Effects of in vitro vitamin D treatment on function of T cells and autophagy mechanisms in high-fat diet-induced obese mice

Min Su Kang 1, Chan Yoon Park 2, Ga Young Lee 1, Da Hye Cho 1, So Jeong Kim 1, and Sung Nim Han 1,3

1Department of Food and Nutrition, College of Human Ecology, Seoul National University, Seoul 08826, Korea
2Department of Food & Nutrition, College of Health Science, The University of Suwon, Hwaseong 18323, Korea
3Research Institute of Human Ecology, Seoul National University, Seoul 08826, Korea

ABSTRACT

BACKGROUND/OBJECTIVES: Obesity is associated with the impaired regulation of T cells characterized by increased numbers of Th1 and Th17 cells and the dysregulation of vitamin D metabolism. Both obesity and vitamin D have been reported to affect autophagy; however, a limited number of studies have investigated the effects of vitamin D on T cell autophagy in obese mice. Therefore, we aimed to determine whether in vitro treatment with vitamin D affects the proliferation, function, and autophagy of T cells from obese and control mice.

MATERIALS/METHODS: Five-week-old male C57BL/6 mice were fed control or high-fat diets (10% or 45% kcal fat: CON or HFDs, respectively) for 12 weeks. Purified T cells were stimulated with anti-CD3 and anti-CD28 monoclonal antibodies and cultured with either 10 nM 1,25(OH)2D3 or 0.1% ethanol (vehicle control). The proliferative response; expression of CD25, Foxp3, RORγt, and autophagy-related proteins (LC3A/B, SQSTM1/P62, BECLIN-1, ATG12); and the production of interferon (IFN)-γ, interleukin (IL)-4, IL-17A, and IL-10 by T cells were measured.

RESULTS: Compared with the CON group, T cell proliferation tended to be lower, and the production of IFN-γ was higher in the HFD group. IL-17A production was reduced by 1,25(OH)2D3 treatment in both groups. The LC3 II/I ratio was higher in the HFD group than the CON group, but P62 did not differ. We observed no effect of vitamin D treatment on T cell autophagy.

CONCLUSIONS: Our findings suggest that diet-induced obesity may impair the function and inhibit autophagy of T cells, possibly leading to the dysregulation of T cell homeostasis, which may be behind the aggravation of inflammation commonly observed in obesity.

Keywords: Obesity; vitamin D; T lymphocytes; autophagy

INTRODUCTION

Obesity is characterized by the disruption of T cell homeostasis, which may cause tissue and systemic inflammation [1]. Several studies have reported increased numbers of Th1 and Th17 cells in the adipose tissue and splenocytes of obese mice [2-5]. Interferon (IFN)-γ, which is...
secreted by Th1 cells, is closely related to metabolic dysfunction and autoimmunity [6,7]. Winer et al. [5] reported that insulin resistance was induced by IFN-γ-producing Th1 cells in obese mice. The IFN-γ levels in serum and IFNG messenger RNA (mRNA) levels in peripheral blood mononuclear cells (PBMCs) were higher in lupus patients compared with healthy people [8,9]. In type 1 diabetes patients, PBMCs that were reacted with insulinoma associated-2 peptides, natural epitopes of a single islet autoantigen, exhibited extreme polarization to IFN-γ positive cells, and this might have been a CD4 T cell response because it disappeared when CD4 T cells were depleted [10]. Interleukin (IL)-17 is also associated with autoimmunity, as an excessive increase in Th17 cells in obese mice contributed to the development and maintenance of experimental autoimmune encephalomyelitis (EAE) and accelerated the progression of colitis [11]. Taken together, these findings show obesity-induced systemic metabolic dysfunction may be caused by the abnormal regulation of T cell homeostasis.

The dysregulation of vitamin D metabolism has been observed in obesity [12,13]. T cells express the vitamin D receptor (VDR) and are known to be the direct and indirect targets of vitamin D [14]. Vitamin D has been reported to inhibit the differentiation of T cells into Th1 and Th17 cells and the production of IFN-γ, IL-17A, and IL-22 [15,16]. Bruce et al. [17] reported that CD4 T cells from VDR-knockout mice showed increased development into Th17 cells compared with wild-type mice under in vitro Th17- and Treg-culture conditions, suggesting that vitamin D is critical in regulating the distribution of T cell subsets.

Autophagy, which is important for maintaining cell and energy homeostases, is an intracellular degradation system [18]. Autophagy regulates the stimulation, proliferation, differentiation, and function of T cells [19]. T cells have a basal level of autophagy, which is induced in response to T cell receptor and common-γ-chain cytokine receptor signals. The regulation of autophagy in obesity has been investigated in several organs, including the pancreas, liver, heart, and adipose tissue [20-22], and many studies have reported an association between autophagy and vitamin D. Zhao et al. [23] found that the mRNA levels of LC3 in PBMC were lower in a severely vitamin-D-deficient group (serum 25(OH)D3 levels < 10 ng/mL) compared with a vitamin-D-insufficient group (serum 25(OH)D3 levels 10–30 ng/mL). According to Yuk et al. [24], 1,25(OH)2D3 treatment upregulated the mRNA levels of BECN1 and ATG5 in human monocytes and the fusion of autophagosomes and lysosomes in human monocytes and macrophages was also upregulated by 1,25(OH)2D3. These results imply that vitamin D affects autophagy in immune cells. However, a limited number of studies have investigated the effects of vitamin D on autophagy in T cells.

The aim of this study was to investigate the effects of in vitro vitamin D treatment on the function of T cells and the expression of proteins involved in autophagy in high-fat diet-induced obese mice. We evaluated whether in vitro 1,25(OH)2D3 treatment affects proliferative capacity, the expression of a surface marker and transcription factors, the production of cytokines related to T cell function, and the expression of autophagy-related proteins in the T cells of obese and control mice.

**MATERIALS AND METHODS**

**Animals and diets**

Five-week-old male C57BL/6 mice (Central Laboratory Animal, Inc., Seoul, Korea) were housed in a specific pathogen-free room with an environmentally controlled temperature
After a 5-day adaptation period, the mice were randomized into two groups and fed experimental diets differing in fat content for 12 weeks. The CON group was fed the control diet (10% kcal fat, D12450B, Research Diets, Inc., New Brunswick, NJ, USA) and the HFD group was fed a high-fat diet (45% kcal fat, D12451, Research Diets, Inc.). The experimental diets and drinking water were provided ad libitum. Food intake was recorded four times per week, and body weight was measured once a week. At the end of the experimental period, the mice were fasted for 12 hours and euthanized by CO\(_2\) asphyxiation. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Seoul National University (approval No. SNU-181029-6).

**Preparation of 1,25(OH)\(_2\)D\(_3\), solution**

A stock solution of 10 \(\mu\)M 1,25(OH)\(_2\)D\(_3\) (Sigma Aldrich, St. Louis, MO, USA) was prepared in 99.9% ethanol and filtered through a 0.22-\(\mu\)m filter. Further dilution was performed by diluting with complete Roswell Park Memorial Institute (RPMI) medium containing 10% fetal bovine serum (FBS; GibcoBRL, Grand Island, New York, USA) for cell culture. The complete RPMI was prepared with the addition of 100,000 U/L penicillin (GibcoBRL), 100 mg/L streptomycin (GibcoBRL), 25 mmol/L HEPES (Sigma Aldrich), and 2 mmol/L L-glutamine (GibcoBRL) in RPMI 1640 medium (Lonza, Walkersville, MD, USA).

**Isolation of T cells from spleen**

Spleens were removed and put into sterile complete RPMI. A single-cell suspension was prepared by homogenizing each spleen with sterile frosted glass slides. Splenocytes were centrifuged at 700 rpm for 25 s at 25°C to remove tissue debris, then resuspended in complete RPMI. Red blood cells were lysed using Gey’s solution. Cells were washed twice and suspended in complete RPMI with 10% FBS. Cell viability was determined by the trypan blue exclusion test. To purify T cells, the cell suspension was incubated with a mixture of mAbs (Pan T cell Isolation Kit II or CD4\(^+\) T cell Isolation Kit; Miltenyi Biotec, Bergisch Gladbach, Germany) and negatively selected using the magnetic-activated cell sorter QuadroMACS separator and LS column (Miltenyi Biotec) according to the manufacturers’ instructions.

**In vitro 1,25(OH)\(_2\)D\(_3\), treatment**

Purified T cells were cultured in a 24-well plate (1 \(\times\) 10\(^6\) cells/well) for 72 h with complete RPMI containing 10% FBS. T cells from the same animal were cultured with either 10 nM 1,25(OH)\(_2\)D\(_3\) solution or 0.1% ethanol (vehicle control) and with or without stimulation with 5 \(\mu\)g/mL plate-bound anti-CD3 (clone 145-2C11; BD Biosciences, San Jose, CA, USA) and 2 \(\mu\)g/mL soluble anti-CD28 (clone 37.51; BD Biosciences) antibodies during the entire culture period. Cells were incubated at 37°C in 5% CO\(_2\). After 72 h, the supernatant was collected for enzyme-linked immunosorbent assay (ELISA) analysis, and T cells were harvested for western blot analysis. CD4\(^+\) T cells were used for flow cytometric analysis. The experimental design and cell culture process are depicted in Fig. 1.

**T cell proliferation**

Purified T cells were plated into a 96-well round-bottom cell culture plate (2 \(\times\) 10\(^5\) cells/well; Thermo Fisher Scientific, Waltham, MA, USA), cultured with or without 5 \(\mu\)g/mL plate-bound anti-CD3 mAb (clone 145-2C11; BD Biosciences) and 2 \(\mu\)g/mL soluble anti-CD28 mAb (clone 37.51; BD Biosciences), and treated with 10 nM 1,25(OH)\(_2\)D\(_3\) or a vehicle control for 72 h. Cells were pulsed with 0.5 uCi of \(^{3}H\) TdR (PerkinElmer, Waltham, MA, USA) in 20 \(\mu\)L of complete RPMI for the last 8 h. The cells were harvested on filter paper using a MicroBeta FilterMate-96...
Harvester (PerkinElmer), and the proliferation was quantified by measuring the amount of [\(^3\)H] TdR incorporated into the DNA, as determined by the MicroBeta2 Plate Counter (PerkinElmer). Data are expressed as counts per minute.

**Flow cytometric analysis**

For the FACS analysis, \(1 \times 10^5\) CD4\(^+\) T cells were resuspended in a FACS-staining buffer (0.09% sodium azide, 1% FBS, 1 x PBS based, 0.22-\(\mu\)m filter-sterilized) and stained with fluorescence-labeled antibodies specific for CD25-conjugated FITC (clone 7D4; BD Biosciences) or the isotype control at 4°C for 30 min. Then, cells were incubated with Fixation/Perm working buffer for intracellular staining (Foxp3/Transcription Factor Staining Buffer Set; eBioscience, Inc., San Diego, CA, USA). After incubation, the cells were resuspended in the perm diluent and reacted with the antibodies ROR\(\gamma\)-conjugated PerCP- 

Vitamin D and T cell function and autophagy in obese mice

**Quantification of cytokine production**

Supernatant was collected after 72 h of cell culture. The levels of IFN-\(\gamma\), IL-17A, IL-4, and IL-10 produced by T cells were determined using Mouse ELISA IL-17A (Invitrogen, Carlsbad, CA, USA), IFN-\(\gamma\), IL-4, and IL-10 kits (BD Biosciences) according to the manufacturers’ instructions. The absorbance was measured at 450 nm with a microplate spectrophotometer (Spectramax iD3; Molecular Devices, San Jose, CA, USA).

**Western blot analysis**

Protein was extracted from T cells using radio-immunoprecipitation assay buffer (50 mM Tris-HCl [pH 7.4], 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM Na\(_3\)VO\(_4\), 1 mM NaF, 1 mM Na-pyrophosphate, 1 mM \(\beta\)-glycerophosphate, 10% glycerol, protease inhibitor cocktail tablet [Roche, Basel, Switzerland]). Protein lysates (30 \(\mu\)g) were electrophoresed on 13% SDS-PAGE gels and transferred to a polyvinylidene difluoride membrane. The membrane was incubated with the following antibodies: LC3A/B (1:1,000), SQSTM1/P62 (1:1,000), BECLIN-1 (1:1,000), and ATG12 (D88H11) (1:1,000), followed by
HRP-conjugated anti-rabbit IgG (1:3,000). All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Specific bands on the membrane were visualized with chemiluminescence luminol reagent (Santa Cruz Biotechnology, Dallas, TX, USA) and developed using the JP-33 automatic X-ray film processor (JPI Healthcare, Seoul, Korea). The target bands were visualized and analyzed using Quantity One software (Bio-Rad, Hercules, CA, USA).

**Statistical analyses**
Statistical analyses were conducted using SPSS statistics software version 25 (IBM SPSS Statistics, Chicago, MI, USA). The student’s t-test was used to determine the effect of the dietary fat amount. A paired t-test was performed to determine the effect of *in vitro* 1,25(OH)$_2$D$_3$ treatment. If the data did not follow normality, nonparametric tests, i.e., Mann-Whitney U test or Wilcoxon signed-rank test, were performed. All data are presented as mean ± SE and statistical significance was set at $P < 0.05$.

**RESULTS**

**Body weight, weight change, WAT weight, and food intake**
There was no significant difference in the body weights at 0 week between the CON and HFD groups. After 12 weeks of feeding, the body weight of the HFD group was 30.7% higher than the CON group ($P < 0.001$). Weight gain ($P < 0.001$) and white adipose tissue weight ($P < 0.001$) were significantly higher in the HFD group than the CON group. The average food intake was significantly lower and average energy intake was significantly higher in the HFD group (both $P < 0.001$) compared with the CON group (**Table 1**).

**T cell proliferation**
The proliferative capacity of T cells from the HFD group tended to be lower than that of T cells from the CON group (39.5% lower, $P = 0.069$). *In vitro* 1,25(OH)$_2$D$_3$ treatment had no significant effect on T cell proliferation in both the CON and HFD groups (**Fig. 2**).

**Expression of surface marker and transcription factors of CD4$^+$ T cells**
The proportions of Th17 (ROR$\gamma^+$) cells and Treg (CD25$^+$ Foxp3$^+$) cells were unaffected by either fat amount or *in vitro* 1,25(OH)$_2$D$_3$ treatment (**Fig. 3**).

**Production of cytokines by T cells**
The production of IFN-γ by T cells was significantly higher in the HFD group compared with the CON group (2.6-fold higher, $P < 0.05$ (**Fig. 4A**)). However, *in vitro* 1,25(OH)$_2$D$_3$ treatment

**Table 1. Body weight, weight gain, adipose tissue weight, and food intake of mice in the CON and HFD groups$^{1-4}$**

| Parameters                          | CON (n = 26) | HFD (n = 31) | $P$-value |
|-------------------------------------|-------------|-------------|-----------|
| Body weight at 0 week (g)           | 19.4 ± 0.3  | 19.6 ± 0.2  | 0.562     |
| Body weight at 12 week (g)          | 31.6 ± 0.4  | 41.3 ± 0.5  | < 0.001   |
| Weight gain (g)                     | 12.3 ± 0.4  | 21.8 ± 0.5  | < 0.001   |
| WAT weight (g)$^3$                  | 3.3 ± 0.2   | 6.6 ± 0.7   | < 0.001   |
| Average food intake (g/day)$^4$     | 2.8 ± 0.03  | 2.7 ± 0.04  | < 0.001   |
| Average energy intake (kcal/day)$^4$| 10.8 ± 0.1  | 12.5 ± 0.2  | < 0.001   |

CON, control; HFD, high-fat diet.

$^1$The data are presented as mean ± SE.

$^2$Student’s t-test was used to determine the effect of HFD. If the data does not follow the normality, Mann-Whitney test was performed.

$^3$WAT includes perirenal, intraperitoneal, epididymal, and subcutaneous fat.

$^4$Calculated from the average food intake and the energy density of the CON diet (3.82 kcal/g) and HFD (4.7 kcal/g).
had no significant effect on IFN-γ production by T cells. IL-17A levels were not significantly different between the CON and HFD groups; however, *in vitro* treatment with 10 nM 1,25(OH)_2D_3 significantly decreased IL-17A levels in both the CON (34.8% less, *P* < 0.001) and HFD (41.6% less, *P* < 0.001) groups compared with the respective vehicle controls (Fig. 4B). IL-4 and IL-10 production was not affected by either fat amount or *in vitro* 1,25(OH)_2D_3 treatment (Fig. 4C and D).
The LC3 II/I ratio was significantly higher in the HFD group compared with the CON group (57.4% higher, \( P < 0.05 \)). In vitro treatment with 10 nM 1,25(OH)\(_2\)D\(_3\) did not significantly affect the LC3 II/I ratio in both CON and HFD groups. The expression levels of SQSTM1/P62, BECLIN-1, and ATG12 were not significantly different between the CON and HFD groups, and in vitro treatment with 10 nM 1,25(OH)\(_2\)D\(_3\) did not have a significant effect on these proteins (Fig. 5).

**DISCUSSION**

In this study, we investigated the effects of in vitro vitamin D treatment on the proliferation, differentiation, and autophagy of splenic T cells in obese and control mice. T cell proliferation tended to decrease with obesity; however, the production of IFN-\(\gamma\) was higher in the obese mice compared with the lean controls. This increased IFN-\(\gamma\) production seemed to be partially related to the higher LC3 II/I ratio in the obese mice.

Our results show that the proliferation of T cells tended to be lower in the HFD group (39.5% lower). Several previous studies have reported lower proliferation levels of splenocytes and T cells from obese mice compared with those from control mice, which
implies that obesity induces the dysfunction of immunity [25-27]. Conflicting results have been observed for the effect of vitamin D on the proliferation of T cells. Lacey et al. [28] reported that when D10.G4.1 cells—the Th2 cell line of murine lymph nodes—were treated with 10 nM 1,25(OH)$_2$D$_3$, or vehicle CON, and stimulated with anti-CD3 mAb (5 μg/mL) and anti-CD28 mAb (2 μg/mL) for 72 h. Cells were harvested, and analyzed on western blot. The intensity of LC3, ATG12, BECLIN-1, SQSTM1/P62 in T cells was densitometrically measured and normalized with β-ACTIN. Data are presented as mean ± SE (n = 5 per group). Student t-test and paired t-test was used to determine the effects of HFD and in vitro 1,25(OH)$_2$D$_3$ treatment. CON, control; HFD, high-fat diet; mAb, monoclonal antibody.

Our results show that the production of IFN-γ by T cells was higher in the obese mice compared with the controls. Consistent with our results, the proportion of IFN-γ$^+$ cells in splenic CD4$^+$ T cells stimulated with anti-CD3/anti-CD28 and the IFN-γ levels produced by PHA-stimulated splenocytes were reported to be higher in obese mice than control mice [2,4]. IFN-γ, which is secreted by CD4$^+$ Th1 cells, CD8$^+$ cytotoxic T cells, and various other immune cells, promotes macrophage activation, enhances antigen presentation, and regulates T cell subset differentiation [31,32]. IFN-γ priming induces post-transcriptional changes that cause macrophage activation and inflammatory responses triggered by Toll-like

Fig. 5. Effects of HFD and in vitro 1,25(OH)$_2$D$_3$ treatment on expression of proteins associated with autophagy in total T cells (A) Immunoblotting for LC3, ATG12, BECLIN-1, SQSTM1/P62, and β-ACTIN, and (B) densitometric analysis of protein expression. Total T cells purified from splenocytes of CON and HFD groups were incubated with 10 nM 1,25(OH)$_2$D$_3$ or vehicle CON, and stimulated with anti-CD3 mAb (5 μg/mL) and anti-CD28 mAb (2 μg/mL) for 72 h. Cells were harvested, and analyzed on western blot. The intensity of LC3, ATG12, BECLIN-1, SQSTM1/P62 in T cells was densitometrically measured and normalized with β-ACTIN. Data are presented as mean ± SE (n = 5 per group). Student t-test and paired t-test was used to determine the effects of HFD and in vitro 1,25(OH)$_2$D$_3$ treatment. CON, control; HFD, high-fat diet; mAb, monoclonal antibody. *P < 0.05.
receptor ligands [33]. Furthermore, excess IFN-γ in the culture medium leads to the impaired development of memory CD8+ T cells during infection, and increased protein expression of IFN-γ may contribute to insulin resistance [5,34]. According to a report by Vandanmagsar et al. [35], higher levels of Nlrp3 mRNA in the adipose tissue of obese mice induced the mRNA and protein expression of Th1 cytokine IFN-γ, which increased the activation of M1 macrophages and caused both insulin resistance and inflammation. This study confirmed there is an increased production of IFN-γ, specifically by T cells, in obesity. This could be one of the contributing factors aggravating the macrophage activation and inflammation often observed with obesity.

In vitro treatment with 1,25(OH)2D3 decreased the IL-17 production by T cells from both control and obese mice, but there was no difference in levels of IL-17 produced between control and obese mice. Obesity has been reported to promote an expansion of the Th17 T cell sublineage, and this has been suggested as the reason for more pronounced autoimmune disease associated with obesity [11]. However, it seemed that divergence exists among tissues with obesity regarding Th17 cytokine as IL-17 was increased in the liver while decreased in the ileum, colon, and white adipose tissue [36]. T cells purified from spleens of obese mice did not exhibit difference in IL-17 production compared with the lean mice in this study. In vitro treatment with 1,25(OH)2D3 inhibited differentiation of Th17 cells and oral treatment with 1,25(OH)2D3 in animal experimental model of EAE inhibited the onset of EAE and IL-17 positive cells in the splenocytes [37]. In our study, IL-17 production by purified T cells was inhibited by vitamin D in both control and obese mice. The significance of this needs to be investigated in the future study.

The ratio of LC3-II to LC3-I was higher but p62 expression was comparable in obese mice compared with the lean controls in our study. The increase in LC3 indicates there was increased autophagosome formation; however, the similar p62 levels in the two groups suggests that initiation of autophagy was inhibited because of reduced availability of autophagy precursors [38,39]. Impaired autophagy reduces the clearance of apoptotic cells, resulting in tissue inflammation and the dysregulation of T cell homeostasis [40]. While IFN-γ induces autophagy by inducing LC3 puncta and LC3-positive phagosomes to eliminate intracellular antigens in macrophages [40-42]. Considering the involvement of IFN-γ in macrophage autophagy, higher IFN-γ production by T cells in the obese mice in this study might be related to the higher LC3 II/I ratio. Although the mechanisms by which IFN-γ induces autophagy in macrophages have not been completely revealed, the p38 MAPK signaling pathway and the pathway involving immunity-related GTPase family M member 1 have been proposed to be the autophagy activation pathways induced by IFN-γ [43,44]. Rincon et al. [45] showed that the production of IFN-γ by Th1 cells was significantly reduced when ConA-stimulated CD4+ T cells were treated with a p38 MAPK inhibitor, whereas IFN-γ production by Th1 cells was increased in constitutively activated MKK6 (upstream activator of p38 MAPK) transgenic mice. From these results, it appears autophagy in the T cells of obese mice may be activated through the pathway that activates autophagy in macrophages, involving the increased production of Th1 cytokines.

Vitamin D has been shown to regulate autophagy in many tissues and cancer cells and to accelerate anti-mycobacterial activity by increasing the mRNA levels of BECN1 and ATG5 and the fusion of autophagosomes and lysosomes in human monocytes and macrophages [24,46]. Although autophagy has been shown to affect the regulation of T cell metabolism, studies investigating the effects of vitamin D on autophagy in T cells have been limited. The
decreased expression of LC3 in PBMC in vitamin D-deficient patients suggests that vitamin D is important for autophagy in T cells [23]. Klug-Micu et al. [47] reported that soluble CD40-ligand-activation of human monocytes cultured in vitamin D-sufficient human serum upregulated the expression of CYP27B1 and VDR, and increased the proportion of LC3-II+ cells. Autophagy in monocytes was enhanced when CD3-activated T-cell clones were co-incubated with primary human monocytes in 10% vitamin D-sufficient human serum, suggesting that the T cell-mediated pathway might be associated with the vitamin D-dependent antimicrobial mechanism in human monocytes. However, we observed no significant effect of 1,25(OH)2D3 treatment on autophagy in T cells in this study.

In this study, only male mice were used. Sex differences in HFD induced obesity and metabolic syndrome as well as the T cell population have been reported [48]. Male mice developed adipose tissue inflammation, glucose intolerance, hyperinsulinemia with HFD feeding, while female mice were protected against HFD-induced metabolic changes and maintained an anti-inflammatory environment in the intra-abdominal adipose tissue with expanded Treg cell population. We anticipated that vitamin D’s effect on T cell function would be more evident in male mice which are more vulnerable to the HFD-induced changes. Non the less, effects of vitamin D on T cell function need to be determined in female mice in the future study.

In conclusion, our findings suggest that HFD-induced obesity affects the proliferation, cytokine production, and autophagy of T cells. Proliferation tended to decrease, while the production of IFN-γ and the LC3 II/I ratio increased, with HFD-induced obesity. The elevated Th1 cytokine production in obese mice compared with control mice may have contributed to increased levels of LC3. These results suggest that diet-induced obesity impairs T cell function and inhibits autophagy, resulting in the dysregulation of T cell homeostasis, which may contribute to the exacerbation of inflammation commonly observed with obesity. Vitamin D treatment did not affect autophagy of T cells, but inhibited the production of IL-17.

REFERENCES

1. Touch S, Clément K, André S. T cell populations and functions are altered in human obesity and type 2 diabetes. Curr Diab Rep 2017;17:81.

2. Mito N, Hosoda T, Kato C, Sato K. Change of cytokine balance in diet-induced obese mice. Metabolism 2000;49:1295-300.

3. Endo Y, Yokote K, Nakayama T. The obesity-related pathology and Th17 cells. Cell Mol Life Sci 2017;74:1231-45.

4. Surendar J, Frohberger SJ, Karunakaran I, Schmitt V, Stamminger W, Neumann AL, Wilhelm C, Hoerauf A, Hübner MP. Adiponectin limits IFN-γ and IL-17 producing CD4 T cells in obesity by restraining cell intrinsic glycolysis. Front Immunol 2019;10:2555.

5. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, Dorfman R, Wang Y, Zielenki J, Mastronardi F, Maezawa Y, Drucker DJ, Engleman E, Winer D, Dosch HM. Normalization of obesity-associated insulin resistance through immunotherapy. Nat Med 2009;15:921-9.

6. Baccala R, Kono DH, Theofilopoulos AN. Interferons as pathogenic effectors in autoimmunity. Immunol Rev 2005;204:9-26.
7. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. J Clin Invest 2011;121:2111-7.
PUBMED | CROSSREF

8. Preble OT, Black RJ, Friedman RM, Klippel JH, Vilcek J. Systemic lupus erythematosus: presence in human serum of an unusual acid-labile leukocyte interferon. Science 1982;216:429-31.
PUBMED | CROSSREF

9. Csizár A, Nagy G, Gergely P, Pozsonyi T, Pócsik E. Increased interferon-gamma (IFN-gamma), IL-10 and decreased IL-4 mRNA expression in peripheral blood mononuclear cells (PBMC) from patients with systemic lupus erythematosus (SLE). Clin Exp Immunol 2000;122:464-70.
PUBMED | CROSSREF

10. Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, Roep BO, Peakman M. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. J Clin Invest 2004;113:451-63.
PUBMED | CROSSREF

11. Winer S, Paltser G, Chan Y, Tsui H, Engleman E, Winer D, Dosch HM. Obesity predisposes to Th17 bias. Eur J Immunol 2009;39:2629-35.
PUBMED | CROSSREF

12. Park JM, Park CY, Han SN. High fat diet-Induced obesity alters vitamin D metabolizing enzyme expression in mice. Biofactors 2015;41:175-82.
PUBMED | CROSSREF

13. Jung YS, Wu D, Smith D, Meydani SN, Han SN. Dysregulated 1,25-dihydroxyvitamin D levels in high-fat diet-induced obesity can be restored by changing to a lower-fat diet in mice. Nutr Res 2018;53:51-60.
PUBMED | CROSSREF

14. Cantorna MT. Why do T cells express the vitamin D receptor? Ann N Y Acad Sci 2011;1217:77-82.
PUBMED | CROSSREF

15. Boonstra A, Barrat FJ, Crain C, Heath VL, Savelkoul HF, O’Garra A. 1alpha,25-Dihydroxyvitamin d3 has a direct effect on naive CD4(+) T cells to enhance the development of Th2 cells. J Immunol 2001;167:4974-80.
PUBMED | CROSSREF

16. Palmer MT, Lee YK, Maynard CL, Oliver JR, Bikle DD, Jetten AM, Weaver CT. Lineage-specific effects of 1,25-dihydroxyvitamin D3 on the development of effector CD4 T cells. J Biol Chem 2011;286:9974004.
PUBMED | CROSSREF

17. Bruce D, Yu S, Ooi JH, Cantorna MT. Converging pathways lead to overproduction of IL-17 in the absence of vitamin D signaling. Int Immunol 2011;23:519-28.
PUBMED | CROSSREF

18. Yoshii SR, Mizushima N. Monitoring and measuring autophagy. Int J Mol Sci 2017;18:1865.
PUBMED | CROSSREF

19. Dowling SD, Macian F. Autophagy and T cell metabolism. Cancer Lett 2018;419:20-6.
PUBMED | CROSSREF

20. Abe H, Uchida T, Hara A, Mizukami H, Komiya K, Koike M, Shigihara N, Toyofuku Y, Ogihara T, Uchiyama Y, Yagihashi S, Fujitani Y, Watada H. Exendin-4 improves β-cell function in autophagy-deficient β-cells. Endocrinology 2013;154:4512-24.
PUBMED | CROSSREF

21. López-Vicario C, Alcaraz-Quiles J, García-Alonso V, Rius B, Hwang SH, Titos E, Lopategi E, Arroyo V, Claria J. Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. Proc Natl Acad Sci U S A 2015;112:536-41.
PUBMED | CROSSREF

22. Cao L, Qin X, Peterson MR, Haller SE, Wilson KA, Hu N, Lin X, Nair S, Ren J, He G. CARD9 knockout ameliorates myocardial dysfunction associated with high fat diet-induced obesity. J Mol Cell Cardiol 2016;92:185-95.
PUBMED | CROSSREF

23. Zhao M, Duan XH, Gao CC, Wang N, Zheng ZH. Severe vitamin D deficiency affects the expression of autophagy related genes in PBMCs and T-cell subsets in active systemic lupus erythematosus. Am J Clin Exp Immunol 2017;6:43-51.
PUBMED

24. Yuk JM, Shin DM, Lee HM, Yang CS, Jin HS, Kim KK, Lee ZW, Lee SH, Kim JM, Jo EK. Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin. Cell Host Microbe 2009;6:231-43.
PUBMED | CROSSREF

25. Sato Mito N, Suzuki M, Yashino H, Kaburagi T, Sato K. Long term effects of high fat and sucrose diets on obesity and lymphocyte proliferation in mice. J Nutr Health Aging 2009;13:602-6.
PUBMED | CROSSREF
26. Lewis ED, Ren Z, DeFuria J, Obin MS, Meydani SN, Wu D. Dietary supplementation with blueberry partially restores T-cell-mediated function in high-fat-diet-induced obese mice. Br J Nutr 2018;119:1393-9.

27. Odaka Y, Nakano M, Tanaka T, Kaburagi T, Yoshino H, Sato-Mito N, Sato K. The influence of a high-fat dietary environment in the fetal period on postnatal metabolic and immune function. Obesity (Silver Spring) 2010;18:1688-94.

28. Lacey DL, Axelrod J, Chappell JC, Kahn AI, Teitelbaum SL. Vitamin D affects proliferation of a murine T helper cell clone. J Immunol 1987;138:1680-6.

29. Rigby WF, Stacy T, Fanger MW. Inhibition of T lymphocyte mitogenesis by 1,25-dihydroxyvitamin D3 (calcitriol). J Clin Invest 1984;74:1451-5.

30. Cha KS, Park CY, Lee SE, Kim TY, Han SN. The effects of 1,25-dihydroxyvitamin D3 on markers related to the differentiation and maturation of bone marrow-derived dendritic cells from control and obese mice. J Nutr Biochem 2020;85:108464.

31. Tau G, Rothman P. Biologic functions of the IFN-gamma receptors. Allergy 1999;54:1233-51.

32. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 2004;75:163-89.

33. Borges da Silva H, Fonseca R, Alvarez JM, D’Impiéro Lima MR. IFN-γ priming effects on the maintenance of effector memory CD4(+) T cells and on phagocyte function: evidences from infective diseases. J Immunol Res 2015;2015:202816.

34. Zhang X, Starnbach MN. An excess of the proinflammatory cytokines IFN-γ and IL-12 impairs the development of the memory CD8+ T cell response to Chlamydia trachomatis. J Immunol 2015;195:1665-75.

35. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Ravussin E, Stephens JM, Dixit VD. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nat Med 2011;17:179-88.

36. Cavallari JF, Denou E, Foley KP, Khan WI, Schertzer JD. Different Th17 immunity in gut, liver, and adipose tissues during obesity: the role of diet, genetics, and microbes. Gut Microbes 2016;7:82-9.

37. Chang JH, Cha HR, Lee DS, Seo KY, Kweon MN. 1,25-Dihydroxyvitamin D3 inhibits the differentiation and migration of T(H)17 cells to protect against experimental autoimmune encephalomyelitis. PLoS One 2010;5:e12925.

38. Gottlieb RA, Andres AM, Sin J, Taylor DP. Untangling autophagy measurements: all fluxed up. Circ Res 2015;116:504-14.

39. Klionsky DJ, Abieliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, Baba M, Baehrecke EH, Bahr BA, Ballabio A, Bamber BA, Bassham DC, Bergamini E, Bi X, Biard-Piechaczyk M, Blum JS, Bredesen DE, Brodsky JL, Brunell JH, Brunk UT, Bursch W, Camougrand N, Cebollero E, Cecconi F, Chen Y, Chin LS, Choi A, Chu CT, Chung J, Clarke PG, Clark RS, Clarke SG, Clavé C, Cleveland JL, Codogno P, Colombo MI, Coto-Montes A, Cregg JM, Cuervo AM, Debnath J, Demarchi F, Dennis PB, Dennis PA, Deretic V, Devenish RJ, Di Sano F, Dice JF, Difiglia M, Dinesh-Kumar S, Distelhorst CW, Djavaheri-Mergny M, Dorsey FC, Droge W, Dron M, Dunn WA Jr, Duszenko M, Eissa NT, Elazar Z, Esclatine A, Eskelinen EL., Fésus L, Finley KD, Fuentes JM, Fueyo J, Fujisaki K, Galliot B, Gao FB, Gewirtz DA, Gibson SB, Gohla A, Goldberg AL, Gonzalez R, Gonzalez-Estévez C, Gorski S, Gottlieb RA, Häussinger D, He YW, Heidenreich K, Hill JA, Hoyer-Hansen M, Hu X, Huang WP, Iwasaki A, Játtelá M, Jackson WT, Jiang X, Jin S, Johansen T, Jönk JU, Kadowaki M, Kang C, Kelekari A, Kessel DH, Kiel JA, Kim HP, Kimchi A, Kinsella TJ, Kiseljoy K, Kimataki K, Knecht E, Komatsu M, Kominami E, Kondo S, Kovacs AL, Kroemer G, Kucyan C, Kumar R, Kundu M, Landry J, Laporte M, Le W, Leitão V, Lenardo MJ, Levine B, Lieberman A, Lim KL, Lin FC, Liu W, Liu LF, Lopez-Berestein G, López-Otín C, Lu B, Macleod KF, Malorni W, Martiniert W, Matsuoka K, Mautner J, Meléndez A, Michels P, Mirotta G, Mjolsness LM, Monesteroli M, Moore MN, Moreira PI, Moriyasu Y, Motyl T, Münz C, Murphy LO, Naqvi NI, Neufeld TP, Nishino I, Nixon RA, Noda T, Nürnberg B, Ogawa M, Oleinick NL, Olsen LJ, Ozpolat B, Paglin S, Palmer GE, Papassideri I, Parkes M, Perlmutter DH, Perry G, Piacentini M, Pinkas-Kramarski R, Prescott M,
Vitamin D and T cell function and autophagy in obese mice

https://e-nrp.org

https://doi.org/10.4162/nrp.2021.15.6.673

685