Thesis for the Degree of Master of Science

The effect of bee venom phospholipase A2 on high fat diet-induced obesity in mice

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Abstract

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Obesity is a complex metabolic disorder that is characterized by excess body fat accumulation and is often complicated by cardiovascular disease, high blood pressure, type 2 diabetes, and high cholesterol. Obesity is also accompanied by an accumulation of inflammatory immune cells in white adipose tissue (WAT), leading to dysfunction of adipose cell metabolism. Bee venom is a complex mixture of proteins and compounds and has been reported to have anti-tumor, anti-inflammatory, and anti-obesity functions. However, the specific compound in bee venom that is responsible for its anti-obesity effect is unknown. In this study, the therapeutic effects of bee venom phospholipase A2 (bvPLA2) on high-fat diet (HFD)-induced obesity were evaluated. The results showed that bvPLA2 markedly decreased body and WAT weight and significantly lowered liver and kidney weight in HFD-fed mice. Administration of bvPLA2 in HFD-fed mice also decreased lipid accumulation of liver and reduced kidney inflammation. In addition, bvPLA2 decreased M1 pro-inflammatory macrophages in adipose tissue and alleviated M1/M2

imbalance *in vivo*. bvPLA2 also stimulated M2 differentiation while inhibiting M1 polarization, but did not directly inhibit adipogenesis *in vitro*. Thus, bvPLA2 shows potential as a therapeutic agent for obesity by regulating immune homeostasis.

Keywords: Obesity, High-fat diet, bvPLA2, White adipose tissue, Adipose tissue macrophages

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I. Introduction

Obesity is a growing health problem worldwide that is characterized by the excess accumulation of body fat and a high body mass index (BMI ≥ 30) (Bhurosy & Jeewon, 2014; Chan, Yan, & Payne, 2013). Obesity is a life-threatening disease that reduces quality of life and increases the mortality rate by increasing insulin resistance and the risk of significant complications, such as type 2 diabetes cases, cardiovascular disease, hypertension, cancer, polycystic osteoarthritis, and metabolic syndrome. Obesity requires long-term care accompanied by lifestyle management (healthy eating and exercise) and can be extremely difficult to control. Therefore, the development of novel treatments without adverse effects is essential to cure obesity (Cannon & Kumar, 2009; Fuentes, Fuentes, Vilahur, Badimon, & Palomo, 2013; Kaplan, Huguet, Newsom, McFarland, & Lindsay, 2003).

In obesity, excessive fat accumulation in white adipose tissue (WAT) triggers the improper release of adipokines, free fatty acids (FFAs), and triglycerides (TG) from adipocytes and accelerates the activation of IκB kinase (IKK), c-Jun N-terminal kinase (JNK), and the Nuclear factor kappa B (NF-κB) pathway, resulting in insulin resistance and chronic inflammation (Gómez-Hernández, Beneit, Díaz-Castroverde, & Escribano, 2016; McArdle, Finucane, Connaughton, McMorrow, & Roche, 2013). Further, it has been reported that the accumulation of immune cells, such as macrophages, monocytes, and lymphocytes, in adipose tissue (AT) is increased in the obese state. Most of the immune cells infiltrating the AT are macrophages (Catrysse & van Loo, 2018; Odegaard & Chawla, 2011). Adipose tissue macrophages (ATMs) have

been known to be rapidly increased in the early stage of obesity, inducing inflammation and adipocyte apoptosis (Russo & Lumeng, 2018; Singer & Lumeng, 2017; Zheng et al., 2016). There are two types of ATMs, classically activated M1 macrophages and alternatively activated M2 macrophages (Braune et al., 2017). M1 ATMs release pro-inflammatory cytokines, such as TNF-α, INF-γ, IL-1β, IL-6, and MCP-1. The recruitment of M1 ATMs in the AT leads to insulin resistance and adipocyte dysfunction (Sartipy & Loskutoff, 2003). In contrast, M2 ATMs secrete anti-inflammatory cytokines, such as IL-4, IL-10, and IL-13, and highly express Arg1 and Ym1 (Castoldi, Naffah de Souza, Camara, & Moraes-Vieira, 2015; Fujisaka et al., 2009). In the lean state, most of the resident macrophages in AT are polarized to the M2 phenotype, and they maintain normal adipocyte function via tissue remodeling and angiogenesis (Lumeng, Bodzin, & Saltiel, 2007). In AT, an M1/M2 imbalance toward the M1 phenotype results in inflammation and adipocyte dysfunction, whereas switching of M1 ATMs to the M2 phenotype improves the browning of WAT and insulin sensitivity (Liu, Lin, Burton, & Wei, 2015). Hence, targeting ATMs might be an important therapeutic strategy for the treatment of obesity (Chylikova, Dvorackova, Tauber, & Kamarad, 2018; Shaul, Bennett, Strissel, Greenberg, & Obin, 2010; Wellen & Hotamisligil, 2003).

Phospholipase A2 (PLA2) is one of the major components of honeybee (*Apis mellifera*) venom. Previous studies reported that bee venom PLA2 (bvPLA2) has therapeutic effects on Parkinson's disease, Alzheimer's disease, pulmonary inflammation, and atopic dermatitis by reducing inflammation (E. S. Chung et al., 2015; Jung et al., 2017; Jung et al.,

2016; Ye et al., 2016). As bvPLA2 was previously reported to bind to dendritic cells via CD206 mannose receptors, which are also abundant on macrophages, bvPLA2 is also predicted to strongly bind to macrophages. Thus, I investigated whether bvPLA2 had therapeutic effects on obesity by reducing inflammation in high fat diet (HFD)-induced obesity model mice.

II. Materials and Methods

1. Materials

Bee venom phospholipase A2 (bvPLA2, *Apis mellifera*), 3-Isobutyl-1-methylxanthine (IBMX), Dexamethasone (DEX), insulin and Oil red O were purchased from Sigma-aldrich (St. Louis, MO, USA).

2. Cell culture and differentiation

3T3-L1 pre-adipocytes were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). The cells were cultured in DMEM (Welgene, Daegu, Korea) supplemented with 10% bovine calf serum (BCS) until confluent, and the medium was exchanged every 2–3 days. To induce adipocyte differentiation, the 3T3-L1 cells were seeded in a 96-well plate, and at confluence, differentiation was induced by changing the medium to MDI medium (DMEM containing IBMX, 111 μg/mL; DEX, 2 μM; and insulin, 2 μg/mL). For experiments, the cells were treated with bvPLA2 or PBS (vehicle) at the same time. The differentiation medium (MDI) was exchanged every 2 days. After 4 days of differentiation, fully differentiated cells were analyzed using Oil red O staining.

3. Oil red O staining

Differentiated 3T3-L1 cells were washed twice with PBS and fixed in buffered 4% paraformaldehyde for 20 min. Oil Red O (Sigma-Aldrich St. Louis, MO, USA) was dissolved in 60% isopropanol, and the cells were stained with this Oil Red O solution for 20 min at RT. After the cells were washed twice with distilled water, the OD at 510 nm was measured.

4. Experimental animals

Male C57BL/6 mice (age, 5 weeks; weight, 18–20 g) were purchased from Daehan Biolink, Co. (Seoul, Korea). After 1 week of adaptation, mice were fed either a normal diet (ND, 10 kcal%) or a high-fat diet (HFD, 60 kcal%) for 15 weeks (Research Diets, NJ, USA). The mice were intraperitoneally injected (i.p.) with PBS or bvPLA2 (0.5 mg/kg) every 3 days starting on week 5, for 11 weeks.

5. Quantitative real-time PCR

Total RNA was isolated from WAT samples using the easy-BLUE RNA extraction kit (iNtRON Biotechnology, Korea) and cDNA was synthesized using Cyclescript reverse transcriptase (Bioneer, Korea). The synthesized cDNA was used for quantitative real-time PCR with the SensiFAST SYBR no-Rox kit (Bioline, Korea) (Cycling conditions: 95 for 15 s, 55 for 10 s and 72 10 s). Each reaction was performed in triplicate. The base sequences of the primers are as follows: GAPDH: forward, 5’-CCCAGAAGACTGTGGATGG-3’; reverse, 5’-CACATTGGGGGTAGGAACAC-3’. TNF-α: forward, 5’-TTCTGTCTACTGAACTTCGGGGTGATCGGTCC-3’; reverse, 5’-GTATGAGATAGCAAATCGGCTGACGGTGTGGG-3’. IL-1β: forward, 5’-GGACAGAATATCAACCAACAAGTGATA-3’; reverse, 5’-GTGTGCCGTCTTTCATTACACAG-3’. IL-12a: forward, 5’-GCTCTAGACCCTGTGCCTTG-3’; reverse, 5’-GAAGGCTTACCTGCA TCAGC-3’. IL-4: forward, 5’-ACGAAGAACACCACAGAG-3’; reverse, 5’-TGATGTGGACTTGGACTC-3’. CD206: forward, 5’-

AGTGGCAGGTGGCTTATG-3’; reverse, 5’-GGTTCAGGAGTTGTTGTG-3’. Ym1: forward, 5’-CATTCAGTCAGTTATCAGATTCC-3’; reverse, 5’-AGTGAGTAGCAGCCTTGG-3’. PPAR γ: forward, 5’-GAAGGCTGAAGTCACCAAGC-3’; reverse, 5’-TCAGCCTTGCCAGAGTTTTT-3’. C/EBP α: forward, 5’-TTACAACAGGCCAGGTTTCC-3’; reverse, 5’-CTCTGGGATGGATCGATTGT-3’. UCP-1: forward, 5’-TCTCAGCCGGCTTAATGACT-3’; reverse, 5’-GCTGGGTGTATGTGCCTTTT-3’.

6. H&E staining

Liver and kidney tissues were fixed with 4% paraformaldehyde overnight. After dehydration and paraffin embedding, the tissues were sectioned (4 μm thick). The sections were deparaffinized with xylene and rehydrated with graded ethanol (100%, 90%, 80%, and 70%). The rehydrated tissue sections were washed under tap water and stained with hematoxylin solution for 5 min. After dipping in 1% acid alcohol, the sections were stained with eosin solution for 3 min. Then, the tissues were dehydrated with graded ethanol (70%, 80%, 90%, and 100%) and cleared with xylene for mounting.

7. FACS staining and flow cytometry

WAT samples were digested with collagenase B (1 mg/ml; Roche), and DNaseI (1 Unit/ml; Roche) in serum-free DMEM (Welgene) medium for 20 min at 37 ℃ in a shaking incubator. Then, the tissue was dissociated

using a gentleMACS Dissociator and a MACS C tube (Milteny Biotec). The obtained isolated cells were filtered through a 100 μm strainer (falcon) and centrifuged. After the red blood cells were removed by incubation in RBC lysis buffer, single cells were washed and stained with the following antibodies: CD45-FITC, CD11b-V510, CD206-APC, CD11c-APC/cy7. All antibodies were purchased from e-bioscience (San Diego, CA, USA). Flow cytometry analysis was performed using a FACSLyric system (BD bioscience, CA, USA).

8. Statistical analysis

The results are expressed as the mean and standard error. Statistical significance was analyzed by one-way ANOVA and the Newman-Keuls test using Prism 5 software.

III. Results

1. bvPLA2 reduces body weight and adipose tissue weight in HFD-induced in obesity.

To assess the therapeutic effect of bvPLA2 on obesity, C57BL/6 mice were fed a ND or HFD for 15 weeks. After 4 weeks of ND or HFD feeding, PLA2 or PBS vehicle injection was initiated on week 5 (Fig. 1A). At termination of the study, the adipose tissue weight and body weight of the ND- and HFD-fed mice with or without bvPLA2 were measured. The body weight of HFD mice was higher than that of ND mice. The HFD+PLA2 mice had significantly lower body weights than HFD mice, indicating that PLA2 prevented this increase in body weight (Fig. 1B and 1C). Food uptake did not differ among the groups, indicating that bvPLA2 did not affect appetite (Fig. 1D). The weights of the epididymal WAT (eWAT) and inguinal WAT (iWAT) were remarkably increased in the HFD group compared to those in the ND group, whereas these weights were significantly decreased in the HFD+bvPLA2 group when compared to the HFD group (Fig. 1E, F). Therefore, administration of bvPLA2 significantly reduced body weight and adipose tissue weight, without affecting food uptake.

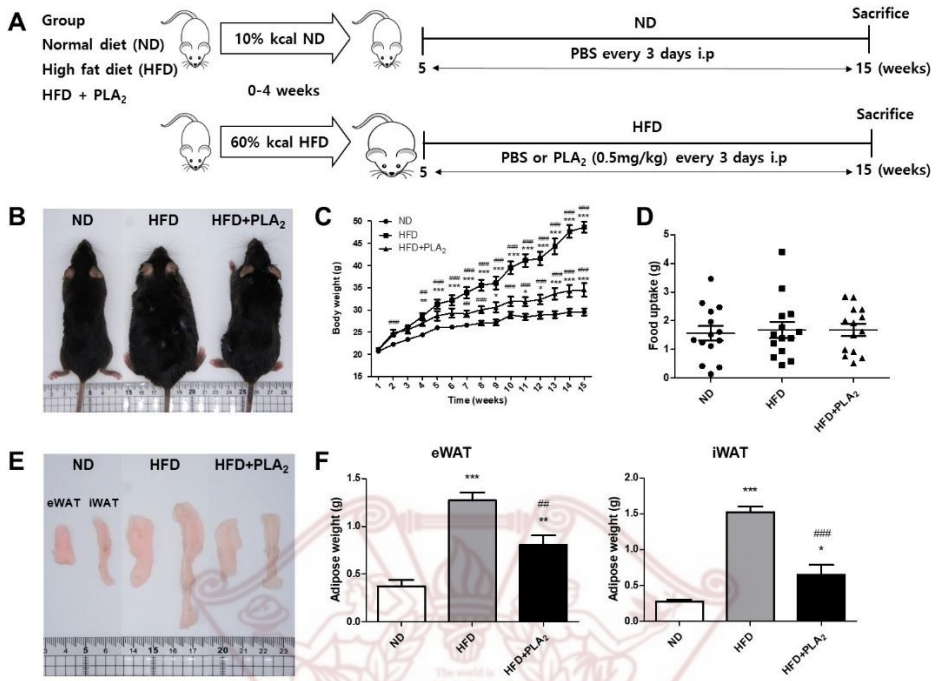

Figure 1. bvPLA2 inhibited HFD-induced obesity. (A) Experimental schedule. Five-week-old mice were divided into three groups: the normal diet (ND), high-fat diet (HFD), and HFD plus bvPLA2 (HFD+PLA2) groups. The ND and HFD groups were treated with PBS vehicle only. (B) Representative pictures of mice in the ND, HFD, and HFD+PLA2 groups after 15 weeks. (C-D) Comparisons of (C) body weight and (D) weekly food uptake in the ND, HFD, and HFD+PLA2 groups. (E) Representative pictures of epididymal white adipose tissue (eWAT) and inguinal white adipose tissue (iWAT) and (F) the eWAT and iWAT weights of ND, HFD, and HFD+PLA2 mice after 15 weeks. All data represented as the means ± SEMs; *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ versus the ND group; ##$P < 0.01$, ###$P < 0.0001$ versus the HFD group.

2. bvPLA2 decreases hepatic steatosis and kidney inflammation in obese mice.

To determine whether bvPLA2 attenuates HFD-induced hepatic steatosis and kidney inflammation, liver and kidney tissues from the mice were stained with H&E. Compared to the ND group, the size and weight of the liver in the HFD group were increased due to hepatomegaly, associated with lipid droplet accumulation. However, bvPLA2 treatment attenuated these increases in HFD-fed mice (Fig. 2A, B). The HFD group showed significantly greater lipid droplet accumulation in the liver when compared to the ND group, and lipid accumulation was significantly reduced in the HFD+PLA2 group when compared to the HFD group (Fig. 2C, D). Next, I confirmed the glomerular diameter indicating initial symptom of diabetic nephropathy by kidney damage. Hypertrophy of the kidney due to lipid accumulation was observed in HFD mice, while both the ND and HFD+PLA2 mice did not show hypertrophy, i.e., an increase in glomerulus diameter (Fig. 2E, F). These data indicated that bvPLA2 prevented hepatic steatosis and kidney damage by reducing lipid droplet accumulation.

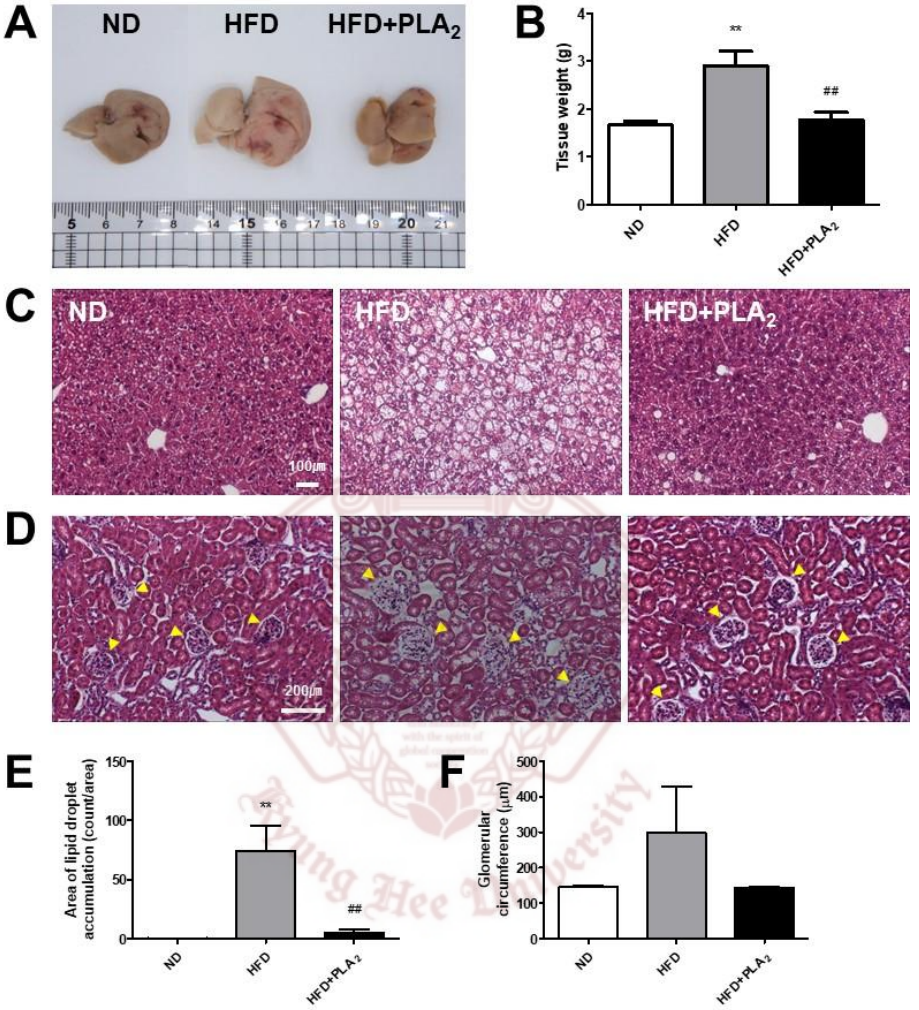

Figure 2. Histological analysis of liver and kidney tissue. (A-B) Representative images of the liver and measurements of liver weight. (C-D) Histology of H&E-stained (C) liver and (D) kidney sections. (E) The area of lipid droplet accumulation in the liver and (F) glomerular circumference expansion in the kidney. All data represented as the means ± SEMs; $^{**}P < 0.01$ versus the ND group; $^{\#\#}P < 0.01$ versus the HFD group.

3. Effect of bvPLA$_2$ treatment on the serum lipid profile of HFD-induced mice.

To determine the effect of bvPLA2 on liver and kidney function, serum levels of aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatine (Crea) were measured. After HFD feeding, aspartate, AST, ALT, and Crea levels were significantly increased compared to the levels in ND mice. The increased levels of AST, ALT, and Crea in HFD mice were reduced by bvPLA2 treatment. BUN levels were slightly higher in the ND and HFD+PLA2 groups than in the HFD group; however, all levels were in the normal range (Fig. 3A). Triglycerides (TG), glucose (GLU), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), and hormone levels were analyzed to assess the effects of bvPLA2 on metabolic syndrome. The levels of GLU, HDL-C, and LDL-C were markedly higher in HFD mice when than in ND mice, and were significantly lower in HFD+PLA2 mice than in HFD mice. Also, TG was increased in HFD group compared to ND group, and decreased in HFD+PLA2 group (Fig. 3B). Plasma insulin and leptin levels were higher in the HFD group than in the ND group, and were significantly lower in the HFD+PLA2 group than in the HFD group. However, adiponectin levels did not differ significantly among the groups (Fig. 3C). These results indicated that bvPLA2 alleviated HFD-induced metabolic dysfunction.

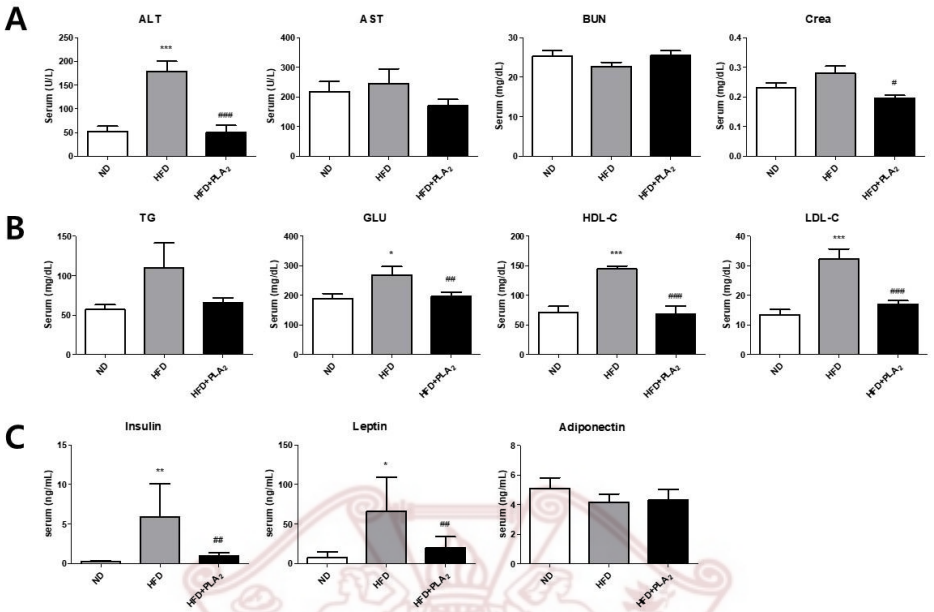

Figure 3. Metabolic regulation by bvPLA2 in HFD-induced obese mice. Analysis of serum levels of (A) ALT, AST, BUN, and Crea; (B) TG, GLU, HDL-C, and LDL-C; and (C) the hormones insulin, leptin, and adiponectin. All data represented as the means ± SEMs; **P* < 0.05, ***P* < 0.01, ****P* < 0.0001 versus the ND group; #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.0001 versus the HFD group.

4. bvPLA2 treatment prevent macrophages infiltration in AT.

Given that obesity is characterized by the localization of crown like structures (CLS), which are formed by pro-inflammatory macrophages surrounding dead adipocytes in AT (Cinti et al., 2005), I next assessed whether bvPLA2 reduced inflammatory macrophage infiltration in WAT by histological examination of H&E-stained sections. ND mice showed few infiltrating inflammatory macrophages in WAT. In comparison, HFD mice showed significantly increased CLS formation, whereas bvPLA2-treated mice (HFD+PLA2 group) showed decreased macrophage infiltration and CLS formation in AT (Fig. 4A, B). To clarify whether bvPLA2 directly reduced adipogenesis, preadipocytes (3T3-L1 cells) were differentiated into adipocytes *in vitro* and treated with bvPLA2 (Fig. 4C, D). No significant difference was observed between the control and bvPLA2-treated groups, suggesting that bvPLA2 did not directly inhibit the differentiation of adipocytes.

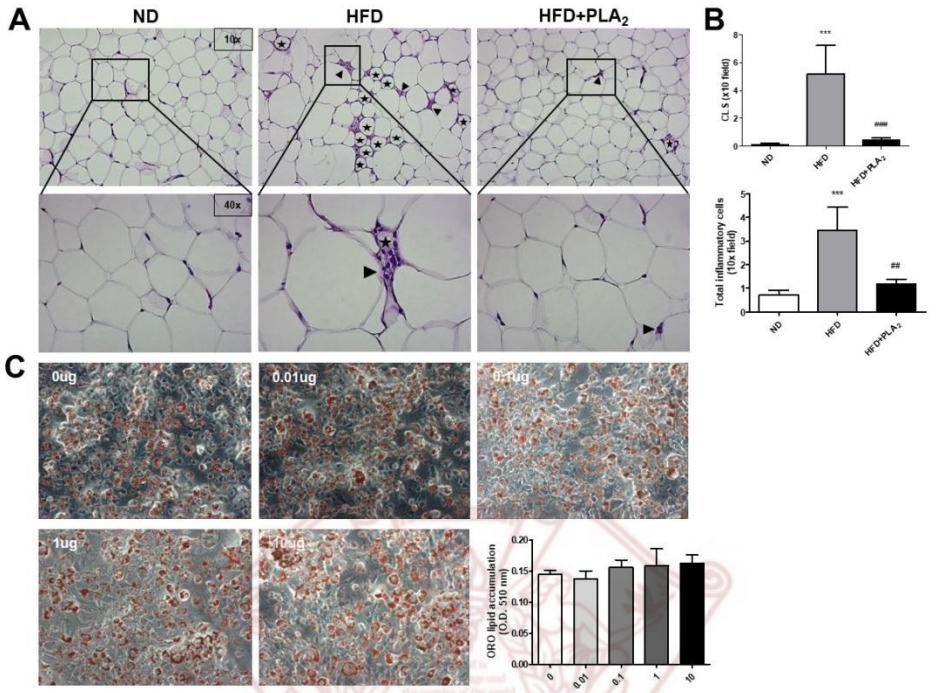

Figure 4. Treatment of bvPLA2 decreased the accumulation of macrophages in AT, not directly affecting adipocytes. (A) Representative H&E-stained WAT sections. The images in the black-lined box are magnified (bottom panel). Crown like structures (CLS) formed by dead adipocytes are marked with stars, and infiltrated macrophages are indicated with black arrows. (B) The number of CLS and infiltrated macrophages in adipose tissue. (C-D) Effect of bvPLA2 on lipid accumulation *in vitro*. 3T3-L1 cells were differentiated, and lipid droplets were stained with Oil Red O. Data are normalized to the absorbance of the 0 μg group. All data represented as the means ± SEMs; ***$P < 0.0001$ versus the ND group; ##$P < 0.01$, ###$P < 0.0001$ versus the HFD group.

5. bvPLA2 regulates the M1 or M2 phenotypic markers and adipogenic factors.

In the previous experiment, I investigated the change in infiltration of macrophages in the AT. Next, the changes in the phenotypes of macrophages were determined by quantitative real-time PCR analysis. The expression of TNF-α, IL-1β, and IL-12a was measured to verify the effect of bvPLA2 on M1-related markers. The expression levels of TNF-α was higher in HFD mice than in ND and HFD+PLA2 mice. Although IL-1β and IL-12a levels showed no significances, the levels were slightly higher in HFD group than in ND or HFD+PLA2 group (Fig. 5A). The level of TNF-α was significantly increased in HFD mice compared to ND mice, but this was markedly downregulated in HFD+PLA2 mice compared to HFD mice. In contrast, the M2-related markers IL-4, CD206, and Ym1 were remarkably increased in the HFD+PLA2 group when compared to the levels in the ND and HFD groups (Fig. 5B).

Next, the markers associated with lipid accumulation and adipogenesis such as PPARγ and C/EBPα (Rosen et al., 2002) were confirmed to investigate the effect of PLA2 on adipocytes; C/EBP α was significantly decreased in the HFD+PLA2 group when compared with the level in HFD group, and PPAR γ showed no significant differences among all groups. On the other hand, UCP-1, the marker of thermogenesis (Fromme & Klingenspor, 2011), was significantly lower in HFD mice than in ND mice, but increased in HFD+PLA2 mice compared to HFD mice (Fig. 5C).

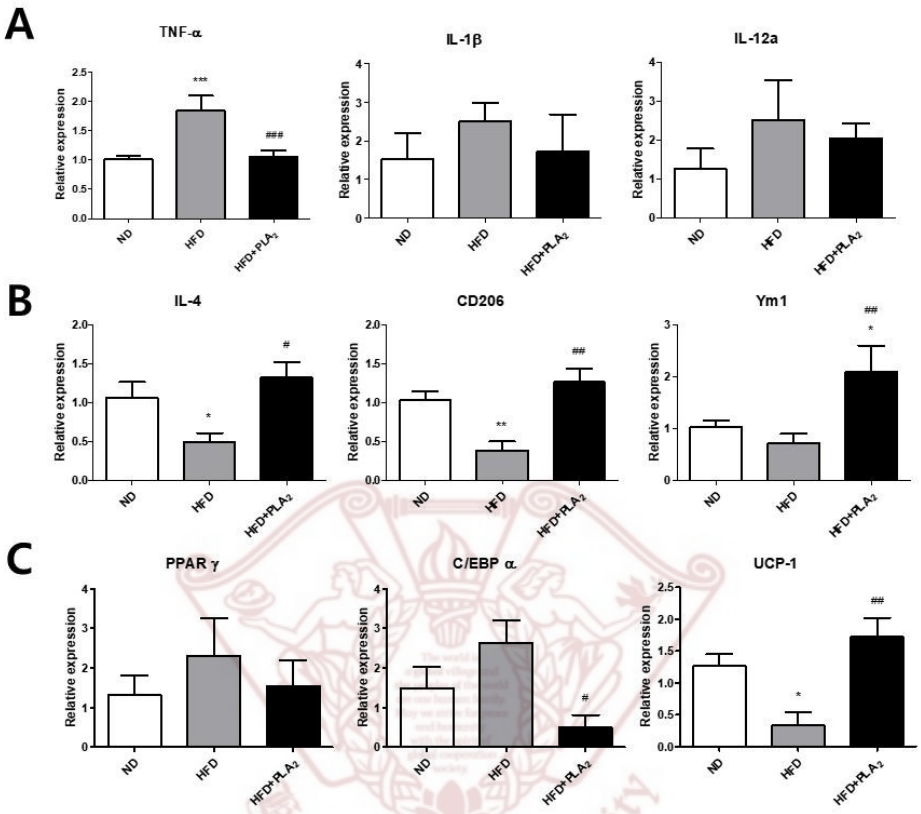

Figure 5. Effect of bvPLA2 on M1/M2 macrophage and adipogenic gene expression in WAT. (A-B) Relative expression levels of the M1-like markers TNF-α, IL-1β, and IL-12a and the M2-like markers IL-4, CD206, and Ym1 in adipose tissue were assessed by quantitative real-time PCR. (C) The adipogenic markers PPARγ, C/EBPa, and UCP-1 in adipose tissue were analyzed by quantitative real-time PCR. All data represented as the means ± SEMs; *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ versus the ND group; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.0001$ versus the HFD group.

6. Treatment of bvPLA2 decreases M1/M2 macrophage ratio in AT.

Next, to confirm the effect of bvPLA2 on the M1/M2 ATM ratio in AT *in vivo*, single cells were isolated from eWAT. Then, the percentages of $CD11c^{+}CD206^{-}$ M1 ATMs and $CD11c^{-}CD206^{+}$ M2 ATMs among the $CD45^{+}CD11b^{+}$ cells, gated on live cells were analyzed. Flow cytometry analysis showed that the M1/M2 macrophage ratio in the HFD group was higher than that in the ND, and this imbalance was effectively reversed by administration of bvPLA2 (Fig. 6). These results suggest that bvPLA2 regulates the M1/M2 balance of ATMs.

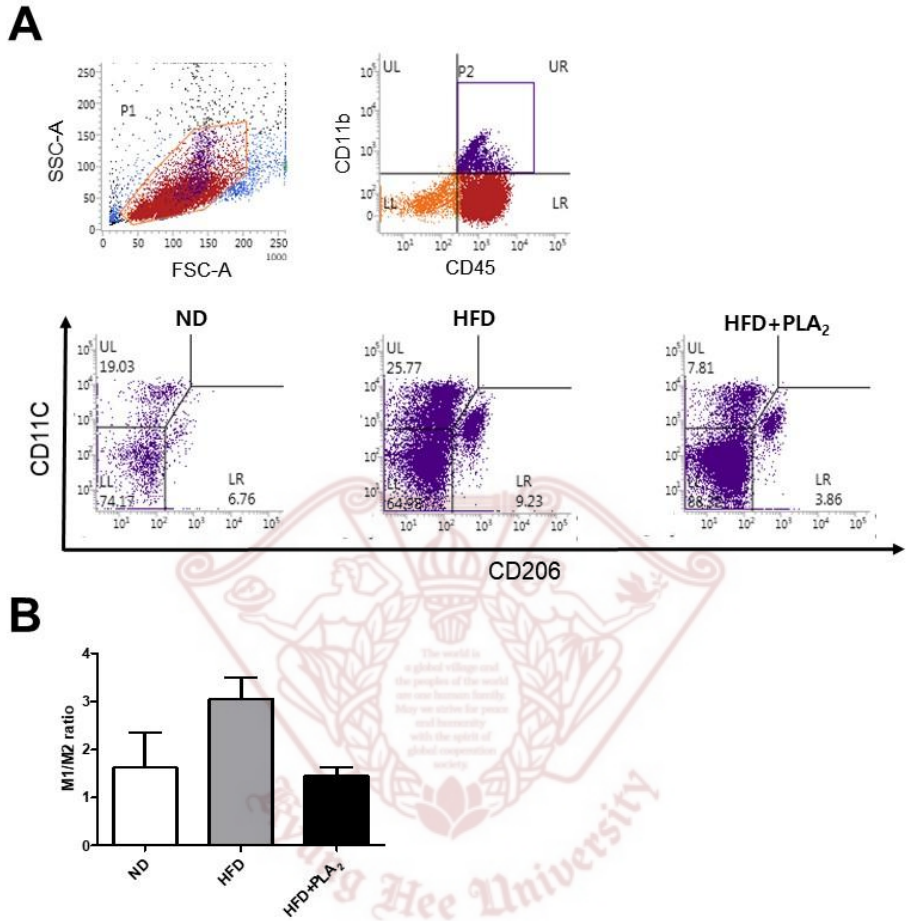

Figure 6. bvPLA2 treatment decreased the M1/M2 ratio in HFD-induced obese mice. ATMs were stained with antibodies against CD45, CD11b, CD11c, and CD206 and examined by FACS analysis. Plots showing the percentages of M1/M2-like ATMs among the $CD45^{+}CD11b^{+}$ cells. (A) $CD11c^{+}CD206^{-}$ (M1) and $CD11c^{-}CD206^{+}$ (M2) macrophages in lean (ND) and obese (HFD) mice. (B) The ratio of classically activated M1-like ATMs ($CD11c^{+}CD206^{-}$) and alternatively activated M2-like ATMs ($CD206^{+}CD11c^{-}$) measured by FACS.

7. $bvPLA_2$ increased M2-like macrophage polarization and decreased M1-like macrophage polarization.

The effect of bvPLA2 on M1/M2 macrophage polarization was evaluated by measuring M1 and M2 differentiation markers *in vitro*. Bone marrow cells were differentiated into M0 macrophages by M-CSF supplementation. Then, the M0 macrophages were treated with LPS or IL-4 to stimulate polarization into M1 or M2 macrophages, respectively. LPS-treated M1 macrophages showed significantly higher TNF-α and IL-12a mRNA expression compared to M0 macrophages, and the expression of these markers was decreased by bvPLA2 treatment. In contrast, IL-4-treated M2 macrophages showed higher mRNA expression of CD206 and Ym1 than control M0 macrophages, and the levels of these M2 markers were increased by bvPLA2 treatment. These results showed that bvPLA2 inhibited M1 macrophage polarization and stimulated M2 macrophage polarization (Fig. 7).

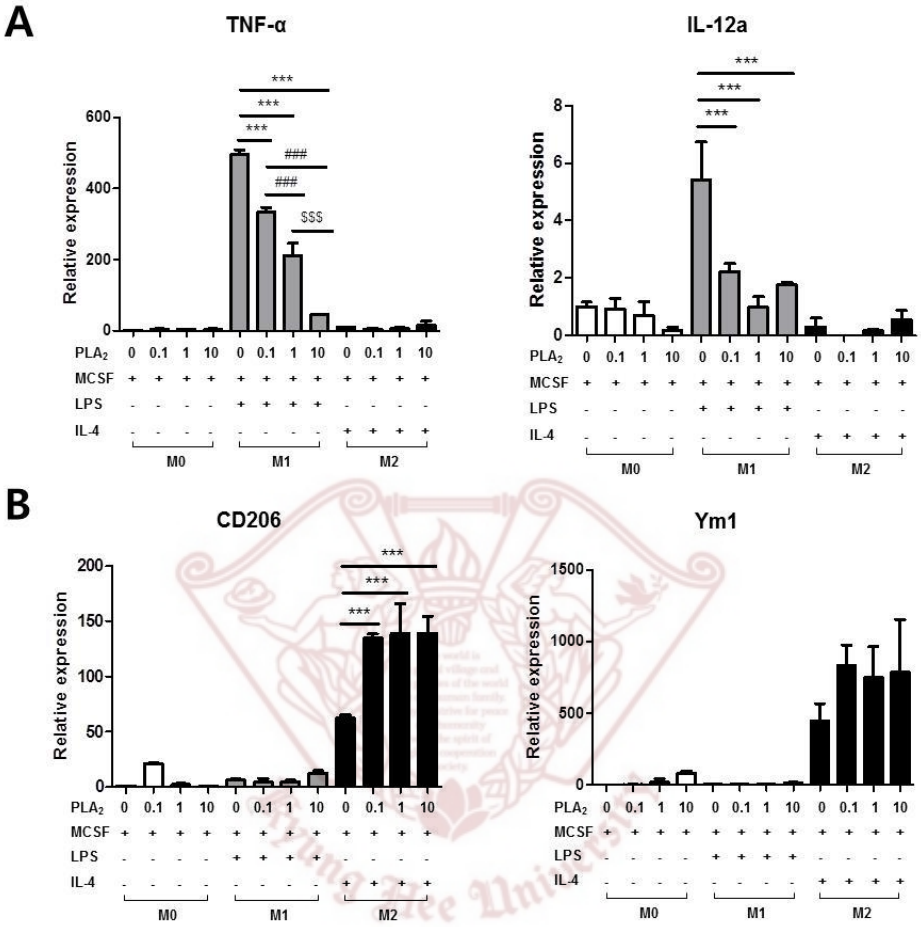

Figure 7. bvPLA2 alters macrophage polarization. M1- or M2-differentiated bone marrow-derived macrophages were treated with bvPLA2, and respective marker gene expression was evaluated by quantitative real-time PCR. mRNA expression levels of (A) the M1-phenotypic markers TNF-α and IL-12a, and (B) the M2-phenotypic markers CD206 and Ym1. All data represented as the means ± SEMs; ***$P < 0.0001$ compared with the LPS or IL-4 only treated groups; ###$P < 0.0001$ compared with the LPS 0.1 or 1 treated group; \$\$\$$P < 0.0001$ compared with the LPS 1 treated group.

IV. Discussion

In this study, I demonstrated the therapeutic effects of bvPLA2 on obesity for the first time. The results showed that bvPLA2 prevented rapid body weight gain, hepatic steatosis, and glomerular hypertrophy *in vivo*. bvPLA2 treatment also reduced adipocyte size and significantly reduced the obesity-induced increases in macrophage infiltration into WAT and subsequent CLS formation and adipocyte death. In addition, bvPLA2 treatment reduced liver and kidney toxicity, which can result in metabolic dysfunction, and inhibited the elevation of glucose, cholesterol, and insulin levels in serum.

Previous studies reported that bvPLA2 can directly bind to the mannose receptor CD206. When bvPLA2 binds to the surface of dendritic cells, prostaglandin E2 (PGE2) secretion is increased via stimulation of cyclooxygenase-2 (COX-2), resulting in the differentiation of $Foxp3^+$ regulatory T cells via EP2 receptor signaling in Th0 cells (Eun Sook Chung et al., 2015). Given that bvPLA2 binds to CD206, which is highly expressed on the surface of macrophages as well as dendritic cells, bvPLA2 also likely directly binds to CD206 on macrophages. Here, I newly reported that bvPLA2 stimulated M2-phenotypic macrophage differentiation *in vitro*, as shown in Figure 7. I also demonstrated that $CD11c^+CD163^-$ inflammatory ATMs were significantly decreased, while $CD11c^-CD163^+$ M2 ATMs were increased by bvPLA2 *in vivo*, as shown in Figure 6, suggesting that bvPLA2 stimulates M2 phenotypic differentiation by binding to CD206. It is predicted bvPLA2 might increase the Treg population in HFD-induced obese mice and further study is needed to confirm this change in the Treg population in AT.

AT, which is composed of adipocytes, is the main site for fat storage and metabolic control (Wijayatunga et al., 2018). AT is largely divided into two types, WAT and brown adipose tissue (BAT) (Nakamura, Hongo, Kodama, & Hiramatsu, 2011). WAT contains large lipid droplets and releases adipokines, such as FFAs, TNF-α, IL-6, IL-10, resistin, leptin, and adiponectin, to maintain metabolic homeostasis. WAT also stores TG for future energy production during periods of malnutrition. In contrast, BAT contains multiple small lipid droplets and maintains body temperature and weight by converting consumed food into heat through UCP-1-mediated thermogenesis (Kuryszko, Slawuta, & Sapikowski, 2016; Shabalina et al., 2013). Given that BAT can eliminate circulating glucose and TG during heat production, the browning of WAT, which means the switching of WAT to a BAT-like phenotype, might be a useful therapeutic strategy for obesity. It has been reported that PGE2 induces UCP-1 in WAT and promotes thermogenesis. PGE2 was also shown to increase adipolysis and regulate metabolic homeostasis in WAT (S. H. Kim & Plutzky, 2016; Nasrallah, Hassouneh, & Hébert, 2016; Xue, Xu, Zhang, Farokhzad, & Langer, 2016; Zhang et al., 2018). As shown in Figure 5, bvPLA2 significantly increased UCP-1, which can mediate the browning of WAT. However, as shown in Figure 4, bvPLA2 did not directly affect adipocyte differentiation. Thus, further study is needed to verify whether the increase in UCP-1 induced by bvPLA2 is related to the increase in PGE2 via the phenotypic change of M1 to M2 macrophages.

Obesity is a serious problem both nationally and globally, and the currently available treatments are ineffective or have a high risk of

adverse effects. Currently, three drugs are approved by the U.S. FDA for the treatment of obesity, orlistat, diethylpropion, and phentermine (Joo & Lee, 2014). However, long-term treatment with these drugs is associated with several serious side effects, such as liver injury, kidney stones, and pancreatitis (Ioannides-Demos, Piccenna, & McNeil, 2011). In addition, most anti-obesity drugs decrease body weight by inhibiting the absorption of fat from foods, resulting in steatorrhea (G. W. Kim, Lin, Blomain, & Waldman, 2014). Although regulating the inflammatory state is critical for the prognosis of obese patients, there is no currently available treatment for reducing inflammation in obesity. Therefore, it is necessary to develop a curative treatment for obesity. Here, I used a long-term, 15-week treatment mouse model of HFD-induced obesity with bvPLA2 administration every 3 days. The AST/ALT and BUN/CREA analysis showed that long-term treatment with bvPLA2 did not induce any side effects, including hepatic or renal damage. Thus, these results suggest that bvPLA2 could be used as a therapeutic drug to prevent obesity and associated complications, such as diabetes, and maintain homeostasis.

V. Conclusion

These results indicate that bvPLA2 alleviated HFD-induced obesity by improving the balance of M1/M2 macrophages in AT. Thus, bvPLA2 is a promising treatment for obesity and obesity-related metabolic diseases.

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국문초록

고지방식이로 유도 된 마우스 모델에서의 봉독 조성물 포스포리파아제 A2 ($bvPLA_2$)의 효과

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지도 교수: 배현수

비만은 복잡한 대사 장애로 체지방이 과도하게 축적되는 것이 특징이며 심혈관 질환, 고혈압, 제 2형 당뇨병 및 고 콜레스테롤에 의해 복잡해지는 경우가 많다. 비만은 또한 백색 지방 조직(WAT)에 염증성 면역세포가 축적되어 지방세포의 기능장애로 이어진다. 봉독은 단백질과 화합물이 복합적으로 혼합된 것으로 항 종양, 항 염증 및 항 비만 기능이 있는 것으로 보고 되었다. 그러나 비만에서의 항 비만 효과를 일으키는 봉독의 구체적인 화합물은 아직 알려지지 않았다. 본 연구에서는, 봉독 포스포리파아제 A2(bvPLA2)가 고지방식이(HFD) 유도 비만에 대한 치료 효과를 확인하였다. 결과적으로, bvPLA2는 체중과 WAT 무게를 현저하게 감소시키고 HFD를 섭취한 생쥐의 간과 신장의 체중을 현저히 낮춘 것으로 나타났다. HFD 섭취 생쥐에 bvPLA2 를 투여하였을 때 간에서의 지질 축적을 줄이고 신장

염증을 감소시켰다. 또한, bvPLA2는 지방조직의 M1 전-염증성 대식세포를 감소시키고 생체 내에서 M1/M2 불균형을 완화시켰다. bvPLA2는 또한 M1 분화를 억제하면서 M2 분화를 자극 하였지만 시험관 내에서는 지방 생성을 직접 억제하지는 못하였다. 따라서, bvPLA2는 면역 항상성을 조절함으로써 비만 치료제로서의 가능성을 나타낸다.

키워드: 비만, 고지방식이, 봉독 포스포리파아제 A2, 백색 지방조직, 지방조직 대식세포