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Experimental food allergy leads to adipose tissue inflammation, systemic metabolic alterations and weight loss in mice

Luana Pereira Antunes Dourado [a,b], Maria de Lourdes Meirelles Noviello [a], Débora Moreira Alvarenga [a], Zélia Menezes [b,c], Denise Alves Perez [a], Nathália Vieira Batista [a], Gustavo Batista Menezes [a,b], Adaliene Versiani Matos Ferreira [b,d], Danielle da Glória de Souza [b,c], Denise Carmona Cara [a,*]

[a] Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Imunofarmacologia, Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[c] Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[d] Departamento de Enfermagem Básica, Escola de Enfermagem, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

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ABSTRACT

To investigate the consequences of food allergy in adipose tissue and metabolism, we used a murine model in which mice have been sensitized subcutaneously with ovalbumin and further received antigen-containing diet. Allergic mice presented a significant weight loss 7 days after oral challenge with a concomitant decrease in epididymal adipose tissue mass. This decrease was associated with increased lipolysis and local inflammation. In adipose tissue of allergic mice there were increased leukocyte rolling and adhesion in the microvasculature, increased number of leukocytes in the tissue, especially macrophages ($F4/80^+$ cells) and increased pro-inflammatory cytokines levels, including TNF-α, IL-6 and CCL2. In addition, we observed low serum concentrations of triglyceride, glucose, total cholesterol and free fatty acids in the allergic mice. Our results suggest that the induction of food allergy in mice leads to adipose tissue inflammation and systemic metabolic alterations that contribute to the weight loss observed.

1. Introduction

Food allergy is defined as an adverse immunological response (hypersensitivity) to food proteins. The normal immune response to dietary proteins is associated with the induction of oral tolerance, which involves a modification of the antigen in the lumen by gastrointestinal enzymes, the posterior contact with specific antigen-presenting cells with distinct activation requirements, and activation of regulatory T cells. It is well accepted that a breakdown in oral tolerance mechanism or a failure of induction of oral tolerance results in food allergy [1]. Food antigen induced allergic immune responses are generally characterized by the production of T-helper type 2 (Th2) cytokines, an increase in IgE antibody levels and infiltration of eosinophils and/or mast cells into the intestinal tract. Immunoglobulin E (IgE)-mediated food allergy (type I food allergy) accounts for the majority of food allergic reactions and the onset of symptoms can occur immediately after the allergen ingestion [2].

There is a growing body of evidence that the prevalence of sensitization to common allergens has increased markedly over the last half century [3,4] and it is of considerable interest that food allergy is particularly frequent among children [5]. The main treatment strategy for most food allergies is based on allergen avoidance, which may present potential adverse nutritional deficiencies related to inadequate growth, neurological development and cardiovascular health [6,7]. The understanding of the mechanisms involved in allergic inflammation may provide useful insights into disease pathophysiology and aid in the development of novel therapeutic strategies.

Several experimental murine models have been conducted for the study of mechanisms involved in food allergy. Also, our group has developed an experimental model of food allergy in which ovalbumin (Ova)-sensitized BALB/c mice are given the antigen orally. This model mimics several pathological changes that occur in patients with food allergy (e.g. increased anti-Ova IgE and IgG1 production, intestinal edema and eosinophil infiltration in the jejunum). One of the most remarkable systemic alterations observed in the Ova-sensitized mice is an accentuated weight loss after the chronic antigen ingestion, which started in the first week after antigen ingestion, maintaining the reduced weight loss for at least 21 days [8]. Although similar significant weight reduction has been observed in other murine models of food allergy, the mechanisms underlying the weight loss deserve further investigation [9,10].

* Corresponding author. Address: Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Avenida Antônio Carlos, 6627 Bairro Pampulha, 31. 270-901 Belo Horizonte, Minas Gerais, Brazil. Fax: +55 31 3409 2810.
E-mail address: deniseccm@icb.ufmg.br (D.C. Cara).

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Body mass is controlled by the balance of energy intake and energy expenditure, which is mainly regulated by a balance between carbohydrate and lipid metabolic pathways. Fatty acids (FAs), stored in adipose tissue as triacylglycerols (TAGs), are important lipid fuel reserves [11] that are dependent on the availability and uptake of exogenous nonesterified fatty acids. FAs are released to metabolism from plasma lipoproteins by lipoprotein lipase (LPL) or by de novo synthesis (lipogenesis), which uses glucose as the main substrate [12]. The amount of FAs released to the blood stream from stored TAG is mainly dependent on the rate of lipolysis, mediated by hormone-sensitive lipase [13,11].

Body weight reduction may be observed in various chronic diseases (e.g. cancer, arthritis and inflammatory bowel disease) [14–16]. The excessive body weight loss observed in these conditions may be related to increased energy demand required for such exuberant inflammatory response, which prioritize substrates to generate energy for cells involved in inflammation. This process especially involves the mobilization of lipids stored in the adipose tissue [17,18].

The aim of this study was to evaluate if the inflammatory process induced by experimental food allergy in mice may have metabolic consequences that contribute to the significant weight loss.

2. Materials and methods

2.1. Animals

Male BALB/c mice at 6 weeks of age were obtained from our animal facility (ICB/UFMG). All mice have received standard (Purina, Belo Horizonte, MG, Brazil) mouse chow until the antigen challenge. The investigations were in accordance with the Ethical Principles in Animal Experimentation, adopted by the Ethics Committee in Animal Experimentation of our Institution (CETEA/UFMG).

2.2. Mice sensitization

The control group received 0.2 ml saline (0.9%) with adjuvant [1 mg $Al(OH)_3$] on day 0 and saline on day 14. The allergic group received 0.2 ml saline (0.9%) with adjuvant and 10 μg Ova (five times crystallized hen's egg albumin; Sigma, St. Louis, MO, USA) on day 0 and saline with 10 μg soluble Ova on day 14. All injections were performed subcutaneously. Seven days after the secondary sensitization (day 21), the standard chow was replaced with 14% Ova containing diet for a period of 1 week (day 21 until 28). This diet was prepared using a lyophilized egg white (Salto's, Belo Horizonte, MG, Brazil) and nutrient contents were the same specified for AIN-93G [19].

2.3. Evaluation of the animals and tissue collection

Body weight was determined once a week throughout the 4 weeks. Food consumption was assessed daily by weighting the remaining chow, in the cage of six mice, and comparing its weight with the previous day. Data was reported as percentage of diet consumption (allergic group/control group), considering diet consumption by control group as 100%.

By using disease activity index (DAI) score [20], the body weight and stool were scored every day after antigen challenge (day 0–7 post challenge) as follows: body weight score (zero – no weight loss; one – 1–5% weight loss; two – 6–10% weight loss; three – 11–15% weight loss; four – above 15% weight loss); stool viscosity (zero – normal; two – fluffy; four – diarrhea); stool hemorrhage (zero – normal; two – apparent hemorrhage).

After 7 days of continuous antigen challenge, mice were anesthetized with 10 mg/kg xylazine and 100 mg/kg ketamine hydrochloride – intraperitoneal (i.p.) and serum samples were obtained from all groups. Later, under deep anaesthesia, mice were euthanized, and the epididymal fat and the muscle gastrocnemius were collected and weighted. The weight of these tissues was correlated to body weight. Epididymal fat fragments were immediately frozen in liquid nitrogen and stored at −80°C and or fixed in 10% neutral formalin for posterior analysis.

2.4. Serum anti-Ova IgE evaluation

Anti-Ova IgE was measured by capture-ELISA using plates coated with rat anti-mouse IgE, 50 μl of serum and biotinylated Ova, as previously described [21]. The results for both antibodies are reported in arbitrary units using a positive reference serum (1000 A.U).

2.5. Histological evaluation of epididymal adipose tissue

Epididymal fat pads from allergic and control mice were fixed in 10% neutral formalin for 24 h, dehydrated in absolute ethanol, cleared in xylene and then embedded in paraffin. The histological sections (5 μm) sections were stained with Harris haematoxylin counterstained with eosin, and then evaluated by light microscopy. Sections were analyzed by using a microscope (Olympus BX41) equipped with a digital camera (Moticam 2500) for quantification of adipocyte size. The cell area was measured in 50 adipocytes per animal, using the Image J software.

To evaluate the number of mast cells, epididymal fat pads were fixed in Carnoy for 1 h, dehydrated in absolute ethanol, cleared in xylene and then embedded in paraffin. The histological sections (7 μm) were stained with Toluidine blue and evaluated by light microscopy. Ten sequential images (×40 objective, 0.2 mm^2) were analyzed to assess mast cell number. Data were reported as number of mast cells/field.

2.6. Intravital microscopy visualization of epididymal adipose tissue microvasculature

Intravital microscopy was performed in the epididymal adipose tissue microcirculation. Briefly, mice were anesthetized with 10 mg/kg xylazine and 100 mg/kg ketamine hydrochloride i.p. The right jugular vein was cannulated and rhodamine 6G (Sigma, St. Louis, MO, USA) was injected intravenous (i.v.; 0.15 mg/kg) to visualize the leukocyte – endothelial cell interactions. Rhodamine epi-illumination was achieved with 150 W variable HBO mercury lamp in conjunction with a Zeiss filter set 15 (546/12 nm band – pass filter, 580 nm Fourier transforms, 590 nm late potentials; Zeiss, Wetzlar, Germany). The microscopic images were captured using a Nikon eclipse 50i (Nikon Instruments Inc., Japan) microscope (×20 objective) with a video camera (5100 HS; Panasonic, Secaucus, NJ) and recorded digitally using both filters blocks consecutively. Data analysis was performed off-line.

Rolling leukocytes were defined as those cells moving slower than the cells at a regular flux in a given vessel. The flux of rolling cells was measured as the number of rolling cells passing by a given point in the venule per minute, with results expressed as cells/minute. A leukocyte was considered to be adherent if it remained stationary for at least 30 s, and total leukocyte adhesion was quantified as the number of adherent cells within a 100 μm length of venule, with results expressed as cells/100 μm.

2.7. Adipocyte isolation

Adipocytes were isolated from epididymal fat pads as previously described [22]. Briefly, digestion was carried out at 37 °C, with constant shaking for 45 min. Cells were filtered through nylon

mesh and washed three times with buffer containing (in mmol/l): 137 NaCl, 5 KCl, 4.2 $NaHCO_3$, 1.3 $CaCl_2$, 0.5 $MgCl_2$, 0.5 KH_2PO_4, 20 HEPES (pH 7.4), and 1% BSA.

2.8. Analysis of adipose tissue derived stromal vascular cells by flow cytometry

Epididymal fat from mice was digested as described above. Adipose tissue derived stromal vascular cells were washed with DMEM supplemented with 10% FCS, counted, and 10^6 cells/group were labeled with F4/80-PE, CD11b-PerCP, CD11c-FITC, CD3-Cy5, CD4-PE, CD8b-PE or their respective isotype control (BD Biosciences), and analyzed with a FACScan. Flow analyses were completed after 30,000 detected events.

2.9. Adipose tissue cytokine and chemokine measurement

Epididymal adipose tissue extracts were obtained during the necropsy and were stored on ice. Thereafter, using Ultra-Turrax, the tissue was homogenized in extraction solution (100 mg of tissue per 1 ml) containing 0.4 M NaCl, 0.05% Tween 20, 0.5% BSA, 0.1 mM phenylmetilsulfonil fluoride, 0.1 mM benzetonio chloride, 10 mM EDTA and 20 KIU aprotinin. The adipose tissue homogenate was spun at 10000*g* for 10 min at 4 °C and the supernatants were stored at −70 °C. The levels of TNF-α, IL-6, IL-10 and CCL-2 were measured by ELISA in supernatants of epididymal adipose tissue, at 1:3 dilution in PBS containing 1% BSA, using commercially available antibodies and according to the procedures supplied by the manufacturer (R&D Systems, Minneapolis, MN, USA).

2.10. Lipolysis measurements

Lipolysis was measured by following the rate of glycerol release, as previously described [23]. After an initial wash, adipocytes were incubated at 37 °C in a water bath for 60 min in the presence or absence of isoproterenol (ISO, 0.1 μmol/L). At the end of incubation period, and aliquot of the infranatant was removed for enzymatic determination of glycerol released into the incubation medium (LABTEST, Lagoa Santa, MG, Brazil).

2.11. Serum analysis

Serum triglyceride, total cholesterol and glucose were assayed by conventional enzymatic methods using kits (KATAL, Belo

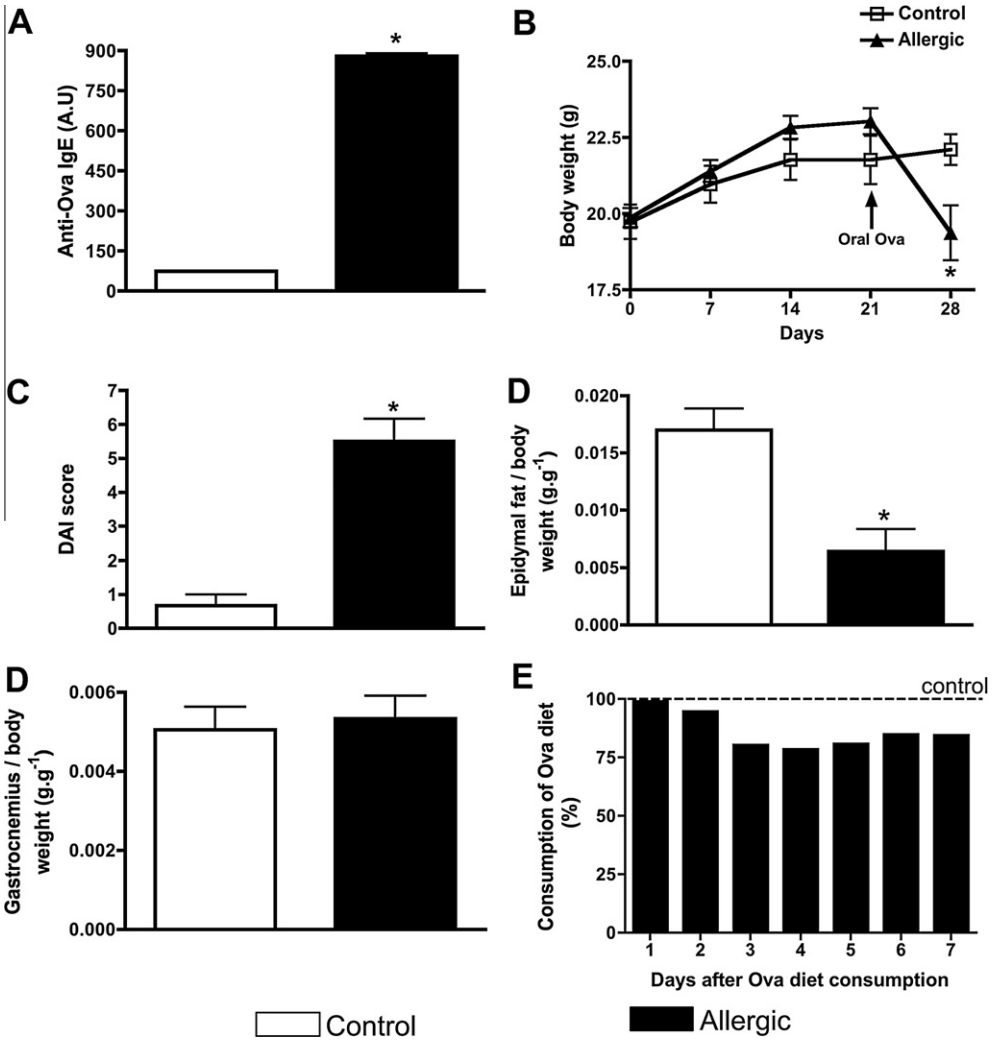

Fig. 1. Allergic mice lose body weight and epididymal adipose tissue mass. After 7 days of ingestion of the ovalbumin (Ova) containing diet (day 28), serum was collected for anti-Ova IgE measurement by ELISA (A). Body weight was assessed weekly (B) throughout the experiment. Disease activity index (DAI) was score on day 28 (C). The epididymal fat (D) and gastrocnemius muscle (E) were collected, weighted and correlated to body weight. Food intake was assessed every day during the antigenic challenge (day 21–28) and the data was reported as percentage of diet consumption (allergic group/control), considering diet consumption by control group as 100% (F). All data, except food consumption, are reported as means ± SEM for six mice in each group. $^*P < 0.05$ compared to control group. (Student *t*-test).

Horizonte, MG, Brazil). Free fatty acid (FFAs) were estimated using commercial kit from Wako (Pure Chemical Industries, Japan).

2.12. Statistical analysis

Data were analyzed statistically by one-way analysis of variance (ANOVA – Tukey) or by the Student *t*-test when appropriate. The level of significance was set at $P < 0.05$.

3. Results

3.1. Food allergy leads to the loss of body weight and decreased fat mass in mice

The allergic group showed significant increase of serum anti-Ova IgE antibodies compared with the control group (Fig. 1A). Allergic but not control mice lost approximately 16% of their body weight after 7 days of Ova oral challenge (Fig. 1B). The stools of allergic mice were fluffy after 3 days and diarrhea was found after 6 days of antigen ingestion, although no hemorrhage was apparent. DAI scores of body weight and stools were significantly increased compared with control group on day 7 after antigen challenge (Fig. 1C). We have also observed marked reduction of epididymal adipose tissue mass in the allergic mice (Fig. 1D), while the gastrocnemius muscle weight remained unchanged (Fig. 1E). After 3 days of continuous challenge, allergic mice displayed 18% decrease in Ova diet consumption comparing to the control group (Fig. 1F).

3.2. Epididymal adipose tissue from allergic mice has decreased adipocyte area and increased infiltration of macrophages and mast cells

Morphometric analysis has revealed that the adipocyte size, determined as cell area (Fig. 2A), was strongly reduced in allergic mice (Fig. 2D), when compared to controls (Fig. 2C). Furthermore,

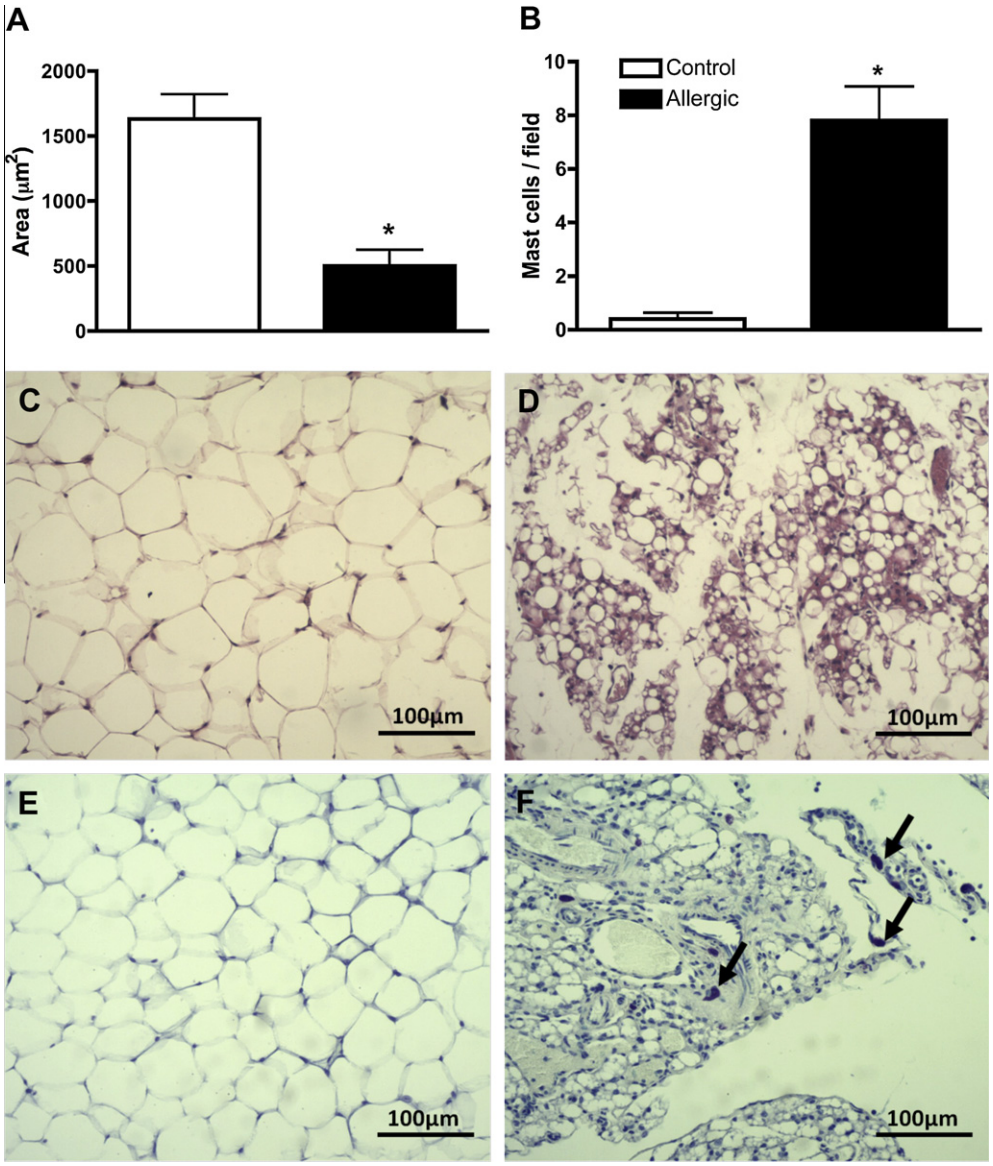

Fig. 2. Epididymal adipose tissue of allergic mice have decreased adipocyte area and increased number of mast cells. After 7 days of ingestion of the ovalbumin (Ova) containing diet, epididymal adipose tissue was collected for histological analysis. The area of fifty adipocytes from each animal was measured in hematoxylin–eosin stained sections (A, C and D) and the number of mast cells was evaluated in ten consecutives fields of Toluidine blue stained sections (B, E and F). C and E: illustrative pictures of epididymal adipose tissue from control mice. D and F: illustrative pictures of epididymal adipose tissue from allergic mice. Arrows indicate mast cell. Data are reported as means ± SEM for six mice in each group. $^{*}P < 0.05$ compared to control group. $^{**}P < 0.05$ compared to allergic basal group (Student *t*-test/ANOVA – Tukey).

histological analysis of the adipose tissue revealed an increased inflammatory cells infiltration, characterized by lymphocytes and predominantly macrophages (Fig. 2D). In addition, the number of mast cells (Fig. 2B) infiltrating the epididymal adipose tissue from allergic mice (Fig. 2F) was higher than the observed in controls (Fig. 2E).

3.3. Leukocyte rolling and adhesion are increased in the epididymal adipose tissue of allergic mice

Epididymal adipose tissue microcirculation was visualized by intravital microscopy to investigate whether inflammatory cells were being recruited from microcirculation rather then a local proliferation. In control mice, few adhered and rolling leukocytes are seen in venular microcirculation (Sup. material video 1). However, a significant increase in both rolling and adhered leukocytes was observed in allergic mice (Sup. material video 2), as shown in Fig. 3A and B.

3.4. Epididymal adipose tissue from allergic mice exhibits high frequency of macrophages amongst stromal cells

In order to characterize the leukocyte infiltrating population in adipose tissue of allergic mice, we performed flow cytometry analysis. The number of total leukocytes in the stroma was increased in adipose tissue of allergic mice (Fig. 4A) with a higher percentage of macrophages in comparison to controls. Although there were more leukocytes in allergic adipose tissue, the profile of macrophages (M1 or M2) and lymphocytes was equivalent to control group (Fig. 4B and C). Furthermore, the activation status of lymphocytes was similar in both groups, as assessed by the CD69 expression (data not shown).

3.5. Alterations in adipose tissue from allergic mice are associated with increased levels of TNF-α, IL-6 and CCL2

TNF-α, IL-6 and CCL2 levels were determined to evaluate whether adipose tissue derived cytokines and chemokines were involved in the alterations induced in the epididymal fat of allergic mice. We found increased production of all the cytokines or chemokine investigated in the epididymal adipose tissue of allergic mice when compared to control mice (Fig. 5A, B and C).

3.6. Epididymal adipose tissue from allergic mice shows increased lipolytic activity

Adipocytes were incubated in basal or ISO-stimulated conditions to determine the effect on lipolysis of the antigen ingestion in sensitized mice. The adipocytes from allergic mice showed an increased basal lipolysis relative to control. The presence of ISO produced a significant increase in lipolytic rate in both groups (Fig. 5D).

3.7. Triglyceride, glucose, free fatty acid and total cholesterol are decreased in the serum of allergic mice

Fasting serum triglyceride (Fig. 6A), free fatty acid (Fig. 6B), total cholesterol (Fig. 6C) and glucose levels (Fig. 6D) were significantly decreased in allergic mice after the antigen ingestion when compared with the control animals. Blood glucose levels also had a significant reduction (approximately 12%) in allergic animals (data not shown).

4. Discussion

In the present work, we used an experimental model of food allergy in which Ova-sensitized mice were chronically fed with antigen [8]. Our results showed that the allergic mice demonstrated an accentuated weight loss during antigen ingestion. Moreover, these mice showed a significant reduction in epididymal adipose tissue mass, metabolic changes and an exuberant adipose tissue inflammatory process.

Changes in body weight reflect the energy homeostasis by integrating the intake of nutrients with an effective utilization of ingested calories, either by storage or by expenditure as cellular fuel. In our model, we observed a tenuous decrease in food intake in allergic group compared to control, which is related to an immunological food aversion [24,25]. On the other hand, we have previously shown that if the antigen is in a liquid source instead of in the chow, the food consumption is equivalent to control group and 20% of weight loss is still observed in allergic mice [8]. In agreement with this data, it has been shown that 60% restriction in food intake during 7 days was necessary to induce 18% body weight loss in mice [26]. Also, in another study, in which the body weight fat was measured, it was shown that mice held on 30% of diet restriction decreased 15% of their body weight fat [27]. In this sense, 18% reduction in food consumption could not itself explain the decreased body weight (16%) and decreased epididymal fat

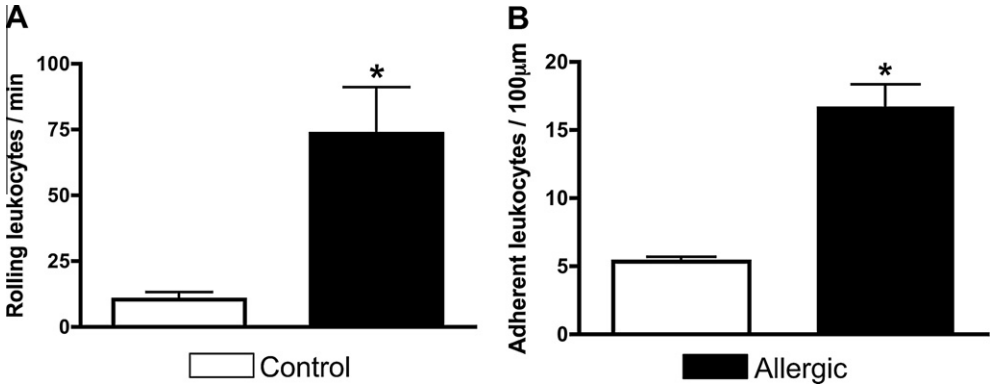

Fig. 3. Leukocyte rolling and adhesion are increased in the epididymal adipose tissue microvasculature of allergic mice. After 7 days of ingestion of the ovalbumin (Ova) containing diet, the leukocyte rolling (A) and adhesion (B) to the microvasculature of epididymal adipose tissue were assayed by intravital microscopy. Data are reported as means ± SEM for three mice in each group. $^{*}P < 0.05$ compared to control group. (Student *t*-test).

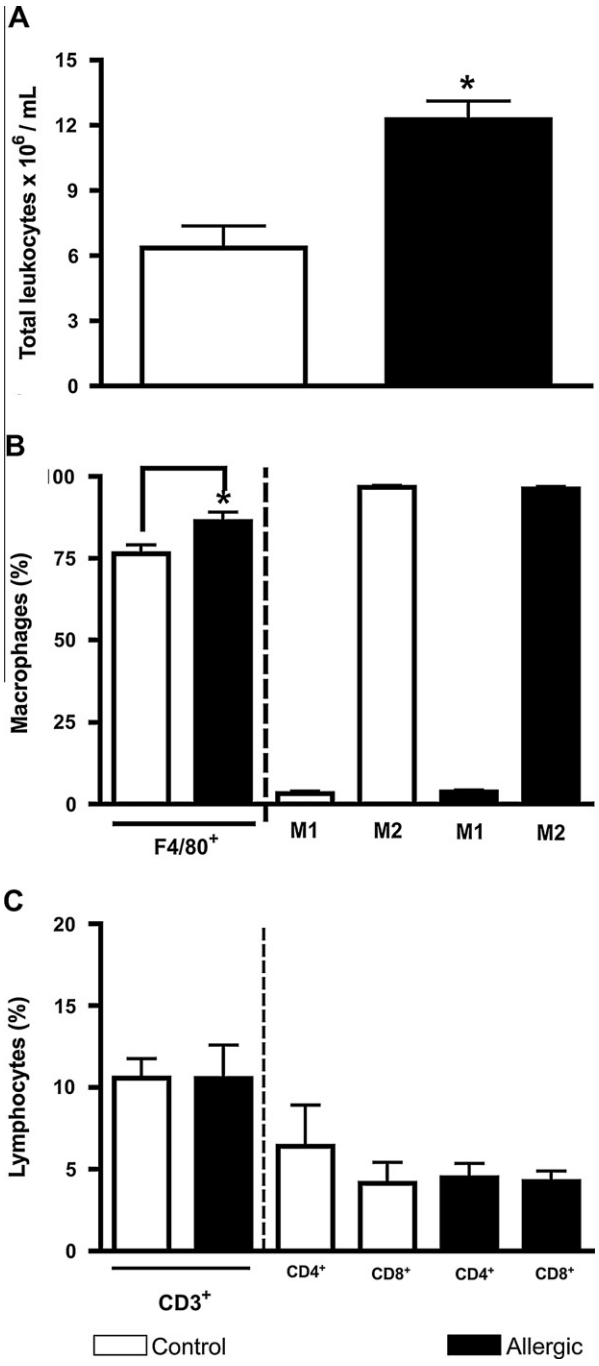

Fig. 4. Epididymal adipose tissue of allergic mice exhibits increased frequency of macrophages. After 7 days of ingestion of the ovalbumin (Ova) containing diet, the stromal vascular cells from epididymal adipose tissue was collected and the total leukocytes were counted (A). Flow cytometric analysis were performed to investigate the frequency of macrophages ($F4/80^+$), M1 macrophages ($F4/80^+CD11b^+CD11c^+$), M2 macrophages ($F4/80^+CD11b^+CD11c^-$), $CD3^+$ cells, $CD4^+$ T cells and $CD8^+$ T cells in stromal vascular fraction. Numbers on the graph indicate the percentage of cells among gated cells (B and C). Data are reported as means ± SEM for five mice in each group. $^*P < 0.05$ compared to control group. (ANOVA – Tukey).

(40%) observed in allergic mice. Another food allergy sign that may contribute to weight loss is the diarrhea, which is characterized by imbalanced ion exchange and water transfer [28]. Although this sign is not accompanied by dehydration in this model [8], diarrhea may impair nutrient absorption and contribute to weight loss.

In addition to the presence of food aversion and diarrhea, we investigated if an inflammatory response could be associated with the weight loss, as energy redistribution and particularly the lipids mobilization may occur to redistribute nutrients to the cells involved in the inflammatory response [15,17,18,29]. Histology analysis revealed an increased number of mast cells in epididymal adipose tissue of allergic mice. As there are higher levels of circulating anti-Ova specific IgE, we suggest that adipose tissue mast cells can be sensitized with anti-Ova IgE through FcεRI receptors. Ova can be partly transported on chylomicrons, which are prominently cleared in adipose tissue [30], and when in contact with sensitized mast cells, can induce degranulation and release of various pro-inflammatory mediators, including TNF-α, IL-1 and IL-6 [31,32]. According to this possible mechanism, we have shown increased TNF-α and IL-6 levels in the epididymal adipose tissue from allergic mice which may induce and exacerbate the local inflammation. As TNF-α up-regulates endothelial adhesion molecules allowing leukocytes recruitment to sites of inflammation [33], we hypothesized that a higher production of TNF-α seen in the adipose tissue from allergic mice contributes to increased leukocyte migration to adipose tissue. The production of TNF-α and IL-6 by adipocytes and stromal cells can be up-regulated and contribute to the pro-inflammatory milieu in the adipose tissue [34] and this may explain the increased leukocyte rolling, adhesion and emigration observed in adipose tissue during food allergy.

In addition to increased mast cell numbers, macrophage emigration was also higher in allergic mice, which was confirmed by flow cytometry analysis ($F4/80^+$ cells) [35]. The concomitant presence of mast cells and macrophages in adipose tissue has previously been described, and depletion of mast cells resulted in reduced adipose tissue macrophage content, suggesting that mast cells may be involved in adipose tissue macrophage (ATM) recruitment [36].

ATMs exhibit an M1–M2 polarization state in which M1 macrophages are defined as classically activated, pro-inflammatory cells, while M2 macrophages comprise an alternatively activated, anti-inflammatory population [37]. Although a higher number of macrophages were found in allergic adipose tissue, further flow cytometry analysis of macrophage polarization have not shown any differences between allergic and control group. In this sense, it is reasonable to suggest that in despite of a maintained M1/M2 proportion, a probably relevant M1 population is expected in allergic mice, as a clear pro-inflammatory profile is observed during food allergy, or, regulatory mechanisms can be involved to control this observed adipose tissue inflammation.

The increase in macrophage infiltration in adipose tissue of allergic mice was associated with higher local levels of the chemokine CCL2. These findings are in agreement with previous studies, which have established that a higher expression of CCL2 in adipose tissue may result in increased macrophage recruitment [38,39]. In this sense, emigrated macrophages represent a prominent source of inflammatory mediators in adipose tissue [37,40] that can both initiate and maintain the inflammatory milieu during allergy.

Previous in vitro data from co-culture system using adipocytes and macrophages demonstrated that a paracrine loop involving saturated fatty acids and TNF-α derived, respectively, from adipocytes and macrophages, establishes a vicious cycle that causes inflammatory changes that lead to up-regulation of pro inflammatory adipocytokines including CCL2, TNF-α and IL-6. TNF-α, which is derived mostly from infiltrated macrophages, induces adipocyte lipolysis and such released saturated fatty acids activate macrophages via Toll like receptor 4, increasing adipose tissue inflammation [41]. Interestingly, recombinant TNF-α administration in human subjects can promote 40% increase in whole body lipolysis [42].

Related weight loss diseases are frequently associated to elevated IL-6 levels. For example, body weight loss in mice with acute colitis was associated with higher serum levels of IL-6. IL-6 may

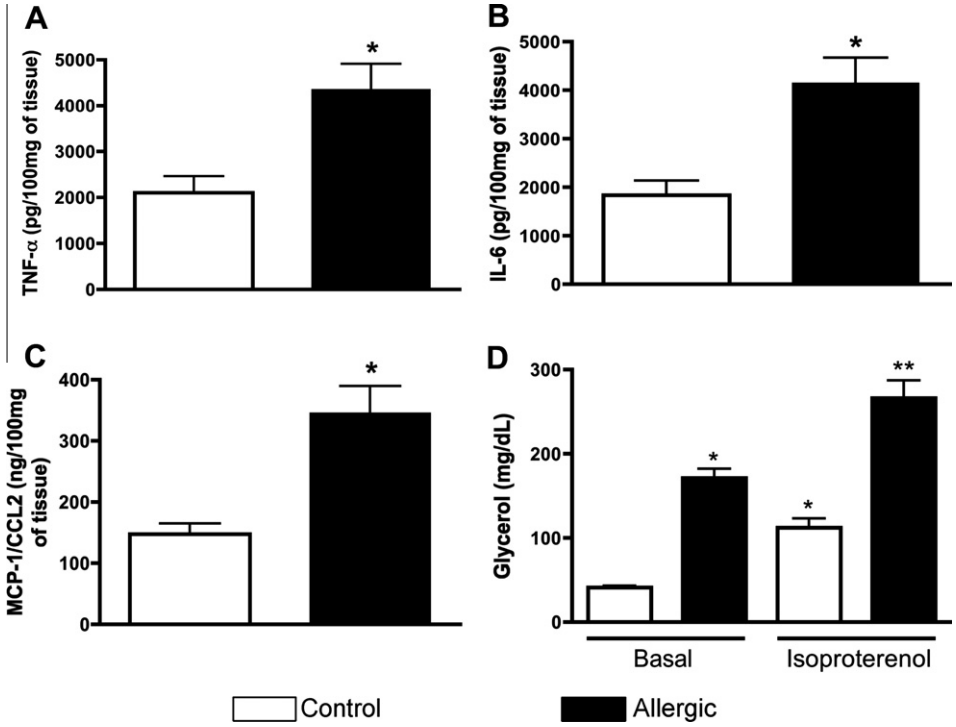

Fig. 5. Epididymal adipose tissue of allergic mice have elevated TNF-α, IL-6 and CCL2 levels and increased lipolysis. After 7 days of ingestion of the ovalbumin (Ova) containing diet, the epididymal adipose tissue was collected and assayed for TNF-α (A), IL-6 (B) and CCL2 (C) levels by ELISA. The glycerol release by isolated epididymal adipocytes were measured in the absence (basal) and presence of isoproterenol (D). Data are reported as means ± SEM for six mice in each group. $^{*}P < 0.05$ compared to control group. (Student *t*-test for the ELISA experiments; ANOVA – Tukey for the lipolysis experiments).

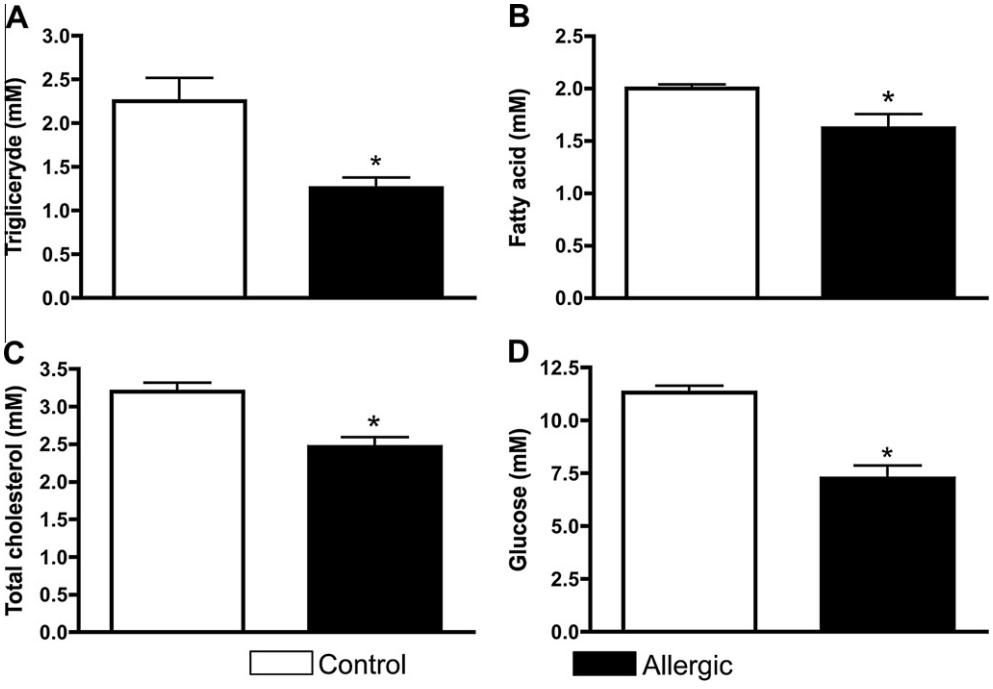

Fig. 6. Serum of allergic mice have decreased triglyceride, free fatty acid, total cholesterol and glucose levels. After 7 days of ingestion of the ovalbumin (Ova) containing diet, the serum was collected and the concentrations of triglyceride (A), free fatty acid (B), total cholesterol (C) and glucose (D) were measured in overnight fasted mice. Data are reported as means ± SEM for six mice in each group. $^{*}P < 0.05$ compared to control group. (Student *t*-test).

have a paracrine action in adipose tissue [29], since IL-6 levels in the human adipose tissue was 100-fold higher than that in serum [43]. In our study, we have shown that increased levels of IL-6 were found in epididymal adipose tissue from allergic mice, which corroborated the lipolytic action with weight-reducing properties of this cytokine [44]. Also, fully differentiated 3T3-L1 adipocytes have increased lipolysis after IL-6 treatment [45]. Therefore, it is very reasonable that both TNF-α and IL-6 can contribute to the

increased basal lipolytic activity seen in adipose tissue from allergic mice in our study. We have also demonstrated that the adipocyte area is decreased in the epididymal adipose tissue from allergic mice. Adipocyte atrophy may be attributable to a decrease in lipid deposition or an increase in the lipolysis rate [46]. We can infer that the increased lipolysis contributes to the reduced adipocyte size in allergic mice.

In summary, the inflammatory process induced by food allergy probably is associated with metabolic changes, increased lipolysis activity, reduction in the adipose tissue mass and decreased adipocyte size. Together, these alterations resulted in a significant body weight loss after antigen ingestion by allergic mice. In this sense, our study provides important contribution about the mechanisms involved in the body weight loss in animal models of food allergy and suggests an interplay between immune system and systemic metabolism.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.cellimm.2011.05.008.

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