Nutrition 32 (2016) 273–280

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Immunologic and metabolic effects of high-refined carbohydrate-containing diet in food allergic mice

Letícia Tamie Paiva Yamada Ph.D. [a], Marina Chaves de Oliveira M.Sc. [b,c], Nathália Vieira Batista M.Sc. [c,d], Roberta Cristelli Fonseca B.B.I. [d], Rafaela Vaz Sousa Pereira B.B.I. [d], Denise Alves Perez M.Sc. [d], Mauro Martins Teixeira Ph.D. [c], Denise Carmona Cara Ph.D. [d,†], Adaliene Versiani Matos Ferreira Ph.D. [b,c,*,†]

[a] Faculty of Nutrition, Universidade Federal de Alfenas, Alfenas, Brazil
[b] Department of Nutrition, Nursing School, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[c] Immunopharmacology, Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[d] Department of Morphology, Biological Sciences Institute, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

ARTICLE INFO

Article history:
Received 13 May 2015
Accepted 14 August 2015

Keywords:
Food allergy
High-carbohydrate diet
Gut
Adipose tissue
Metabolism
Inflammation

ABSTRACT

Objective: Allergic mice show a reduction in body weight and adiposity with a higher inflammatory response in the adipose tissue similar to obese fat tissue. This study aimed to evaluate whether the low-grade inflammatory milieu of mice with diet-induced mild obesity interferes with the allergic response induced by ovalbumin (OVA).
Methods: BALB/c mice were divided into four groups: 1) non-allergic (OVA−) mice fed chow diet, 2) allergic (OVA+) mice fed chow diet, 3) OVA− mice fed high-refined carbohydrate-containing (HC) diet, and 4) OVA+ mice fed HC diet. After 5 wk, allergic groups were sensitized with OVA and received a booster 14 d later. All groups received an oral OVA challenge 7 d after the booster.
Results: Allergic groups showed increased serum levels of total IgE, anti-OVA IgE, and IgG1; a high disease activity index score; aversion to OVA; and increased intestinal eosinophil infiltration. Non-allergic mild-obese mice also showed aversion to OVA and an increased number of eosinophils in the proximal jejunum. After the allergic challenge, OVA+ mice fed chow diet showed weight loss and lower adiposity in several adipose tissue depots. OVA+ mice fed HC diet showed a loss of fat mass only in the mesenteric adipose tissue. Furthermore, increased levels of TNF, IL-6, and IL-10 were observed in this tissue.
Conclusions: Our data show that mild-obese allergic mice do not present severe pathologic features of food allergy similar to those exhibited by lean allergic mice. Mild obesity promoted by HC diet ingestion causes important intestinal disorders that appear to modulate the inflammatory response during the antigen challenge.

Introduction

Food allergy can affect the intestinal mucosa and cause local inflammation [1]. The main food allergy treatment is to avoid the food with the allergen that has a negative impact on the quality of life [2]. Despite great advances in our understanding of mucosal immunology, the causes underlying the increased rates of allergy remain unknown. Complex factors, such as environmental factors and modern lifestyle, mainly associated with the incidence of obesity, can initiate an allergic process [2,3].

Obesity is a risk factor for the development of allergic reactions [3]. Such an association may be related to the chronic low-grade inflammatory feature of obesity [4,5]. It has been demonstrated that the frequency of specific positive IgE levels in obese individuals is threefold higher than that found in non-obese [6]. According to Hancox et al. [7], 28% of patients with allergic asthma exhibit a positive correlation with overweight and obesity. Obesity may contribute to the increased

* Corresponding author. Tel./fax: +55 31 3409 8028.
E-mail address: adaliene@gmail.com (A. V. M. Ferreira).
† Co-senior authors.

http://dx.doi.org/10.1016/j.nut.2015.08.011
0899-9007/

prevalence of allergic diseases, particularly food allergy, because of systemic inflammation [8].

A study on patients with asthma found that weight loss lessened the symptoms of asthma, independent of the IgE levels, by modulating the action of T cells and thus the profile of proinflammatory cytokines [9]. Leptin, an adipocytokine that increases during obesity, increased twofold in overweight children with asthma compared with overweight children without asthma [10]. Other important cytokines in the inflammation process, such as TNF, IL-6, and eotaxin, are also modified in allergic individuals with obesity [9,11]. Despite strong evidence supporting obesity as a risk factor for allergy, this relationship remains to be clarified because of controversial results [12,13]. Researchers found no strong correlation between atopy or asthma and obesity in children [14]. Sidoroff et al. [15] concluded that a previous or current overweight state does not increase the risk of asthma or allergy in school-aged children but obesity may decrease allergic risk after bronchiolitis in infancy.

Our group has reported that allergic mice, despite having a reduction in body weight and adiposity, show a higher inflammatory response in the adipose tissue and a metabolic dysfunction [16,17]. This phenotype is quite similar to the adipose tissue inflammation observed in obese mice [4,18,19]. In fact, our group has previously found that a high-refined carbohydrate-containing (HC) diet increases adiposity without significant differences in overall body weight, causing mild obesity. In this model, the fat mass expansion is accompanied by an early induction of adipose tissue inflammation and metabolic dysfunction. On the basis of these results, the aim of the present study was to evaluate whether the low inflammatory milieu of mild-obese mice interferes with the allergy response induced by ovalbumin (OVA). In the present study, mild obesity promoted by the HC diet does not exacerbate the immunologic and metabolic response in allergic mice.

Materials and methods

Male BALB/c mice at 4 to 5 wk of age were obtained from our animal care center (Centro de Bioterismo [CEBIO]/Universidade Federal de Minas Gerais [UFMG]) and maintained in an environmental-controlled room under a 14-h light/10-h dark cycle. The animals had free access to food and tap water, and were maintained in accordance with the guidelines of the Ethics Committee on Animal Use of our institution (Comissão de Ética no Uso de Animais [CEUA]/UFMG Protocols 060/2010 and 299/2007). The mice were fed standard chow (Labina) or HC diet for 8 wk as described previously [4]. The HC diet contained at least 30% refined sugars, mostly sucrose. The animals were divided into four groups: 1) non-allergic (OVA−) mice fed chow diet, 2) allergic (OVA+) mice fed chow diet, 3) OVA− mice fed HC diet, and 4) OVA+ mice fed HC diet.

The weight of the mice was measured once a week and the food consumption was assessed twice a week. During the week of the oral challenge (eighth to ninth week), the weight was measured on the first day and 7 d after the antigen challenge (see experimental design in Fig. 1). At the end of the treatment, the animals were anesthetized with ketamine (130 mg/kg) and xylazine (0.3 mg/kg), and euthanized.

Mice sensitization

After 5 wk of continuous intake of different diets, the animals were subjected to the allergy protocol (Fig. 1). For sensitization, the OVA− mice fed with chow or HC diet received a subcutaneous injection of 0.2 mL of saline (0.9%) and adjuvant [1 mg of $Al(OH)_3$]. OVA+ groups received 0.2 mL of saline (0.9%), adjuvant [1 mg of $Al(OH)_3$], and 10 μg of OVA (five-times-crystallized Hen's egg albumin; Sigma, St. Louis, MO, USA). Fourteen days after the sensitization, the OVA− groups received a subcutaneous injection with only 0.2 mL of saline (0.9%), and allergic mice received a booster of 0.2 mL of saline (0.9%) and 10 μg of soluble OVA. One week after the administration of the booster, the oral challenge was conducted. For all groups, drink water was replaced with a 20% OVA solution for 1 wk and quantified each day to verify aversion. This solution was prepared using a lyophilized egg white (Salto's, Belo Horizonte, Brazil) [2,20].

Disease activity index score

For the assessment of the disease activity index (DAI) score [16], body weight and stool were scored every day after antigen challenge as follows: body weight score (0 = no weight loss; 1 = 1–5% weight loss; 2 = 6–10% weight loss; 3 = 11–15% weight loss; 4 = >15% weight loss); stool viscosity (0 = normal; 2 = fluffy; 3 = pasty and liquid consistency; 4 = diarrhea); and stool occult blood (0 = normal; 1 = positive fecal blood; 2 = hemorrhage) by Hexagon OBScreen kit (Human GmbH, Wiesbaden, Germany).

Evaluation of serum total IgE, anti-OVA IgE, and IgG1

Total IgE, anti-OVA IgE, and IgG1 levels were measured by capture-ELISA using plates coated with rat antimouse IgE or IgG1, 50 μL of serum, and

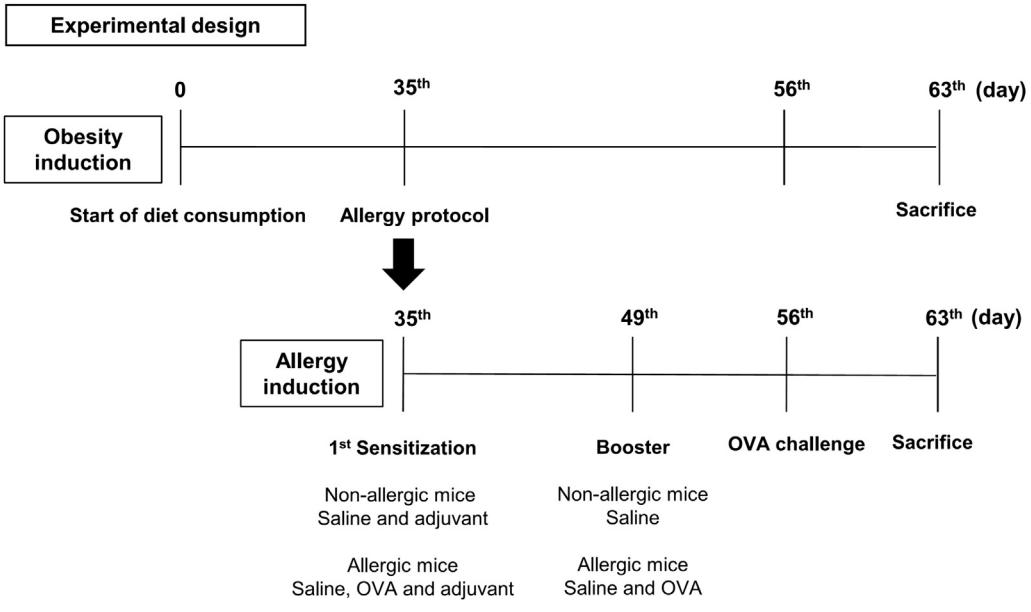

Fig. 1. Experimental design of obesity and food allergy induced by antigen in mice. According to the obesity protocol, the mice were fed chow or HC diet for 63 d. After 35 d, allergy induction was initiated through two immunizations. The challenge was performed through OVA ingestion. HC, high-refined carbohydrate-containing; OVA, ovalbumin.

biotinylated OVA, as described previously [21]. The results for both antibodies are reported in arbitrary units (A.U.) using a positive reference serum (1000 A.U).

Intravital microscopy observation of adipose tissue microvasculature

Intravital microscopy was performed for the epididymal and mesenteric adipose tissue microcirculation. Briefly, mice were anesthetized with xylazine 10 mg/kg and ketamine hydrochloride i.p. 100 mg/kg. The adipose tissue was exposed, and Rhodamine 6G (Sigma) was injected intravenously (0.15 mg/kg) to observe the leukocyte–endothelial cell interactions. The microscopy images were captured using a Nikon Eclipse 50i (Nikon Instruments Inc., Tokyo, Japan) microscope (×20 objective) with a video camera (5100HS; Panasonic, Secaucus, NJ, USA) and recorded digitally using both filter blocks consecutively. A leukocyte was considered to be adherent if it remained stationary for at least 30 s, and the total leukocyte adhesion was quantified as the number of adherent cells within a 100-μm length of the venule; these data were expressed as cells/100 μm.

Histologic evaluation of tissues

Tissues from the mice were fixed in 4% neutral formalin, dehydrated in absolute ethanol, cleared in xylene, and then embedded in paraffin. The histologic sections (5 μm) were stained with hematoxylin–eosin, and the proximal jejunum was stained with periodic acid–Schiff (PAS) for mucus analysis. The sections were analyzed using a microscope (Olympus BX41) equipped with a digital camera (Moticam 2500). Ten fields of the gut tissue were randomly chosen from each animal and the images (40×) were captured for quantification of the eosinophil infiltration, and the data were reported as number of eosinophils per field. For mucus analysis, three sections of the jejunum stained with PAS were subjected to morphometric analysis using an image analysis program running on an IBM computer. Images were obtained at 40×; for the determination of goblet cell volume, all pixels with green hues were selected for the creation of a binary image and subsequent calculation of the total area; data were reported as % PAS/field. For the quantification of adipocyte size, a cell area of 50 adipocytes per animal was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Determination of serum parameters

Fasting glucose, cholesterol, and triacylglycerol levels were assayed using enzymatic kits (Katal, Belo Horizonte, Brazil). The fasting serum levels of adiponectin and resistin were determined by ELISA (R&D Systems Europe Ltd., Abington, UK).

Total and differential blood cell counts

Blood was collected from the tail vein of mice, and the total white blood cells were counted using a Neubauer chamber. Peripheral blood smears were stained with May-Grünwald Giemsa staining, and the differential white blood cell count was determined under oil immersion (1000×).

Oral glucose tolerance and insulin sensitivity tests

For the oral glucose tolerance test, D-glucose (2 mg/g body weight) was administered orally to mice that were fasted overnight; this test was performed on the ninth week. The glucose levels were monitored using an Accu-Check glucometer (Roche Diagnostics, Indianapolis, IN, USA) in tail blood samples collected 0, 15, 30, 60, and 90 min after glucose overload.

ELISA assay

The levels of IL-6, TNF, and IL-10 in the gut, epididymal, and mesenteric adipose tissue were determined using DuoSet ELISA kits according to the manufacturer's instructions (R&D Systems, Inc., Minneapolis, MN, USA).

Statistical analysis

The results are expressed as mean ± SEM and analyzed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA, USA). All data were analyzed for normality of distribution by the Kolmogorov–Smirnov test and were found to be normal. Comparisons between two groups were performed using the Student's t test, and multiple comparisons were performed using one-way ANOVA with Newman–Keuls post hoc analysis. Statistical significance was set at $P < 0.05$.

Results

Allergic response in mice fed chow or HC diet

Sensitized mice (OVA+) exhibited higher serum concentrations of total IgE (Fig. 2A), anti-OVA IgE (Fig. 2B), and anti-OVA IgG1 (Fig. 2C) compared with OVA− mice fed chow or HC diet. The higher levels of these immunoglobulins showed the development of allergy to the OVA protein. Sensitized mice (OVA+) also showed aversion to the antigen in water. In fact, allergic groups reduced the consumption of water containing OVA on the fifth day of the oral challenge, which was maintained until the end of the allergic challenge. Non-allergic mice fed HC diet also showed aversion to the OVA solution (Fig. 2D).

The body weight gain and food intake were similar between the groups before the allergic challenge (Table 1). Also, there was no difference between caloric intakes. After the allergic challenge, only OVA-sensitized mice fed chow diet showed weight loss (Fig. 2E).

The analysis of the clinical parameters of food allergy revealed that allergic mice fed chow diet had a higher DAI score compared with non-allergic mice. Non-allergic mice fed HC diet had a higher DAI score compared with mice fed chow diet. Therefore, we found no difference between the allergic and non-allergic mice fed HC diet (Fig. 2F).

Effect of food allergy and HC diet on gut parameters

The gut eosinophilic infiltrate was twofold higher in the allergic mice fed with chow diet compared with non-allergic mice. Similarly, obese non-allergic animals exhibited higher numbers of eosinophils in the jejunum compared with non-allergic mice fed chow diet. However, allergic mice fed HC diet showed no further increase in eosinophils compared with non-allergic mice fed the same diet (Fig. 3A, B).

The gut photomicrographs stained with PAS showed that allergic mice fed chow diet presented with increased mucus in the intestinal mucosa compared with non-allergic mice. Non-allergic obese mice also showed increased PAS-positive cells compared with non-allergic lean mice (Fig. 3B, C).

To evaluate whether mice fed with HC diet showed higher numbers of gut eosinophils because of OVA ingestion or as a result of nutrient overload, we performed a set of experiments in which mice were fed chow or HC diet without any allergic stimulus (OVA ingestion) in the water. Mice fed HC diet showed an increase in eosinophil numbers per field compared with mice fed chow diet (chow [C] = 5.18 ± 0.4 versus HC = 13.73 ± 0.6); however, no differences in the numbers of PAS-positive cells were found (C = 6.00 ± 1.1 versus HC = 6.47 ± 0.6). In addition, the intestinal mucosa levels of the cytokines TNF, IL-6, and IL-10 did not change (data not shown).

Adipose tissue response after allergy challenge in mice fed chow or HC diet

Allergic mice fed chow diet exhibited lower adiposity compared with non-allergic mice fed the same diet (Fig. 4A–D). However, allergic mice fed HC diet showed unaltered adiposity in most tissues (Fig. 4A, C, D) compared with non-allergic group fed HC diet, with the exception of the mesenteric adipose tissue, which showed a remarkable reduction (Fig. 4B). These data were confirmed through a histologic analysis of the epididymal and

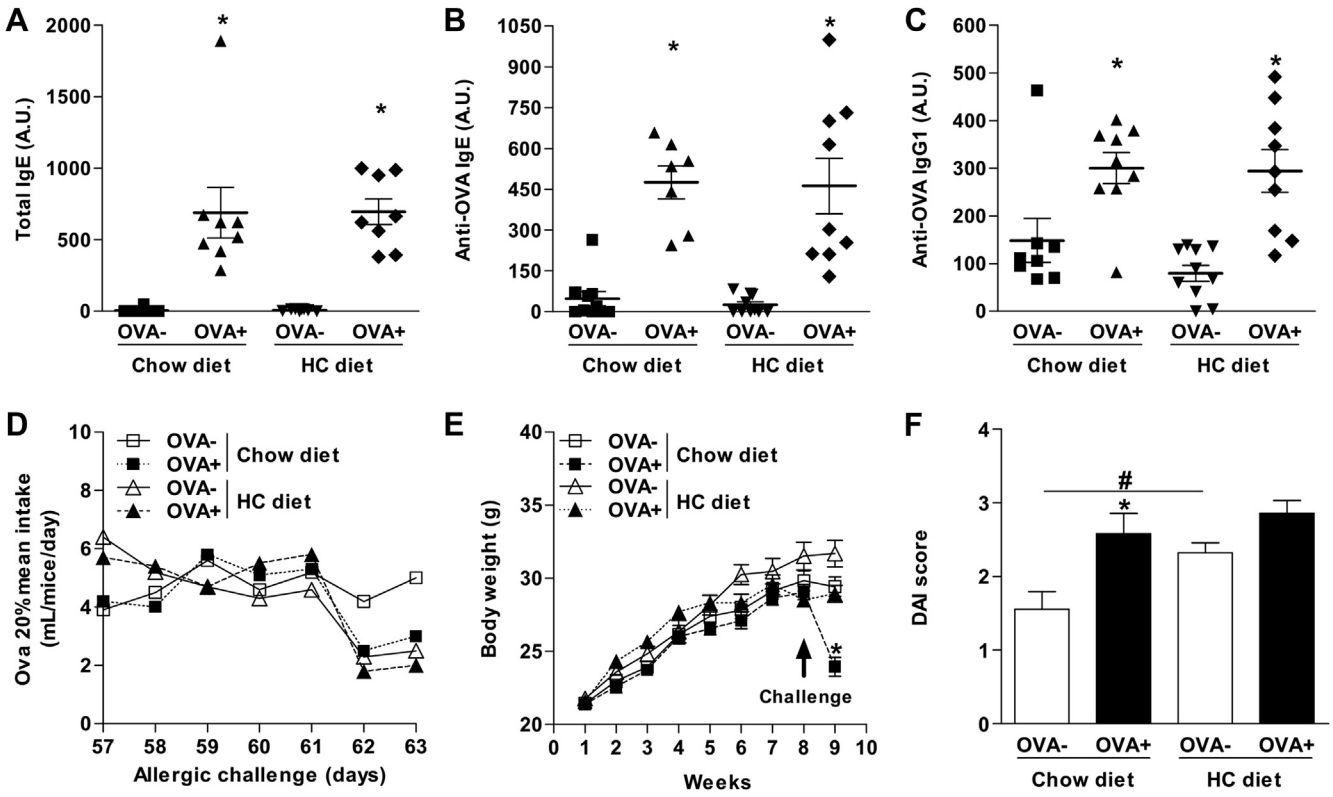

Fig. 2. Markers of allergy after ovalbumin consumption in OVA− and OVA+ mice fed chow or HC diet. (A) Total IgE, (B) anti-OVA IgE, and (C) anti-OVA IgG1 levels. (D) Ingestion of ovalbumin solution, (E) body weight gain, and (F) DAI score. The data are expressed as mean ± SEM (n = 8–10 per group). $^{*}P < 0.05$ compared with the OVA− group; $^{\#}P < 0.05$ compared with the respective OVA+ or OVA− group fed chow diet. HC, high-refined carbohydrate-containing; OVA−, non-allergic; OVA+, allergic.

mesenteric adipose tissues (Fig. 4E–G). The epididymal adipocytes from allergic mice fed HC diet showed a similar area compared with those from obese non-allergic mice, whereas the mesenteric adipocytes from allergic mice fed HC diet presented a smaller area.

Allergic mice fed chow diet had more adhered leukocytes in the epididymal and mesenteric adipose tissues than had non-allergic mice. Allergic mice fed HC diet had no changes in adhered leukocytes compared with non-allergic mice fed the same diet (Fig. 5A, B).

The number of leukocytes in the blood was increased in the allergic group fed chow diet compared with non-allergic mice. Mice fed HC diet exhibited higher leukocyte numbers; however, the number of these cells did not increase after the allergic challenge. The same profile was observed in the numbers of mononuclear blood cells (data not shown).

We also analyzed the adipose tissue cytokines TNF, IL-6, and IL-10 and observed no differences in the epididymal adipose tissue between the allergic groups fed HC or chow diet (Fig. 5C). In contrast, the levels of IL-6 and IL-10 in the mesenteric adipose tissue of allergic mice fed HC diet were higher than those found in the allergic mice fed chow diet (Fig. 5D).

Although allergy and obesity change the metabolic profile of mice, obese allergic mice did not show any further metabolic dysfunction (Table 1).

Discussion

Diseases such as allergy and obesity induce metabolic dysfunction and adipose tissue inflammation [4,18,19]. However, it is not clear whether low-grade inflammation in a context of mild obesity influences the allergic response. The major findings

Table 1
Serum analysis of non-allergic and allergic mice fed chow or high-refined carbohydrate-containing diet

Parameters evaluated	C (OVA−)	C (OVA+)	HC (OVA−)	HC (OVA+)
Food intake (g/d/mice)	5.3 ± 0.4	5.3 ± 0.5	5.0 ± 0.3	4.7 ± 0.4
Body weight after 8 wk of diet (g)	29.8 ± 0.7	29.5 ± 0.5	30.9 ± 0.9	28.5 ± 0.4
Serum triacylglycerols (mg/dL)	48.6 ± 5.3	68.7 ± 2.8	84.0 ± 12.1*	88.6 ± 13.5
Serum total cholesterol (mg/dL)	76.2 ± 4.4	75.5 ± 7.1	96.8 ± 4.5*	92.0 ± 2.1
Serum glucose (mg/dL)	208.4 ± 8.2	148.3 ± 6.7†	196.9 ± 13.5	215.3 ± 23.1*
Area under the curve OGTT	14054 ± 300.3	12253 ± 1617	17112 ± 372.5*	16742 ± 886.1*
Adiponectin (μg/mL)	1.1 ± 0.1	1.0 ± 0.4	1.1 ± 0.1	1.1 ± 0.04
Resistin (ng/mL)	246.1 ± 34.8	341.0 ± 47.3	314.2 ± 17.7	365.4 ± 13.1

C, chow; HC, high-refined carbohydrate-containing; OGTT, oral glucose tolerance test; OVA−, non-allergic; OVA+, allergic
Data represent mean ± SEM (n = 7–8 per group)
* $P < 0.05$ compared with the respective OVA− or OVA+ group fed chow diet.
† $P < 0.05$ compared with the OVA− group.

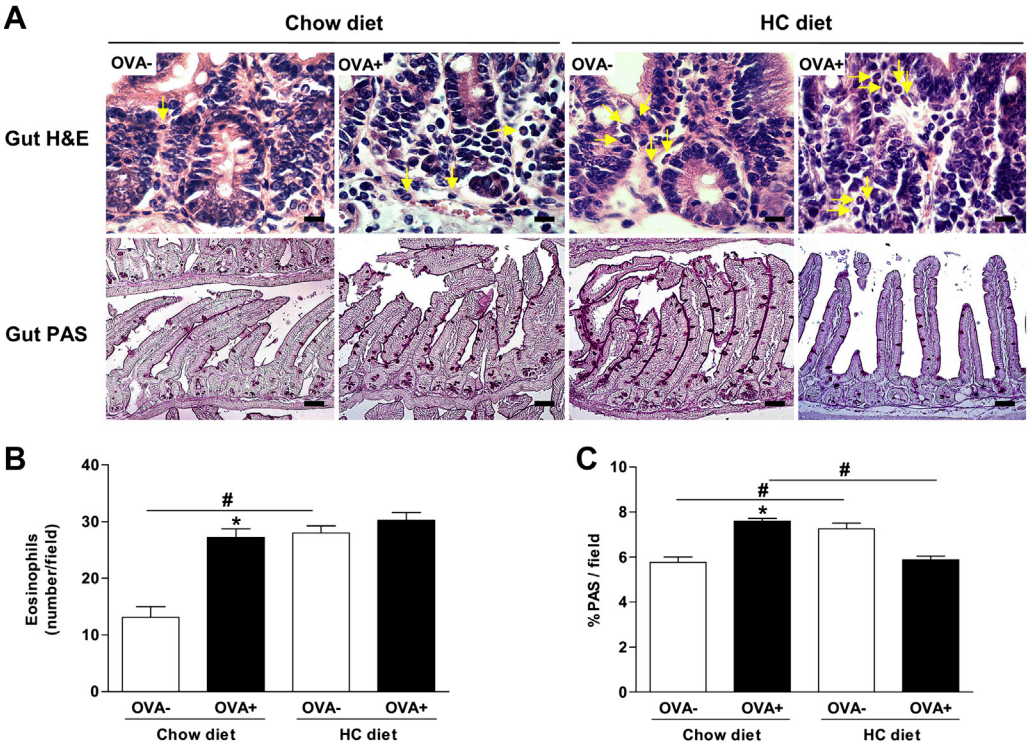

Fig. 3. Small intestine (jejunum) histologic analysis of eosinophil infiltrate and PAS-positive cells in OVA− and OVA+ mice fed chow or HC diet. (A) Representative photomicrographs of hematoxylin–eosin-stained intestine showing eosinophils in the jejunum (400×). PAS staining was used to determine the mucus production (goblet cells in evidence; 100×). (B) Eosinophil count per field and (C) percentage of PAS-positive cells by field. The data are expressed as mean ± SEM (n = 8–10 per group). $^{*}P < 0.05$ compared with the OVA− group; $^{\#}P < 0.05$ compared with the respective OVA+ or OVA− group fed chow diet. HC, high-refined carbohydrate-containing; OVA−, non-allergic; OVA+, allergic; PAS, periodic acid–Schiff.

of our study can be summarized as follows: 1) Mild obesity induced by HC diet did not worsen the parameters of food allergy in mice; in fact, the weight and fat pad mass loss after antigen exposure were decreased in these animals, except in mesenteric adipose tissue. 2) The closer location of the mesenteric fat pad to the intestine may be related with a higher inflammation and susceptibility to lose fat in this tissue. 3) Non-allergic mice fed HC diet exhibited aversion to antigen (OVA), increased numbers of eosinophils and PAS-positive cells in the gut, and clinical signs of intestinal inflammation.

The production of IgE is the main marker for immediate hypersensitivity reactions, commonly called allergy or atopy [22]. After the production of IgE, this protein binds with high affinity to the receptor on the surface of mast cells and basophils [23]. In this study, we found that the oral ingestion of the antigen characteristically increased the concentrations of total IgE, anti-OVA IgE, and IgG1 in allergic mice, regardless of the diet composition. These data suggest that the HC diet does not cause a further increase in the immunoglobulin levels, indicating that diet-induced mild obesity does not worsen the allergic response. In contrast, it was proposed that obesity promotes immunologic changes that result in decreased immune tolerance to antigens, making these individuals more susceptible to immune-mediated diseases [3]. Previous studies assessing allergic asthma in obese mice fed a high-fat diet reported that increased body weight increases the levels of specific IgE and IgG [24–26]. It has also been demonstrated that a high-fat diet affects the immune response to antigens by modulating their uptake and transport through intestinal epithelial cells [27]. The allergy manifestations at the intestinal level promote a change in mucosa that leads to diarrhea and increased permeability to macromolecules [23]. These controversial data suggest that diet composition may modulate the OVA absorption and consequently the immune response. This finding may explain, at least in part, why the mice fed HC diet did not show any further increase in immunoglobulin levels after the antigen challenge, as previously shown in mice fed a high-fat diet. Moreover, mild-obese mice did not show a further increase in the symptoms of food allergy. Thereby, our data suggest that the distinct diet composition may influence the susceptibility to food allergy at least by altering differentially the production of IgE.

The clinical signs of food allergy, in addition to the measurement of the levels of IgE and IgG, are important for the evaluation of the allergic response to antigen. Allergic mice presented a higher DAI score and aversion to OVA, regardless of their diet composition. Non-allergic obese mice also showed those dysfunctions. The DAI score combines three manifestations observed in food allergy: 1) weight loss after challenge, 2) occurrence of diarrhea, and 3) presence of blood in the feces. Even though obese mice did not show weight loss, these mice presented a higher DAI score, akin to allergic mice. Moreover, non-allergic obese mice also showed food aversion, which was unrelated to the IgE levels. The decision of non-allergic obese mice to avoid drinking water containing OVA may be an adaptive response resulting from the negative stimulus on the intestinal mucosa because of diarrhea and bleeding discomfort [28]. In fact, this finding suggests that the previous gut alteration induced by HC diet sensitizes the mice to exhibit an aversive response to the OVA antigen.

A cluster of factors is involved in the aversion phenomenon [29–31]. Some immune cells and cytokines appear to be key regulators of the antigen-induced aversion. The gut eosinophils produce damage to the tissue and trigger diarrhea and intestinal

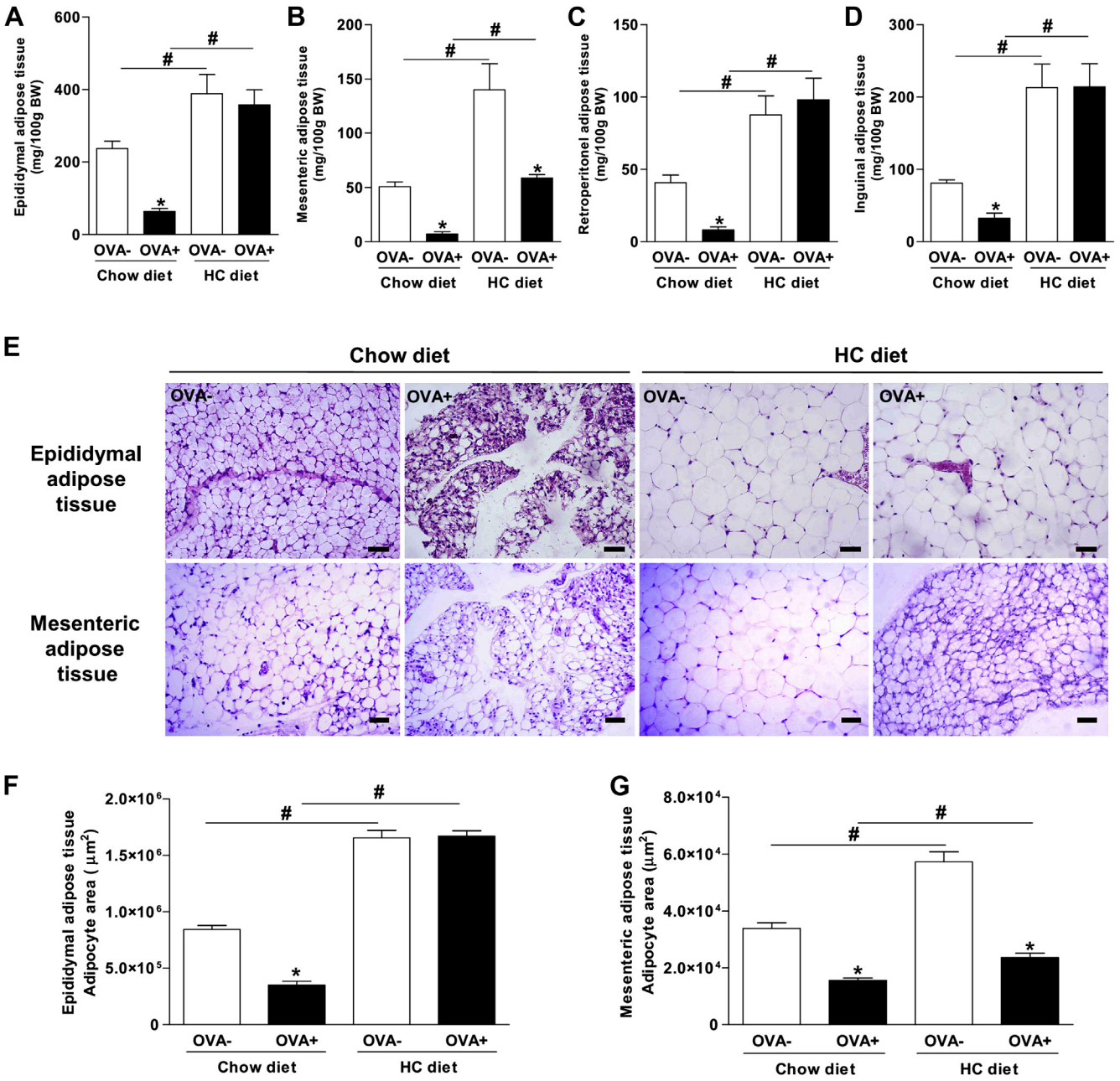

Fig. 4. Adipose tissue weight in different sites: (A) epididymal, (B) mesenteric, (C) retroperitoneal, and (D) inguinal adipose tissue. (E) Representative photomicrographs of hematoxylin–eosin-stained epididymal and mesenteric adipose tissue used to determine the epididymal (F) and mesenteric (G) adipocyte areas (μm^2) of OVA− and OVA+ mice fed chow or HC diet (100×). The data are expressed as mean ± SEM (n = 8–10 per group). $^{*}P < 0.05$ compared with the OVA− group; $^{\#}P < 0.05$ compared with the respective OVA+ or OVA− group fed chow diet. HC, high-refined carbohydrate-containing; OVA−, non-allergic; OVA+, allergic.

bleeding, which may contribute to the antigen aversion phenomenon [23,28]. In fact, the gut of non–OVA-sensitized mild-obese mice showed an increase in eosinophil infiltration in the mucosa before the oral challenge, which may induce signs of food intolerance. Moreover, the adipose tissue from obese mice produces a milieu of cytokines that can influence the intestinal immune response to a given antigen. Indeed, the adipose tissue is closely linked with the lymphoid structure [32]. A new type of innate lymphocyte that is present in a novel lymphoid structure associated with adipose tissues that produce large amounts of Th2 cytokines, such as IL-5, IL-6, and IL-13 [32] was recently discovered. A previous study found that the serum IL-5 levels are maintained by long-lived type 2 innate lymphoid cells resident in peripheral tissues that secrete IL-5 constitutively and are induced to co-express IL-13, resulting in localized eotaxin production and eosinophil accumulation [33]. The researchers also reported that type 2 innate lymphoid cells in the small intestine co-express IL-5 and IL-13, and that this co-expression is enhanced after caloric intake [33]. The close interconnection between adipose tissue, lymphoid organs, and intestine may explain the increased number of eosinophils in the gut of non-allergic mild-obese mice.

A previous study from our group reported that food allergy triggers an important reduction in body weight, mainly because of fat mass loss [16]. Indeed, in this study, we found that food allergy decreases the visceral fat tissue. The lower adiposity

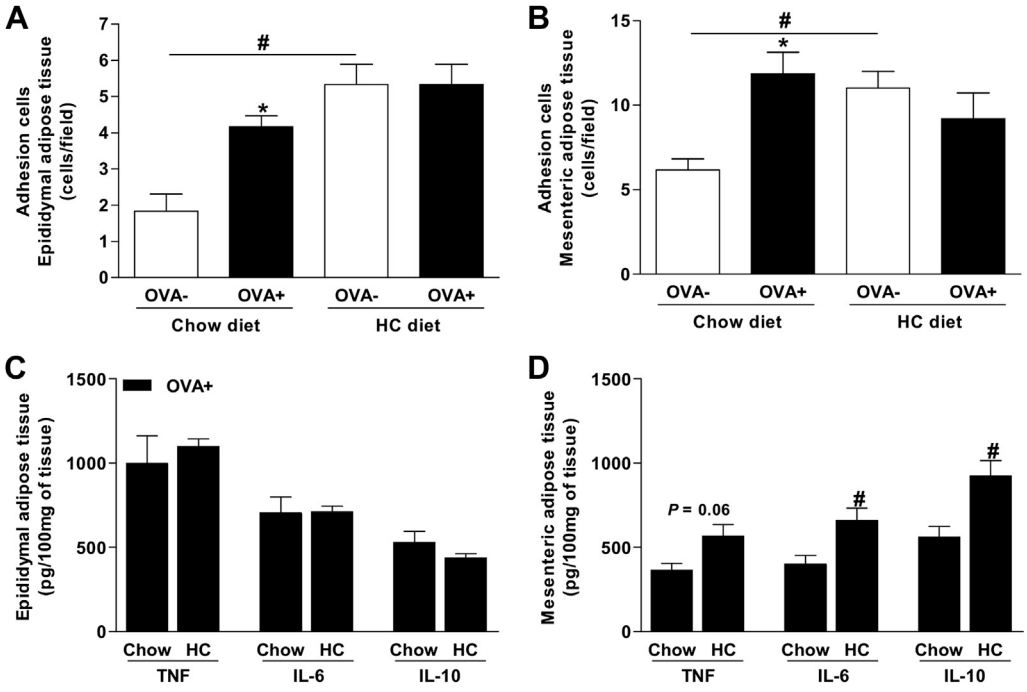

Fig. 5. Observation of leukocyte–endothelium interaction in epididymal and mesenteric adipose tissues in OVA− and OVA+ mice fed chow or HC diet. Intravital microscopy was used to assess the adhesion of leukocytes in (A) epididymal and (B) mesenteric adipose tissue vessels in vivo. Cytokines levels in the (C) epididymal and (D) mesenteric adipose tissue of OVA− and OVA+ mice fed chow or HC diet. The data are expressed as mean ± SEM (n = 6 per group). $^{*}P < 0.05$ compared with the OVA− group; $^{\#}P < 0.05$ compared with the respective OVA+ or OVA− group fed chow diet. HC, high-refined carbohydrate-containing; OVA−, non-allergic; OVA+, allergic.

observed in allergic mice may be related to higher adipocyte lipolysis, which is necessary to respond to the high energy demand during the inflammatory process [16]. As we (and other researchers) have previously shown, lipolysis appears to be driven by the increased numbers of leukocytes and cytokine levels in adipose tissue [16,34,35]. Surprisingly, although mild-obese allergic mice also showed signs of food allergy, these mice lose fat mass only in the mesenteric adipose tissue, which is located near the intestine. Furthermore, allergic mild-obese mice showed a higher cytokine content in the mesenteric adipose tissue. The higher cytokine levels in mesenteric adipose tissue may be related with the fat loss, particularly in this tissue. The proximity of the mesenteric adipose tissue to the intestine and its readiness to respond to the energy demand during an inflammatory response [36] indicate its participation as an active energy provider during the allergic inflammatory response.

Conclusions

Our data show that mice fed a high-carbohydrate–containing diet did not worsen the food allergy response induced by OVA. However, these mild-obese mice exhibit important intestinal disorders that appear to dampen the inflammatory response during the antigen challenge and any further increase in eosinophils infiltrate, DAI score, mucus, and aversion phenomenon. Mild obesity induced by the HC diet in non-allergic mice, although not associated with IgE levels, causes pathologic features similar to those found in lean allergic mice.

Acknowledgments

The financial assistance from Pró-Reitoria de Pesquisa da UFMG, Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) is gratefully acknowledged. Some of the authors are also recipients of CNPq research fellowships (N.V.B., R.C.F., R.V.S.P., and D.C.C.) and CAPES research fellowships (D.A.P.).

References

[1] Macdonald TT, Monteleone G. Immunity, inflammation, and allergy in the gut. Science 2005;307:1920–5.
[2] Ferreira CT, Seidman E. Food allergy: a practical update from the gastroenterological viewpoint. J Pediatr (Rio J) 2007;83:7–20.
[3] Hersoug LG, Linneberg A. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? Allergy 2007;62:1205–13.
[4] Oliveira MC, Menezes-Garcia Z, Henriques MC, Soriani FM, Pinho V, Faria AM, et al. Acute and sustained inflammation and metabolic dysfunction induced by high refined carbohydrate-containing diet in mice. Obesity (Silver Spring) 2013;21:E396–406.
[5] Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 2007;56:16–23.
[6] Vieira VJ, Ronan AM, Windt MR, Tagliaferro AR. Elevated atopy in healthy obese women. Am J Clin Nutr 2005;82:504–9.
[7] Hancox RJ, Milne BJ, Poulton R, Taylor DR, Greene JM, McLachlan CR, et al. Sex differences in the relation between body mass index and asthma and atopy in a birth cohort. Am J Respir Crit Care Med 2005;171:440–5.
[8] Visness CM, London SJ, Daniels JL, Kaufman JS, Yeatts KB, Siega-Riz AM, et al. Association of obesity with IgE levels and allergy symptoms in children and adolescents: results from the National Health and Nutrition Examination Survey 2005-2006. J Allergy Clin Immunol 2009;123:1163–9. 1169.e1–4.
[9] Dixon AE, Shade DM, Cohen RI, Skloot GS, Holbrook JT, Smith LJ, et al. Effect of obesity on clinical presentation and response to treatment in asthma. J Asthma 2006;43:553–8.
[10] Mai XM, Bottcher MF, Leijon I. Leptin and asthma in overweight children at 12 years of age. Pediatr Allergy Immunol 2004;15:523–30.
[11] Lang A, Carlsen KH, Haaland G, Devulapalli CS, Munthe-Kaas M, Mowinckel P, et al. Severe asthma in childhood: assessed in 10 year olds in a birth cohort study. Allergy 2008;63:1054–60.
[12] Tantisira KG, Weiss ST. Complex interactions in complex traits: obesity and asthma. Thorax 2001;56(Suppl 2):ii64–73.
[13] Chinn S. Obesity and asthma: evidence for and against a causal relation. J Asthma 2003;40:1–16.

[14] Leung TF, Kong AP, Chan IH, Choi KC, Ho CS, Chan MH, et al. Association between obesity and atopy in Chinese schoolchildren. Int Arch Allergy Immunol 2009;149:133–40.
[15] Sidoroff V, Hyvarinen MK, Piippo-Savolainen E, Korppi M. Overweight does not increase asthma risk but may decrease allergy risk at school age after infantile bronchiolitis. Acta Paediatr 2012;101:43–7.
[16] Dourado LP, Noviello Mde L, 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.
[17] Batista NV, Pereira RV, Noviello ML, Dourado LP, Perez DA, Foureaux G, et al. Prolonged ingestion of ovalbumin diet by sensitized mice improves the metabolic consequences induced by experimental food allergy. Clin Exp Immunol 2014;178:416–27.
[18] Lee YS, Li P, Huh JY, Hwang IJ, Lu M, Kim JI, et al. Inflammation is necessary for long-term but not short-term high-fat diet-induced insulin resistance. Diabetes 2011;60:2474–83.
[19] Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, et al. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. Metabolism 2008;57:1071–7.
[20] Saldanha JC, Gargiulo DL, Silva SS, Carmo-Pinto FH, Andrade MC, Alvarez-Leite JI, et al. A model of chronic IgE-mediated food allergy in ovalbumin-sensitized mice. Braz J Med Biol Res 2004;37:809–16.
[21] Russo M, Nahori MA, Lefort J, Gomes E, de Castro Keller A, Rodriguez D, et al. Suppression of asthma-like responses in different mouse strains by oral tolerance. Am J Respir Cell Mol Biol 2001;24:518–26.
[22] Ishizaka K, Ishizaka T. Mechanisms of reaginic hypersensitivity and IgE antibody response. Immunol Rev 1978;41:109–48.
[23] Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol 2010;125:S73–80.
[24] Calixto MC, Lintomen L, Schenka A, Saad MJ, Zanesco A, Antunes E. Obesity enhances eosinophilic inflammation in a murine model of allergic asthma. Br J Pharmacol 2010;159:617–25.
[25] Mito N, Kitada C, Hosoda T, Sato K. Effect of diet-induced obesity on ovalbumin-specific immune response in a murine asthma model. Metabolism 2002;51:1241–6.
[26] de Vries A, Hazlewood L, Fitch PM, Seckl JR, Foster P, Howie SE. High-fat feeding redirects cytokine responses and decreases allergic airway eosinophilia. Clin Exp Allergy 2009;39:731–9.
[27] Wang Y, Ghoshal S, Ward M, de Villiers W, Woodward J, Eckhardt E. Chylomicrons promote intestinal absorption and systemic dissemination of dietary antigen (ovalbumin) in mice. PLoS One 2009;4:e8442.
[28] Garcia J, Lasiter PS, Bermudez-Rattoni F, Deems DA. A general theory of aversion learning. Ann N Y Acad Sci 1985;443:8–21.
[29] Cara DC, Conde AA, Vaz NM. Immunological induction of flavor aversion in mice. Braz J Med Biol Res 1994;27:1331–41.
[30] Berin MC, Mayer L. Immunophysiology of experimental food allergy. Mucosal Immunol 2009;2:24–32.
[31] Dourado LP, Saldanha JC, Gargiulo DL, Noviello Mde L, Brant CC, Reis ML, et al. Role of IL-4 in aversion induced by food allergy in mice. Cell Immunol 2010;262:62–8.
[32] Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, et al. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+) Sca-1(+) lymphoid cells. Nature 2010;463:540–4.
[33] Nussbaum JC, Van Dyken SJ, von Moltke J, Cheng LE, Mohapatra A, Molofsky AB, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. Nature 2013;502:245–8.
[34] Kuhnke A, Burmester GR, Krauss S, Buttgereit F. Bioenergetics of immune cells to assess rheumatic disease activity and efficacy of glucocorticoid treatment. Ann Rheum Dis 2003;62:133–9.
[35] Straub RH, Cutolo M, Buttgereit F, Pongratz G. Energy regulation and neuroendocrine-immune control in chronic inflammatory diseases. J Intern Med 2010;267:543–60.
[36] Sackmann-Sala L, Berryman DE, Munn RD, Lubbers ER, Kopchick JJ. Heterogeneity among white adipose tissue depots in male C57 BL/6 J mice. Obesity (Silver Spring) 2012;20:101–11.