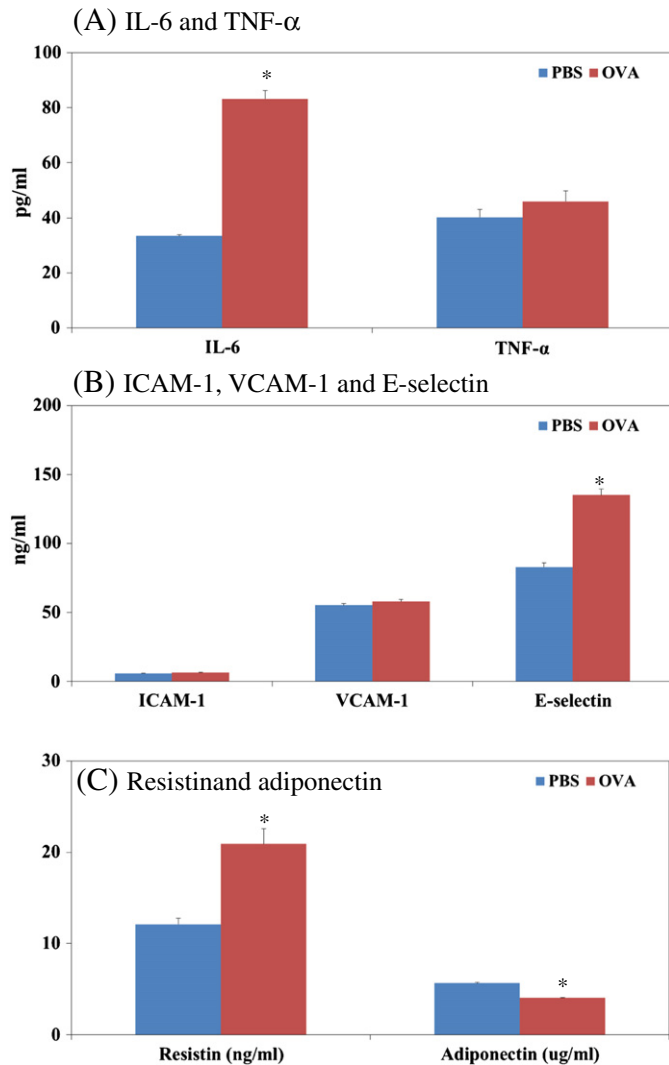


Fig. 3. Serum level of inflammatory biomarkers. (A) IL-6 and TNF- α , (B) ICAM-1, VCAM-1 and E-selectin and (C) resistin and adiponectin (* $p < 0.05$ when compared with PBS) ($n = 4$ –6/group).

3.4. Adipose tissue weight and inflammation

Since the abundance of circulating adipokines and E-selectin appeared to be influenced by OVA exposure, we wondered if the characteristics of adipose tissue were affected concurrently. We dissected the adipose tissue, and analyzed the tissue weight and the expression profile of pro-inflammatory (macrophage M1) and anti-inflammatory (macrophage M2) markers by qPCR. As shown in Table 2, the weight proportions of inguinal and gonadal adipose tissues were substantially lower in OVA-challenged mice than those in the control group ($p < 0.05$), but the brown adipose tissue (BAT) weight did not show a detectable difference. Moreover, there are more inflammation expressions of adipose tissues in OVA-challenged mice compared to the PBS-challenged mice, suggesting that there is obvious inflammation in allergen mice (Fig. 5). We also found that mice with OVA-challenge have higher expressions of F4/80 in adipose tissues than those with PBS-challenge, demonstrating that there was obvious ATM accumulation in OVA challenged mice (Fig. 6). For gene expression in the gonadal adipose tissue, we found elevated TNF- α and IL-1 β mRNA expressions ($p < 0.05$) (Fig. 7A) and decreased IL-10 and Mgl2 in OVA-challenged mice ($p < 0.05$) (Fig. 7B). For the inguinal adipose tissue (Fig. 8), we detected higher IL-6 and IL-1 β mRNA expressions ($p < 0.05$), likewise, lowered IL-10 and Mgl2 in OVA-challenged mice ($p < 0.05$). These

results indicate that exposure to OVA allergen induces reduction in white adipose tissue weight, up-regulation of pro-inflammatory gene expressions, and down-regulation of anti-inflammatory gene expressions.

To investigate whether fluctuated serum IL-6, adipokine and E-selectin levels are linked to adipose tissue inflammation, we had analyzed the relationship between these circulating factors and the expression of pro-inflammatory genes in adipose tissues. As shown in Table 3, we found significant associations in gonadal adipose tissue between resistin and TNF- α ($p < 0.05$), between adiponectin and TNF- α and IL-1 β ($p < 0.05$), between E-selectin and IL-1 β and TNF- α ($p < 0.05$), and between IL-6 and TNF- α and IL-1 β . In inguinal adipose tissue, we found that resistin was associated with IL-6 and IL-1 β ($p < 0.05$), E-selectin was associated with IL-1 β ($p < 0.05$), while adiponectin was negatively correlated with IL-6 and IL-1 β ($p < 0.05$). Taken together, these results demonstrate that OVA exposure induced adipose tissue inflammation and has considerable influence on the serum level of adipokines and E-selectin.

4. Discussions

This is the first study that reveals a potential connection between allergen exposure and insulin resistance and suggests adipose tissue inflammation as a possible mediator. Allergen exposure induces adipose tissue inflammatory phenotypic change; the gene expressional changes correlate with circulating inflammatory markers E-selectin and adipokines that are shown to participate in the development of insulin resistance. Therefore, based on our experimental model, we propose that allergen exposure may enhance the risk of insulin resistance.

We have validated that allergen exposure successfully induced hyper-reactivity of the immune system in the airway of OVA-challenged mice. As immune cell counts in BALF and serum level of total IgE are common indications for airway inflammation [31–34], we have observed a substantially increased number of immune cells in BALF and elevated IgE level in blood (Table 1). Moreover, we found that the predominant cell type in BALF was neutrophils, which was shown as an important index for severe asthma [35,36]. Therefore, our results suggest that long-term exposure of allergen could cause serious airway inflammation.

We recently found that acute exposure to allergen can up-regulate the inflammatory marker TNF- α in adipose tissues and down-regulate the level of adiponectin [28]. Adiponectin is known to play a protective role in insulin resistance, because it may increase glucose uptake and inhibit the activity of NF- κ B, a transcription factor intimately involved with inflammation [18–20,37]. Therefore, we speculate that allergen-elicited adipose tissue inflammation and reduction of adiponectin may involve insulin resistance. Besides, previous studies indicated that adipose tissue inflammation was concurrent with increased inflammatory markers IL-6, TNF- α and resistin, and these molecules could activate c-Jun NH(2)-terminal kinase (JNK) and I κ B kinase- β (IKK β) [38]. JNK, a kinase that mediates inflammatory responses, phosphorylates insulin receptor substrate-1 (IRS-1) and negatively regulates insulin receptor; while IKKi, a kinase that targets the inhibitor of nuclear factor kappa B (NF- κ B), enables NF- κ B activation and causes insulin resistance [7]. Furthermore, the inflammatory mediators, such as IL-6 and TNF- α , can induce local inflammatory responses and stimulate expression of adhesion molecules, such as VCAM-1, ICAM-1 and E-selectin, on endothelial cells of nearby blood vessels [39,40]. Adhesion molecules can promote the production of inflammatory indexes and participate in the development of insulin resistance [7]. Therefore, the current study is set out to test our hypotheses that exposure to allergen may cause insulin resistance through adipose tissue inflammation, and that adipokines and adhesion molecules are potentially important mediators.

We have taken OGTT, HOMA-IR, serum level of adhesion molecules and adipokines, and Pearson correlation to evaluate our hypotheses. In research and clinical practice, the OGTT and fasting HOMA-IR are often used to identify individuals of abnormal glucose intolerance and insulin

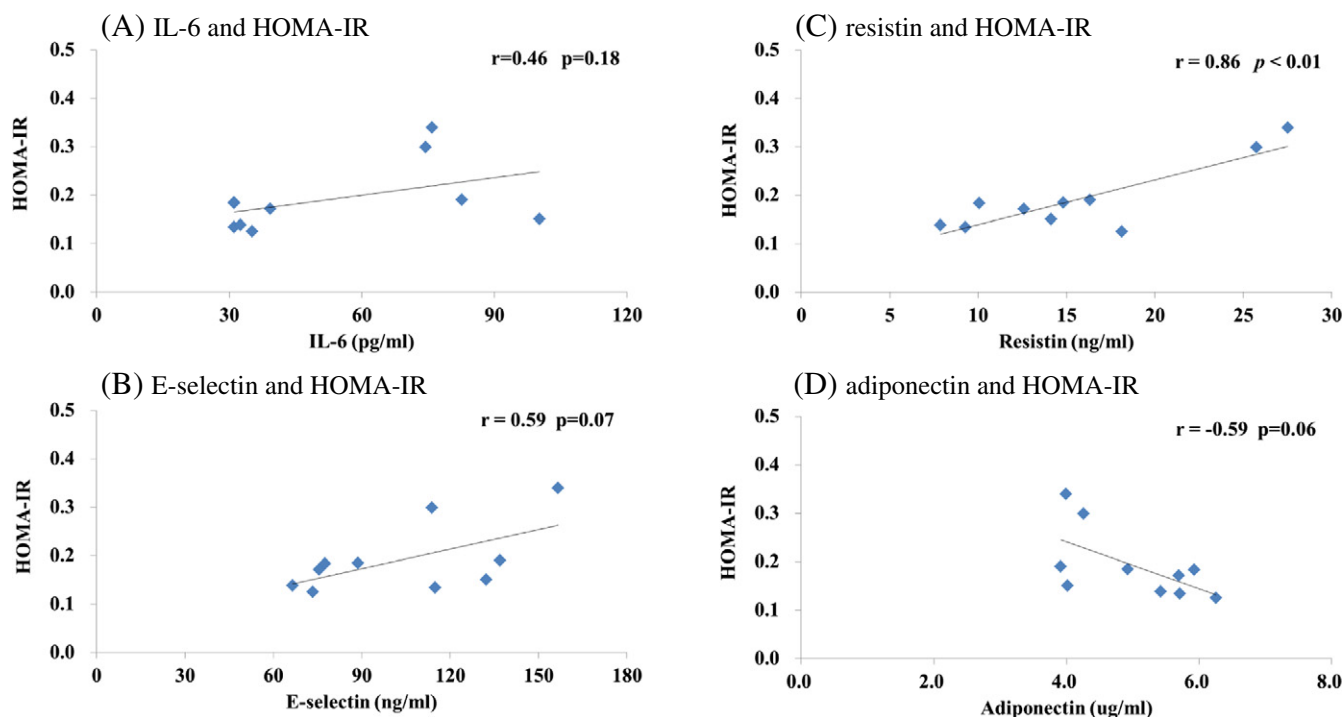


Fig. 4. Correlation of HOMA-IR with IL-6 (A), E-selectin (B), resistin (C) and adiponectin (D) (statistical significant was at $p < 0.05$).

resistance [29,41]. In the current study, we measured the level of glucose and insulin and calculated fasting HOMA-IR to test whether exposure to allergen causes insulin resistance. Fig. 1 shows that there are higher levels of glucose and insulin at the 30th min in OVA-challenged mice compared to the PBS-challenged mice, suggesting that the OVA allergen exposure can be involved in the pathogenesis of insulin resistance. After 60 min, there was an indication of liver β -cell function leading to production of insulin, while other studies also suggested similar study results [42]. In Fig. 2, although there are no significant differences between glucose and insulin in blood in OVA- and PBS-challenged mice, there is a significantly higher level of HOMA-IR in mice with OVA-challenge. These findings reflect that OVA allergen exposure can impair glucose tolerance. On the other hand, many studies performed OGTT by mice fed with high-fat diet [11,21]. It is known that obesity can induce the inflammation reactions in adipose tissues. To avoid the obesity effect from interfering with the examination between allergen exposure and adipose tissue inflammation, the study provided normal chow to mice. This is the reason why there was no significant difference in insulin and glucose levels at various study intervals when compared to other studies.

Besides, we found higher levels of IL-6, E-selectin and resistin and a lower level of adiponectin from the serum of OVA-challenged mice than those of PBS-treated mice (Fig. 3). To test if these biomolecules are mediators for allergen-caused insulin resistance, we further analyzed the relationship between HOMA-IR and these biomolecules by Pearson correlation. We found that HOMA-IR was significantly associated with resistin, yet possibly associated with E-selectin and adiponectin

(Fig. 4). Resistin can be secreted from adipose tissues, and serves as an important regulator in the pathogenesis of glucose metabolism. Our data also demonstrated that resistin level was higher in OVA-challenged mice compared to the PBS-challenged mice, and resistin was associated with HOMA-IR. In Fig. 4B, E-selectin plays an indirect role in regulating the metabolism of glucose [7], and this may be why there is no significant association between HOMA-IR and E-selectin level. Although Fig. 4D does not support that adiponectin is associated with HOMA-IR, previous studies have demonstrated that the level of adiponectin in blood was lower in subjects with diabetes [27,43,44], and those findings were consistent with our study. Taken together, our data suggest that exposure to OVA allergen elicits changed serum level of inflammatory molecules, and these molecules are likely mediators for allergen-induced insulin resistance.

Adipose tissue inflammation is characterized by induction of the inflammatory M1 polarity of adipose tissue macrophages [17,38]. In the current study, we examined the expression of pro-inflammatory M1- and anti-inflammatory M2-related genes in dissected adipose tissues, and showed in Figs. 7 and 8 that M1 genes were up-regulated while M2 genes were down-regulated in OVA-challenged mice. In addition, we found that the weight of adipose tissues in OVA-exposed mice was substantially reduced (Table 2), which agreed with a previous finding that adipose tissue weight was for environmental stimulus [45]. Moreover, HE method indicated that there were more obvious inflammation in the OVA-challenged mice than in the PBS-challenged mice (Fig. 5). Moreover, HE and IHC methods indicated that there were more obvious inflammations in the OVA-challenged mice than in the PBS-challenged mice (Figs. 5 and 6). Most strikingly, our analysis of correlation reveals a link between the gene expressional changes in adipose tissue and the circulating inflammatory biomarkers (Table 3). Taken together, our data indicate that exposure to allergen induces a pro-inflammatory phenotype of adipose tissues, and the phenotypic change is significantly correlated with the serum level of inflammatory molecules of our study. More remarkably, these inflammatory molecules are possible mediators for insulin resistance (Fig. 4). Previous studies found that Th2 cells, such as IL-4, IL-5 and eosinophils, play a critical role in enhancing glucose tolerance [46–48]. Our study displayed that there were higher Th1 cell

Table 2

Body weight and weight percentage of adipose tissue ($n = 4-6/\text{group}$).

Category	PBS	OVA
Body weight (g)	23.2 $\pm$ 1.6	19.3 $\pm$ 0.9*
BAT (%) ^a	0.31 $\pm$ 0.08	0.30 $\pm$ 0.04
Inguinal (%) ^a	0.32 $\pm$ 0.07	0.09 $\pm$ 0.04*
Gonadal (%) ^a	1.37 $\pm$ 0.29	0.09 $\pm$ 0.03*

^a (Adipose tissue weight / body weight of mouse) $\times$ 100.

* $p < 0.05$ when compared with PBS.

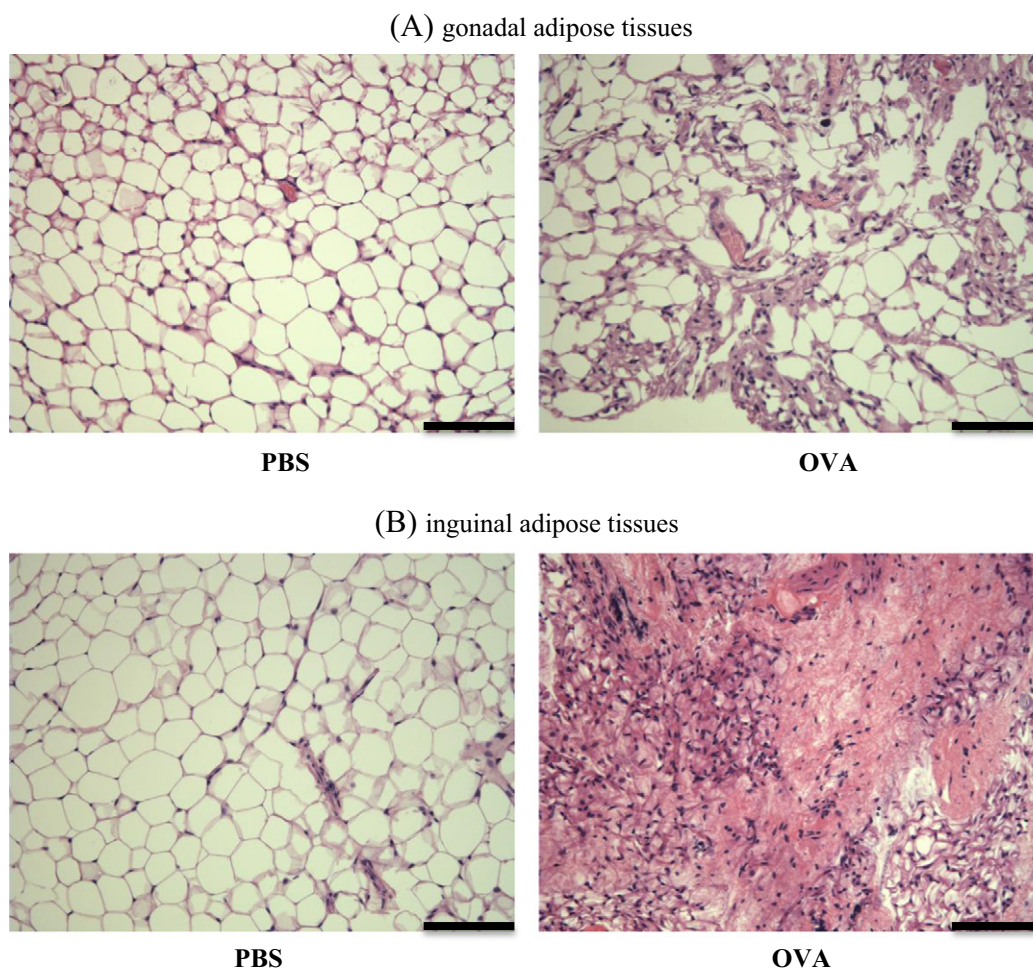


Fig. 5. Adipose tissue HE photo from PBS and OVA-challenged mice. (A) gonadal adipose tissues and (B) inguinal adipose tissues (scale bar: 100 μ m).

expressions in adipose tissues in the OVA-challenged mice than in the PBS-challenged mice. These results suggested that long-term OVA exposure corresponds to inflammatory condition in adipose tissues that promote the production of pro-inflammatory factors involved in the pathogenesis of insulin resistance. Previous research has also suggested that higher Th1 cell expressions, such as IL-6 or TNF- α , is involved in the pathogenesis of insulin resistance; while IL-4, IL-5 and IL-13 are predominant cells in adipose tissues, which further generate M2 cell and enhance glucose tolerance [49]. Obesity is definitely one risk factor leading to induce inflammation reactions in adipose tissues. However, obesity was not the central concern; rather, this study aimed to investigate whether OVA allergen exposure may induce inflammation in adipose tissue. The lower body weight of the OVA-challenged mice may be attributable to the fact that allergen exposure may lead to inflammation in adipose tissues which play a key role in energy homeostasis, and therefore results in a decrease of food intake [50]. These combined evidences should strongly imply that the induction of OVA allergen exposure in mice may be able to derive at adipose tissue inflammation and system metabolic alternations.

To this date, no epidemiologic study has investigated the direct relationship between exposure to allergen and insulin resistance. However, there were higher levels of IL-4, IL-5, IL-6, leptin and HOMA-IR in blood found in subjects with allergic rhinitis than those in a healthy population, and HOMA-IR was associated with IL-6 and TNF- α [50]. Moreover, Ciprandi and co-workers found that there was a higher level of resistin in subjects with moderate–severe allergic rhinitis than those with a mild one [51]. Ciprandi and co-workers also indicated that patients with allergic rhinitis had a higher leptin level than healthy subjects

during pollen season [52]. Therefore, allergen exposure is one potential risk factor of insulin resistance, while there is a lack of understanding of the mechanism on the pathogenesis of insulin resistance after allergen exposure. This study is the first to demonstrate that allergen exposure can induce inflammation in adipose tissues and blood, and is later involved in the pathogenesis of insulin resistance by animal model. Some studies found that exposure to allergic pollen can change the levels of adiponectin and lerpain in blood and therefore participates in the development of insulin resistance [52,53]. Both allergic pollen and OVA allergen are proteins, and are viewed as having similar biological characteristics. Moreover, OVA allergen is often used to study the health effects of exposure to allergen [31–34,54]. Therefore, the experimental results from OVA allergen are presumed able to produce similar results with other allergens. Fig. 9 shows that OVA allergen can be inhaled into the lung (1), and OVA allergen can go to adipose tissues through blood (2, 3). OVA allergens can then promote inflammatory condition in adipose tissues (high M1, and low M2), (4) further secrete inflammatory mediators into the blood (5) (high resistin, IL-6, E-selectin, and low adiponectin) and be involved in the pathogenesis of glucose tolerance (6, 7).

This is the first study to discover a correlation between allergen-induced adipose tissue inflammation and insulin resistance, leading to our conclusion that allergen exposure might be one risk factor for insulin resistance. On the basis of the current study, the same analysis on human subjects will be highly desired. Additionally, further studies on allergen-induced signaling pathways will be essential to understand the molecular mechanism that gives rise to insulin resistance and to develop therapeutic approaches to the highly prevalent pathological condition.

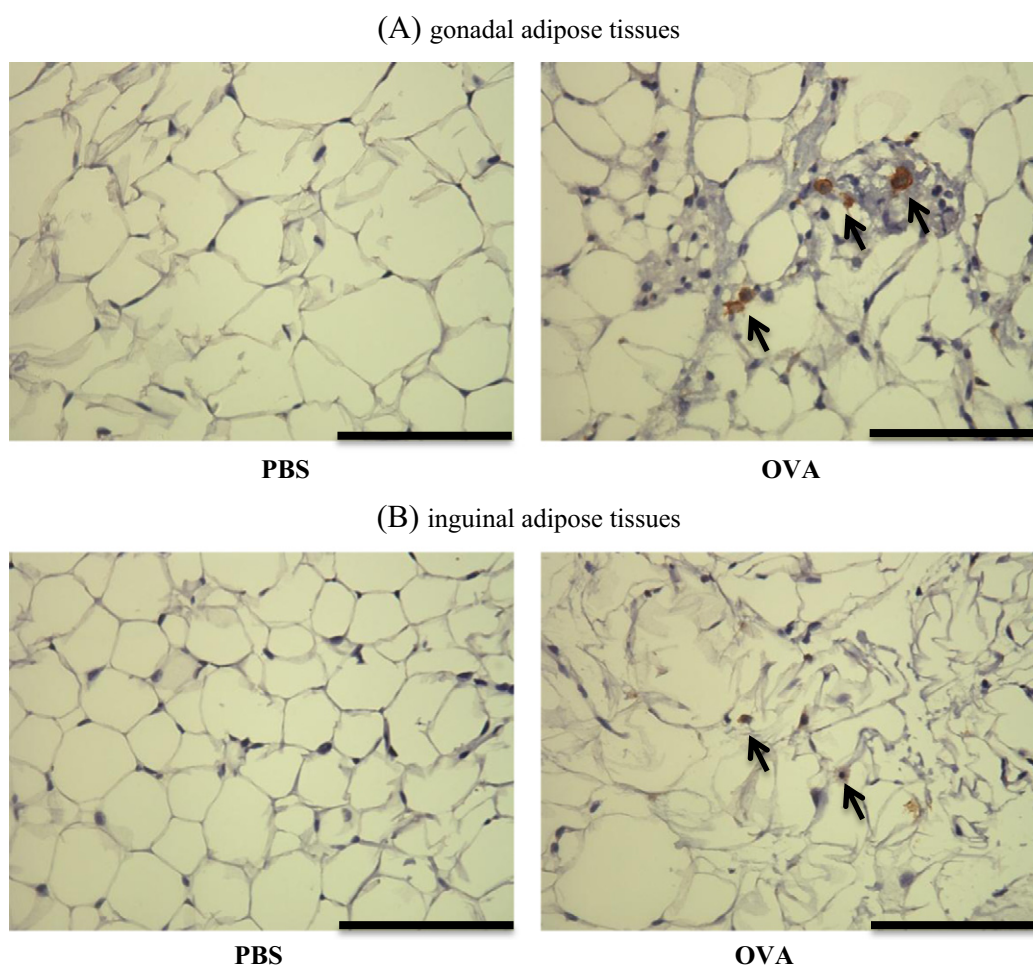


Fig. 6. Results of IHC with F4/80 antibody in adipose tissue. (A) gonadal adipose tissues and (B) inguinal adipose tissues. Arrows indicate F4/80 (scale bar: 100 μ m).

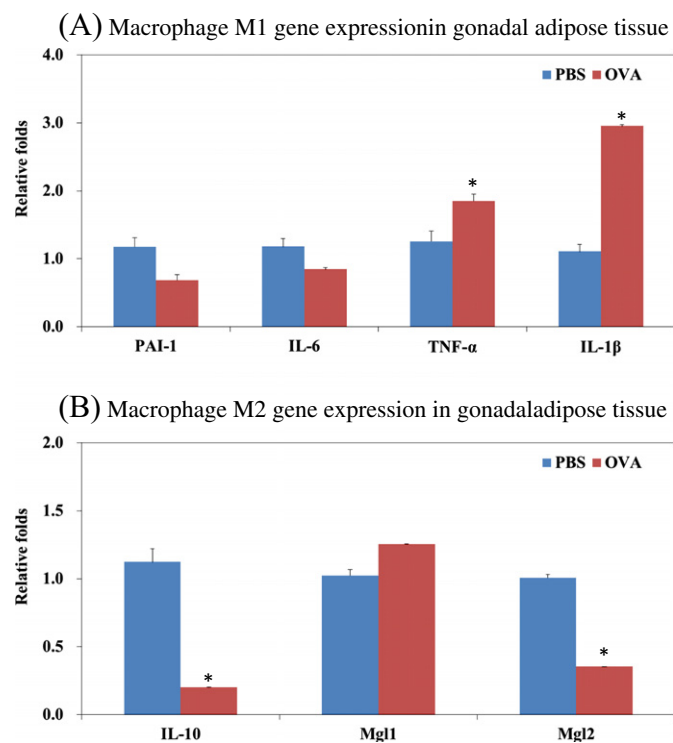


Fig. 7. Macrophage M1/M2 gene expression in gonadal adipose tissue. (A) M1 and (B) M2 (* p < 0.05 when compared with PBS) (n = 4–6/group).

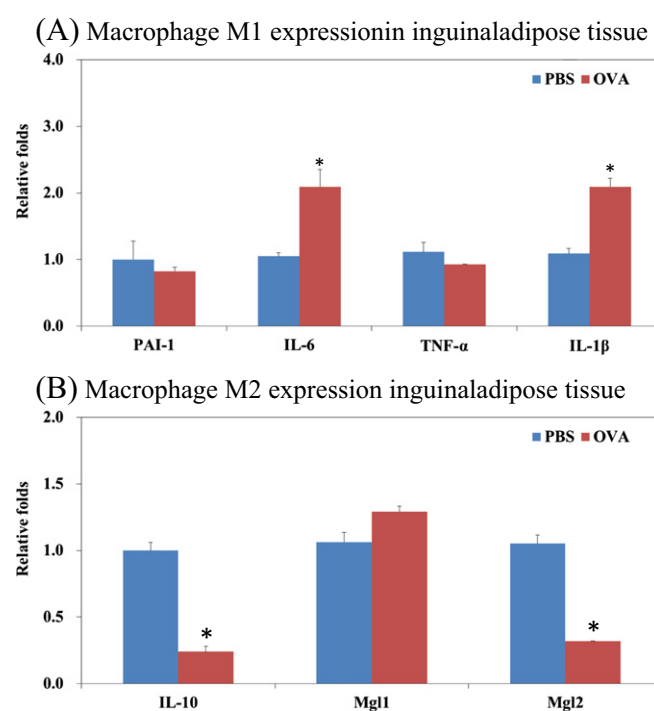
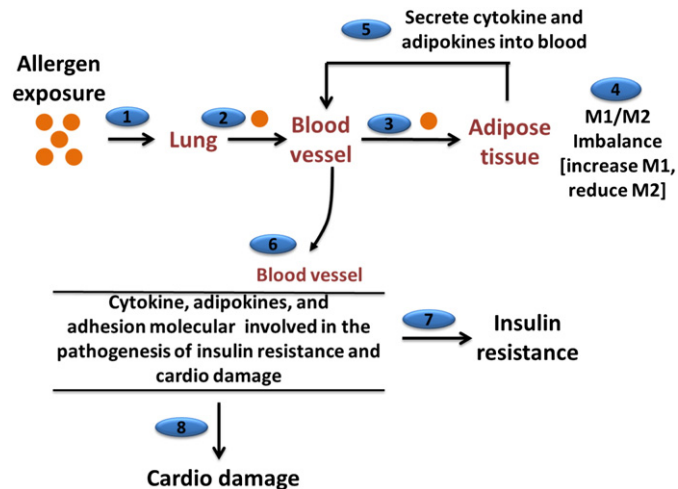


Fig. 8. Macrophage M1/M2 gene expression in inguinal adipose tissue. (A) M1 and (B) M2 (* p < 0.05 when compared with PBS) (n = 4–6/group).

Table 3Correlation between circulating resistin, adiponectin, E-selectin and IL-6 and adipose tissue pro-inflammatory gene expressions (p -value*).

Adipose tissue mRNA expression		Circulating factors			
		Resistin (ng/ml)	Adiponectin (μ g/ml)	E-selectin (ng/ml)	IL-6 (pg/ml)
Gonadal adipose tissue	PAI-1	−0.27 (0.45)	0.45 (0.19)	−0.28 (0.44)	−0.44 (0.20)
	IL-6	−0.29 (0.42)	0.08 (0.84)	−0.22 (0.53)	−0.31 (0.39)
	TNF- α	0.89 (<0.01)	−0.75 (<0.05)	0.75 (<0.05)	0.67 (<0.05)
	IL-1 β	0.62 (0.06)	−0.98 (<0.001)	0.77 (<0.01)	0.86 (<0.01)
Inguinal adipose tissue	PAI-1	0.23 (0.55)	0.44 (0.24)	−0.31 (0.41)	−0.26 (0.51)
	IL-6	0.86 (<0.01)	−0.63 (<0.05)	0.60 (0.07)	0.48 (0.16)
	TNF- α	−0.38 (0.28)	0.42 (0.22)	0.03 (0.94)	−0.05 (0.88)
	IL-1 β	0.77 (<0.01)	−0.76 (<0.05)	0.78 (<0.01)	0.62 (0.06)

* Statistical significance at $p < 0.05$.**Fig. 9.** Summary of study results.

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References

- [1] Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010;87:4–14.
- [2] Bi YF, Wang TG, Xu M, Xu Y, Li MA, Lu JL, et al. Advanced research on risk factors of type 2 diabetes. *Diabetes Metab Res Rev* 2012;28:32–9.
- [3] Hectors TLM, Vanparys C, van der Ven K, Martens GA, Jorens PG, Van Gaal LF, et al. Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. *Diabetologia* 2011;54:1273–90.
- [4] Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care* 2011;34:1249–57.
- [5] Navas-Acien A, Silbergeld EK, Streeter RA, Clark JM, Burke TA, Guallar E. Arsenic exposure and type 2 diabetes: a systematic review of the experimental and epidemiologic evidence. *Environ Health Perspect* 2006;114:641–8.
- [6] Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004;25:4–7.
- [7] Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* 2006;116:1793–801.
- [8] Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 2005;11:191–8.
- [9] Cai DS, Yuan MS, Frantz DF, Melendez PA, Hansen L, Lee J, et al. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappa B. *Nat Med* 2005;11:183–90.
- [10] Madonna R, Massaro M, de Caterina R. Insulin potentiates cytokine-induced VCAM-1 expression in human endothelial cells. *Biochim Biophys Acta (BBA) — Mol Basis Dis* 2008;1782:511–6.
- [11] Sun Q, Yue P, Deiluiis JA, Lumeng CN, Kampfrath T, Mikolaj MB, et al. Ambient air pollution exacerbates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. *Circulation* 2009;119:538–46.
- [12] Matsumoto K, Sera Y, Nakamura H, Ueki Y, Miyake S. Serum concentrations of soluble adhesion molecules are related to degree of hyperglycemia and insulin resistance in patients with type 2 diabetes mellitus. *Diabetes Res Clin Pract* 2002;55:131–8.
- [13] Taniguchi A, Fukushima M, Nakai Y, Kuroe A, Yamano G, Yanagawa T, et al. Soluble E-selectin, leptin, triglycerides and insulin resistance in nonobese Japanese type 2 diabetic patients. *Metabolism* 2005;54:376–80.
- [14] Targher G, Bonadonna RC, Alberiche M, Zenere MB, Muggeo M, Bonora E. Relation between soluble adhesion molecules and insulin sensitivity in type 2 diabetic individuals — role of adipose tissue. *Diabetes Care* 2001;24:1961–6.
- [15] Fujisaka S, Usui I, Kanatani Y, Ikutani M, Takasaki I, Tsuneyama K, et al. Telmisartan improves insulin resistance and modulates adipose tissue macrophage polarization in high-fat-fed mice. *Endocrinology* 2011;152:1789–99.
- [16] Han MS, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA, et al. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. *Science* 2013;339:218–22.
- [17] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* 2007;117:175–84.
- [18] Combs TP, Pajvani UB, Berg AH, Lin Y, Jelicks LA, Laplante M, et al. A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology* 2004;145:367–83.
- [19] Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 2002;277:25863–6.
- [20] Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002;8:731–7.
- [21] Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, et al. Regulation of fasted blood glucose by resistin. *Science* 2004;303:1195–8.
- [22] Muse ED, Obici S, Bhanot S, Monia BP, McKay RA, Rajala MW, et al. Role of resistin in diet-induced hepatic insulin resistance. *J Clin Invest* 2004;114:232–9.
- [23] Chen BH, Song YQ, Ding EL, Roberts CK, Manson JE, Rifai N, et al. Circulating levels of resistin and risk of type 2 diabetes in men and women: results from two prospective cohorts. *Diabetes Care* 2009;32:329–34.
- [24] Yin JH, Gao HF, Yang J, Xu L, Li M. Measurement of salivary resistin level in patients with type 2 diabetes. *Int J Endocrinol* 2012;2012.
- [25] Hanley AJG, Wagenknecht LE, Norris JM, Bergman R, Anderson A, Chen YI, et al. Adiponectin and the incidence of type 2 diabetes in Hispanics and African Americans. *Diabetes Care* 2011;34:2231–6.
- [26] Hivert MF, Sullivan LM, Shrader P, Fox CS, Nathan DM, D'Agostino RB, et al. Insulin resistance influences the association of adiponectin levels with diabetes incidence in two population-based cohorts: the Cooperative Health Research in the Region of Augsburg (KORA) S4/F4 Study and the Framingham Offspring Study. *Diabetologia* 2011;54:1019–24.
- [27] Zhu N, Pankow JS, Ballantyne CM, Couper D, Hoogeveen RC, Pereira M, et al. High-molecular-weight adiponectin and the risk of type 2 diabetes in the ARIC Study. *J Clin Endocrinol Metab* 2010;95:5097–104.
- [28] Jung CC, Chang CC, Tsai YS, Su HJ. Allergen exposure induces inflammation and affects adiponectin levels in adipose tissue. *Toxicol Lett* 2013;223:88–95.
- [29] Akagiri S, Naito Y, Ichikawa H, Mizushima K, Takagi T, Handa O, et al. A mouse model of metabolic syndrome; Increase in visceral adipose tissue precedes the development of fatty liver and insulin resistance in high-fat diet-fed male KK/Ta mice. *J Clin Biochem Nutr* 2008;42:150–7.
- [30] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(−delta delta C) method. *Methods* 2001;25:402–8.
- [31] Gueders M, Paulissen G, Crahay C, Quesada-Calvo F, Hacha J, Van Hove C, et al. Mouse models of asthma: a comparison between C57BL/6 and BALB/c strains regarding bronchial responsiveness, inflammation, and cytokine production. *Inflamm Res* 2009;58:845–54.
- [32] Medoff BD, Okamoto Y, Leyton P, Weng M, Sandall BP, Raher MJ, et al. Adiponectin deficiency increases allergic airway inflammation and pulmonary vascular remodeling. *Am J Respir Cell Mol Biol* 2009;41:397–406.
- [33] Shore SA, Schwartzman IN, Mellema MS, Flynt L, Imrich A, Johnston RA. Effect of leptin on allergic airway responses in mice. *J Allergy Clin Immunol* 2005;115:103–9.
- [34] 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.
- [35] Baines KJ, Simpson JL, Bowden NA, Scott RJ, Gibson PG. Differential gene expression and cytokine production from neutrophils in asthma phenotypes. *Eur Respir J* 2010;35:522–31.

- [36] Djukanovic R, Uddin M, Nong G, Ward J, Seumois G, Prince LR, et al. Prosurvival activity for airway neutrophils in severe asthma. *Thorax* 2010;65:684–9.
- [37] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11:85–97.
- [38] Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med* 2008;14:741–51.
- [39] Adams DH, Shaw S. Leukocyte–endothelial interactions and regulation of leukocyte migration. *Lancet* 1994;343:831–6.
- [40] Tedder TF, Steeber DA, Chen A, Engel P. The selectins — vascular adhesion molecules. *FASEB J* 1995;9:866–73.
- [41] Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006;444:840–6.
- [42] Andrikopoulos S, Blair AR, Deluca N, Fam BC, Proietto J. Evaluating the glucose tolerance test in mice. *Am J Physiol Endocrinol Metab* 2008;295:E1323–32.
- [43] Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115:911–9.
- [44] Lindsay RS, Funahashi T, Hanson RL, Matsuzawa Y, Tanaka S, Tataranni PA, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002;360:57–8.
- [45] Harris RBS, Mitchell TD, Kelso EW, Flatt WP. Changes in environmental temperature influence leptin responsiveness in low- and high-fat-fed mice. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R106–15.
- [46] Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPAR gamma controls alternative activation and improves insulin resistance. *Nature* 2007;447:1116–U12.
- [47] Ricardo-Gonzalez RR, Eagle AR, Odegaard JI, Jouihan H, Morel CR, Heredia JE, et al. IL-4/STAT6 immune axis regulates peripheral nutrient metabolism and insulin sensitivity. *Proc Natl Acad Sci U S A* 2010;107:22617–22.
- [48] Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. *Science* 2011;332:243–7.
- [49] Patel Payal S, EDB, Balasubramanyam Ashok. The role of the immune system in obesity and insulin resistance. *J Obes* 2013;2013.
- [50] Arshi M, Cardinal J, Hill RJ, Davies PSW, Wainwright C. Asthma and insulin resistance in children. *Respirology* 2010;15:779–84.
- [51] Ciprandi G, De Amici M, Tosca MA, Marseglia G. Serum resistin in persistent allergic rhinitis: preliminary data in adults. *Int Immunopharmacol* 2010;10:371–2.
- [52] Ciprandi G, De Amici M, Marseglia G. Serum adipon levels in patients with seasonal allergic rhinitis: preliminary data. *Int Immunopharmacol* 2009;9:1460–3.
- [53] Sood A, Qualls C, Seagrave J, Stidley C, Archibeque T, Berwick M, et al. Effect of specific allergen inhalation on serum adiponectin in human asthma. *Chest* 2009;135:287–94.
- [54] Dourado LPA, Noviello MDM, Alvarenga DM, Menezes Z, Perez DA, Batista NV, et al. Experimental food allergy leads to adipose tissue inflammation, systemic metabolic alterations and weight loss in mice. *Cell Immunol* 2011;270:198–206.