



## Asthma alleviates obesity in males through regulating metabolism and energy expenditure



Xiaomin Song<sup>a</sup>, Bolun Li<sup>a</sup>, Haoran Wang<sup>a</sup>, Xuan Zou<sup>a</sup>, Ran Gao<sup>a</sup>, Wei Zhang<sup>a</sup>, Ting Shu<sup>a</sup>, Hongmei Zhao<sup>a</sup>, Bin Liu<sup>b</sup>, Jing Wang<sup>a,\*</sup>

<sup>a</sup> State Key Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Department of Pathophysiology, Peking Union Medical College, Beijing, China

<sup>b</sup> Department of Biochemistry and Biophysics, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642, USA

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### ABSTRACT

Many epidemiological studies suggested a correlation between obesity and asthma. However, little is known about the molecular details explaining this correlation. Here, we show that asthma decreased body weight of asthmatic male mice fed with high fat diet via increasing energy expenditure and insulin sensitivity. The increase of energy expenditure was mainly due to upregulation of pAMPK and Sirt1. The activation of AMPK/Sirt1/PGC1 $\alpha$  signaling promoted the expression of the thermogenic genes like *ucp1*, *PRDM16*, *cidea*, *Elovl3*, *PPAR $\alpha$* , which occurred in brown adipocyte tissue and subcutaneous white adipose tissue. Besides, by activating IL33/ILC2/AAMac pathway in subcutaneous white adipose tissue, asthma promoted subcutaneous white adipose tissue into beige fat. In addition, insulin sensitivity was improved in the asthmatic male mice by decreasing the expression of G6Pase in the liver, which was recapitulated in HepG2. In human, we found that Body Mass Index (BMI) and waist circumference were significantly lower in males suffering asthma compared with the control in the National Health and Nutrition Examination Survey (NHANES) cohort. These data together suggest asthma in males decreases obesity by improving the metabolism function of brown and subcutaneous adipose tissue, and decreasing insulin resistant in the liver.

### 1. Introduction

Obesity is considered as an important health problem in recent years for the increasing number of obese people and its complications including type 2 diabetes, cardiovascular disease, non-alcoholic fatty liver disease, etc. [1]. These complications of obesity are closely relevant to the chronic inflammation. Asthma is a chronic airway inflammation [2], and accompanied by other allergic diseases in some cases, which has a high incidence among children [3]. In recent years, increasing number of epidemiological studies focus on obesity and asthma [4–6], The majority of them suggests that asthma positively associated with obesity especially in women than men [7,8]. Though some of them do not support a consistent link between asthma and obesity [9,10]. Negative correlation between asthma and obesity is also reported in literature [10]. However, mechanisms underlying the correlation, no matter positive or negative, are not clear.

The energy metabolism adopts three basic forms including basic metabolism, adaptive heat production, and physical activity [11]. Adipose tissue plays an important role in the procedure of energy

metabolism. White adipocytes store energy in the form of triglycerides, and beige/brown adipocytes are involved in heat metabolism [12–14]. It is known that the expression of bronchial smooth muscle PGC1 $\alpha$  is upregulated under asthma condition [15]. PGC1 $\alpha$  is a main regulator for mitochondrial biogenesis and thermogenesis gene expression in white and brown adipocytes [14,16,17]. PGC1 $\alpha$  activates brown adipocytes and promotes white adipose tissue browning by upregulating UCP1 expression [17,18]. On the upstream of PGC1 $\alpha$ , AMPK and Sirt1 are two important metabolic regulators [19–22]. In skeletal muscle and brown adipose tissue, Sirt1 deacetylates PGC1 $\alpha$ , consequently elevating PGC1 $\alpha$  to enhance the mitochondrial activity and the heat production [23]. In white and brown adipocyte, AMPK/PGC1 $\alpha$  signal can directly induce thermogenic genes and regulate metabolism [24].

Obesity is a chronic inflammation disease in which several inflammatory cytokines are involved in the regulation of metabolism [25]. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is reported upregulated in both obese mice and obese people [26]. The TNF- $\alpha$  mainly comes from the accumulated macrophages in adipose tissue, and contributes to the insulin resistance [27,28]. Besides, other inflammatory cells such as

\* Corresponding author.

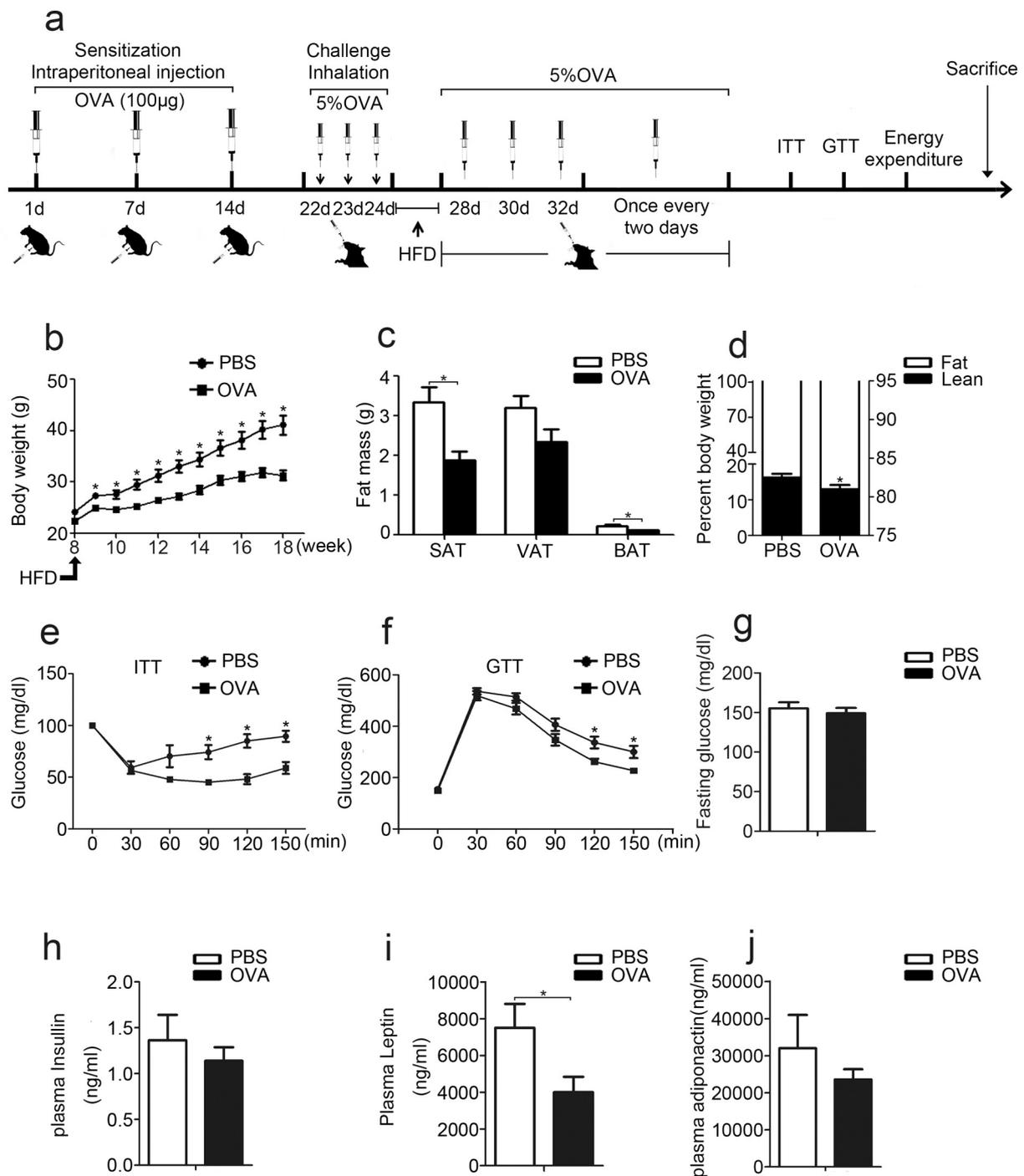
E-mail address: [wangjing@ibms.pumc.edu.cn](mailto:wangjing@ibms.pumc.edu.cn) (J. Wang).

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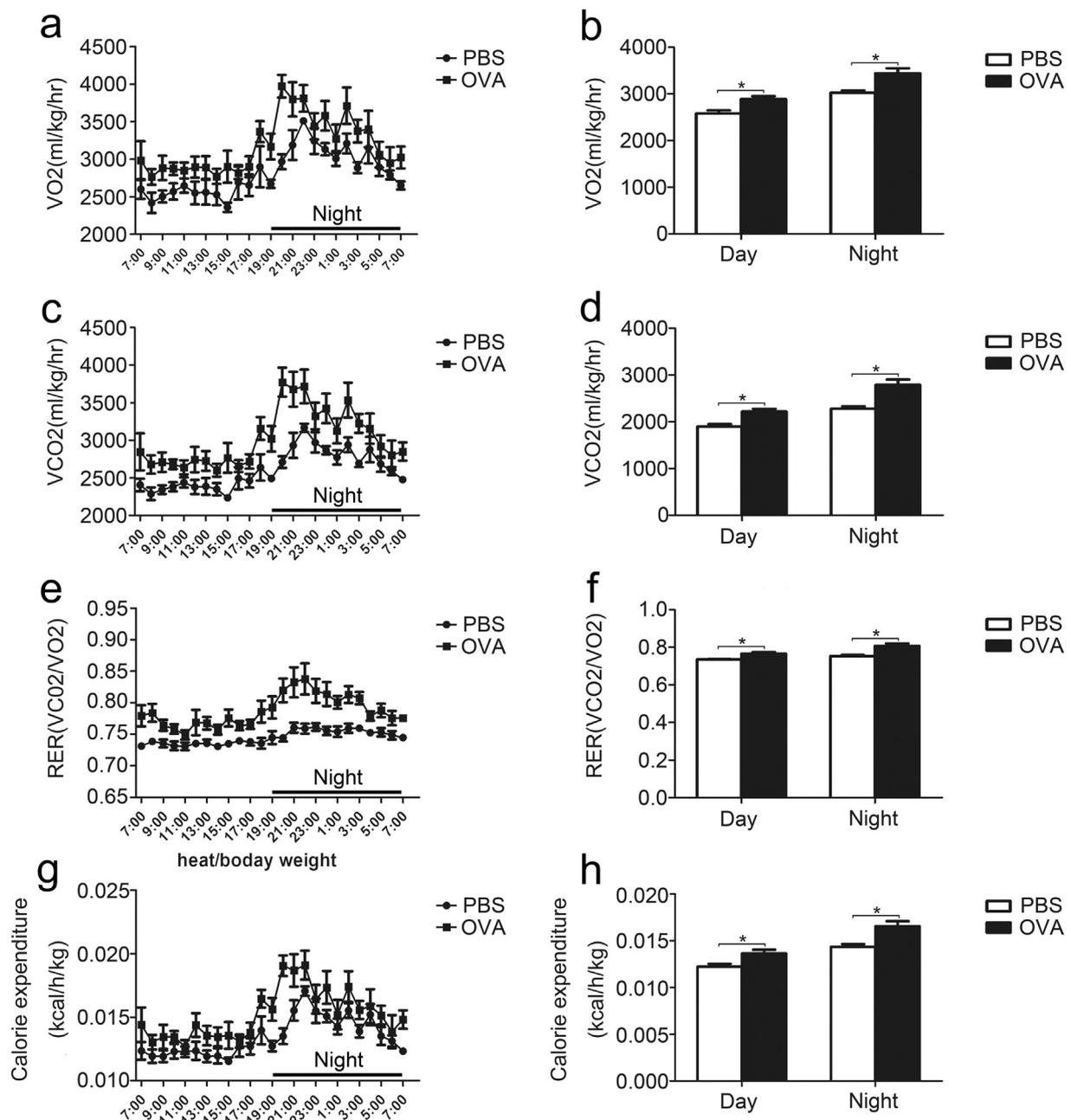
**Fig. 1.** Asthma decreased body weight and improved insulin sensitivity in HFD-fed male mice. **a.** Experimental protocol for a murine model of asthma. **b.** Body weight gain of male mice on a HFD with or without asthma. **c.** All parts of fat weight in mice. **d.** Body composition. **e.** Insulin tolerance tests. **f.** Glucose tolerance tests. **g.** blood glucose level was measured in male mice after fasting overnight. **h.** Plasma serum insulin level determined by ELISA. **i.** Plasma serum leptin level determined by ELISA. **j.** Plasma serum adiponectin level determined by ELISA. Values are expressed as mean ± SE. \*,  $p < 0.05$  vs PBS,  $n = 8$ .

alternatively activated macrophage (AAMac), eosinophil, Group2 innate lymphoid cell (ILC2s), invariant nature killer T (iNKT), T helper type 2 (Th2), and regulatory T (Treg) cells can also regulate obesity by changing energy metabolism [25]. It has been shown that the asthma-induced IL33 activates ILC2, and subsequently promotes type 2 inflammatory response [29–34].

Current epidemiological studies support that allergic asthma is associated with insulin resistance [35,36]. The liver is one of the insulin-sensitive organs that play a vital role in regulating systemic energy

metabolism [37]. With insulin resistance in liver, glycogen synthesis of hepatocytes is increased [37,38]. However, it is not clear how asthma affects liver insulin sensitivity.

This research is designed to study the effect of allergic asthma on obesity and metabolism.



**Fig. 2.** Asthma increased energy expenditure in HFD-fed mice. **a.** oxygen consumption. **c.** carbon dioxide production. **e.** respiratory exchange ratio. **g.** calorie expenditure. The statistics data for each group in the day or night cycle (**b, d, f, h**). Values are expressed as mean  $\pm$  SE. \*,  $p < 0.05$  vs PBS,  $n = 8$ .

## 2. Materials and methods

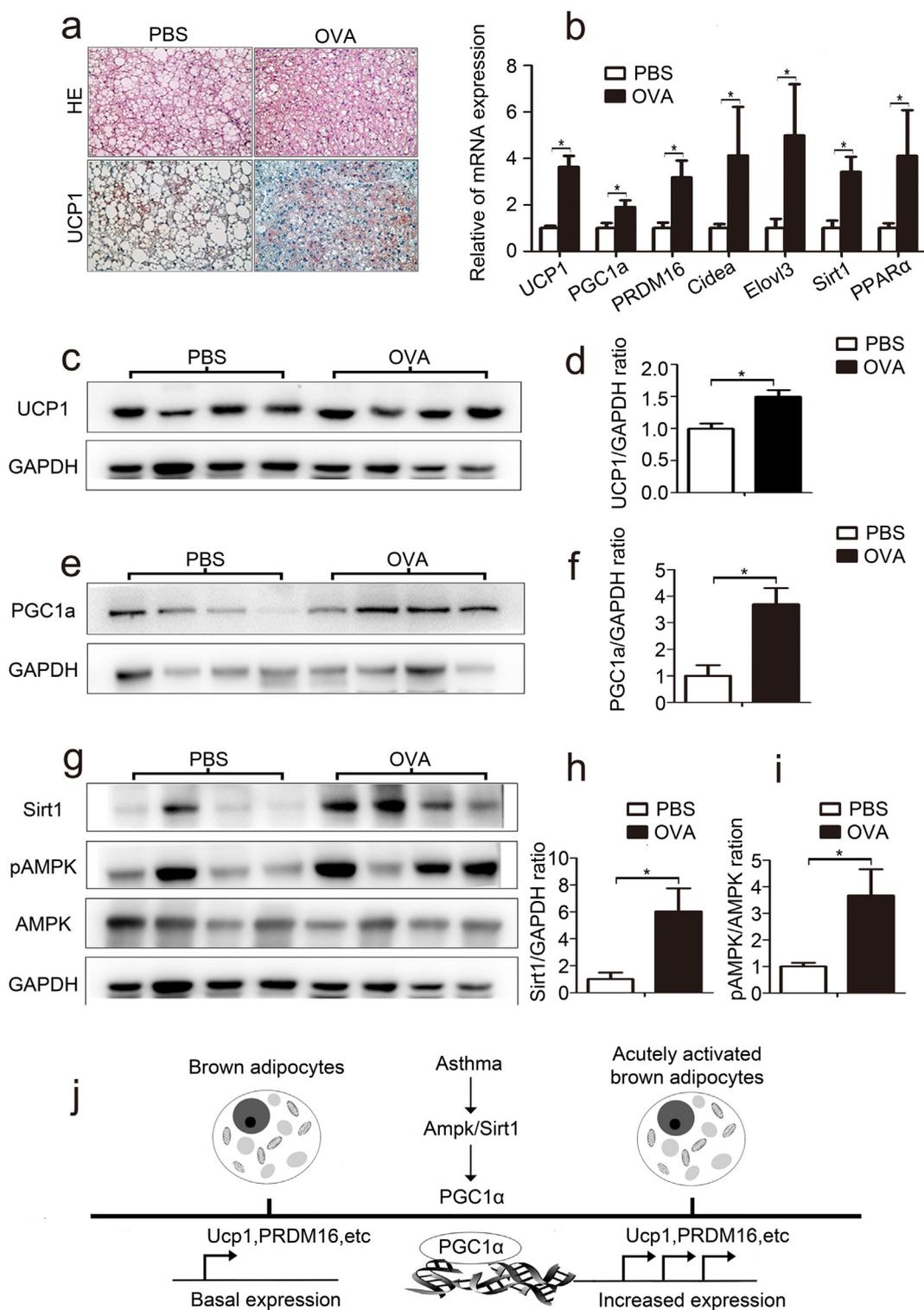
### 2.1. Animal model

Six to eight week old mice (20–22 g) were maintained in a temperature and humidity controlled room with a 12 h light-dark cycle. Mice had free access to tap water and were fed irradiated food. All the procedures were approved by the Animal Care and Use Committee of Peking Union Medical College. Male C57BL/6 J mice (Vitalriver, China) were randomly assigned to the asthma group or control group. Briefly, the experimental mice were sensitized by intraperitoneal injection (i.p.) of 50  $\mu$ g ovalbumin with alum adjuvant on day 0, 7 and 14, while the control mice received i.p. saline with alum. From day 21 onward, the mice were inhaled through the nose 5% OVA (allergen exposure) or saline (sham exposure) 12.5  $\mu$ L/day for 3 days. From the 28th days,

mice were fed a high-fat diet (HFD; D12108C; Research Diets Inc), and mouse were inhale 5% OVA (allergen exposure) or saline (sham exposure) 12.5  $\mu$ L every other day. Mouse body weight was weekly measured. After 16 weeks on a HFD, mice were placed individually in an indirect open circuit calorimeter for 3 days (Oxymax/CLAMS; Columbus Instruments). Oxygen consumption (VO<sub>2</sub>), carbon dioxide production (VCO<sub>2</sub>) and respiratory exchange ratio (VCO<sub>2</sub>/VO<sub>2</sub>) were continuously monitored during the 72 h. At last, the mice were sacrificed and tissues were collected.

### 2.2. Bronchoalveolar lavage, histopathology, and immunohistochemistry

Mice were anesthetized with ketamine through i.p. (60 mg/kg), and the adequacy of anesthesia was continually monitored by assessing reflexes and respiration. After exsanguinations via cardiac puncture,

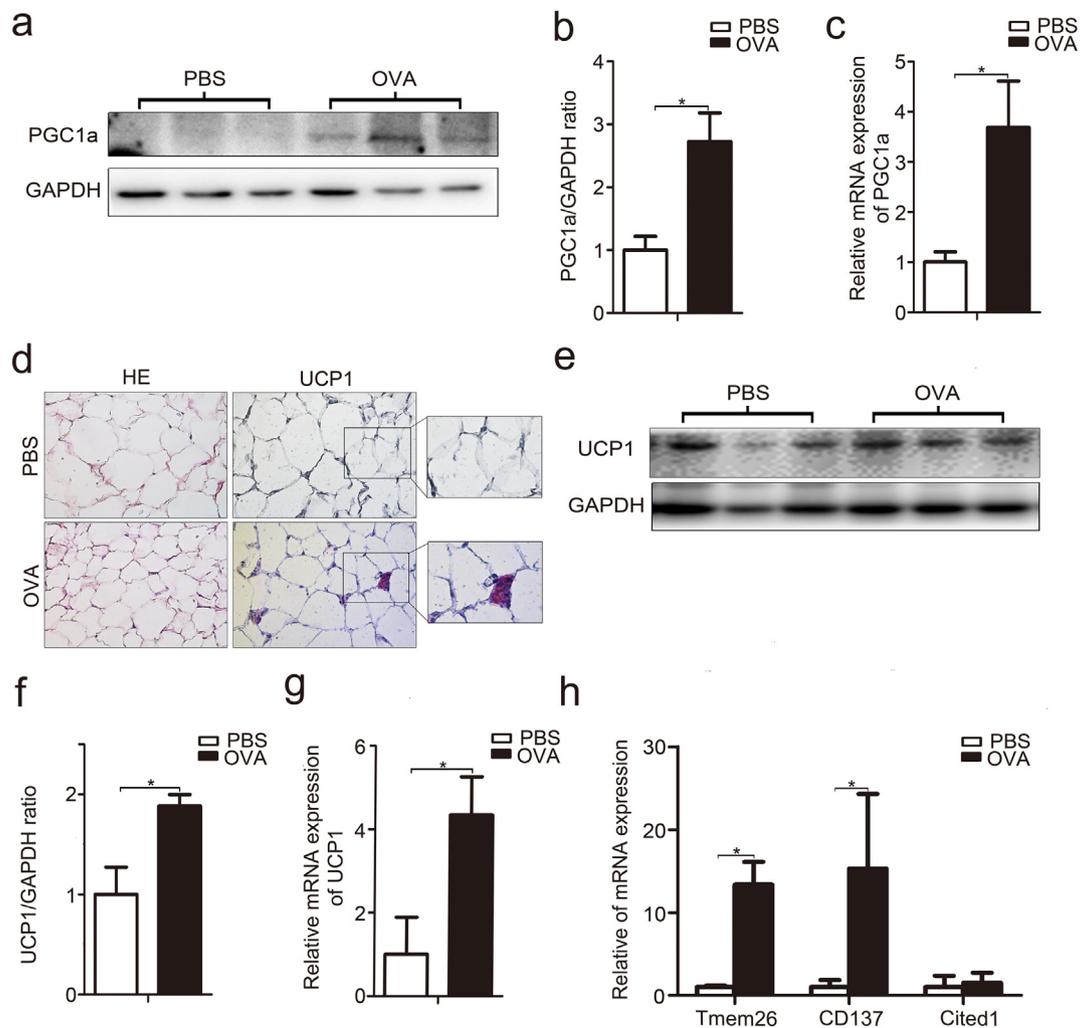


**Fig. 3.** Asthma activated AMPK/PGC1 $\alpha$  signaling in BAT.

**a.** Representative H&E staining and UCP1 staining for BAT. **b.** mRNA level of thermogenic genes in BAT determined by qPCR ( $n = 5/\text{group}$ ). **c.** UCP1 expression in BAT determined by WB ( $n = 4/\text{group}$ ). **d.** Quantification of UCP1. **e.** PGC1 $\alpha$  expression determined by WB ( $n = 4/\text{group}$ ). **f.** Quantification of PGC1 $\alpha$ . **g.** Western blot analyzed of Sirt1 and pAMPK expression ( $n = 4/\text{group}$ ). **h.** Quantification of Sirt1. **i.** Quantification of pAMPK/AMPK expression in BAT ( $n = 4/\text{group}$ ). **j.** Model for the role of asthma-mediated heat production in BAT. Values are expressed as mean  $\pm$  SE. \*,  $p < 0.05$  vs PBS.

bronchialalveolar lavage fluid (BALF) was collected (0.8 mL saline, two times) by tracheal intubation. The death was confirmed by cervical dislocation. Allergic inflammatory responses and lung pathology were assessed in situ by lung histopathology staining (H&E and Masson) [39], and ex vivo by Wright-Giemsa staining eosinophils counts of BALF

according to previously studies [40]. Similarly, liver pathology was examined by histopathology staining (H&E and Masson) [39] and Oil Red O Staining. For Oil Red O staining, liver cryostat sections (6  $\mu\text{m}$ ) were fixed with 10% formaldehyde, stained with Oil Red O (sigma) for 20 min, and counterstained with hematoxylin (Solarbio). For brown



**Fig. 4.** PGC1 $\alpha$  promoted asthma-induced UCP1 expression and adipocyte browning in SAT.

**a.** Western blot analyzed the expression of PGC1 $\alpha$  ( $n = 3$ /group). **b.** Quantification of PGC1 $\alpha$  expression in SAT ( $n = 3$ /group). **c.** mRNA expression of PGC1 $\alpha$  in SAT determined by qPCR ( $n = 5$ /group). **d.** Representative H&E and UCP1 staining for SAT. **e.** Western blot analysis of UCP1 expression ( $n = 3$ /group). **f.** Quantification of UCP1 expression in SAT ( $n = 3$ /group). **g.** mRNA expression of UCP1 in SAT determined by qPCR ( $n = 5$ /group). **h.** mRNA level of beige cell marker gene determined by qPCR in SAT ( $n = 5$ /group). Values are expressed as mean  $\pm$  SE. \*,  $p < 0.05$  vs PBS.

and subcutaneous adipose tissues, Immunohistochemistry was performed on tissue sections using UCP1 antibody (1:100, abcam).

### 2.3. ITT (insulin tolerance tests)

After 14 weeks on a HFD, insulin tolerance tests were performed after a 6 h fasting. Mice were injected with insulin intraperitoneally (0.66 IU/kg body weight). Blood glucose levels were measured from tail vein by a glucose meter (yuwell) at 0, 30, 60, 90, 120, 150 min after injection.

### 2.4. GTT (glucose tolerance tests)

Glucose tolerance tests were performed after overnight fasting. Mice were injected with D-glucose intraperitoneally (2 g/kg body weight). Blood glucose levels were measured from tail vein by a glucose meter (yuwell) at 0, 30, 60, 90, 120, 150 min after injection.

### 2.5. Elisa

Blood samples were taken before the mice were sacrificed, and the serum was prepared from the blood. Circulating IgE, TNF $\alpha$ , Insulin, Leptin and Adiponectin in the serum were measured using mouse

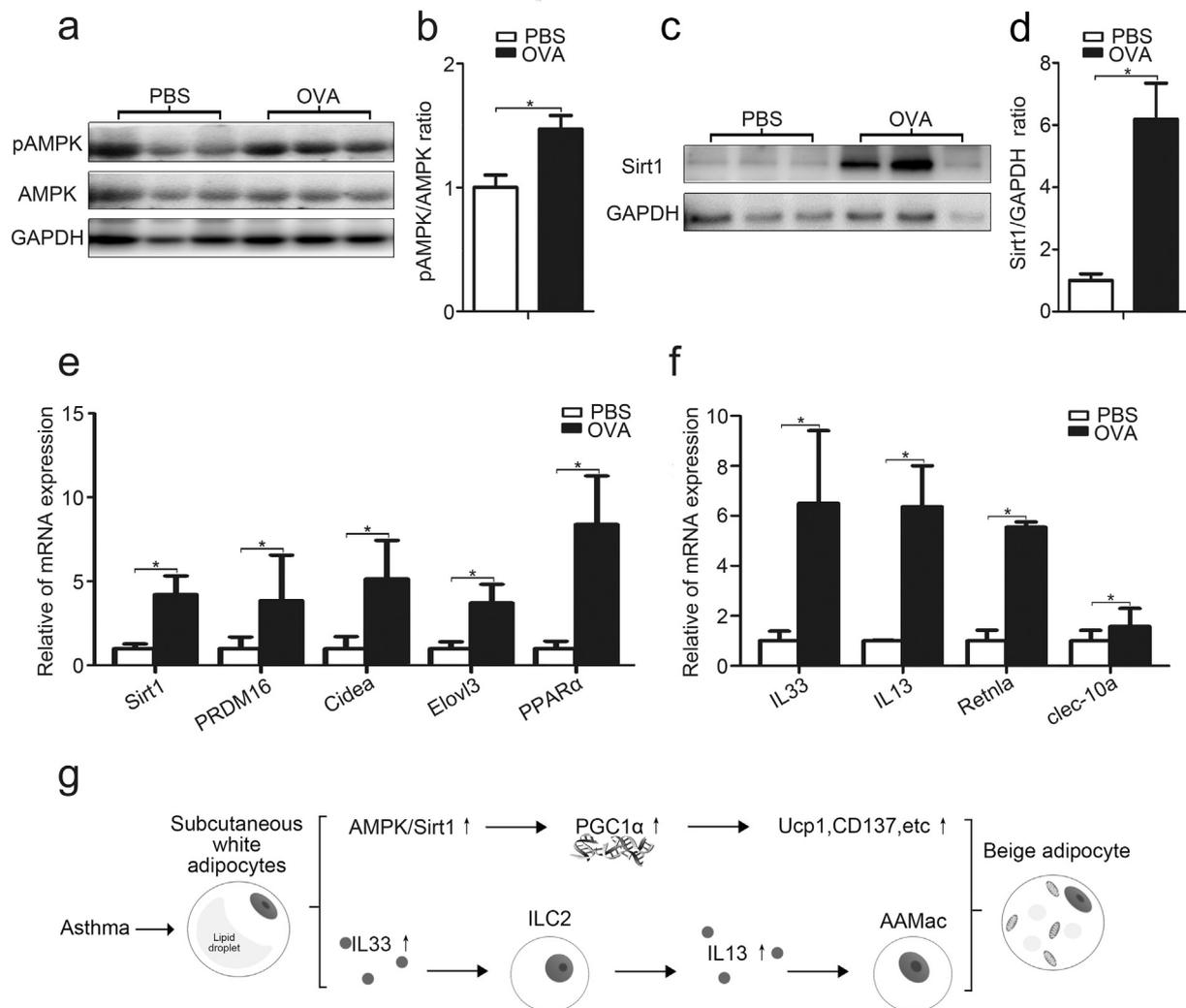
enzyme linked immunosorbent assay according to the manufacturers' instructions (IgE kit was from Cayman Chemical. TNF $\alpha$ , Insulin, Leptin, and Adiponectin kit were from eBioscience).

### 2.6. Biochemical assays

400  $\mu$ L serum collected from each mouse were sent to the Peking Union Medical Hospital clinical laboratory to test AST, ALT, FFA, TG, TC, LDL and HDL.

### 2.7. RNA isolation and quantitative real-time PCR

Total RNA was isolated from the adipose tissues using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. The first strand cDNA was synthesized by M-MLV reverse transcriptase (Invitrogen) from 1  $\mu$ g of total RNA, and analyzed by quantitative real-time PCR with a SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> II RT-PCR Kit (Bio-Rad, USA). All realtime reactions were performed on the iQ5<sup>™</sup> Multicolor Real-Time PCR detection system (Bio-Rad, USA).  $\beta$ -actin was used as internal control.



**Fig. 5.** pAMPK/Sirt1 and IL33/ILC2 were activated in asthma-induced mice.

**a.** Western blot analysis of pAMPK expression (n = 3/group). **b.** Quantification of pAMPK/AMPK expression in SAT (n = 3/group). **c.** Western blot analysis of Sirt1 expression (n = 3/group). **d.** Quantification of Sirt1 expression to GAPDH in SAT (n = 3/group). **e.** mRNA level of thermogenic genes in SAT detected by qPCR (n = 5/group). **f.** mRNA level of inflammatory genes in SAT determined by qPCR (n = 5/group). **g.** Model for the role for asthma in promoting browning in SAT. Values are expressed as mean ± SE. \*,  $p < 0.05$  vs PBS.

## 2.8. Western blot

Proteins were extracted from frozen organ sample in RIPA. A sample containing 35 µg of protein was loaded in each well onto an 8% SDS-polyclonal-amide gel, and separated proteins were transferred to a PVDF membrane. Western blot assays were performed using antibodies specific to UCP1(1:1000, abcam), AMPK (1:1000, cell signaling), pAMPK(1:1000, cell signaling), Sirt1(1:1000, Merck), PGC1α(1:1000, abcam), AKT(1:1000, cell signaling), pAKT (1:1000, cell signaling), G6Pase (1:1000, cell signaling) and GAPDH (1:3000, protech). After primary antibody incubation, membranes were washed with TBST and further incubated with the appropriate horseradish peroxidase-conjugated (HRP-conjugated) secondary antibody at room temperature for 1 h. Immunoreactive bands were visualized with Super Signal West Pico Chemiluminescent Substrate (Pierce). The intensities of the protein bands were analyzed by Image Pro software.

## 2.9. Cell culture

HepG2 cells were cultured in DMEM with 10% fetal bovine serum at 37 °C. The cells were treated with high glucose (30 mM) plus palmitic acid (PA; 0.2 mM) or normal glucose (5 mM glucose) in the absence or

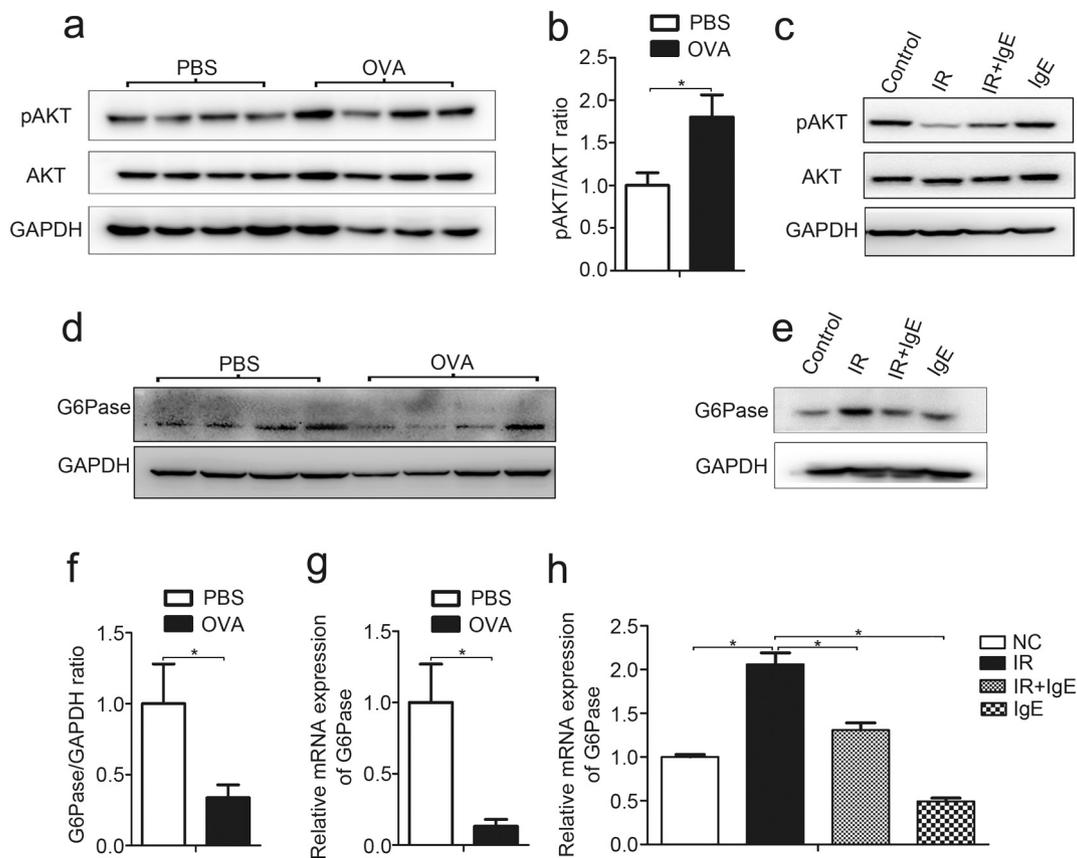
presence of IgE (10 µg/mL) for 24 h [41]. Cells incubated with high glucose and palmitic acid (PA) were used as insulin resistant group.

## 2.10. Metabolic analysis

Glycolytic function analysis in HepG2 cells was performed by Seahorse extracellular analyser (XF24). HepG2 cells were seeded for 20,000 per well and incubated with or without IgE (10 µg/mL) for 24 h. The cells then were washed by Seahorse medium and incubated in a non-CO<sub>2</sub> condition for 1 h at 37 °C. Then the drugs were loaded into the appropriate ports to reach the final concentration, such as 100 mM glucose, 100 µM oligomycin and 500 mM 2-DG. At the end, extracellular acidification rate (ECAR) results were standardized to the concentration of protein.

## 2.11. Statistical analysis

All data were expressed as means ± SE. Nonparametric Mann-Whitney test was used to compare the statistical significance between two animal groups. One-Way ANOVA was used to determine the difference among the cell groups. A level of  $p < 0.05$  was considered statistical significant.



**Fig. 6.** Asthma alleviated insulin resistance in liver.

**a.** Western blot analysis for pAKT in the liver of control (PBS) and asthmatic mice (OVA) ( $n = 4/\text{group}$ ). After overnight fasting, mice were fed with HFD for 30 min next morning. Then the liver tissues were collected from the sacrificed mice. Protein extracts from the liver tissues were used for the western blot analysis. **b.** Quantification of pAKT expression to AKT in liver ( $n = 4/\text{group}$ ). **c.** HepG2 cells were incubated with high glucose (30 mM) plus palmitic acid (PA;0.2 mM) were as insulin resistant (IR). Cells cultured in 5 mM glucose medium as control. Cells were treated with IgE (10  $\mu\text{g}/\text{mL}$ ) for 24 h, then 100 nM insulin for 30 min. Western blot analysis of expression of pAKT in IgE stimulated HepG2 cell. **d.** Western blot analysis of G6Pase expression ( $n = 4/\text{group}$ ). **e.** Western blot analysis of G6Pase in IgE (10  $\mu\text{g}/\text{mL}$ )-stimulated HepG2 cells. **f.** Quantification of G6Pase expression to GAPDH in liver ( $n = 4/\text{group}$ ). **g.** Expression of G6Pase in liver determined by qPCR ( $n = 5/\text{group}$ ). **h.** mRNA level of G6Pase in IgE (10  $\mu\text{g}/\text{mL}$ )-stimulated HepG2 cells determined by qPCR. Values are expressed as mean  $\pm$  SE. \*,  $p < 0.05$  vs PBS.

### 2.12. Study population

The National Health and Nutrition Examination Survey (NHANES) is designed for a stratified multistage probability sampling. In this NHANES (2013–2014), 1375 men were documented as they completed interviews, examinations or laboratory tests related to asthma, hypertension, triglyceride, and survey on other factors including age, race, waist, smoking status, body mass index. Asthma subjects were those who had documented asthma history. Age, waist circumference, hypertension, race, smoking, triglyceride, body mass index (BMI) were considered as potential confounders in the analysis. SAS 9.4 was used for all analysis. Chi-square test or  $t$ -test was used for analyzing demographic characteristics of all the participants with or without asthma. The sampling weights (WTMEC2YR) and design variables (SDMVSTRA; SDMVPSU) were applied to these survey sampling procedures.

## 3. Result

### 3.1. Asthma decreased body weight and improved insulin sensitivity in HFD-fed mice

To determine whether allergic asthma was associated with male mouse obesity, asthma was induced by intraperitoneal injection and inhalation of OVA in C57BL/6J mice. As shown in Supplementary Fig. 1, IgE was significantly increased in the serum of asthmatic mice, and the lung tissue structure was changed significantly in the OVA-

treated mice, suggesting that OVA induced asthma in C57BL/6J male mice successfully. The asthmatic mice were then fed with high fat diet, and their body weight was measured every week (Fig. 1a). The results showed that the body weight of the asthmatic mice was significantly lower than that of the control mice (Fig. 1b), and the weight of subcutaneous white fat and brown fat was correspondingly decreased significantly (Fig. 1c), which resulted in the decrease of fat ratio (Fig. 1d). Moreover, in these asthmatic mice, the insulin sensitivity and glucose tolerance were improved (Fig. 1e,f), while serum Leptin level was decreased significantly (Fig. 1i). No significant difference was detected in fasting blood glucose (Fig. 1g), insulin, and adiponectin (Fig. 1h,j) between the asthmatic and control mice. All these results suggest that asthma decreases the body weight and improve the insulin sensitivity in male mice.

### 3.2. Asthma increased energy expenditure in HFD-fed mice

To determine whether the decrease in body weight of the asthmatic male mice was related to energy metabolism, the metabolism rate of our experimental mice was measured. The results showed that the daily rate of  $\text{O}_2$  consumption (Fig. 2a,b), the  $\text{CO}_2$  production (Fig. 2c,d), the respiratory exchange ratio (RER) (Fig. 2e,f), and heat production per body weight (Fig. 2g,h) were significantly increased in the asthmatic mice compared with control mice, whereas the activity of the mice appeared no difference between the two groups (Supplementary Fig. 2). These data suggest that allergic asthma increases energy metabolism of

the mice.

### 3.3. Asthma activated AMPK/PGC1 $\alpha$ signaling in BAT

The brown adipose tissue is an important energy metabolic tissue, so we tested whether the function of brown adipose tissue in asthmatic mice was changed. Histologic staining showed more brown adipocytes with multilocular appearance in asthmatic mice (Fig. 3a). Moreover, the expression of UCP1 in both mRNA and protein levels was also increased in asthmatic brown adipose tissue (Fig. 3a–d, Supplementary Fig. 3). The increased expression of several thermogenic genes (such as PRDM16, Cidea, Elovl3, PPAR $\alpha$ ) suggests asthma stimulates BAT thermogenic program (Fig. 3b). PGC1 $\alpha$  is an important transcriptional regulator in brown adipose tissue that regulates mitochondrial function and genes related to heat production [17,18]. Our results showed that expression of PGC1 $\alpha$  was significantly increased in asthmatic mice (Fig. 3e, f). And the levels of Sirt1 and pAMPK that are upstream regulators of PGC1 $\alpha$  were also significantly increased (Fig. 3g–i) in asthmatic mice. These data together suggest that AMPK/PGC1 $\alpha$  signaling is activated, which results in the increased expression of the thermogenic genes and energy metabolism in brown adipose tissue of the asthmatic mice (Fig. 3j).

### 3.4. PGC1 $\alpha$ promoted asthma-induced UCP1 expression and adipocyte browning in SAT

In asthmatic mice, the expression of PGC1 $\alpha$  was upregulated in white adipose tissue too (Fig. 4a–c). Histologic further affirmed that asthma-induced remodeling of subcutaneous white adipose tissue into beige fat, as evidenced by the plenty of multilocular (Fig. 4d) and higher expression of UCP1 (Fig. 4e–g) in asthmatic mice. qPCR analysis showed that asthma upregulated the expression of a series of beige cell-selective markers including Tmem26, CD137, Cited1 (Fig. 4h). These data together suggest that asthma promotes SAT to transform into beige fat.

### 3.5. pAMPK/Sirt1 and IL33/ILC2/AAMac were upregulated in asthmatic mice

To elucidate the mechanism by which asthma increases the expression of PGC1 $\alpha$ , we examined the expression of pAMPK and Sirt1. These two proteins contribute to the metabolic homeostasis by regulating the expression of PGC1 $\alpha$  and its downstream thermogenic genes [22,23]. As shown in Fig. 5a–b, the expression of pAMPK was significant increase in asthmatic mice compared with control mice. Similarly, the expression of Sirt1 was also elevated in both protein and mRNA levels (Fig. 5c–d). Meanwhile, their down-stream thermogenic genes like PRDM16, Cidea, Elovl3, and PPAR $\alpha$  were upregulated (Fig. 5e).

Immune cells also play important roles in energy metabolism in adipose tissue [25]. Among them, alternatively activated macrophage (AAMac) is important to maintain metabolic homeostasis [25,42,43]. Group 2 innate lymphoid cells (ILC2) can stimulate AAMac through secreting IL13 [25]. Alternatively, ILC2 can activate eosinophil by secreting IL5, who produce IL4 to sustain AAMac response in SAT [25,44,45]. Since asthma can induce IL33 [29,33,46], which can activate ILC2 [47], we hypothesized that asthma may contribute the energy metabolism by activating ILC2-AAMac cells. To test this hypothesis, we first detected the expression of IL33 and IL13 by qPCR in SAT. As shown in Fig. 5f, asthma significantly induced the expression of IL33 and IL13, but not the other related cytokines such as IL4 and IL5 (supplementary Fig. 4). Consequently, the expression of Retnla and clec-10a, AAMac maker genes, were upregulated in asthmatic mice (Fig. 5f). These data suggest that AMPK/PGC1 $\alpha$  and IL33/ILC2/AAMac pathway together contribute the energy metabolism in SAT (Fig. 5g).

### 3.6. Asthma alleviated insulin resistance in the liver of the mice

To study the effect of asthma on insulin sensitivity in liver, we first examined the expression of pAKT in our experimental mice. We found that the expression of pAKT was significantly increased in the liver of asthmatic mice (Fig. 6a–b). To examine whether this phenomenon is relevant to human, insulin resistance (IR) was induced by high-glucose in HepG2 cell in vitro. As shown in Fig. 6c, the expression of pAKT was increased in HepG2 cells treated with IgE, which is highly induced in asthma mice (Fig. 6c). G6Pase is involved in the regulation of insulin resistance in liver as a rate-limiting gluconeogenic enzyme [48]. Our data showed the expression of G6Pase was significantly decreased in the liver from asthmatic group compared with the control (Fig. 6d–g). Consistently, the expression of G6Pase was also declined in IgE-treated HepG2 cells (Fig. 6e–h). However, IgE didn't affect the processes of glycolysis in HepG2 cells by measuring the extracellular acidification rate (ECAR) (Supplemental Fig. 5). These results suggest that asthma-induced high IgE may contribute to insulin resistance by mainly regulating gluconeogenesis.

On the other hand, our results showed asthma didn't change fatty liver phenotype shown by Oil Red O and Masson staining (Supplementary Fig. 6). Similarly, no change was detected in the serum factors such as AST, ALT, FFA, TG, TC, LDL and HDL (Supplementary Fig. 6) in asthmatic mice by biochemical analysis. These results indicate that asthma has no effect on lipid metabolism in liver.

### 3.7. Association between asthma and male obese in human crowd data

In our analysis of National Health and Nutrition Examination Survey (NHANES) 2013–2014, total of 1375 adult male participants were included. Among them, 174 (12.65%) were asthmatic. As shown in Supplementary Table 1, BMI ( $28.53 \pm 0.73 \text{ kg/m}^2$  vs.  $28.65 \pm 0.23 \text{ kg/m}^2$ ,  $p < 0.0001$ ) and the waist circumference ( $100.42 \pm 2.01 \text{ cm}$  vs.  $101.97 \pm 0.62 \text{ cm}$ ,  $p < 0.0001$ ) were significantly lower in asthma people.

## 4. Discussion

In this study, we find that asthma is negatively associated with obesity in male mice. Allergic asthma decreases body weight, promotes energy metabolism and improves systemic insulin sensitivity in male mice. These events are mainly due to the activation of pAMPK/Sirt1/PGC1 $\alpha$  signaling in brown and white adipose tissue, and upregulation of IL33/ILC2/AAMac in white adipose tissue. In addition, asthma-induced high IgE inhibits G6Pase expression in hepatocytes, which results in the improvement of the insulin resistance in liver. This study provides a support for the association between asthma and obesity in male mice, and the molecular mechanisms involved in the energy metabolism.

Allergic asthma is a chronic respiratory inflammatory disease that affects around 235 million people worldwide [2]. At present, a large number of epidemiological results suggest an association between asthma and obesity [5,49]. Some studies have shown that asthma is a risk factor for obesity that was positively correlated with BMI [4,50]. However, other studies suggested that asthma may not be the risk factor for obesity, especially in men [8,51]. In addition, asthma had a higher incidence among underweight people [52]. So Carpaij O A et al. indicated an explanation that the relationship between asthma and obesity has a U-shaped pattern [53]. Here, we analyzed the data of the men from 2013 to 2014 in NHANES. Our results showed that the asthma group had lower BMI and waist circumference. Consistently, our asthma animal model of male mice showed that the body weight was significantly decreased in the asthmatic male mice. These data together suggest that asthma is negatively associated with body weight in obese male.

The development of obesity is identified closely related to energy

metabolism. Brown adipose tissue is the main heat-producing tissue [13]. UCP1 promotes mitochondrial ATP production, and stimulates a series of respiratory chain reaction, subsequently contributes to thermogenesis in BAT [13,54]. Besides, UCP1 is also expressed in the white adipose tissue and regulates energy metabolism [54]. In this study, we found that the expression of UCP1 was up-regulated, and the thermogenic program was activated in the white adipose tissue. This upregulation of UCP1 is most likely due to the elevated beige cell-selective markers of SAT.

The regulation of heat production in adipocytes is complex, and several transcription factors are involved in the process. PGC1 $\alpha$  was a significant regulator of heat production in brown adipose tissue [55]. In white adipose tissue, PGC1 $\alpha$  can also mediate the expression of thermogenesis genes such as UCP1 and reflects the activity of thermogenic [13]. In our study, the expression of PGC1 $\alpha$  in both brown and subcutaneous white adipose tissues is significantly increased. These results suggested that asthma activates PGC1 $\alpha$  and promotes the heat production. Another involved important transcriptional regulator is PRDM16, which regulates the differentiation of brown adipocytes into mature brown adipocytes. With PRDM16 knocked out, heat production is decreased in BAT [56,57]. PRDM16 is highly expressed in visceral adipocyte tissue (VAT), promotes the development of brown-like adipocytes, and plays a vital role in regulating the metabolism and thermogenesis in SAT [58]. In our study, PRDM16 expression in BAT and SAT of asthmatic mice is significantly increased, which may promote the metabolism and thermogenesis. However, whether asthma-induced high IgE mediates PRDM16 expression is not studied from the cellular perspective.

AMPK is an important energy metabolism regulator that can enhance Sirt1 activity. Both AMPK and Sirt1 mediate the expression of PGC1 $\alpha$  [19–21]. AMPK/Sirt1/PGC1 $\alpha$  constitute an energy sensing network that controls energy expenditure [59]. In our study, asthma-activated AMPK/PGC1 $\alpha$  signaling increases brown fat thermogenesis, and promotes SAT into beige fat. Further study is needed to identify the role of asthma-induced high IgE in the activation of AMPK and Sirt1.

Our results showed no significant difference in fasting blood glucose level and serum insulin level between the control and asthmatic mice, but a significant increase in the systemic insulin sensitivity in asthmatic mice. Insulin mainly acts on the adipose tissue, liver, and muscle. Previous studies showed that IgE inhibited the expression of pAKT in visceral adipose tissue [60], suggesting that visceral white adipose tissue might not be involved in the improvement of insulin sensitivity in asthmatic mice. In our hands, asthma downregulated the expression of liver G6Pase while upregulated expression of liver pAKT. These data together suggest that asthma-induced high IgE may regulate insulin sensitivity by suppressing gluconeogenesis. Reducing hepatic gluconeogenesis is important for enhancing hepatic insulin sensitivity and improving hyperglycemia [61]. However, some reports exist conflicting about glycogen synthesis in liver in diabetes. Some results showing that Hepatic insulin resistance decrease glycogen synthesis [62], and others studies indicate that glycogen synthesis was not affected [63]. In our study, we didn't assess the contents of glycogen in liver between the two groups. The change of glycogen synthesis in the liver of asthmatic mice is still unclear.

In conclusion, this study strongly suggest that asthma is negatively correlated with obesity in the males, and at least 4 signaling pathways with different functions mediate this complicated correlation. These findings provide the fundamental information for studying the effect of asthma on obesity.

## Transparency document

The [Transparency document](#) associated with this article can be found, in online version.

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## Appendix A. Supplementary data

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## References

- [1] C.T. Juel, C.S. Ulrik, Obesity and asthma: impact on severity, asthma control, and response to therapy, *Respir. Care* 58 (2013) 867–873.
- [2] E.D. Bateman, S.S. Hurd, P.J. Barnes, J. Bousquet, J.M. Drazen, M. FitzGerald, P. Gibson, K. Ohta, P. O'Byrne, S.E. Pedersen, E. Pizzichini, S.D. Sullivan, S.E. Wenzel, H.J. Zar, Global strategy for asthma management and prevention: GINA executive summary, *Eur. Respir. J.* 31 (2008) 143–178.
- [3] D.A. Waite, E.F. Eyles, S.L. Tonkin, T.V. O'Donnell, Asthma prevalence in Tokelauan children in two environments, *Clin. Allergy* 10 (1980) 71–75.
- [4] S. Guerra, D.L. Sherrill, A. Bobadilla, F.D. Martinez, R.A. Barbee, The relation of body mass index to asthma, chronic bronchitis, and emphysema, *Chest* 122 (2002) 1256–1263.
- [5] E.S. Ford, D.M. Mannino, S.C. Redd, A.H. Mokdad, J.A. Mott, Body mass index and asthma incidence among USA adults, *Eur. Respir. J.* 24 (2004) 740–744.
- [6] J.M. Smith, Atopy and asthma: an epidemic of unknown cause, *J. Allergy Clin. Immunol.* 116 (2005) 231–232.
- [7] E. Ronmark, C. Andersson, L. Nystrom, B. Forsberg, B. Jarvholm, B. Lundback, Obesity increases the risk of incident asthma among adults, *Eur. Respir. J.* 25 (2005) 282–288.
- [8] C.A. Camargo, S.T. Weiss, S.M. Zhang, W.C. Willett, F.E. Speizer, Prospective study of body mass index, weight change, and risk of adult-onset asthma in women, *Arch. Intern. Med.* 159 (1999) 2582–2588.
- [9] Y. Chen, D. Rennie, Y. Cormier, J. Dosman, Sex specificity of asthma associated with objectively measured body mass index and waist circumference - the Humboldt study, *Chest* 128 (2005) 3048–3054.
- [10] P. Bustos, H. Amigo, M. Oyarzun, R.J. Rona, Is there a causal relation between obesity and asthma? Evidence from Chile, *Int. J. Obes.* 29 (2005) 804–809.
- [11] B.M. Spiegelman, J.S. Flier, Obesity and the regulation of energy balance, *Cell* 104 (2001) 531–543.
- [12] J. Wu, P. Bostrom, L.M. Sparks, L. Ye, J.H. Choi, A.H. Giang, M. Khandekar, K.A. Virtanen, P. Nuutila, G. Schaart, K. Huang, H. Tu, W.D. van Marken Lichtenbelt, J. Hoeks, S. Enerback, P. Schrauwen, B.M. Spiegelman, Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human, *Cell* 150 (2012) 366–376.
- [13] M. Harms, P. Seale, Brown and beige fat: development, function and therapeutic potential, *Nat. Med.* 19 (2013) 1252–1263.
- [14] E.D. Rosen, B.M. Spiegelman, What we talk about when we talk about fat, *Cell* 156 (2014) 20–44.
- [15] T. Trian, G. Benard, H. Begueret, R. Rossignol, P.O. Girodet, D. Ghosh, O. Ousova, J.M. Vernejoux, R. Marthan, J.M. Tunon-De-Lara, P. Berger, Bronchial smooth muscle remodeling involves calcium-dependent enhanced mitochondrial biogenesis in asthma, *J. Exp. Med.* 204 (2007) 3173–3181.
- [16] S. Kajimura, M. Saito, A new era in brown adipose tissue biology: molecular control of brown fat development and energy homeostasis, *Annu. Rev. Physiol.* 76 (2014) 225–249.
- [17] P.J. Fernandez-Marcos, J. Auwerx, Regulation of PGC-1 $\alpha$ , a nodal regulator of mitochondrial biogenesis, *Am. J. Clin. Nutr.* 93 (2011) 884S–890.
- [18] P. Seale, Transcriptional regulatory circuits controlling brown fat development and activation, *Diabetes* 64 (2015) 2369–2375.
- [19] M. Suwa, H. Nakano, S. Kumagai, Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles, *J. Appl. Physiol.* 95 (2003) 960–968.
- [20] S. Jager, C. Handschin, J. St-Pierre, B.M. Spiegelman, AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1 $\alpha$ , *Proc. Natl. Acad. Sci. U. S. A.* 104 (2007) 12017–12022.
- [21] C. Canto, Z. Gerhart-Hines, J.N. Feige, M. Lagouge, L. Noriega, J.C. Milne, P.J. Elliott, P. Puigserver, J. Auwerx, AMPK regulates energy expenditure by modulating NAD<sup>+</sup> metabolism and SIRT1 activity, *Nature* 458 (2009) 1056–1060.
- [22] D.G. Hardie, F.A. Ross, S.A. Hawley, AMPK: a nutrient and energy sensor that maintains energy homeostasis, *Nat. Rev. Mol. Cell Biol.* 13 (2012) 251–262.
- [23] M. Lagouge, C. Argmann, Z. Gerhart-Hines, H. Meziane, C. Lerin, F. Daussin, N. Messadeg, J. Milne, P. Lambert, P. Elliott, B. Geny, M. Laakso, P. Puigserver, J. Auwerx, Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 $\alpha$ , *Cell* 127 (2006) 1109–1122.
- [24] X. Zhang, Q.X. Zhang, X. Wang, L. Zhang, W. Qu, B. Bao, C.A. Liu, J. Liu, Dietary luteolin activates browning and thermogenesis in mice through an AMPK/PGC1 $\alpha$  pathway-mediated mechanism, *Int. J. Obes.* 40 (2016) 1841–1849.

- [25] J.R. Brestoff, D. Artis, Immune regulation of metabolic homeostasis in health and disease, *Cell* 161 (2015) 146–160.
- [26] G.S. Hotamisligil, P. Arner, J.F. Caro, R.L. Atkinson, B.M. Spiegelman, Increased adipose tissue expression of tumor necrosis factor- $\alpha$  in human obesity and insulin resistance, *J. Clin. Invest.* 95 (1995) 2409–2415.
- [27] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante Jr., Obesity is associated with macrophage accumulation in adipose tissue, *J. Clin. Invest.* 112 (2003) 1796–1808.
- [28] H. Xu, G.T. Barnes, Q. Yang, G. Tan, D. Yang, C.J. Chou, J. Sole, A. Nichols, J.S. Ross, L.A. Tartaglia, H. Chen, Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance, *J. Clin. Invest.* 112 (2003) 1821–1830.
- [29] K.R. Bartemes, K. Iijima, T. Kobayashi, G.M. Kephart, A.N. McKenzie, H. Kita, IL-33-responsive lineage-CD25<sup>+</sup> CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs, *J. Immunol.* 188 (2012) 1503–1513.
- [30] T.Y. Halim, C.A. Steer, L. Matha, M.J. Gold, I. Martinez-Gonzalez, K.M. McNagny, A.N. McKenzie, F. Takei, Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation, *Immunity* 40 (2014) 425–435.
- [31] S.J. van Dyken, A. Mohapatra, J.C. Nussbaum, A.B. Molofsky, E.E. Thornton, S.F. Ziegler, A.N. McKenzie, M.F. Krummel, H.E. Liang, R.M. Locksley, Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and gammadelta T cells, *Immunity* 40 (2014) 414–424.
- [32] W.B. Cherry, J. Yoon, K.R. Bartemes, K. Iijima, H. Kita, A novel IL-1 family cytokine, IL-33, potently activates human eosinophils, *J. Allergy Clin. Immunol.* 121 (2008) 1484–1490.
- [33] R. Chai, B. Liu, F. Qi, The significance of the levels of IL-4, IL-31 and TLSP in patients with asthma and/or rhinitis, *Immunotherapy* 9 (2017) 331–337.
- [34] B.W.S. Li, D. Beerens, M.D. Brem, R.W. Hendriks, Characterization of group 2 innate lymphoid cells in allergic airway inflammation models in the mouse, *Methods Mol. Biol.* 1559 (2017) 169–183.
- [35] B.H. Thuesen, L.L. Husemoen, L.G. Hersoug, C. Pisinger, A. Linneberg, Insulin resistance as a predictor of incident asthma-like symptoms in adults, *Clin. Exp. Allergy* 39 (2009) 700–707.
- [36] J.C. Cardet, S. Ash, T. Kusa, C.A. Camargo Jr., E. Israel, Insulin resistance modifies the association between obesity and current asthma in adults, *Eur. Respir. J.* 48 (2016) 403–410.
- [37] R.B. Bazotte, L.G. Silva, F.P. Schiavon, Insulin resistance in the liver: deficiency or excess of insulin? *Cell Cycle* 13 (2014) 2494–2500.
- [38] S.H. Kim, M.J. Park, Effects of growth hormone on glucose metabolism and insulin resistance in human, *Ann. Pediatr. Endocrinol. Metab.* 22 (2017) 145–152.
- [39] L. Song, H. Tang, D. Liu, J. Song, Y. Wu, S. Qu, Y. Li, The chronic and short-term effects of gefinitib on airway remodeling and inflammation in a mouse model of asthma, *Cell. Physiol. Biochem.* 38 (2016) 194–206.
- [40] X. Ge, C. Bai, J. Yang, G. Lou, Q. Li, R. Chen, Effect of mesenchymal stem cells on inhibiting airway remodeling and airway inflammation in chronic asthma, *J. Cell. Biochem.* 114 (2013) 1595–1605.
- [41] F. Yan, G. Dai, X. Zheng, Mulberry anthocyanin extract ameliorates insulin resistance by regulating PI3K/AKT pathway in HepG2 cells and db/db mice, *J. Nutr. Biochem.* 36 (2016) 68–80.
- [42] H. Spits, D. Artis, M. Colonna, A. Diefenbach, J.P. Di Santo, G. Eberl, S. Koyasu, R.M. Locksley, A.N. McKenzie, R.E. Mebius, F. Powrie, E. Vivier, Innate lymphoid cells—a proposal for uniform nomenclature, *Nat. Rev. Immunol.* 13 (2013) 145–149.
- [43] G.F. Sonnenberg, J. Mjosberg, H. Spits, D. Artis, SnapShot: innate lymphoid cells, *Immunity* 39 (2013) 622.
- [44] R.R. Rao, J.Z. Long, J.P. White, K.J. Svensson, J. Lou, I. Lokurkar, M.P. Jedrychowski, J.L. Ruas, C.D. Wrann, J.C. Lo, D.M. Camera, J. Lachey, S. Gygi, J. Seehra, J.A. Hawley, B.M. Spiegelman, Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis, *Cell* 157 (2014) 1279–1291.
- [45] Y. Qiu, K.D. Nguyen, J.I. Odegaard, X. Cui, X. Tian, R.M. Locksley, R.D. Palmiter, A. Chawla, Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat, *Cell* 157 (2014) 1292–1308.
- [46] Y. Wang, L. Wang, S. Hua, Interleukin-33 in children with asthma: a systematic review and meta-analysis, *Allergol. Immunopathol.* 45 (2017) 387–392.
- [47] Y. Bordon, Immunometabolism. ILC2s skew the fat, *Nat. Rev. Immunol.* 15 (2015) 67.
- [48] H. Wu, X. Deng, Y. Shi, Y. Su, J. Wei, H. Duan, PGC-1 $\alpha$ , glucose metabolism and type 2 diabetes mellitus, *J. Endocrinol.* 229 (2016) R99–R115.
- [49] P. Sivapalan, Z. Diamant, C.S. Ulrik, Obesity and asthma: current knowledge and future needs, *Curr. Opin. Pulm. Med.* 21 (2015) 80–85.
- [50] S.O. Shaheen, J.A. Sterne, S.M. Montgomery, H. Azima, Birth weight, body mass index and asthma in young adults, *Thorax* 54 (1999) 396–402.
- [51] S. Kim, C.A. Camargo, Sex-race differences in the relationship between obesity and asthma: the behavioral risk factor surveillance system, 2000, *Ann. Epidemiol.* 13 (2003) 666–673.
- [52] A. Lusky, V. Barell, F. Lubin, G. Kaplan, V. Layani, Z. Shohat, B. Lev, M. Wiener, Relationship between morbidity and extreme values of body mass index in adolescents, *Int. J. Epidemiol.* 25 (1996) 829–834.
- [53] O.A. Carpaij, M. van den Berge, The asthma-obesity relationship: underlying mechanisms and treatment implications, *Curr. Opin. Pulm. Med.* 24 (2018) 42–49.
- [54] A. Vitali, I. Murano, M.C. Zingaretti, A. Frontini, D. Ricquier, S. Cinti, The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes, *J. Lipid Res.* 53 (2012) 619–629.
- [55] P. Puigserver, Z. Wu, C.W. Park, R. Graves, M. Wright, B.M. Spiegelman, A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis, *Cell* 92 (1998) 829–839.
- [56] P. Seale, B. Bjork, W. Yang, S. Kajimura, S. Chin, S. Kuang, A. Scime, S. Devarakonda, H.M. Conroe, H. Erdjument-Bromage, P. Tempst, M.A. Rudnicki, D.R. Beier, B.M. Spiegelman, PRDM16 controls a brown fat/skeletal muscle switch, *Nature* 454 (2008) 961–967.
- [57] P. Seale, S. Kajimura, W. Yang, S. Chin, L.M. Rohas, M. Uldry, G. Tavernier, D. Langin, B.M. Spiegelman, Transcriptional control of brown fat determination by PRDM16, *Cell Metab.* 6 (2007) 38–54.
- [58] P. Seale, H.M. Conroe, J. Estall, S. Kajimura, A. Frontini, J. Ishibashi, P. Cohen, S. Cinti, B.M. Spiegelman, Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice, *J. Clin. Invest.* 121 (2011) 96–105.
- [59] C. Canto, J. Auwerx, PGC-1 $\alpha$ , SIRT1 and AMPK, an energy sensing network that controls energy expenditure, *Curr. Opin. Lipidol.* 20 (2009) 98–105.
- [60] Y.J. Lee, C. Liu, M. Liao, G.K. Sukhova, J. Shirakawa, M. Abdennour, K. Iamarene, S. Andre, K. Inouye, K. Clement, R.N. Kulkarni, A.S. Banks, P. Libby, G.P. Shi, Deficiency of FcR1 increases body weight gain but improves glucose tolerance in diet-induced obese mice, *Endocrinology* 156 (2015) 4047–4058.
- [61] R.B. Bazotte, L.G. Silva, F.P.M. Schiavon, Insulin resistance in the liver: deficiency or excess of insulin? *Cell Cycle* 13 (2014) 2494–2500.
- [62] M. Kusunoki, K. Tsutsumi, T. Hara, H. Ogawa, T. Nakamura, T. Miyata, F. Sakakibara, Y. Fukuzawa, T. Suga, S. Kakumu, Y. Nakaya, Correlation between lipid and glycogen contents in liver and insulin resistance in high-fat-fed rats treated with the lipoprotein lipase activator NO-1886, *Metabolism* 51 (2002) 792–795.
- [63] R. Upton, P.S. Widdowson, S. Ishii, H. Tanaka, G. Williams, Improved metabolic status and insulin sensitivity in obese fatty (fa/fa) Zucker rats and Zucker Diabetic Fatty (ZDF) rats treated with the thiazolidinedione, MCC-555, *Br. J. Pharmacol.* 125 (1998) 1708–1714.