# GALECTIN-3 DELETION ENHANCES VISCERAL ADIPOSE TISSUE INFLAMMATION AND DYSREGULATES GLUCOSE METABOLISM IN MICE ON A HIGH-FAT DIET

Ilija Jeftic[1, 2,*], Marina Miletic-Kovacevic[1, 3,*], Nemanja Jovicic[1, 3], Jelena Pantic[1], Nebojsa Arsenijevic[1], Miodrag L. Lukic[1], Nada Pejnovic[1, 2]
[1] Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[2] Institute of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[3] Institute of Histology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
[*] Authors Ilija Jeftic and Marina Miletic-Kovacevic contributed equally to this study.

# DELECIJA GALEKTINA-3 POSPEŠUJE INFLAMACIJU U VISCERALNOM MASNOM TKIVU I NARUŠAVA HOMEOSTAZU GLUKOZE U MIŠEVA NA ISHRANI BOGATOJ MASTIMA

Ilija Jeftić[1, 2,*], Marina Miletić-Kovačević[1, 3,*], Nemanja Jovičić1[1, 3], Jelena Pantić[1], Nebojša Arsenijević[1], Miodrag L. Lukić[1], Nada Pejnović[1, 2]
[1] Centar za molekulsku medicine I ispitivanje matičnih ćelija, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[2] Institut za patofiziologiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[3] Institut za histologiju, Fakultet medicinskih nauka, Univerzitet u Kragujevcu, Kragujevac, Srbija
[*] Autori Ilija Jeftić i Marina Miletić-Kovačević su podjednako učestvovali u istraživanju.

Received / Primljen: 16. 02. 2016. Accepted / Prihvaćen: 15. 03. 2016.

## ABSTRACT

*Obesity and type 2 diabetes mellitus (T2DM) constitute major health problems worldwide. Increased visceral adiposity enhances the risk of insulin resistance and type 2 diabetes. The mechanisms involved in obesity-associated chronic inflammation in metabolic tissues (metaflammation) that lead to insulin resistance and dysregulated glucose metabolism are incompletely defined. Galectin-3 (Gal-3), a β-galactoside-binding lectin, modulates immune/inflammatory responses and specifically binds to metabolic danger molecules. To dissect the role of Gal-3 in obesity and diabetes, Gal-3-deficient (LGALS3$^{-/-}$) and wild-type (WT) C57Bl/6 male mice were placed on a high-fat diet (HFD, 60% kcal fat) or a standard chow diet (10% kcal fat) for 6 months and metabolic, histological and immunophenotypical analyses of the visceral adipose tissue were performed. HFD-fed LGALS3$^{-/-}$ mice had higher body weights and more body weight gain, visceral adipose tissue (VAT), hyperglycaemia, hyperinsulinemia, insulin resistance and hyperlipidemia than diet-matched WT mice. Compared to WT mice, the enlarged VAT in obese LGALS3$^{-/-}$ mice contained larger adipocytes. Additionally, we demonstrate enhanced inflammation in the VAT of LGALS3$^{-/-}$ mice compared with diet-matched WT mice. The VAT of LGALS3$^{-/-}$ mice fed a HFD contained more numerous dendritic cells and proinflammatory F4/80$^{+}$CD11c$^{+}$CD11b$^{+}$ and F4/80$^{high}$ macrophages. In contrast to WT mice, the numbers of CXCR3$^{+}$ and CD8$^{+}$ T cells were increased in the VAT of Gal-3-deficient mice after 6 months of high-fat feeding. We provide evidence that Gal-3 ablation results in enhanced HFD-induced adiposity, inflammation in the adipose tissue, insulin resistance and hyperglycaemia. Thus, Gal-3 represents an important regulator of obesity-associated immunometabolic alterations.*

**Keywords:** *Galectin-3, obesity, hyperglycaemia, insulin resistance, metaflammation*

## SAŽETAK

*Gojaznost i tip 2 diabetes mellitusa (T2DM) predstavljaju veliki svetski zdravstveni problem. Uvećanje visceralnog masnog tkiva u gojaznosti je povezano sa većim rizikom za nastanak insulinske rezistencije i T2DM. Molekularni mehanizmi povezani sa hroničnom inflamacijom u metabolički aktivnim tkivima (metaflamacijom) u gojaznosti koji leže u osnovi insulinske rezistencije i narušene homeostaze glukoze nisu do kraja razjašnjeni. Galektin-3 je multifunkcionalni lektin sa značajnom ulogom u imunoregulaciji i metaflamaciji. Sa ciljem da se ispita uloga galektina-3 u nastanku gojaznosti i T2DM, galektin-3 deficijentni miševi (LGALS3$^{-/-}$) i miševi divljeg soja (WT) stavljeni su na ishranu sa visokim sadržajem masti (60% kcal od masti) ili standardnu ishranu (10% kcal od masti) u trajanju od 6 meseci nakon čega su ispitivani metabolički parametri, morfologija visceralnog masnog tkiva i fenotipske karakteristike infiltrišućih ćelija. LGALS3$^{-/-}$ miševi na ishrani sa visokim sadržajem masti imali su veću telesnu težinu, veću količinu visceralnog masnog tkiva, hiperglikemiju, hiperinsulinemiju, izraženiju insulinsku rezistenciju i hiperlipidemiju u poređenju sa WT miševima na istom režimu ishrane. Adipociti iz uvećanog visceralnog masnog tkiva LGALS3$^{-/-}$ miševa imali su veći dijametar u poređenju sa adipocitima u WT miševa. Izražena inflamacija u visceralnom masnom tkivu LGALS3$^{-/-}$ miševa na ishrani sa visokim sadržajem masti bila je praćena većom zastupljenošću dendrtičnih ćelija, proinflamatornih F4/80$^{+}$CD11c$^{+}$CD11b$^{+}$ i F4/80$^{high}$ makrofaga, CD3$^{+}$CXCR3$^{+}$ i CD8$^{+}$ T limfocita u poređenju sa WT miševima na istom režimu ishrane. Dobijeni rezultati ukazuju na značajnu protektivnu ulogu galektina-3 u nastanku gojaznosti, insulinske rezistencije i T2DM.*

**Ključne reči:** *Galektin-3, gojaznost, hiperglikemija, insulinska rezistencija, metaflamacija*

DE GRUYTER OPEN

**UDK: 616.98:579.852(497.11)"2014" / Ser J Exp Clin Res 2016; 17 (3): 231-239**
**DOI: 10.1515/SJECR-2016-0030**

**Corresponding author:** Nada Pejnovic, MD, PhD,
Professor of Pathophysiology, Institute of Pathophysiology, Faculty of Medical Sciences, University of Kragujevac,
Svetozara Markovica 69, 34000 Kragujevac, Serbia; Tel: +381 34 306 800, Fax: +38134306800112; E-mail: nada.pejnovic@gmail.com

## INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) constitute a major public health problem worldwide, particularly in developing countries (1). Obesity, mainly abdominal adiposity, is linked to various metabolic alterations that increase the risk for T2DM (2). T2DM is a metabolic disorder characterized by insulin resistance (IR) followed by pancreatic β-cell dysfunction (3). Pancreatic β-cells normally compensate for obesity-associated reduced insulin sensitivity by secreting more insulin to maintain glucose homeostasis. Hyperinsulinemia occurs early in the disease progression and before the β-cell function becomes impaired, leading to late-stage (insulin-dependent) T2DM (4).

Obesity-induced diabetes is associated with low-grade inflammation in the visceral adipose tissue (VAT). Inflammation in the VAT plays an important role in metabolic abnormalities such as IR (5). Antigen-presenting cells, including dendritic cells (DCs) and macrophages, are increased in the VAT in obesity, where they potentiate both innate and adaptive immune/inflammatory responses. Numerous studies have shown the increased infiltration of the visceral adipose tissue by macrophages during obesity (6,7). Macrophages may be derived from blood monocytes and are classified as classically (M1) and alternatively (M2) activated macrophages (8). Recent studies have shown that macrophages are key cells that mediate obesity-induced metabolic abnormalities. In obesity, a shift from alternatively activated M2 to classically activated M1 macrophages, characterized by elevated F4/80, CD11b and CD11c expression, has been demonstrated (9,10). Moreover, the ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin-resistant animals (11). Adipocytes damaged by high lipid intake produce signals that promote a proinflammatory Type 1 immune response. The number of T cells is increased in the enlarged VAT during obesity in response to adipose tissue-specific factors (12). Adipose tissue-associated regulatory T cells (Tregs) protect against (13), while the recruitment of Type 1 CD4 and $CD8^+$ T lymphocytes precedes, metabolic alterations in obesity (14,15). The chemokine receptor CXCR3 is highly expressed in activated T cells and is involved in the regulation of T cell trafficking and maturation. Additionally, it has been shown that CXCR3-positive cells play an important role in the modulation of obesity-induced visceral adipose tissue inflammation and systemic insulin resistance (16).

Galectin-3 (Gal-3) is a β-galactoside-binding lectin that is expressed in different tissues and cells and plays an important role in obesity, T2DM and inflammation (17,18,19). Gal-3 regulates inflammation and adipogenesis, and this lectin is expressed in adipocytes and infiltrating immune cells in the adipose tissue (20). Additionally, Gal-3 regulates adipocyte cell proliferation and differentiation (21) and has a variety of regulatory roles in the innate and adaptive immune response, depending on the disease conditions (18,22,23). However, the role of Gal-3 in type 2 diabetes remains incompletely understood. The Gal-3 levels are increased in the sera of obese subjects and negatively correlate with the levels of glycosylated haemoglobin (24). In contrast with these data, Okhura et al. reported that low serum Gal-3 levels are associated with insulin resistance and T2DM (25). However, controversial results have been reported regarding the effects of Gal-3 ablation in experimental models of diabetes. It has been shown that Gal-3-deficient mice were relatively resistant to diabetogenesis in streptozotocin-induced diabetes (26). On the other hand, Pejnovic et al. reported that obese Gal-3-deficient mice had enhanced adiposity, hyperglycaemia, hyperinsulinemia, IR and systemic inflammation in comparison with their diet-matched wild-type controls (18). Moreover, in a study by Peng et al. (27), Gal-3-null mice fed a HFD had increased adiposity and dysregulated glucose metabolism. In addition, the same authors found that young Gal-3-deficient mice fed a standard diet exhibit altered glucose homeostasis, thus suggesting the modulation of glucose metabolism and possibly β-cell function by Galectin-3 (Gal-3) independently of obesity and inflammation.

To investigate the role of Gal-3 in high-fat diet-induced obesity, we used Gal-3-deficient mice on a C57Bl/6 background. We examined inflammation in the visceral adipose tissue and metabolic abnormalities following long-term HFD exposure in Gal-3-deficient and wild type (WT) mice. We report here that Gal-3 deletion enhanced high-fat diet (HFD)-induced obesity and visceral adiposity, amplified inflammation in the visceral adipose tissue and led to dysregulated glucose metabolism characterized by hyperglycaemia and insulin resistance.

## MATERIALS AND METHODS

### Experimental mice and study design

Gal-3-deficient mice on a C57BL/6 background and their littermate controls were obtained from the University of California Davis (Davis, CA; by courtesy of D.K. Hsu and F.T. Liu) and accommodated in our animal facilities under standard laboratory conditions in a temperature-controlled environment with a 12 h light/darkness cycle. Male 2-month-old wild type (WT) and Gal-3-deficient ($LGALS3^{-/-}$) mice were fed either normal chow or a 60% fat/kcal diet (Mucedola, Italy) *ad libitum*. After 6 months, the mice were sacrificed and blood samples and visceral adipose tissue were collected for analyses. All animal procedures were approved by the ethical committee of the Faculty of Medical Sciences, University of Kragujevac (Permit Number 01-2759/2).

### Body weight and glucose metabolism analyses

Body weights and fasting blood glucose levels were measured once per month and after 6 months on a standard or

HFD. The mice were fasted for 4 h, and their glucose levels (mmol/L) were determined using the Accu-Chek Performa glucometer (Roche Diagnostics, Mannheim, Germany). The serum concentrations of total cholesterol and triacylglycerol were measured using the Olympus AU600 Chemistry Immuno Analyzer (Olympus, Tokyo, Japan) and the fasting insulin was measured using an Insulin ELISA kit (Alpco, Salem, NH, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = [(Glucose mmol/L x Insulin mU/L)] / 22.5.

### Histological analysis of visceral adipose tissue

Visceral adipose tissue (VAT), including the epididymal, mesenteric and renal fat pads, was dissected and weighed as visceral fat content. After weighing, part of the epididymal fat was fixed in 10% buffered formalin. The mean adipocyte size of the visceral fat pad was determined by computer-assisted image analysis of paraffin-embedded adipose tissue sections (5 **µm) stained with** haematoxylin and eosin (H&E). The adipocyte size was measured from a total of 50 cells per mouse in three separate fields using a light microscope (BX51; Olympus) equipped with a digital camera and *ImageJ* software. Analyses were performed in a blinded fashion by two independent observers. The data are expressed as the mean adipocyte diameter (µm) for each tissue in each animal.

### Isolation of visceral adipose tissue stromal ascular fraction cells

Total visceral adipose tissue (VAT) was subjected to the isolation of stromal vascular fraction (SVF) cells. Collagenase digestion (1 mg/ml collagenase type II and 2% BSA (Sigma-Aldrich, St. Louis, MO)) was used to separate the SVF from the adipocytes of the VAT, as previously described (18). SVF was used for flow cytometric analysis, as described below.

### Flow cytometric analyses

Adipose tissue SVF cells were stained with the following fluorescence-tagged monoclonal antibodies: anti-mouse CD45, CD3, CD4, CXCR3, CD8, CD11b, Lineage cocktail (BD Biosciences, San Jose, CA), Sca-1, NK 1.1, F4/80 and CD11c (BioLegend, San Diego, CA) or isotype-matched controls (BD Biosciences). The cells were analysed using a FACSCalibur flow cytometer (BD Biosciences) and FlowJo software (Tree Star).

### Statistical analyses

Statistical analysis was performed using SPSS 13.0. The data are presented as the means ± SEM. Statistical significance was determined by an independent-sample Student's t test and, where appropriate, a Mann-Whitney U test. Statistical significance was assumed at $p<0.05$.

## RESULTS

### Galectin-3 ablation accelerated HFD-induced obesity and obesity-related metabolic alterations

After 6 months, HFD increased the body weight and weight gain in both genotypes of mice, and these parameters were higher in HFD-fed LGALS3$^{-/-}$ mice than in diet-matched WT mice (Fig. 1A). HFD feeding increased the visceral fat mass in both genotypes of mice, which was more pronounced in HFD-fed LGALS3$^{-/-}$ mice than in diet-matched WT mice (Fig. 1B). The serum total cholesterol and triglyceride levels were significantly higher in obese LGALS3$^{-/-}$ mice than in WT mice (Fig. 1C).

### Galectin-3 deletion modulates HFD-induced adipocyte morphology

In view of the fact that Gal-3 is expressed in the adipose tissue and regulates adipocyte cell proliferation and differentiation, we analysed adipose tissue morphology and adipocyte size. The HFD significantly increased the adipocyte size in both genotypes of mice compared with mice on a standard diet. A significantly higher amount of total visceral adipose tissue was accompanied by larger adipocytes in LGALS3$^{-/-}$ mice on a HFD than in diet-matched WT animals (Fig. 1D).

### Gal-3 ablation accelerated HFD-induced hyperglycaemia, hyperinsulinemia and obesity-associated insulin resistance

In addition to accelerated HFD-induced obesity, the fasting blood glucose, insulin and HOMA-IR were significantly higher in obese LGALS3$^{-/-}$ mice than in WT mice (Fig. 2). Additionally, the fasting blood glucose levels, insulin and insulin resistance were significantly higher in LGALS3$^{-/-}$ mice than in WT mice on a standard diet (Fig. 2).

### Increased dendritic cells and proinflammatory macrophages in the VAT in LGALS3$^{-/-}$ mice on a HFD

Because obesity-induced diabetes is strongly associated with adipose tissue inflammation, we first analysed the innate immune cells in the visceral adipose tissue in both genotypes of mice after 6 months on a HFD. DCs and macrophages are known to be increased in the visceral adipose tissue during obesity. The VAT from HFD-fed LGALS3$^{-/-}$ mice contained higher numbers of total CD11c$^{+}$ ($p=0.034$) and CD11c$^{+}$F4/80$^{-}$ ($p=0.043$) DCs than the VAT from HFD-fed WT mice (Fig. 3A). In addition, the subsets of mature proinflammatory F4/80$^{hi}$ ($p=0.034$) and triple-pos-

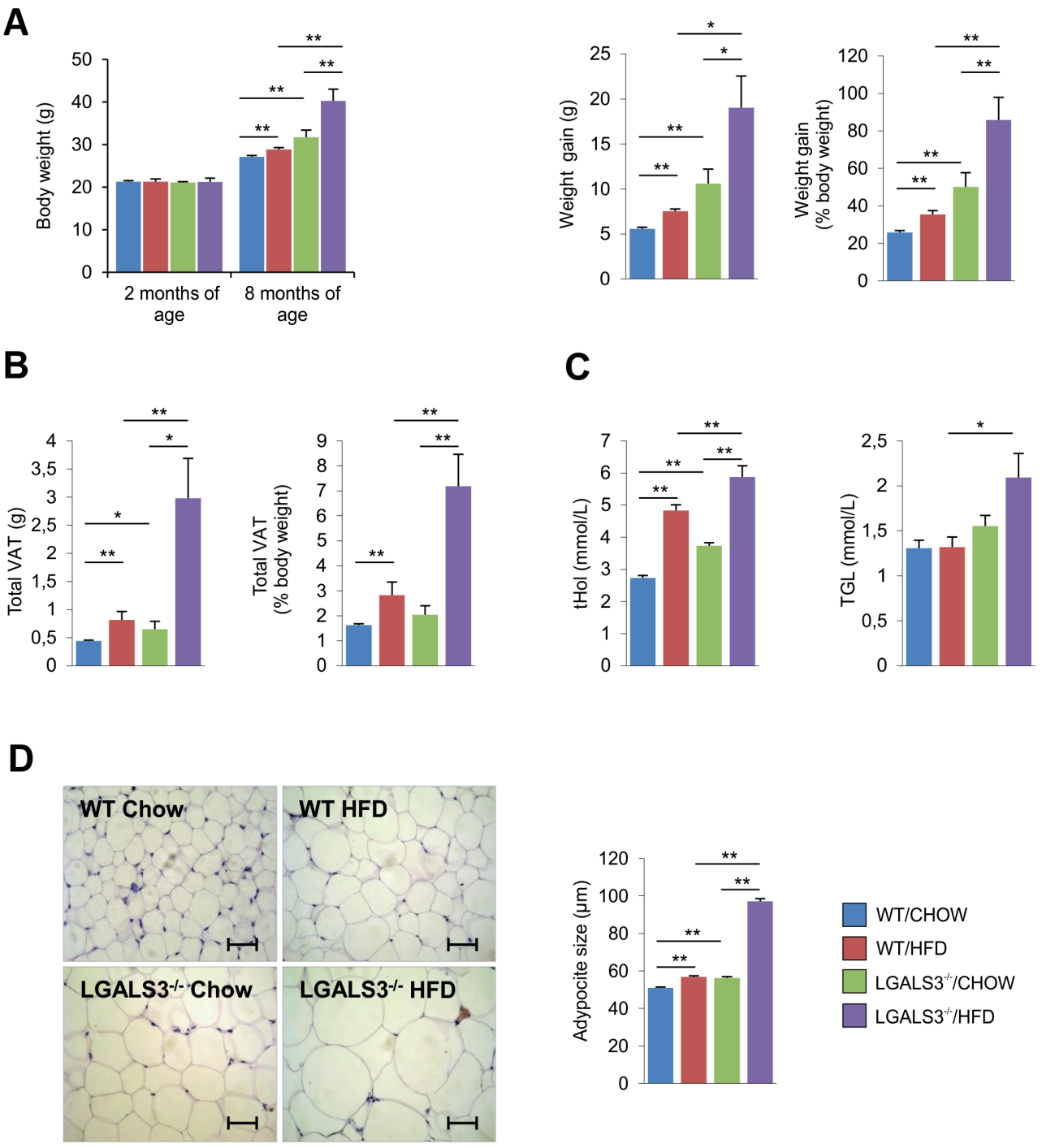


**Figure 1. Galectin-3 ablation accelerated HFD-induced obesity and adipocyte hypertrophy**
Male 2-month-old wild type (WT) and Gal-3-deficient (LGALS3$^{-/-}$) mice were fed either standard chow or a HFD *ad libitum* for 6 months. Metabolic parameters after 6 months on the HFD or standard diet are shown. (A) Body weight and weight gain were higher in HFD-fed LGALS3$^{-/-}$ mice than in diet-matched WT mice. (B) Total VAT and visceral fat mass (% body weight) were significantly higher in LGALS3$^{-/-}$ mice fed a HFD than in diet-matched WT animals. (C) Serum lipid levels were significantly higher in obese LGALS3$^{-/-}$ mice than in WT mice. (D) Representative images depicting the larger adipocyte size in LGALS3$^{-/-}$ mice on a HFD than in diet-matched WT animals (original magnification 40x, scale bar = 50 µm). The results are shown as the means ± SEM (n=5-6 mice/group), *P<0.05, **P<0.01.

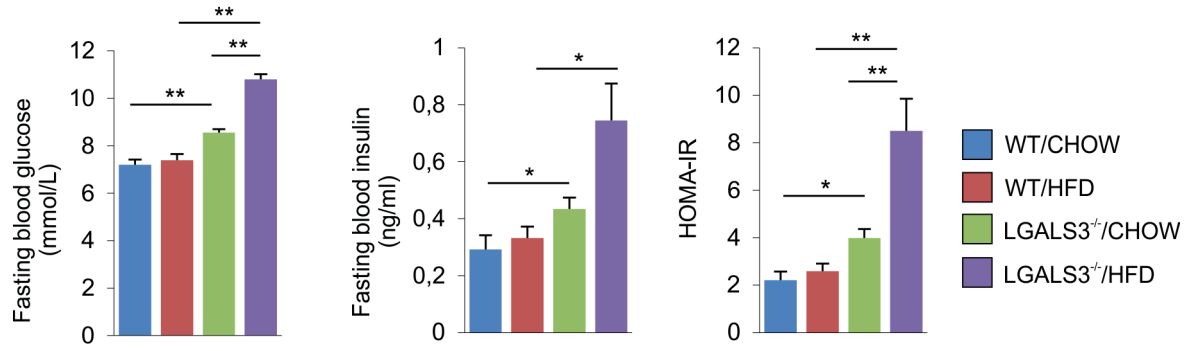


**Figure 2. Galectin-3 ablation accelerated HFD-induced hyperglycaemia, insulinemia and insulin resistance**
The fasting blood glucose, insulin and HOMA-IR were significantly higher in LGALS3$^{-/-}$ mice than in WT mice after 6 months on a HFD. The results are shown as the means ± SEM (n=5-6 mice/group), *P<0.05, **P<0.01.

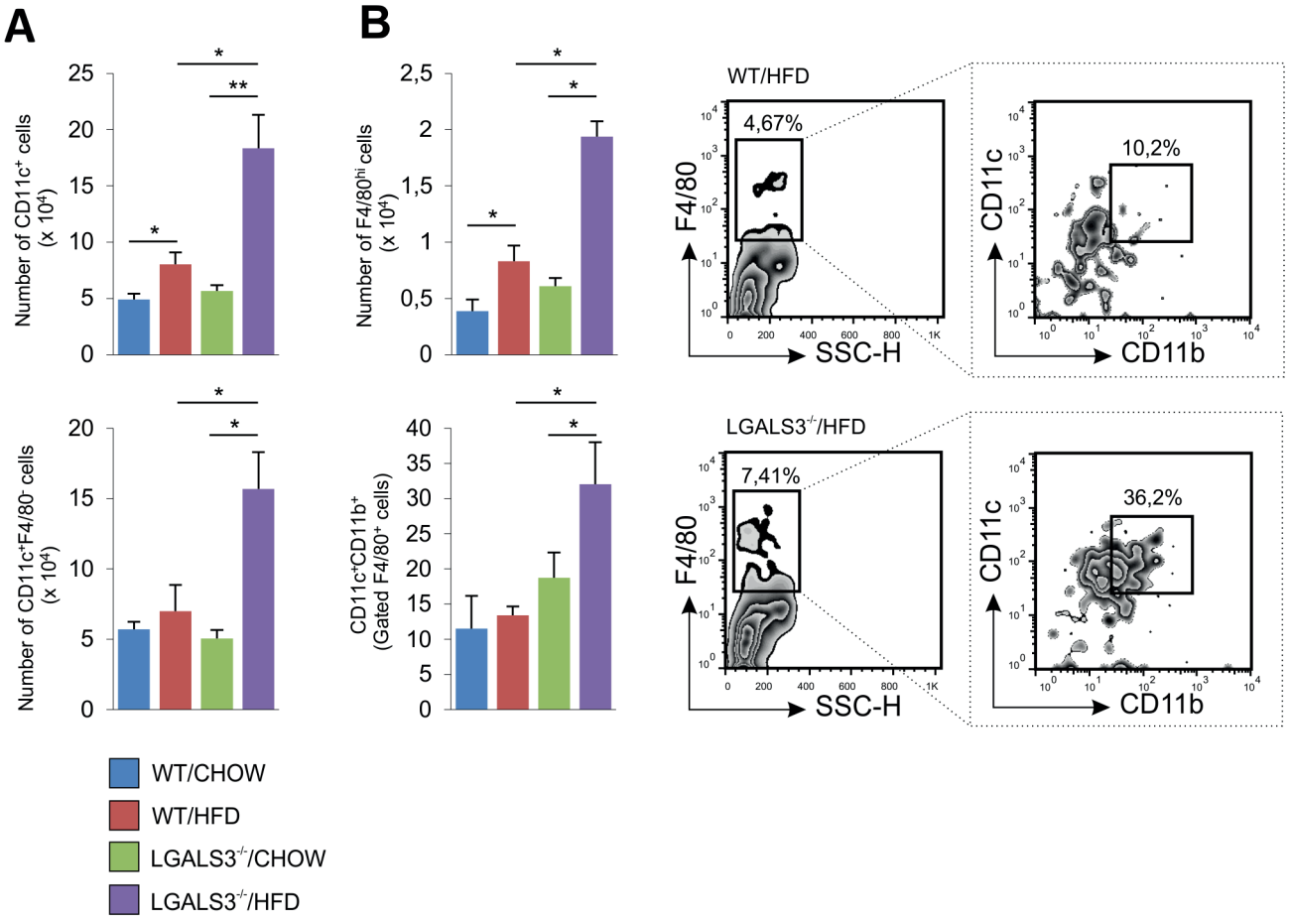


**Figure 3. Increased dendritic cells and proinflammatory macrophages in the visceral adipose tissue of LGALS3$^{-/-}$ mice on a HFD**
Representative images and flow cytometric analysis of VAT stromal vascular fraction cells from WT and LGALS3$^{-/-}$ mice after 6 months on a HFD or standard diet. (A) Dendritic cell numbers were increased significantly in the VAT in obese LGALS3$^{-/-}$ mice. (B) Proinflammatory macrophages were significantly increased in LGALS3$^{-/-}$ mice fed a HFD compared with diet-matched WT mice. Representative FACS plots are shown. The results are shown as the means ± SEM (n=5-6 mice/group), *P<0.05, **P<0.01.

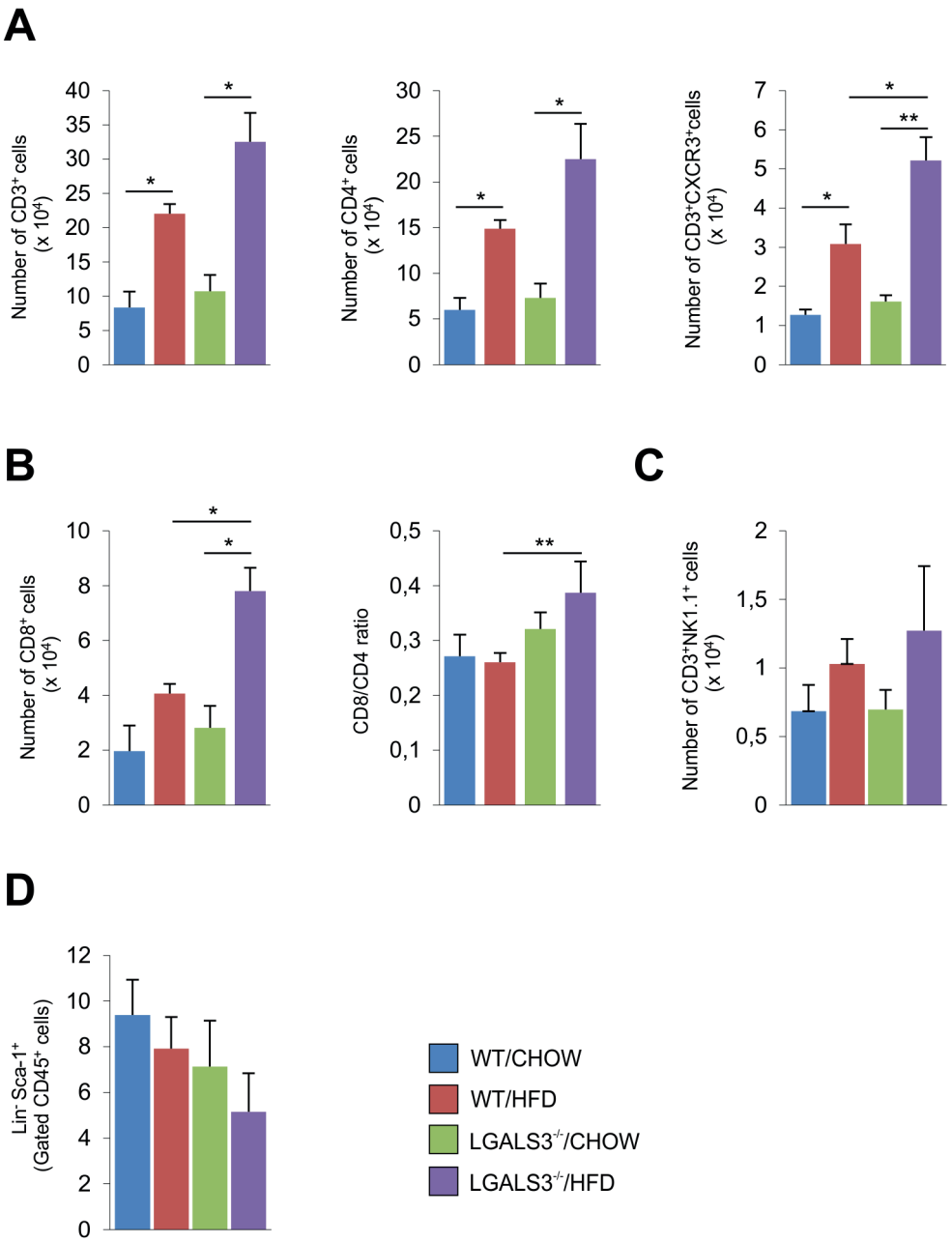


**Figure 4. Increased CXCR3+ and CD8+ T lymphocytes in the visceral adipose tissue of LGALS3$^{-/-}$ mice on a HFD**
Flow cytometric analysis of VAT stromal vascular fraction cells from WT and LGALS3$^{-/-}$ mice after 6 months on a HFD or standard diet. (A) CD3$^{+}$, CD4$^{+}$ and CD3$^{+}$CXCR3$^{+}$ T lymphocytes in the VAT. (B) The number of CD8$^{+}$ T lymphocytes and the CD8/CD4 ratio were higher in Gal-3-deficient mice on a HFD than in diet-matched WT mice. (C) NKT lymphocytes in the VAT (D) Lin$^{-}$ Sca-1$^{+}$ innate lymphoid cells in the VAT. The results are shown as the means ± SEM (n=5-6 mice/group), *P<0.05, **P<0.01.

itive F4/80$^{+}$CD11b$^{+}$CD11c$^{+}$ macrophages (p=0.034) were significantly higher in the VAT of obese LGALS3$^{-/-}$ mice than in WT mice (Fig. 3B).

### Increased CXCR3+ and CD8+ T cells in the VAT in LGALS3$^{-/-}$ mice on a HFD.

Increased Type 1 T cells and cytotoxic CD8$^{+}$ T cells in the VAT are hallmarks of obesity-induced diabetes and are strongly associated with adipose tissue inflammation. Therefore, we performed phenotypic analyses of T lymphocytes in the visceral adipose tissue in both genotypes of mice after 6 months on a HFD.

The HFD significantly increased the number of CD3$^{+}$ and CD4$^{+}$ T lymphocytes in both genotypes of mice compared with mice on a standard diet. There was no significant difference in the number of CD3$^{+}$ and CD4$^{+}$ T cells between the genotypes on a HFD (Fig. 4A). The HFD sig-

nificantly increased the number of $CD3^+CXCR3^+$ T cells in both genotypes of mice compared with mice fed a standard diet. However, the number of $CD3^+CXCR3^+$ cells (p=0.031) was higher in the VAT from $LGALS3^{-/-}$ mice than in the VAT from WT mice, both on a HFD (Fig. 4A). Moreover, the number of $CD8^+$ (p=0.034) T lymphocytes and the CD8-to-CD4 ratio (Fig. 4B) were higher in $LGALS3^{-/-}$ mice fed a HFD than in diet-matched WT mice (p=0.014). There was no difference in the number of NKT cells (Fig. 4C) or the percentage of $CD45^+Lin^-Sca\text{-}1^+$ innate lymphoid cells between the two genotypes of mice on both diets (Fig. 4D).

## DISCUSSION

In this report, we demonstrate increased obesity, visceral adipose tissue inflammation and dysregulated glucose metabolism in $LGALS3^{-/-}$ mice on a long-term high-fat diet. These effects appear to be mediated by both the metabolic and the immunoregulatory effects of Gal-3. After 6 months on a HFD, the body weight, weight gain and amount of total visceral tissue were significantly higher in $LGALS3^{-/-}$ mice than in diet-matched WT mice (Fig. 1A and 1B). HFD feeding in Gal-3-deficient mice resulted in increased weight and visceral fat mass followed by significantly higher serum total cholesterol and triglyceride levels (Fig. 1C). Additionally, the fasting blood glucose and insulin levels, as well as the HOMA-IR, were significantly higher in obese and lean $LGALS3^{-/-}$ mice than in WT mice (Fig. 2).

There is increasing evidence that Gal-3 plays an important role in obesity and T2DM (18). However, controversial results have been reported regarding the effects of Gal-3 in obese patients and experimental models. Our results are in agreement with the study reported by Okhura et al., which demonstrated that low serum Gal-3 levels are associated with insulin resistance in T2DM patients (25). It has been recently demonstrated that obese $LGALS3^{-/-}$ mice have increased fasting blood glucose and insulin levels compared with diet-matched WT animals (18). Additionally, Pejnovic et al. (18) reported significantly increased IFN-γ-producing Type 1 T/NKT cells and pro-inflammatory M1 macrophages and reduced T regulatory cells and alternatively activated M2 macrophages in the VAT of $LGALS3^{-/-}$ mice fed a HFD compared with diet-matched WT animals. Pang et al. (27) reported that Gal-3-deficient mice fed a HFD for 12 weeks develop increased adiposity and systemic inflammation. In addition, the same authors showed that despite the increased adiposity in Gal-3-deficient mice, there was no significant difference in the size of the adipocytes (27). In this study, we demonstrated that LGALS3-/- mice fed a HFD for 6 months developed visceral adiposity, hyperglycaemia and IR. Increased adiposity in Gal-3-deficient mice on a HFD was associated with adipocyte hypertrophy (Fig. 1D).

Inflammation in the VAT during obesity plays a central role in metabolic abnormalities such as IR. We demonstrate that a long-term HFD induced innate and adaptive immune cell infiltration in the VAT (Fig. 3 and 4). It has been reported that DCs, as professional antigen-presenting cells, have an important role in obesity-induced VAT inflammation (28). The obesity-associated increase of $CD11c^+$ cells in the adipose tissue suggests that DCs play a role in macrophage recruitment and activation (29). The VAT from HFD-fed $LGALS3^{-/-}$ mice contained higher numbers of total $CD11c^+$ and $CD11c^+F4/80^-$ DCs than HFD-fed WT animals (Fig. 3A). Additionally, the expression of CD11c is one of the key characteristics of proinflammatory (M1) macrophages in addition to specific markers such as F4/80 and CD11b. Triple-positive $F4/80^+CD11b^+CD11c^+$ macrophages (30) were more numerous in the VAT of $LGALS3^{-/-}$ mice fed a HFD (Fig. 3B) and were recently described as a proinflammatory macrophage subset (10). This macrophage population is increased in the adipose tissue in obese vs. lean mice (10). In addition, the subset of proinflammatory $F4/80^{hi}$ macrophages (30) was significantly higher in obese $LGALS3^{-/-}$ mice than in WT mice (Fig. 3B). This result is in agreement with the reported data of increased proinflammatory $F4/80^+CD11c^+CD206^+$ macrophage numbers in the VAT of $LGALS3^{-/-}$ after 11 weeks on a HFD (18). Type 1 T cells have a major role in obesity-associated chronic inflammation (31). The HFD increased the number of $CD3^+$ lymphocytes in the VAT of both genotypes of mice compared with chow-fed mice (Fig. 4A). CXCR3, a chemokine receptor that is highly expressed on activated T cells, is involved in T cell trafficking and activation (32). $CD3^+$ cells expressing CXCR3 were higher in the VAT from $LGALS3^{-/-}$ mice than in WT mice, both on a HFD (Fig. 4A). Despite the overlapping expression of chemokine receptors, CXCR3-positive cells represent Th1 lymphocytes. Th1 lymphocytes expressing CXCR3 produce more Th1-type cytokines such as IFN-γ and enhance the activity of CD8+ T cell effectors *in vitro* (33). Obesity is associated with increased $CD8^+$ cells in the VAT (33). In our study, the number of $CD8^+$ cells and the CD8/CD4 ratio (Fig. 4B) in the VAT was increased in $LGALS3^{-/-}$ mice fed a HFD compared with diet-matched WT mice, suggesting the role of cytotoxic $CD8^+$ cells in VAT inflammation and related metabolic abnormalities.

In summary, we provide evidence that Gal-3 deletion enhanced long-term HFD-induced adiposity, visceral adipose tissue inflammation, insulin resistance and hyperglycaemia. These data contribute to better understanding of the role Gal-3 in obesity, metabolic inflammation and T2DM.


### Acknowledgments

We thank Ivan Jovanovic and Gordana Radosavljevic for collegial help and Aleksandar Ilic, Katerina Martinova, Sandra Nikolic (Center for Molecular Medicine, Faculty of Medical Sciences, University of Kragujevac) and Zoran Mitrovic (Institute of Pathology, Faculty of Medical Sciences, University of Kragujevac) for technical assistance.

**Financial support**

This study was supported by grants from the Serbian Ministry of Science and Technological Development (175071 and 175069) (Belgrade, Serbia), the Swiss Science Foundation (SCOPES, IZ73Z0_152407) and Faculty of Medical Sciences grants (JP 02-14, JP 03-14, JP 07-12 and MP 01-12), Kragujevac, Serbia.

## REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract 2010;87:4-14
2. Guilherme A, Virbasius J, Vishwajeet P and Czech MP. Adipocyte dysfunction linking obesity to insulin resistance and type 2 diabetes.Nat Rev Mol Cell Biol 2008;9:367-77
3. Masato K. Insulin resistance and pancreatic β cell failure. J Clin Invest 2006;116:1756-60
4. Ferannini E and Mari A. β-cell function in type 2 diabetes. Metabolism 2014;63:1217-27
5. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest. 2003;112:1821-30
6. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL and Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. J. Clin. Invest. 2003;112:1796-1808
7. Neels JG and Olefsky JM. Inflamed fat: what starts the fire? JClin Invest 2006;116:33-35
8. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front. Biosci. 2008;13:453-61
9. Zeyda M and Stulnig TM. Adipose tissue macrophages. Immunol Let 2007;112:61-67
10. Lumeng CN, Bodzin JL and Saltiel AR. Obesity induce a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007;117:175-184
11. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. Cell Metab 2008;8:301-309
12. Harford KA, Reynolds CM, McGillicuddy FC and Roche HM. Fats, inflammation and insulin resistance: insights to the role of macrophage and T-cell accumulation in adipose tissue. Proc Nutr Soc 2011;70:408-17
13. Feuerer M, Herrero L, Cipolletta D, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nat Med 2009;15:930-39
14. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J et al. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med. 2009;15:921-29.
15. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsuqi M et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. Nat Med 2009;15:914-20
16. Deiuliis JA, Oghumu S, Duggineni D, Zhong J, Rutsky J, Banerjee A et al. CXCR3 modulates obesity-induced visceral adipose inflammation and systemic insulin resistance. Obesity (Silver Spring) 2014;22:1264-74
17. Dumic J, Dabelic S and Flögel M. Galectin-3: an open-ended story. Biochim Biophys Acta. 2006;1760:616-35
18. Pejnovic N, Pantic J, Jovanovic I, Radosavljevic G, Milovanovic M, Nikolic I, Zdravkovic NS, Djukic AL, Arsenijevic NN, Lukic ML:Galectin-3 deficiency accelerates high-fat diet induced obesity and amplifies inflammation in adipose tissue and pancreatic islets. Diabetes 2013,62:1932-44.
19. Weigert J, Neumeier M, Wanninger J, Bauer S, Farkas S, Scherer MN et al. Serum galectin-3 is elevated in obesity and negatively correlates with glycosylated hemoglobin in type 2 diabetes. J Clin Endocrinol Metab 2010:95:1404-11
20. Rhodes DH, Pini M, Castellanos KJ, Montero-Melendez T, Cooper D, Perretti M et al. Adipose tissue-speific modulation of galectin expression in lean and obese mice: evidence for regulatory function. 2013;21:310-19
21. Kiwak K, Novak CM, Hsu DK, Liu FT and Levine JA. Galectin-3 stimulates preadipocyte proliferation and is up-regulated in growing adipose tissue. Obesity 2007; 15:32-39
22. Liu FT. Regulatory roles of galectins in the immune response. Int Arch Allergy Immunol 2005;136:385-400
23. Radosavljevic G, Volarevic V, Jovanovic I, Milovanovic M, Pejnovic N, arsenijevic N et al. The roles of Galectin-3 in autoimmunity and tumor progression. Immunol Res. 2010;52:100-10
24. Weigert J, Neumeier M, Wanninger J, Bauer S, Farkas S, et al. (2010) Serum galectin-3 is elevated in obesity and negatively correlates with glycosylated hemoglobin in type 2 diabetes. J Clin Endocrinol Metab 95:1404-11
25. Okhura T, Fujioka Y, Nakanishi R, Shiochi H, Sumi K, Yamamoto N, et al. Low serum galectin-3 concentrations are associated with insulin resistance in patients with type 2 diabetes mellitus Diabetol Metab Syndr 2014;6:106
26. Mensah-Brown EP, Al Rabesi Z, Shahin A, Al Shamsi M, Arsenijevic N, Hsu DK, Liu FT, Lukic ML: Targeted disruption of the galectin-3 gene results in decreased susceptibility to multiple low dose streptozotocin-induced diabetes in mice. Clin Immunol 2009,130:83-88
27. Pang J, Rhodes DH, Pini M, Akasheh RT, Castellanos KJ, Cabay RJ, Cooper D, Perretti M, Fantuzzi G:Increased adiposity, dysregulated glucose metabolism and systemic inflammation in Galectin-3 KO mice.PLoS One 2013,8:e57915
28. Huh JJ, Park YJ, Ham M and Kim JB. Crosstalk between adipocytes and immune cells in adipose tissue Inflam-

mation and metabolic dysregulation in obesity. Mol Cells 2014;37:265-71
29. Stefanovic-Racic M, Yang X, Turner MS, Mantell BS, Stolz DB, Sumpter TL, et al. Dendritic Cells Promote Macrophage Infiltration and Comprise a Substantial Proportion of Obesity-Associated Increases in CD11c+ Cells in Adipose Tissue and Liver. Diabetes 2012;61:2330-9
30. Nguyen MT, Nguyen AK, Reichart D, Scott PA, Jenn A, Liu-Bryen R et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. J Biol Chem. 2007;282:35279-92
31. Strissel KJ, DeFuria J, Shaul ME, Bennett G, Greenberg AS, Obin MS. T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. Obesity (Silver Spring) 2010;18:1918–25
32. Yoon SH, Yun SO, Park JY, Won HY, Kim EK, Sohn HJ, et al. Selective addition of CXCR3 (+)CCR4(-)CD4(+) Th1 cells enchances generation of cytotoxic T cells by dendritic cells in vitro. Exp Mol Med 2009;41:161-70
33. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes 2008 32:451–63