

High-Fat Diet-Induced Obesity Aggravates Food Allergy by Intestinal Barrier Destruction and Inflammation

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Keywords

Food allergy · Obesity · Intestinal barrier · Intestinal immunity

Abstract

Introduction: The increase in high-fat diet (HFD)-induced obesity and food allergy leads to an assumption that the 2 are related. This study aims to (1) systematic verification of HFD-induced obesity aggravates food allergy and (2) explore the correlation and molecular mechanisms of HFD-induced obesity promotes food allergy. **Methods:** Female BALB/c mice are divided into the control group (control), the ovalbumin (OVA)-sensitized group (OVA), the HFD-induced obesity group (HFD), and HFD-induced allergic obesity group (HFD + OVA). **Results:** In vivo data showed that HFD feed enhance clinical symptoms and intestinal mucosa villi shed on allergic mice. Moreover, we found that HFD and OVA irritation enhanced levels of mast cell degranulation and Th2 humoral response. Additionally, Western blot analysis showed the potentiation of peroxisome proliferator-activated receptor γ (PPAR γ) remarkably reduced on intestinal in HFD and OVA group, thereby inhibiting the expression of nuclear factor kappa B (NF- κ B)/PPAR γ signal the phosphorylation of NF- κ B P65. **Conclusions:** Overall, our results suggest that HFD-induced obesity is a potential risk factor for food allergy, which related to intestinal barrier destruction and inflammation through the PPAR γ /NF- κ B signaling pathway.

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Introduction

In recent years, improved living standards have led to changes in diet composition. The prevalence of obesity has increased significantly [1]; however, increased obesity has also led to an increased incidence in food allergies [2]. The increased risks of obesity make obesity a major global health challenge [3]. Increasing epidemiological evidence suggests that obesity is a potential risk factor for allergic diseases. Obesity also has an important effect on the treatment and prognosis of allergic diseases [4–6]. Food allergy is a common allergic disease that seriously affects quality of life. Symptoms vary from mild abdominal discomfort to life-threatening anaphylaxis [7]. Diet-induced obesity may affect the incidence of food allergies. However, the relationship between these 2 factors remains to be studied.

Obesity was termed “metaflammation,” which can induce the release of pro-inflammatory cytokines and inhibit interferon (IFN)- γ release [8]. Food allergy is characterized by the elevated immunoglobulin E (IgE) levels and the degranulation of mast cells, which is accompanied by the release of histamine and other allergy mediators [9]. Antigen-specific CD4⁺ T cells initiate and regulate IgE production by B cells [10]. The synthesis of IgE is promoted by cytokines such as interleukin-4 (IL-4), interleu-

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kin-5 (IL-5), and tumor necrosis factor- α (TNF- α), whereas IFN- γ inhibit IgE production [11, 12]. Therefore, high-fat diet (HFD) induces obesity may disrupt the balance between Th1/Th2 cytokines and food allergy.

Changes in intestinal barrier function during obesity are a potential link between obesity and food allergies. The sensitization mechanism of food allergens is relevant to intestinal permeability, which has a critical effect on allergen sensitization ability [13, 14]. Recent research has shown that the intestinal ecological imbalance, caused by the destruction of intestinal barrier, may have a role in HFD-induced obesity in mice [15–18].

Food allergy is an inflammatory disease that begins in the gastrointestinal tract and has an important relationship with intestinal inflammation [19]. Obesity weakens the function of the intestinal barrier, which is associated with intestinal inflammation induced by HFD [17, 20]. The composition of the gut microbiota is altered by saturated fatty acids and cholesterol in HFD, which stimulates intestinal epithelial cells to release inflammatory mediators, and directly increases intestinal permeability [21]. Recent studies have demonstrated that HFD-induced intestinal barrier dysfunction has a significant overlap in mechanism in which TNF- α has an important role [22]. TNF- α disrupts the tight junctions of Caco-2 cells, which increase epithelial barrier permeability [23]. Nuclear factor kappa-B (NF- κ B) can induce the production of the TNF- α which has a significant increase in expression with HFD feeding [24]. Research has shown that feeding with HFD for 2 weeks can decrease the transcription factor NF- κ B in intestine, and this process continues until the end of the HFD feeding [20]. A speculated is that the increased expression of NF- κ B induced by the HFD can promote intestinal inflammation by enhancing the production of TNF- α , which destroys the intestinal barrier and thereby increases the incidence of food allergy.

Moreover, mast cells can act as effector cells and release inflammatory mediators when food allergic reactions occur, and thereby cause various gastrointestinal symptoms [25]. Studies have reported that HFD induces an increase in mast cell activation in mice and humans [26]. Peroxisome Proliferator-activated receptor γ (PPAR γ) is expressed in immune cells and regulates the receptors of immune system and inflammation-related genes [27]. It can play an anti-inflammatory role by inhibiting pro-inflammatory genes [28]. PPAR γ has a variety of anti-inflammatory mechanisms, including the inhibition of NF- κ B transcription factors [29]. In inflammatory bowel disease, PPAR γ agonists can improve disease symptoms by inhibiting NF- κ B activation and reducing the expression of the inflammatory cyto-

kine TNF- α in colonic epithelial cells [30]. Therefore, HFD-induced intestinal inflammation may mediate the relationship between obesity and food allergy, and NF- κ B, PPAR γ , and mast cells may be the key factors.

Previous studies on obesity and food allergy have focused on epidemiological evidences [31], diet, and associated gut microbial alterations [32]. How immunological mechanisms and signaling pathway in systemic metabolism induced by obesity affect food allergy has not been reported. Thus, the mechanisms by which obesity increases food allergy are crucial to elucidate. In our study, we established an obesity allergy model in BALB/c mice to study the relationship between obesity and food allergies. We found that obese mice had more severe symptoms of food allergy. Further studies have demonstrated that obesity can promote food allergy by promoting intestinal inflammation in BALB/c mice and destroying intestinal physical barrier. In this study, we investigated whether HFD-induced obesity aggravates food allergy and explored the correlation and molecular mechanisms of HFD-induced obesity that promote food allergy.

Materials and Methods

Animals

In this study, female BALB/c mice (4-week-old) were purchased from Vital River laboratory in Beijing, China, and admitted to the special pathogen-free Animal Laboratory of School of food science and Nutritional Engineering, China Agricultural University (Beijing, China). The animal room was kept at $22 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity, 12 h light/dark cycle, 15 times/h air exchange. All animal experiments were carried out under the scheme approved by the animal experiment welfare and ethics Inspection Committee of China Agricultural University and in accordance with the ethical standard guidelines of China Agricultural University. All the efforts aim to minimize the pain of experimental animals.

Establishment of the Animal Model

After adaptive feeding for a week, according to their body weight, female BALB/c mice were divided into control and HFD group. The control group was fed with normal diet (standard diet [SD]), and the obesity group was fed with HFD. SD and HFD was obtained from Beijing HFK Bioscience Co. In HFD (h10060), 60% of the calories are provided by fat (lard), 20% by protein, and the remaining 20% by carbohydrate. Mice were weighed 8 times a month and maintained on the above diets for 8 weeks. The mice had unlimited access to food and water for 8 weeks. Measured the body length and body mass index (BMI) of BALB/c mice. At the same time, blood samples were collected from the inner canthus vein plexus to determine the levels of triglyceride (TG), leptin, and adiponectin.

According to the results of weight, BMI, hematoxylin-eosin (HE) staining of adipose tissue, particle size distribution of adipocytes, TG, leptin, and adiponectin after 8 weeks of feeding, mice that successfully constructed an obesity model were selected. Ran-

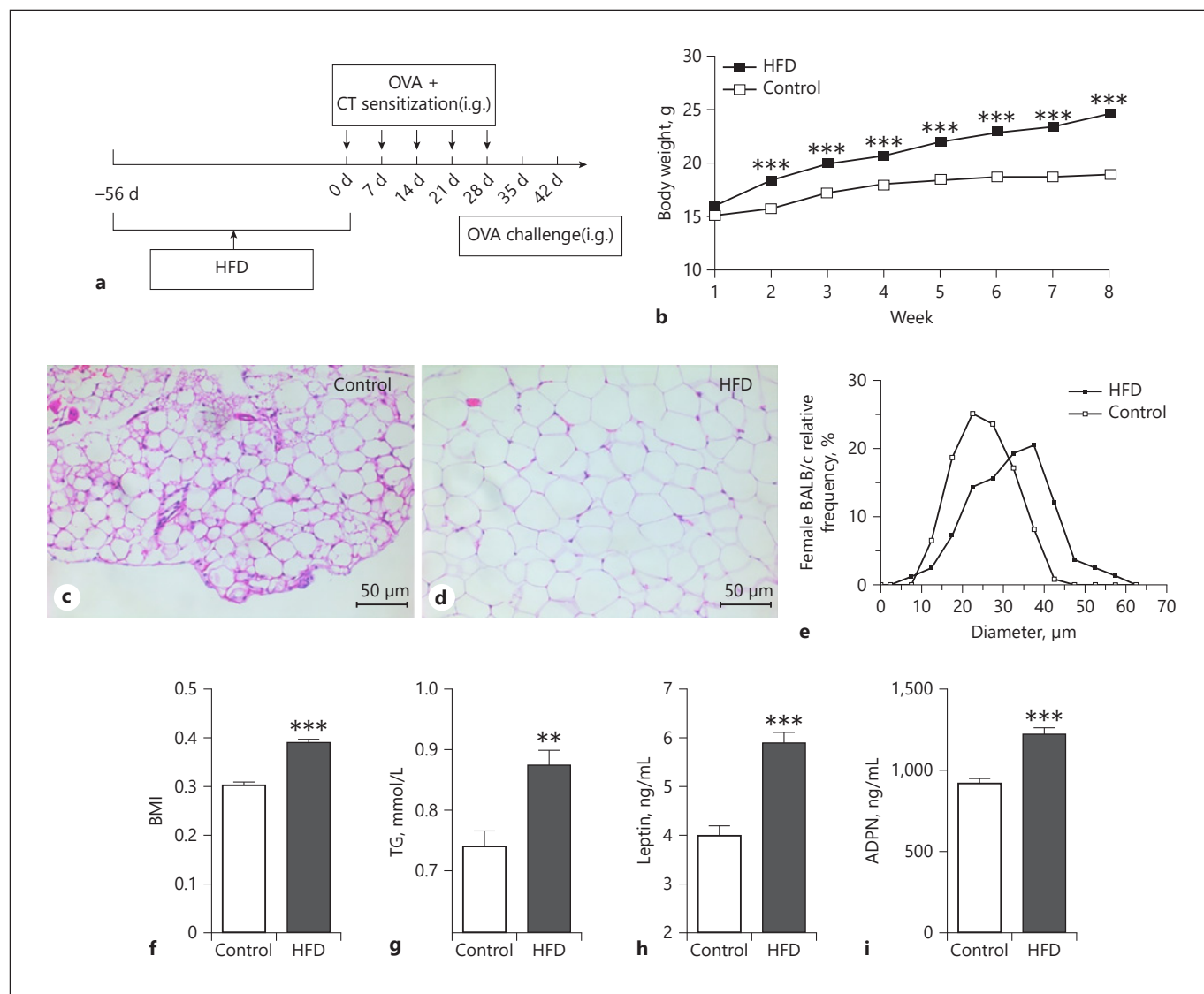


Fig. 1. Establishment of the HFD-induced obesity model. **a** The experimental protocol. **b** Body weight of the BALB/c mice during the SD feeding and HFD feeding. **c** HE staining of visceral adipose tissue of animals fed with SD (scale bar, 50 μ m). **d** HE staining of visceral adipose tissue fed with HFD (scale bar, 50 μ m). **e** Distribution of adipocyte size of visceral adipose tissue, as determined by using histomorphometric analyses. **f** BMI of the BALB/c mice

at the eighth weeks of SD and HFD feeding. **g** The TG levels. **h, i** Adipocytokine levels: leptin levels (**h**) and ADPN levels (**i**). The error bars indicate the mean $\pm$ the standard error of the mean. * p < 0.05 versus the control group, ** p < 0.01 versus the control group, *** p < 0.001 versus the control group. i.g., Intragastric; HFD, high-fat diet; SD, standard diet; HE, hematoxylin-eosin; BMI, body mass index; TG, triglyceride; ADPN, adiponectin.

domly divided into obese group and obese food allergy group according to body weight, 7 animals in each group ($n = 7$). The control group was randomly divided into control group and food allergy group by body weight ($n = 7$). Mice with food allergy were gavaged with ovalbumin (OVA), as shown in Figure 1a. One milligram OVA and 1 mg cholera toxin (Sigma Aldrich, St. Louis, MO, USA) dissolved in 100 μ L saline were given by oral gavage on days 0, 7, 14, 21, and 28, respectively. After fasting overnight on day 42, mice were challenged with 15 mg OVA in 200 μ L saline via oral gavage. The rectal temperature was measured by digital thermom-

eter at 0.5 h after challenge. The scoring criteria of allergic symptoms were as follows: 0, no symptoms; 1, scratching at nose and head; 2, puffiness around eyes and mouth; 3, wheezing, labored respiration, cyanosis around mouth and tail; 4, no activity after stimulation or convulsion; 5, death.

Pathological Section and Mast Cell Staining Analysis

Visceral fat tissues (uterine fat) and intestinal tissues were fixed with 4% paraformaldehyde, then paraffin embedded and stained with HE. Tissues sections were digitalized by using microscope

(DM2500; Laica). Meanwhile, the small intestine mast cells were stained with toluidine blue, and the number of mast cells and the degranulation reaction were observed. The images were obtained from a fluorescent microscope.

ELISA

On the 35th day, the female mice collected serum through the orbital vein and stored at -80°C . Before use, OVA-specific IgE and IgG1 levels were measured by ELISA. Add $100\text{ }\mu\text{g}$ OVA into each well, which dissolved in 0.1 M sodium carbonate ($\text{pH } 9.5$), and incubate the microtitration plate at 4°C overnight. On the second day, the plates were washed 5 times with PBST (0.05% Tween 20/PBS) and incubated with 1% bovine serum albumin in PBS for 1 h. Then, serum samples were added to the plate and incubated at room temperature (RT) for 2 h. The plate was incubated with biotin-labeled antibody (Abcam) in RT for 1 h. Next, washing the plate for 5 times and add TMB substrate solution ($100\text{ }\mu\text{L}$ per/well). Measuring the absorbance at 450 nm . The serum histamine, lepin, adiponectin, and cytokine levels were quantified using the commercial histamine ELISA kit (Neogen Corporation, Lexington, KY, USA). The ELISA analysis mentioned above was carried out according to the manufacturer's protocol.

Immunohistochemistry

For paraffin-embedded small intestinal tissue sections, the antigen was first exposed to 95°C buffer for 10 min. The endogenous peroxidase was then quenched in 3% hydrogen peroxide for 10 min. After blocking with 5% normal goat serum, the sections were cultured overnight in primary antibody at 4°C . Finally, HRP combined with secondary antibody and diaminobenzidine showed the expression and localization of antigen.

Western Blot

Cold RIPA lysis buffer (Beyotime, Shanghai, China) containing a protease inhibitor (BioDee, Beijing, China) was used to homogenize intestinal and splenic tissues to obtain protein extracts. The samples were loaded into 10% 12 sodium sulfate-polyacrylamide gel electrophoresis gel and transferred to poly (vinylidene fluoride 2) film. Then, the membrane was sealed in tris buffer saline tween containing 5% bovine serum albumin for 1 h, gently shaken, and incubated with the primary antibodies of TNF- α , PPAR γ , NF- κ B p65, and phosphorylated p65 (p-p65) at 4°C overnight. The membrane was incubated with horseradish peroxidase bound anti-rabbit IgG secondary antibody at RT for 1 h and then washed again. After treatment with enhanced chemiluminescence system reagent, ImageJ software was used for analysis.

RBL-2H3 Cell-Based Immunological Assay

As described in the previous studies in our laboratory [33], the cells were pre incubated with $1\text{ }\mu\text{g/mL}$ anti-DNP IgE for 2 h. Then, using PBS to wash, ROSI, GW9662, DPN, and PHDTT with final concentration of 0.1 , 1 , or $10\text{ }\mu\text{M}$ were added and incubated for 1 h. Finally, cells were stimulated with $50\text{ }\mu\text{L}$ DNP-human serum albumin (100 ng/mL) at 37°C for 45 min.

After stimulated by DNP-human serum albumin, $30\text{ }\mu\text{L}$ supernatant was transferred to 96-well plate and incubated with $50\text{ }\mu\text{L}$ p-nitrophenyl-n-acetyl- β -D-glucosamine (1.3 mg/mL in 0.1 M citric acid buffer, $\text{pH } 4.5$) at 37°C for 1 h. Then $200\text{ }\mu\text{L}$ termination solution (0.1 M $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$, $\text{pH } 10.0$) was added. The absorbance of each well was measured at 405 nm using a Thermo Science

Varioskan flash microtitration plate reader (Thermo, Waltham, MA, USA). The calculation formula of β -hex is as follows:

$$\beta\text{-hex release (100\%)} = \frac{\text{absorbance of test samples} - \text{absorbance of Tyrode's solution}}{\text{absorbance of total samples} - \text{absorbance of Tyrode's solution}}$$

Statistical Analysis

All data were expressed as the mean $\pm$ standard SEM of 6 independent samples. The statistical significance was determined by one-way ANOVA using Graphpad Prism 5.01 (Graphpad Software Company, San Diego, CA, USA). $p < 0.05$ was considered to be statistically significant.

Results

Establishment of Obesity Model Induced by HFD

The incidence rate of obesity and food allergies is increasing; therefore, we suspect that these processes may be related. Obesity is associated with increased IgE response and allergic symptoms; however, whether obesity is likely to increase the risk of food allergy remains to be tested. To investigate whether a HFD induces obesity and increases the risk of food allergy, female BALB/c mice were fed with HFD for 8 weeks before inducing experimental food allergy.

As shown in Figure 1b, after 8 weeks of HFD consumption, the HFD mice gained 30% more body weight compared to the control mice ($p < 0.001$), which was much higher than the modeling standard of 20% [34]. HE staining showed that the adipocyte size (i.e., diameter) in the HFD group was primarily in the range of $20\text{--}45\text{ }\mu\text{m}$, whereas the adipocyte size in the control group was primarily in the range of $15\text{--}35\text{ }\mu\text{m}$ (shown in Fig. 1c–e). The BMI levels increased in the HFD-fed mice, which were expected (shown in Fig. 1f, $p < 0.001$). TG and serum leptin levels were higher in the HFD group than in the control group (shown in Fig. 1g, h). Significantly increased levels of serum TG in HFD-fed mice enter adipose tissue with blood circulation, thereby resulting in excess energy. On day 0, the leptin level of HFD mice reached 5.90 ng/mL (shown in Fig. 1g). A notable finding was that the serum adiponectin levels did not decrease after 8 weeks of HFD feeding, which was similar to the results of Acín et al. [35]. This finding may be associated with disordered adipose tissue metabolism in obese BALB/c mice (shown in Fig. 1i) [36, 37].

Effects of HFD Induces Obesity on Susceptibility to Food Allergy

Eight weeks after establishing the BALB/c obesity model by using the HFD, the mice were intragastrically

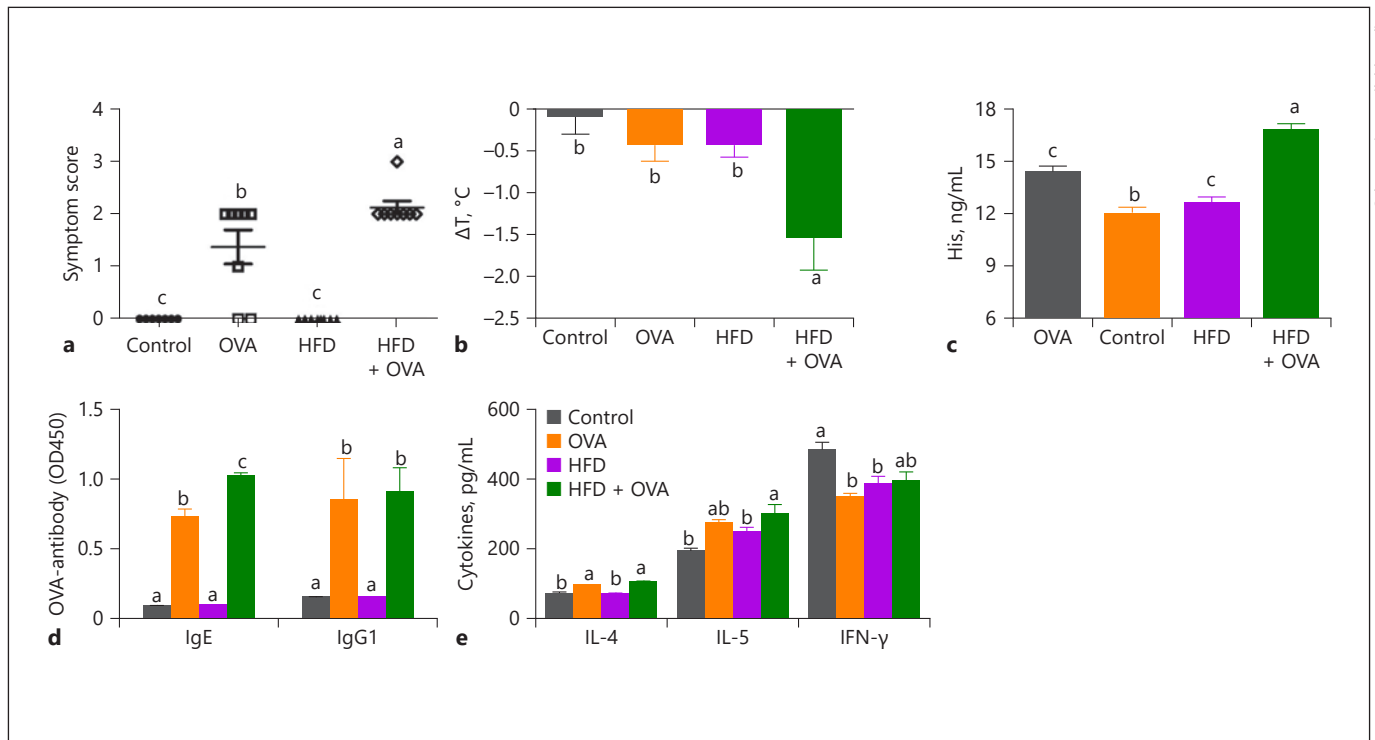


Fig. 2. Effects of HFD induce obesity on susceptibility to food allergy and effects of HFD induce obesity on Th1/Th2 cytokines. **a** Clinical allergy score of control mice and mice with food allergy. **b** Rectal temperature. **c** Histamine levels. **d** OVA-specific IgE and IgG1 levels. **e** Cytokines level of IL-4, IL-5, and IFN- γ . Different

lowercase letter indicates significance differences between groups ($p < 0.05$). The error bars indicate the mean $\pm$ the standard error of the mean. $p < 0.05$, $n = 7$. OVA, ovalbumin; IFN, interferon; IgE, immunoglobulin E; HFD, high-fat diet.

administered OVA to induce food allergy. HFD-fed food allergy mice had higher food allergy scores than did the SD-fed food allergy mice (shown in Fig. 2a). After 42 days, the body temperature of the mice was measured within 30 min after stimulation with OVA. As shown in Figure 2b, the body temperature of OVA mice and HFD-fed OVA mice decreased significantly after the last stimulation. Moreover, weight loss in the HFD-induced allergic mice was 3 times than that of allergic mice. An important finding is that mice with food allergies that were fed the HFD exhibited higher histamine and allergen-specific serum IgE and IgG1 levels than mice fed a SD (shown in Fig. 2c–e). The IgE levels in the serum of the OVA-sensitized mice were significantly increased by the HFD ($p < 0.05$), and the serum IgE and IgG1 levels in OVA-sensitized HFD mice increased by 40.4 and 5.9%, respectively, compared to these levels in the OVA-sensitized mice (shown in Fig. 2d). Thus, our data suggest that long-term HFD feeding increases susceptibility to food allergies.

Effects of HFD Induces Obesity on Th1/Th2 Cytokines

To further explore the effects of the HFD and food allergy on the differentiation of Th1 and Th2 cells in BALB/c mice, we detected Th2-related cytokines (i.e., IL-4 and IL-5) and Th1-related cytokines (i.e., IFN- γ). A striking finding was that, when food allergy was induced in HFD-fed mice, the IL-4 and IL-5 levels increased significantly, compared to their levels in the control animals ($p < 0.05$), whereas IFN- γ was significantly decreased (shown in Fig. 2e, $p < 0.05$). The balance of Th1/Th2 is apparently disrupted, and the immune response is inclined toward the Th2 cell response.

HFD Induces Obesity Promotes Mucosal Mast Cell Accumulation

Given the increase in the food allergy score and the influence of lipid composition on allergic diseases, we hypothesized that effector mast cells in the intestine (especially jejunum) would become dysregulated because of the HFD and food allergy. To verify this hypothesis, we examined the frequency and number of mast cells in the

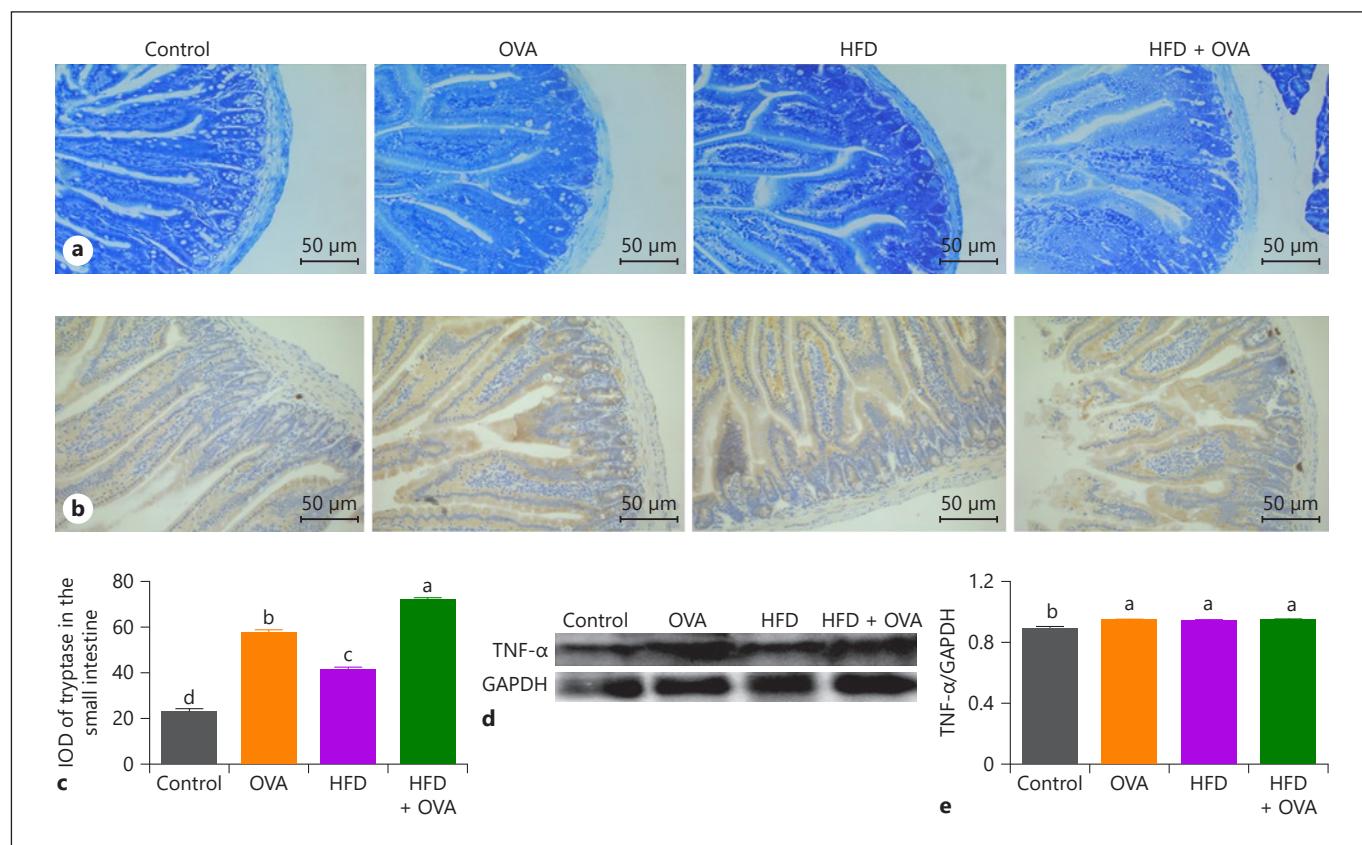


Fig. 3. HFD induces obesity promotes mucosal mast cell accumulation. **a** Toluidine blue staining of the BALB/c small intestine (scale bar, 50 μ m). **b** Immunohistochemical staining of mast cell tryptase of female the BALB/c small intestine (scale bar, 50 μ m). **c** Quantitative analysis of immunohistochemical staining results of

Figure 3b. **d** Western blotting of TNF- α of the BALB/c small intestine. **e** Quantitative analysis of Western blot results of Figure 3d. Different lowercase letter indicates significance differences between groups ($p < 0.05$). The error bars indicate the mean $\pm$ the standard error of the mean. HFD, high-fat diet.

jejunum. The mast cells increased in the HFD-fed nonallergic mice, compared to SD-fed nonallergic mice. Food allergies were remarkable when OVA-sensitized mice were fed an SD or an HFD, and intestinal mast cell accumulation was significantly increased in HFD-fed mice, compared to its accumulation in the control animal (shown in Fig. 3a).

Tryptase is a sign of mast cell activation. As shown in Figure 3b, HFD and OVA stimulation significantly increased the expression of tryptase ($p < 0.05$). The level of tryptase was 24.6% higher in the obese food allergy group than in the food allergy group (shown in Fig. 3b, c). After mast cells are activated by food allergens, mast cells in the intestine degranulate and release inflammatory factor TNF- α , which has a significant role in allergic inflammation [38, 39]. The expression level of TNF- α protein was significantly higher in the small intestine of food allergy group and obesity group than in the control group ($p <$

0.05). This finding indicated that obesity-induced an increase in intestinal inflammatory mediators, similar to food allergy (shown in Fig. 3d, e).

HFD-Induced Obesity Promotes Mechanical Barrier Destruction

Previous studies have shown that HFD-induced intestinal barrier dysfunction and inflammatory bowel disease-induced barrier dysfunction have a mechanism overlap. Thus, obesity may also promote food allergy by damaging intestinal mechanical barrier function. We found mucosal erosion and villi shedding in HFD-fed mice (shown in Fig. 4a). The change in intestinal barrier integrity in HFD-fed food allergic mice is associated with a decrease in Diamine oxidase (Dao) expression level in serum (shown in Fig. 4c) and tight junction protein 1 (ZO-1) (shown in Fig. 4b, d). Food allergy resulted in a more significant reduction level of ZO-1 pro-

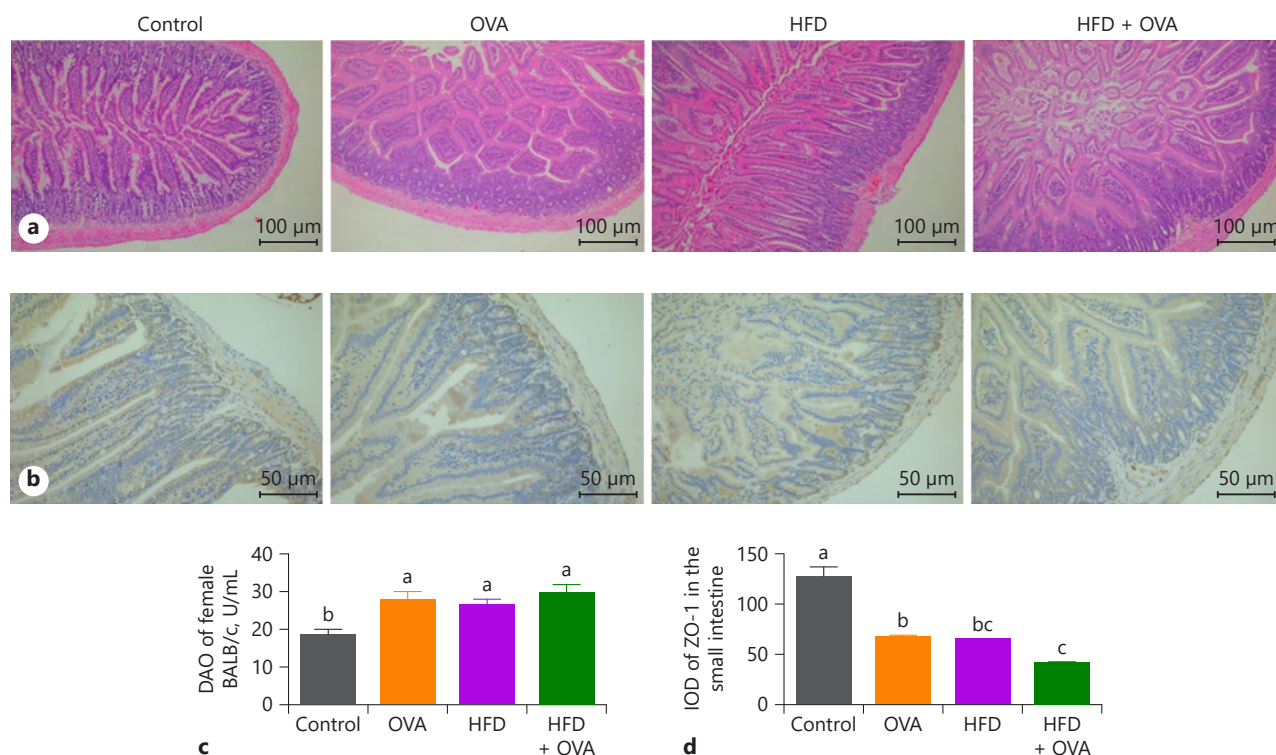


Fig. 4. HFD-induced obesity promotes mechanical barrier destruction. **a** HE staining of BALB/c small intestine (scale bar, 100 μ m). **b** Immunohistochemical staining of ZO-1 of small intestine (scale bar, 50 μ m). **c** DAO level of the BALB/c mice. **d** Quantitative analysis of the immunohistochemical staining results of Figure 4b. Different lowercase letter indicates significance differences between groups ($p < 0.05$). The error bars indicate the mean $\pm$ the standard error of the mean. DAO, diamine oxidase.

tein. Compared to the ZO-1 level in food allergic mice, the ZO-1 level of HFD-induced food allergy mice was decreased by 39.6% (shown in Fig. 4d). Thus, obesity promotes mucosal mast cell accumulation and mechanical barrier destruction, which may aggravate food allergies by increasing intestinal permeability to food allergens.

HFD-Induced Obesity Promotes Food Allergy by Changing PPAR γ /p65 Complex and Reducing NF- κ B Signal Transduction

Owing to the pro-inflammatory effect of NF- κ B in obesity-induced intestinal inflammation and the regulatory effect of PPAR γ on intestinal inflammation by inhibiting NF- κ B [40, 41], we evaluated the expression levels of PPAR γ and NF- κ B in intestine of the HFD mice and the control mice. P65 is one of the important members of NF- κ B transcription factor family [42], so the ex-

pression of NF- κ B was evaluated by measuring the level of p65.

The expression of intestinal PPAR γ was downregulated in the HFD mice and OVA mice (shown in Fig. 5a, b). In these groups, NF- κ B signaling was activated and the intestinal NF- κ B p65 expression was significantly increased (shown in Fig. 5a, b). In addition, the expression of NF- κ B p65 in allergic mice fed the HFD was 8.9% higher than that in nonallergic mice fed the HFD. To further prove whether the mechanism of obesity-induced intestinal inflammation is related to the activation of NF- κ B, the phosphorylation level of p65 (i.e., p-p65) in the intestine of mice was determined. Obesity significantly increased the expression of p-p65 in the intestine of female mice, which indicated that obesity promotes the activation of NF- κ B in the intestine of female mice, which may induce intestinal inflammation and increase the risk of food allergy (shown in Fig. 5c, d). The expression levels

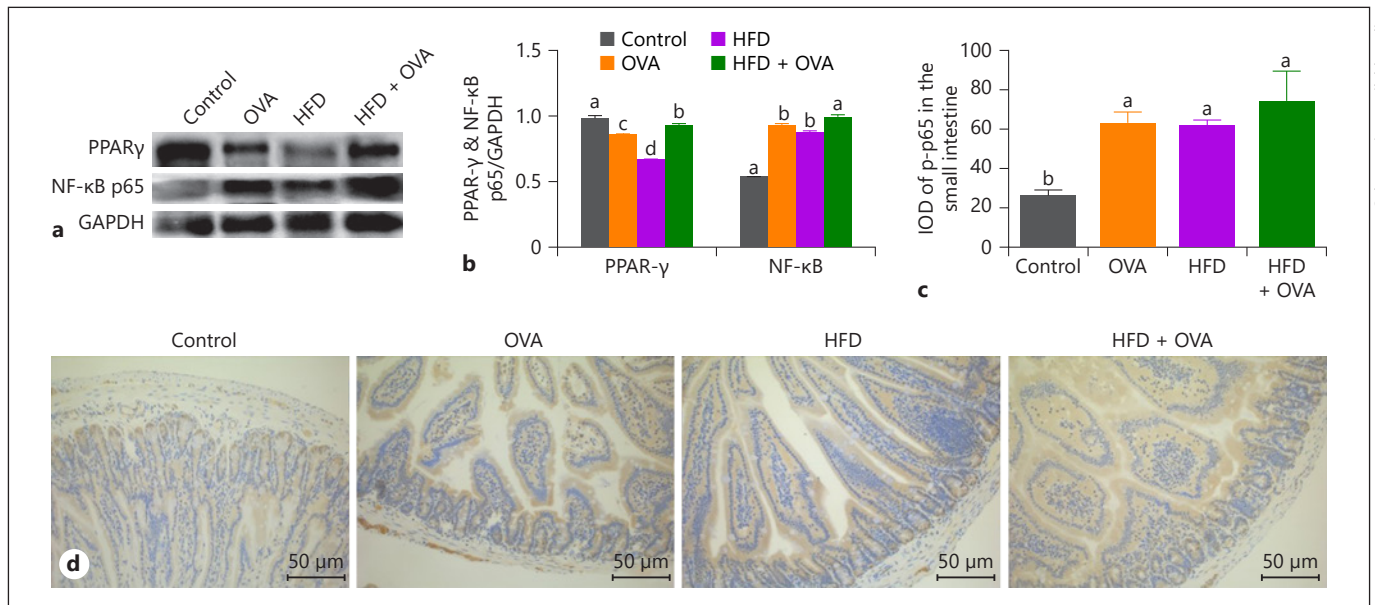


Fig. 5. HFD-induced obesity promotes food allergy by changing PPAR γ and NF- κ B signal transduction. **a** Western blotting for PPAR γ and NF- κ B p65 in the BALB/c small intestine. **b** Quantitative analysis of PPAR γ and NF- κ B p65 immunoblotting results. **c** Quantitative analysis of the immunohistochemical staining results of Figure 5d. **d** Immunohistochemical staining of p-p65 of the

female BALB/c small intestine. Different lowercase letter indicates significance differences between groups ($p < 0.05$). The error bars indicate the mean $\pm$ the standard error of the mean. HFD, high-fat diet; PPAR γ , peroxisome proliferator-activated receptor γ ; NF- κ B, nuclear factor kappa-B.

of PPAR γ , NF- κ B p65, and p-p65 in spleen reflect the mechanism of systemic inflammation, and the results were consistent with those in intestine (shown in Fig. 6a–d).

PPAR γ Inhibited the Release of β -Hex from RBL-2H3 Cells

To further verify the function of PPAR γ on food allergy, we use a PPAR γ agonist (ROSI) and a PPAR γ inhibitor (GW9662) to explore the effect on the release of β -hex from RBL-2H3 cells stimulated. By using a formula, β -hex release by ROSI and GW9662 was calculated (Table 1). As shown in Table 1, the release rates of β -hex of RBL-2H3 cells decreased to 48.2, 39.9 or 35.9% after treating the cells with 0.1, 1 or 10 μ M/L, respectively, of the ROSI extract. GW9662 treatment significantly promoted the release of β -hex. After treated with 0.1, 1, or 10 μ M/L of GW9662, the maximum release of β -hex, 162.1%, occurred with 10 μ M/L GW9662 (Table 1). Thus, the inhibition of PPAR γ promoted mast cell degranulation, which indicated that the effect of obesity on PPAR γ expression is the possible that obesity promotes intestinal inflammation and increases the risk of food allergy.

Discussion/Conclusion

HFD-induced obesity is a common method used to construct animal models obesity. By referring to studies Hussain et al. [43] and Tomas et al. [44], we successfully induced obesity in mice by feeding them the HFD for 8 weeks. Body weight, BMI, physiological and metabolic changes in adipose tissue showed that the mice had obesity characteristics. The negative effects of obesity on the immune system led to the mice being susceptible to food allergies. Previous studies have found that obesity can affect the accumulation of immune cells in adipose tissue, thereby making the body in a state of chronic low-grade inflammation, which is associated with food allergy [8]. In the present study, obesity increased mast cell activation, which released more histamine, and led to more severe food allergy symptoms with OVA stimulation. Obesity significantly increased serum TG level. Medium-chain fatty acids produced by TG reduce the amount of allergens entering the blood; however, they increase the number of allergens entering the Peyer's lymph nodes, which may increase the presentation of intestinal dendritic cells to allergens [2].

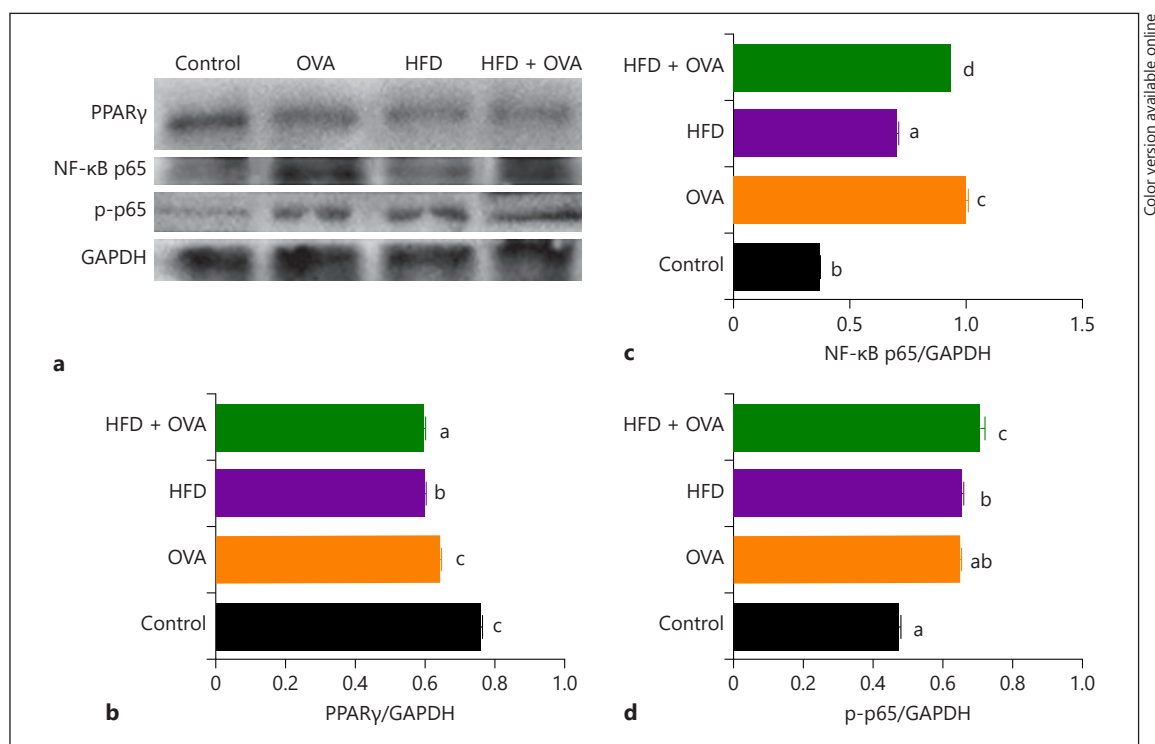


Fig. 6. Western blotting of PPAR γ , NF- κ B p65, p-p65 in spleen. **a** Western blotting of PPAR γ , NF- κ B p65, p-p65 in spleen. **b** Quantitative analysis of PPAR γ (see online suppl. Fig. 6b; see www.karger.com/doi/10.1159/000517866 for all online suppl. material). **c** Quantitative analysis of NF- κ B p65 (online suppl. Fig. 6c).

d Quantitative analysis of p-p65 (online suppl. Fig. 6d). Lowercase letters indicate significant differences between groups. The error bars indicate the mean $\pm$ the standard error of the mean. PPAR γ , peroxisome proliferator-activated receptor γ .

Table 1. HFD-induced obesity inhibited the release of β -hex from RBL-2H3 cells

	Control	ROSI			Gw9662		
		0.1 μ M	1 μ M	10 μ M	0.1 μ M	1 μ M	10 μ M
Release rate, %	100	51.8	60.1*	64.1**	115.5	147.3*	162.1

ROSI and GW9662 release rate of β -hex in RBL-2H3. * $p < 0.05$ versus control, ** $p < 0.01$ versus control, *** $p < 0.001$ versus control. HFD, high-fat diet.

HFD-induced obesity can also affect food allergies by affecting the metabolic homeostasis of adipose tissue. Adipose tissue, as an important endocrine organ, can balance the secretion of pro-inflammatory factor leptin and anti-inflammatory factor adiponectin under normal conditions. When adipose tissue expansion leads to adipocyte dysfunction (e.g., overnutrition or lack of physical activity), the imbalance of adipokines may have local or systemic effects on the inflammatory response and lead to inflammation-related pathological processes [36]. In the present study, leptin levels in HFD-induced obese mice were significantly increased. This change may de-

crease immune tolerance to allergens and promote food allergy. A speculation is that this finding may be related to a disorder of adipose tissue metabolism in obese BALB/c mice, and the difference between human and mouse strains [36, 37].

In this study, we successfully established a BALB/c mouse model of obesity. Compared to the control group mice, the obesity mice were more susceptible to OVA and had more severe food allergy symptoms. Vieira et al. [45] found that the frequency of the positive rate of specific IgE is 3 times higher in obese people than in normal people. Hussain et al. [32] reported that high dietary fat

intake promotes food allergies. This finding may be associated with the impact of HFD [46]. First, lard is the main energy-supplying lipid in HFD. Long-chain TGs are one of the main components in lard, which can promote the secretion of chylomicrons [46]. Chylomicrons combine with dietary allergens to protect them from decomposition, thereby increasing the absorption of allergens. When more complexes are captured by macrophages, the immune response inclines to the Th2 cell response, thereby promoting food allergy [46, 47]. In addition, obesity decreases the Th1-type immune response. However, obesity had no effect on the Th2-type immune response in female mice. Therefore, obesity leads to food allergies in mice by disrupting Th1/Th2 balance.

Similar to food allergy, obesity-induced enteritis can increase the accumulation and activation of intestinal mast cells in mice. This finding confirms conclusion in a previous study [26] that HFD can increase the activation level and quantity of mast cells. Mast cells are effector cells in food allergies, which can release inflammatory mediators and cause gastrointestinal symptoms [48]. In OVA-induced food allergy model, the number of mast cells in colonic mucosa of mice increased [49]. The increase of tryptase induced by HFD can lead to mast cells being more prone to degranulation during OVA stimulation, and thereby promote food allergy. However, it can also activate protease-activated receptor-2 and increase intestinal epithelial permeability [50], thereby increasing the susceptibility to food allergy. Moreover, obesity also increased the expression of TNF- α released by mast cells in the small intestine of mice and promoted intestinal inflammation. These findings are consistent with those of Lam et al. [24]. Studies [23] have shown that TNF- α participates in the pathogenesis of allergic inflammation by destroying the tight junction of Caco-2 cells and increasing the permeability of epithelial barrier. Some studies [15, 18] have also demonstrated that obesity can reduce the expression of tight junction proteins and increase intestinal permeability, and resulting in impaired intestinal barrier function. The sensitization mechanism of food allergens is closely associated with the function of the intestinal mechanical barrier [13, 14]. Therefore, in obese mice, the destruction of the intestinal mechanical barrier leads to OVA pass through the intestine more easily, thereby causing a food allergy reaction. Our results indicate that obesity can increase inflammatory cytokines, which damage the integrity of intestinal mechanical barrier. The reason has been discussed in previous studies:

the intake of saturated fatty acids and cholesterol from the HFD can change the composition of intestinal microbiota and lead to biosynthesis disorders [22]. The imbalance in intestinal flora reduces the types of bacteria that produce anti-inflammatory metabolites, thereby promoting the inflammatory and immune changes in the gut. Therefore, the damage to the mechanical barrier will further promote inflammation.

HFD-induced obesity promotes intestinal and systemic inflammation because of the decrease in PPAR γ and increases in NF- κ B in intestine and spleen. The PPAR γ transcription level in peripheral blood mononuclear cells of obese patients consuming the HFD is negatively correlated with an increase in plasma TG concentration [51]. Therefore, the increase in TG levels in HFD-fed mice may be the reason for the decrease in PPAR γ expression. The activation of PPAR γ may have an anti-inflammatory effect on Th2-mediated diseases. Natural and synthetic PPAR γ agonists significantly inhibit the production of IL-4 in T cells [52], and PPAR γ agonists reduced the levels of IL-4, IL-5, and IL-13 in vitro and the differentiation of Th2 cells by reducing the expression of Th2-specific transcription factors [53]. PPAR γ can also affect B-cell proliferation, activation, and antibody production by regulating T-cell function. The decreased expression of PPAR γ increases the proliferation of T cells and the production of IFN- γ [54]. These cytokines can directly affect the formation, proliferation, and antibody production of plasma cells [55]. In addition, PPAR γ is expressed in mast cells exposed to food allergy [56], and PPAR γ agonists can reduce the phenotypic markers and viability of mast cells, inhibit degranulation, and induce apoptosis [57]. Therefore, the decrease in PPAR γ expression in the mice small intestine and spleen, induced by obesity, may also increase the food allergy's risk by promoting immune cell inflammation-related to food allergy. PPAR γ , a regulatory factors in intestinal inflammation, has decreased expression in intestinal epithelial cells of patients with inflammatory bowel disease [58], and its agonist can improve disease symptoms by inhibiting the expression of NF- κ B and reduce the expression of inflammatory cytokine TNF- α in colonic epithelial cells [59]. In addition, PPAR γ also has an anti-inflammatory effect on immune cells and is the inhibition of NF- κ B [60]. Therefore, the decrease in PPAR γ induced by obesity indirectly promotes the activation of NF- κ B, which may be the cause of intestinal and splenic inflammation. PPAR γ expression and activation can also induce adipogenesis [61]. The results of this study did not completely confirm that the effect of HFD-

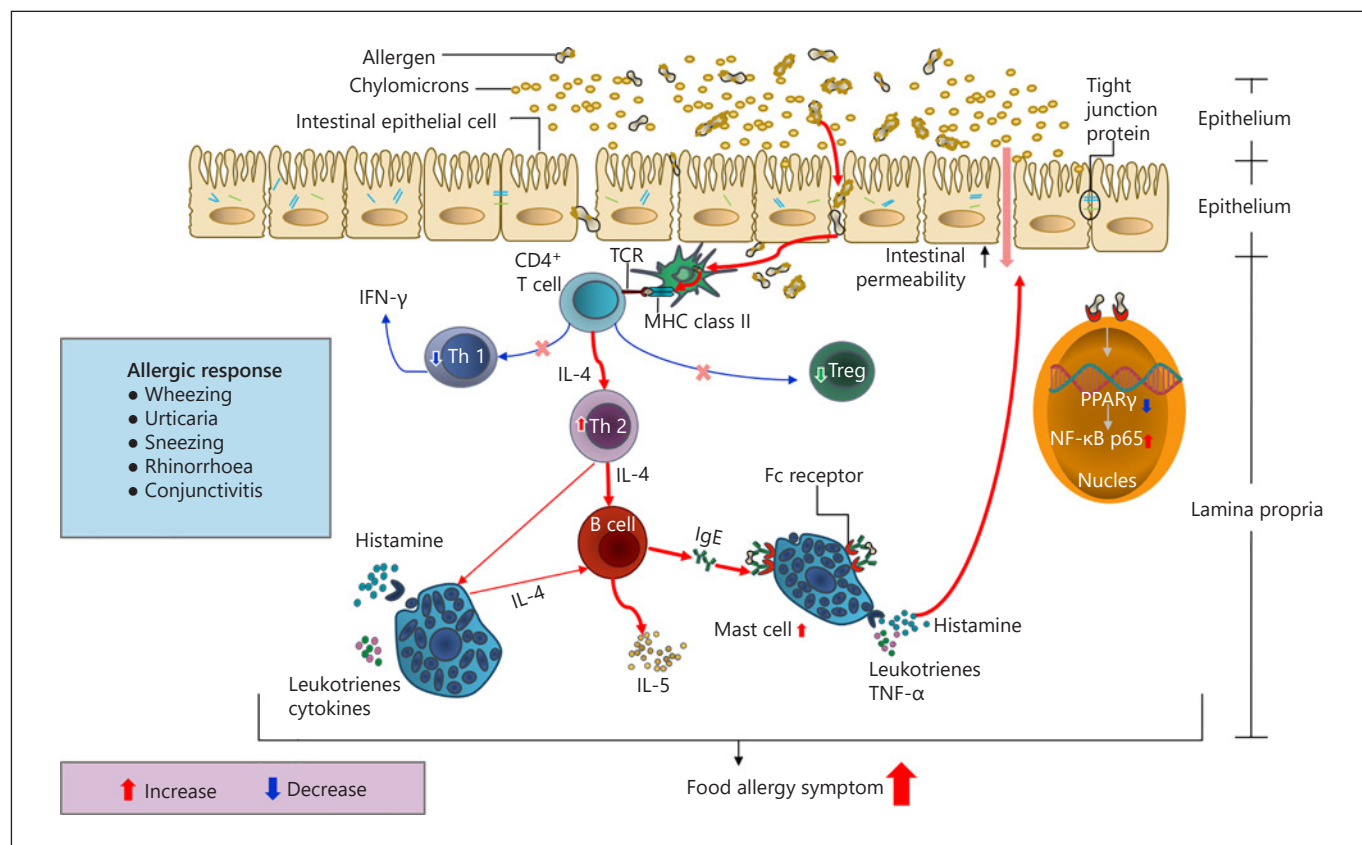


Fig. 7. A model of mechanism underlying the HFD induces obesity on allergy. Obesity through PPAR- γ /NF- κ B signaling pathway promotes the combination of food allergens and chylomicrons into the body, promotes the direct uptake of dendritic cells, induces more mast cell activation and more IL-4, IL-5, and TNF- α secretion, which leads to more severe allergic symptoms. TNF- α , tumor necrosis factor alpha; IL-4, interleukin-4; IL-5, interleukin-5; HFD, high-fat diet; NF- κ B, nuclear factor kappa-B.

induced obesity on food allergy is only associated with the anti-inflammatory effect of PPAR γ , but not associated with its effect on inducing fat formation.

RBL-2H3 cells are rat basophil leukemia cells, which have the characteristics of stable passage, response to antigen stimulation and the release of inflammatory factors [62]. RBL-2H3 cells are often studied for degranulation because their function is similar to that of mast cells [63]. The results of the cell experiment are consistent with those in vivo; thus, HFD-induced obesity may promote food allergy by decreasing the expression of PPAR γ , inducing intestinal inflammation, and further inducing systemic inflammation.

Based on the results of this article, we propose a potential mechanism model HFD induces obesity on allergy as shown in Figure 7. Obesity through PPAR- γ /NF- κ B signaling pathway promotes the combination of food allergens and chylomicrons into the body, promotes the direct

uptake of dendritic cells, induces more mast cell activation and more IL-4, IL-5, and TNF- α secretion, which leads to more severe allergic symptoms.

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Statement of Ethics

Animal experiments in our research were carried out in accordance with the Guide for the Animal Experimental Welfare and Ethical in the Food Science and Nutritional Engineering College

of China Agricultural University and were approved by the Animal Experimental Welfare and Ethical Inspection Committee in China Agricultural University. We promise all animal experiments comply with the ARRIVE guidelines and be carried out in accordance with the in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines. All efforts were made during the animal experiments to minimize suffering and to reduce the number of animals used.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

YanJun Gu and Huilian Che contributed to conceptualization; YanJun Gu and Xiaoya Guo contributed to data curation; Huilian Che contributed to funding acquisition; YanJun Gu, Xiaoya Guo, ShanFeng Sun, and Huilian Che contributed to methodology; YanJun Gu and Huilian Che contributed to writing – original draft; YanJun Gu, Xiaoya Guo, ShanFeng Sun, and Huilian Che contributed to writing – review and editing.

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