이학박사학위 청구논문

# VLDL과 VLDL 수용체를 통한 지방조직 염증반응 및 열생산 조절 연구

Roles of VLDL and VLDL receptor in the Regulation of Adipose Tissue Inflammation and Thermogenesis

2021년 2월

서울대학교 대학원

생명과학부

신경철

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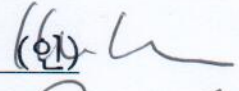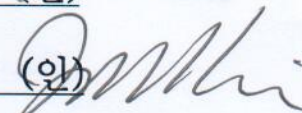
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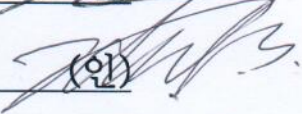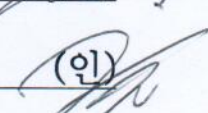
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# Roles of VLDL and VLDL receptor in the regulation of adipose tissue inflammation and thermogenesis

A dissertation submitted in partial fulfillment of

the requirement for degree of

## DOCTOR OF PHILOSOPHY

To the Faculty of the

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At

## SEOUL NATIONAL UNIVERSITY

By

**Kyung Cheul Shin**

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# ABSTRACT

# Roles of VLDL and VLDL receptor in the regulation of adipose tissue inflammation and thermogenesis

**Kyung Cheul Shin**

Triglyceride is a key metabolite for cell growth, division, and being used as an energy source. In plasma, lipid metabolites, including triglycerides, are circulated in the form of lipoproteins. Among various lipoproteins,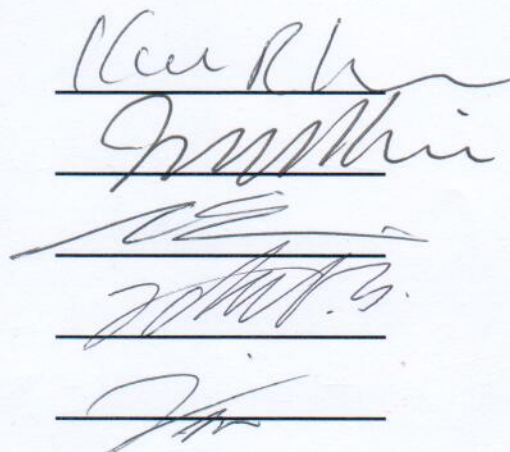 very low density lipoprotein (VLDL) contains large portion of triglyceride, and VLDL receptor (VLDLR) is involved in uptake and storage of circulating VLDL. VLDLR is highly expressed in adipose tissue. It has been reported that VLDLR KO mice exhibit reduced adipose tissue mass compared to control mice. Although VLDLR appears to play key roles in VLDL uptake and lipid homeostasis in fat tissues, it is largely unknown whether adipose tissue VLDLR could contribute to systemic energy homeostasis under metabolic alterations.

In the chapter one, I have demonstrated that elevated VLDLR in white adipose tissue (WAT) could aggravate adipose tissue inflammation and glucose intolerance in obese mice. Moreover, VLDL administration upregulated intracellular levels of C16:0 ceramides in a

VLDLR-dependent manner, potentially leading to pro-inflammatory responses by promoting M1-like macrophage polarization. Adoptive transfer of VLDLR KO bone marrow to wild type (WT) mice relieved inflammatory responses and improved glucose intolerance in diet-induced obese (DIO) mice. These data suggest that increased VLDL-VLDLR signaling in WAT is responsible for aggravation of adipose tissue inflammation and insulin resistance in obesity.

In the chapter two, I have shown that VLDLR-mediated VLDL uptake in brown adipose tissue (BAT) would contribute to adaptive thermogenesis upon cold exposure. By blocking VLDL secretion, WT mice became cold-intolerant. In VLDLR KO mice, thermogenic capacity was impaired in BAT. In brown adipocytes, VLDL treatment enhanced thermogenic gene expression as well as simultaneously potentiated mitochondrial oxygen consumption in a VLDLR-dependent manner. In VLDLR KO mice, the expression levels of PPARδ target genes were downregulated in BAT, while administration of PPARδ agonist restored thermogenic abnormality and mitochondrial dysfunction. Moreover, VLDL-dependent thermogenic activation was not affected in brown adipocyte-specific PPARδ KO mice. These data indicate that VLDL-VLDLR axis in BAT is crucial for thermogenic regulation via PPARδ activation during cold acclimation.

Taken together, these data suggest that adipose tissue VLDLR could mediate VLDL uptake, leading to modulation of not only lipid metabolism but also the manipulation of

cellular signaling. Furthermore, VLDLR in WAT would aggravate pro-inflammatory responses upon obesity and VLDLR in BAT could facilitate adaptive thermogenesis under cold stimulation.

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLE

# BACKGROUND

## 1. Adipose tissue as an energy storage organ

## 1) White adipose tissue on energy storage

Living organisms obtain energy source from their environment and utilize them for survival. Storage of excess energy is an essential physiological activity for survival during periods of starvation. Multicellular organisms evolve specialized cells or organs to store extra nutrients as lipids due to that lipids have higher calories than other carbohydrates or proteins (Hardie, 2012). For instance, *Caenorhabditis elegans* stores surplus energy such as lipids in intestinal cells, while *Drosophila* and shark accumulate excess lipids in fat body and liver, respectively (McKay et al., 2003; Miller et al., 2002; Van Vleet et al., 1984). In case of other organisms, including various fish and higher vertebrates, adipose tissue functions as a specialized energy reservoir (Birsoy et al., 2013). In mammals, adipose tissue is distributed throughout whole-body and capable of expanding for accommodation of excess energy in the form of lipid droplets (Gesta et al., 2007). Most mammals have two major types of adipose tissue, white adipose tissue (WAT) and brown adipose tissue (BAT) (Gesta et al., 2007). Anatomically, WAT is comprised of two major depots, including subcutaneous WAT and visceral WAT around internal organs (Item and Konrad, 2012). These adipose tissues secrete

hormones, cytokines, and metabolites (termed as adipokines) that regulate energy homeostasis by controlling appetitive signals in central nervous system as well as metabolic activity in peripheral tissues (Choe et al., 2016). Thus, adipose tissue is well known as a central metabolic organ in the regulation of systemic energy metabolism under various metabolic conditions.

## 2) Obesity-induced adipose tissue inflammation and insulin resistance

Obesity is characterized by imbalanced expansion of fat tissues. It has been reported that obese fat tissues often exhibit chronic and low grade inflammation, which is associated with metabolic disorders including type 2 diabetes, and insulin resistance (Donath and Shoelson, 2011; Olefsky and Glass, 2010; Wellen and Hotamisligil, 2003). In obesity, adipose tissue appears to be primary tissue to initiate inflammatory system by responding extra nutrients and/or metabolites (Donath and Shoelson, 2011; Olefsky and Glass, 2010; Wellen and Hotamisligil, 2003). For instance, saturated free fatty acids (FFAs) from excess nutrients stimulate toll-like receptor (TLR) 4 in adipocytes (Saberi et al., 2009; Shi et al., 2006; Suganami et al., 2007). Activated TLR4 signaling by saturated FFAs subsequently modulates mitogen-activated protein kinase (MAPK) signaling cascades, which could eventually potentiate pro-inflammatory cytokines, including tumor necrosis factor α, interleukin (IL)-1 beta, and monocyte chemoattractant protein-1 in obese adipose tissue (Saberi et al., 2009; Shi

et al., 2006).

Cumulative evidence has shown that pro-inflammatory cytokines are significantly associated with insulin resistance in obesity (Donath and Shoelson, 2011). It has been shown that activation of IkB kinase-beta (IKKβ) and c-Jun N-terminal kinases (JNK) by pro-inflammatory cytokines results in repression of insulin signaling in adipocytes (Donath and Shoelson, 2011; Olefsky and Glass, 2010; Wellen and Hotamisligil, 2003). In addition, activation of these stress-activated kinases induces serine phosphorylation of insulin receptor substrate 1 (IRS1) and IRS2, the key downstream mediators of insulin receptor, and thus interfering with proper insulin signaling, subsequently leading to insulin resistance (Austin et al., 2008; Hirosumi et al., 2002; Nguyen et al., 2005; Wellen and Hotamisligil, 2005; Yuan et al., 2001; Zhang et al., 2011).

## 3) Adipose tissue macrophage and M1/M2 polarization

Macrophage is a predominant cell type among various immune cells constituting adipose tissue. Although there are resident adipose tissue macrophages (ATMs) of lean subjects, obese adipose tissue exhibits that new macrophages are recruited and subsequently accumulated around adipocytes, a phenomenon that is called 'crown-like structure' (Lumeng et al., 2007b; Weisberg et al., 2003; Xu et al., 2003) (Figure 1). These accumulated macrophages are characterized by scavenging of dead adipocytes, secretion of certain
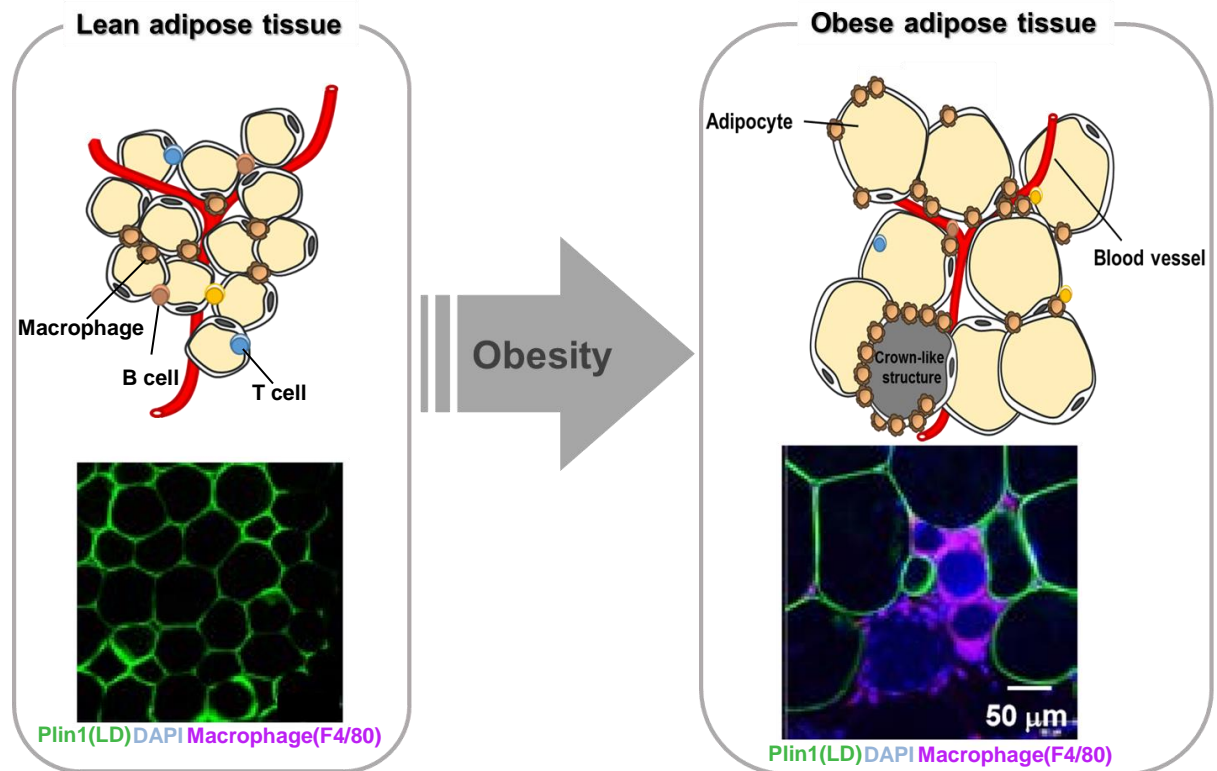
**Figure 1. Obesity induces adipose tissue inflammation with macrophages recruitments.**
Graphical images reveal that adipose tissue consists of adipocytes and various immune cells (Up).
Fluorescent microscopic images of whole-mount immunohistochemistry in adipose tissues upon obesity (Green:Perilipin_lipid droplet_LD,Blue:DAPI_Nucleus,Purple:F4/80_Macrophages) (Down)

cytokines, and eventual regulation of systemic energy homeostasis (Lumeng et al., 2007b; Weisberg et al., 2003; Xu et al., 2003).

ATMs are largely divided into classically activated (M1-like) macrophages and alternatively activated (M2-like) macrophages. In lean animals, the large number of ATMs is composed of M2-like macrophages expressing high levels of IL-4, -10, -13, and arginase (ARG) 1 that are related to insulin sensitivity (Fujisaka et al., 2009; Lumeng et al., 2007a; Sun et al., 2011). In contrast, population of M1-like macrophages rapidly increased in obese adipose tissue (Odegaard and Chawla, 2011; Wynn et al., 2013). M1-like ATMs secrete various pro-inflammatory cytokines that aggravate adipose tissue inflammation and insulin resistance in obesity (Odegaard and Chawla, 2011; Wynn et al., 2013). Therefore, imbalance between M1- and M2-like ATMs plays a crucial role to regulate inflammatory responses and insulin sensitivity in obese adipose tissue.

## 2. Adipose tissue as a thermal regulator

### 1) Brown adipose tissue and thermogenesis

BAT is characterized by rich vascularization, small-multilocular lipid droplets, and abundant mitochondria (Richard and Picard, 2011; Smith and Horwitz, 1969). Unlike WAT, BAT has a prominent function in associated with thermal regulation through heat generation

by fuel oxidation (Cannon and Nedergaard, 2004). In BAT, heat production is activated by sympathetic nervous system through norepinephrine, leading to activation of β-adrenergic signaling and subsequent uncoupling of ATP production from mitochondrial respiration. In mitochondria, uncoupling protein (UCP) 1, which is highly expressed in brown adipocyte, mediates adaptive thermogenesis under cold stimuli (Cannon and Nedergaard, 2004).

## 2) Thermogenic brown adipose tissue and fuel utilization

Upon cold exposure, the dissipation of energy substrates is required for heat-production (Cannon and Nedergaard, 2004) (Figure 2). Circulating carbohydrates (e.g., glucose) and lipids (e.g., fatty acid) are primary energy sources in the regulation of thermogenic BAT (Dallner et al., 2006; Hankir and Klingenspor, 2018; Ouellet et al., 2012). On one hand, it has been reported that brown adipocytes take up large amounts of glucose, concurrently leading to an upregulation of glycolytic activity during cold exposure (Jeong et al., 2018). On the other hand, thermogenic BAT has been investigated to possess a capacity of fatty acid β-oxidation in both rodents and human, which subsequently enhances heat generation and eventually maintains core body temperature (Albert et al., 2016; Lee et al., 2016). Recently, it has been demonstrated that lipid metabolites derived from fatty acids were demonstrated to modulate signaling cascades to mediate thermogenic activity in BAT upon
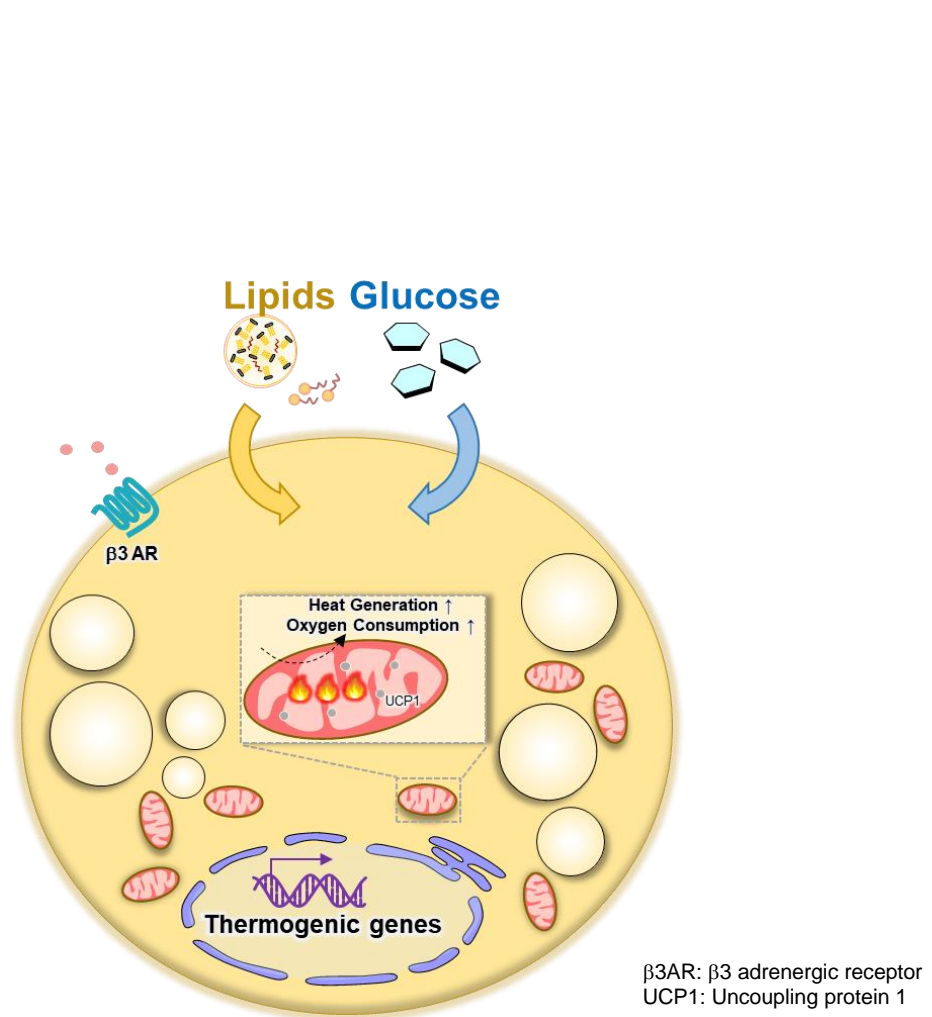
**Figure 2. In BAT, cold exposure requires energy substrates for adaptive thermogenesis.** BAT has a prominent function in associated with thermal regulation through heat generation by fuel oxidation. Circulating carbohydrates (e.g., glucose) and lipids (e.g., fatty acid, and triglyceride-rich lipoproteins) are major energy sources for thermogenesis. In BAT, heat production is activated by sympathetic nervous system through norepinephrine, leading to activation of β-adrenergic signaling and subsequent uncoupling of ATP production from mitochondrial respiration by uncoupling protein (UCP) 1 upon cold exposure.

cold exposure (Heeren and Scheja, 2018). Thus, it is likely that distinct function of substrate modulation needs to be tightly regulated for thermogenesis in BAT.

## 3. Lipoproteins as triglyceride carriers in blood

### 1) Triglyceride and Lipoproteins

Triglycerides are key metabolites synthesized from glycerol and three fatty acids; they are main constituents of adipocyte in humans and other animals as well as vegetable fat (Marcelin and Chua, 2010). In blood, triglycerides are circulated in the form of lipoproteins (Gofman et al., 1950; Minehira et al., 2008). Besides, triglycerides from circulated lipoproteins are essential metabolites in association with cell growth, cell division as well as being used as a precursor of hormone, which is crucial for energy homeostasis (Wollam and Antebi, 2011).

Since triglycerides are insoluble in water, they cannot be transported on their own in extracellular water, including blood plasma (Marcelin and Chua, 2010). Thus, triglycerides are surrounded by hydrophilic external apo-proteins that function as a carrier, so called lipoproteins. Lipoproteins have classified into five groups based on the ratio of internal lipid composition and external apo-proteins (Pownall et al., 2016). As the lipid-to-protein ratio

increases, lipoproteins tend to be larger in size and lower in density (Figure 3).

## 2) Lipoprotein receptors and VLDLR

Circulating lipoproteins are often recognized as membrane receptors (Gofman et al., 1950). Low density lipoprotein receptor (LDLR) is a member of LDLR family, which includes very low density lipoprotein receptor (VLDLR), apolipoprotein E receptor 2 (ApoER2) as well as other lipoprotein related proteins (LRPs) (Takahashi et al., 2003). These lipoprotein receptor family members consist of four major domains: cysteine-rich complement-type repeats, epidermal growth factor precursor-like repeats, transmembrane domain, and cytoplasmic domain (Figure 3). Each module serves distinct and important functions, and specific ligands are bound by these receptors.

The group of Dr. Tokuo T. Yamamoto has reported that VLDLR, one of the LDLR family, would be receptor for VLDL (Takahashi et al., 1992). Human VLDLR gene is located on chromosome 9 as well as its genomic organization to be similar to that of the LDLR and adpoER2. VLDLR displays approximately 95% amino acid conservation among various animals including rabbits, human, rats, and mice (Takahashi et al., 2003). Therefore, VLDLR is well a conserved membrane receptor related to lipid metabolism throughout evolution.

## Lipoprotein



## LDL receptor family
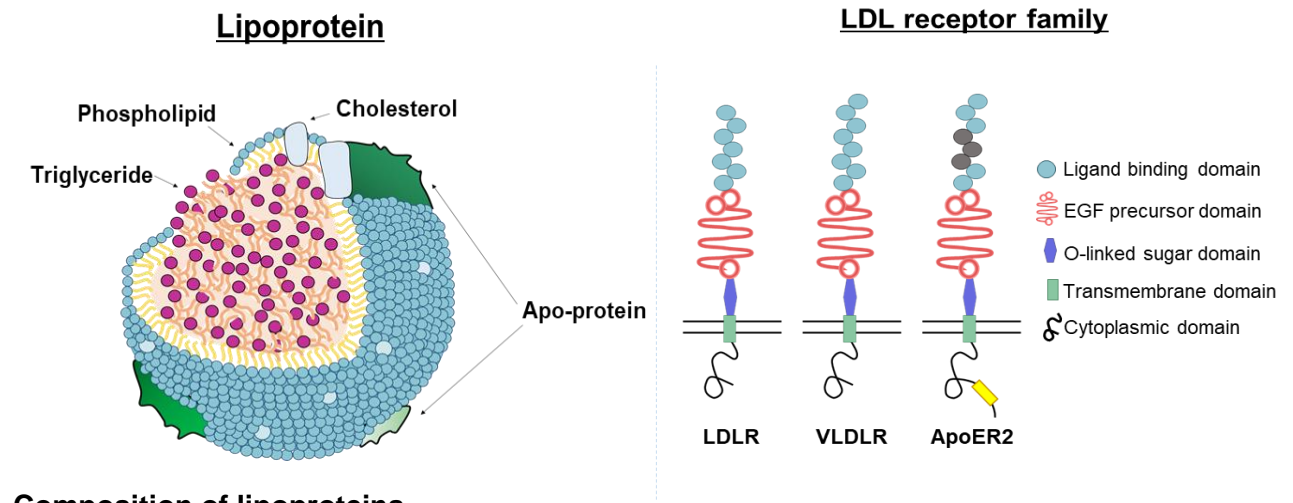


## Composition of lipoproteins

|  | HDL | LDL | IDL | VLDL | Chylomicrons |
|---|---|---|---|---|---|
| Size (nm) | 5-12 | 18-30 | 25-35 | 30-80 | 75-1200 |
| Density (g/ml) | 1.063-1.210 | 1.019-1.063 | 1.006-1.019 | 0.95-1.006 | <0.95 |
| Cholesterol (%dry weight) | 10-20 | 35-50 | 22 | 10-22 | 2-5 |
| Triglyceride (%dry weight) | 2-7 | 4-10 | 20-30 | 45-65 | 80-95 |
| Major apoprotein | ApoA1, 2, 4 ApoC1, 2, 3 ApoD, ApoE | ApoB100 ApoC, ApoE | ApoB100 ApoC1, 2, 3 ApoE | ApoB100 ApoC1, 2, 3 | ApoA1, 2, 3 ApoB100 ApoC1, 2, 3 ApoE |

**Figure 3. Lipoprotein and LDL receptor family.** Lipoproteins have classified into five groups based on the ratio of internal lipid composition and external apo-proteins. Lipoprotein receptor family members consist of five major domains: cysteine-rich complement-type repeats, epidermal growth factor precursor-like repeats, O-linked sugar domain, transmembrane domain, and cytoplasmic domain.

## 3) VLDLR in adipose tissue

It has been reported that VLDLR has important roles in the uptake of VLDL through receptor-mediated endocytosis or lipoprotein lipase (LPL)-dependent lipolysis (den Hartigh et al., 2014; Takahashi et al., 2004). VLDLR is abundantly expressed in heart, kidneys, skeletal muscle and adipose tissue (Takahashi et al., 2004). In order to investigate physiological roles of VLDLR in energy homeostasis, VLDLR-deficient mice have been generated, and initial studies with VLDLR knockout (KO) mice have shown that there were no alterations in plasma lipoprotein levels (Frykman et al., 1995). Nonetheless, adipose tissue of VLDLR KO mice exhibits reduced compared with that of control mice (Goudriaan et al., 2001; Nguyen et al., 2014; Perman et al., 2011). These findings imply that adipose tissue VLDLR might play certain roles in lipid metabolism of adipose tissue.

# 4. Purposes

Circulating VLDL consists of hydrophobic triglycerides, and hydrophilic apo-protein (Gofman et al., 1950), and VLDLR is involved in the clearance of circulating VLDL (Takahashi et al., 2003). Although VLDLR seems to play certain roles for VLDL uptake and adipose tissue mass, it has not been thoroughly understood whether adipose tissue VLDLR might contribute to energy homeostasis under various environmental stimuli.

In this thesis, I have investigated the roles of VLDLR in the aspects of adipose tissue inflammation in white adipose tissue (WAT) and thermogenesis in brown adipose tissue (BAT). In the chapter one, VLDLR was increased in obese WAT, and promoted pro-inflammatory responses through ceramide production, accompanied with enhanced M1-like macrophage polarization. Moreover, bone marrow transplantation from VLDLR KO mice into WT recipient mice attenuated insulin resistance in diet-induced obesity (DIO), simultaneously with reduction of adipose tissue inflammation. These data suggest that upregulated VLDLR in WAT could provoke insulin resistance by enhancing pro-inflammatory signaling pathways, accompanied by altered lipid profiles under lipid-rich condition. In the chapter two, I observed that VLDLR expression in BAT was elevated in cold condition, eventually promoting adaptive thermogenesis through PPARδ activation. VLDLR KO mice were intolerant upon cold exposure, whereas administration of synthetic PPARδ

agonists led to restore thermogenic abnormality and improve mitochondrial dysfunction. Moreover, VLDL-dependent thermogenic regulation was not activated in brown adipocyte-selective PPARδ knockout mice. These data suggest that upregulated VLDL-VLDLR axis in BAT would trigger thermogenic activity by modulating PPARδ signaling pathways under cold stimuli.

Collectively, I would like to propose the idea that VLDLR would promote insulin resistance in obese WAT through alteration of macrophage polarization and enhance adaptive thermogenesis in BAT by facilitating thermogenic system.

# CHAPTER ONE:

# In white adipose tissue, VLDLR regulates obesity-induced insulin resistance with adipose tissue inflammation

**Abstract**

In obesity, increased adipose tissue macrophages (ATMs) have been implicated in pro-inflammatory responses and systemic insulin resistance with altered lipid metabolism. Very low-density lipoprotein receptor (VLDLR), one of the lipoprotein receptor family proteins, is involved in lipoprotein uptake and storage. However, it is not well understood whether lipid uptake via VLDLR in macrophages is involved in obesity-induced inflammatory responses and insulin resistance. In this study, I demonstrate that elevated VLDLR in ATMs could accelerate adipose tissue inflammation and glucose intolerance in obesity. The expression of VLDLR was increased in ATMs from obese mice. In VLDL-treated macrophages, the levels of C16:0 ceramides were elevated through VLDLR, which potentiates pro-inflammatory responses and augmented M1-like macrophage polarization. Moreover, adoptive transfer of VLDLR knockout bone marrow to wild-type mice relieved adipose tissue inflammation and improved insulin resistance in diet-induced obesity. Taken together, these findings suggest that increased VLDL-VLDLR axis in ATMs would aggravate adipose tissue inflammation and insulin resistance in obesity.

**Introduction**

Obesity is characterized by chronic and low grade inflammation accompanied with macrophage accumulation in adipose tissue, eventually leading to metabolic disorders including insulin resistance and type 2 diabetes (Hotamisligil et al., 1993; Xu et al., 2003). Adipose tissue macrophages (ATMs) are key players in adipose tissue inflammatory responses in obesity (Donath and Shoelson, 2011; Lee et al., 2011; Weisberg et al., 2003). In lean animals, the large number of ATMs is composed of alternatively activated (M2-like) macrophages expressing high levels of interleukin (IL)-4, 10, 13, and arginase (ARG) 1 that are associated with insulin sensitivity (Fujisaka et al., 2009; Odegaard et al., 2007). Although it has been shown that M2-like macrophages might produce catecholamines to enhance adaptive thermogenesis (Hui et al., 2015; Qiu et al., 2014; Rao et al., 2014), a very recent study reported that M2-like macrophages do not produce catecholamines (Fischer et al., 2017). These controversial findings are needed to be further investigated. In contrast, in obese animals, the population of classically activated (M1-like) macrophages is rapidly increased in adipose tissue (Lumeng et al., 2007a; Lumeng et al., 2007b). M1-like ATMs secrete numerous pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), and MCP-1, which aggravates adipose tissue inflammation and insulin resistance in obesity (Hirosumi et al., 2002; Shoelson et al., 2006). In obese adipose tissue, pro-inflammatory cytokines secreted from M1-like ATMs induce adipokine dysregulation and impair insulin

action to confer systemic insulin resistance (Berg et al., 2002; Yamauchi et al., 2001). Thus, the imbalance between M1- and M2-like ATMs plays an important role to modulate pro-inflammatory responses in obese adipose tissue.

M1- or M2-like polarization of ATMs has been attributed to dynamic changes in adipose tissue microenvironments (Sica and Mantovani, 2012). Concurrent with the expansion of adipose tissue in obesity, ATMs participate in adipose tissue remodeling by storing surplus lipid metabolites, giving rise to a subpopulation of lipid-laden ATMs (Aouadi et al., 2014; Grijalva et al., 2016; Pecht et al., 2016; Shapiro et al., 2013). Recent studies have shown that cytotoxic lipid species, such as free cholesterol, and short-chain saturated fatty acids, are elevated, whereas protective lipid metabolites, such as long-chain polyunsaturated fatty acids, are decreased in the lipid-laden ATMs of obese mice (Prieur et al., 2011; Shapiro et al., 2013). Furthermore, lipid-overloaded macrophages in obese adipose tissue stimulate pro-inflammatory cytokines such as TNFα, steering to insulin resistance (Xiu et al., 2016). Also, it has been reported that macrophages are able to produce anti-inflammatory lipid metabolites such as DHA and EPA (Oishi et al., 2017). These findings suggest that alteration of lipid metabolism in ATMs would be crucial to induce inflammatory responses and insulin resistance in obesity.

In plasma, essential lipid metabolites such as cholesterol and triglycerides are

circulated in the form of lipoproteins (Brown and Goldstein, 1979). Major triglyceride-carrying lipoproteins are very low-density lipoprotein (VLDL) and chylomicron(Hegele, 2009). VLDL receptor (VLDLR) has a pivotal role to uptake VLDL and chylomicron through receptor-mediated endocytosis or lipoprotein lipase (LPL)-dependent lipolysis(Takahashi et al., 2004; Takahashi et al., 2003). VLDLR, a member of the low-density lipoprotein (LDL) receptor (LDLR) family, is abundantly expressed in adipose tissue, heart, kidneys, and skeletal muscle (Takahashi et al., 2004; Takahashi et al., 2003). Patients with VLDLR mutations exhibit low body mass index (BMI) compared to normal subjects(Boycott et al., 2005; Crawford et al., 2008). Similarly, VLDLR-deficient mice are protected from high fat diet (HFD)-induced obesity (Goudriaan et al., 2001). Furthermore, VLDLR-deficient mice exhibit improved glucose intolerance upon HFD, accompanied with alleviated inflammation and ER stress in adipose tissue (Nguyen et al., 2014). In addition, it has been suggested that VLDL might influence cellular inflammatory responses in macrophages, thereby potentiating metabolic complications, such as atherosclerosis and diabetes (den Hartigh et al., 2014; Saraswathi and Hasty, 2006). However, it remains largely unknown whether VLDLR-mediated VLDL uptake in macrophages is an important factor in mediating adipose tissue inflammation and insulin resistance in obesity.

In this study, I demonstrate that VLDLR is elevated in obese ATMs, and promotes adipose tissue inflammation by upregulating ceramide production and facilitating M1-like

macrophage polarization. Moreover, bone marrow transplantation (BMT) from VLDLR knockout (KO) mice into wild-type (WT) recipient mice attenuates insulin resistance in diet-induced obesity (DIO), simultaneously with reduced adipose tissue inflammation. Altogether, our data suggest that upregulated macrophage VLDLR could provoke insulin resistance by enhancing pro-inflammatory signaling pathways, accompanied with altered lipid profiles under lipid-rich conditions in obesity.

## Materials and Methods

**Animals and treatment.** All animal experiments were approved by the Seoul National University Animal Experiment Ethics Committee (SNU-130611-2). C57BL6/J and VLDLR-deficient mice were obtained from The Jackson Laboratory (Bar Harbor, ME; strain 002529 B6;127S7-Vldlr <tm1Her>J) and housed in colony cages under 12-h light/12-h dark cycles. All mice were genotyped by PCR (Jo et al., 2013) and VLDLR-heterozygous mice were bred to generate WT and VLDLR-deficient littermates. 8-week-old male mice were maintained on a normal chow diet (Zeigler, Gardners, PA) or 60% high fat diet (Research Diet, New Brunswick, NJ). For intraperitoneal glucose tolerance test (GTT), NCD- or HFD-fed mice were fasted for 6 h, blood samples were collected, glucose was administered (2 g/kg body weight), and blood samples were drawn at 15, 30, 60, 90, and 120 min to measure plasma glucose level. For insulin tolerance test (ITT), HFD-fed mice were fasted for 3 h and then administered insulin (0.75 unit/kg body weight; Lilly, Indianapolis, IN). For bone marrow transplant experiments, 8-week-old recipient male mice were lethally irradiated two times (total 5 Gy) by means of a $^{137}$Cs source at an interval of 4 h. After irradiation of the bone marrow cells of the recipient mice, these mice received a transplant of $5 \times 10^6$ bone marrow cells from 8-week-old VLDLR KO male mice or their WT littermates via tail-vein injection (Ham et al., 2016).

**Cell culture.** Mice were intraperitoneally injected with sterile thioglycolate solution (3 ml per mouse) to harvest peritoneal macrophages (Ham et al., 2013). Harvested peritoneal macrophages were cultured in RPMI 1640 medium (HyClone, Logan, UT) with 10% fetal bovine serum (HyClone, Logan, UT), 100 U/ml penicillin, and 100 mg/ml streptomycin. Bone marrow was flushed from the femur and tibia, dispersed, and cultured in DMEM medium containing 10% FBS and 20% L929 conditioned medium for 7 days. To promote M1- or M2-derived macrophages, bone marrow-derived macrophages (BMDMs) were stimulated with LPS (10 ng/ml) and IFN $\gamma$ (100 ng/ml) for M1-driving condition, and IL-4 (20 ng/ml) for M2-driving condition. To test effects of lipoproteins, human VLDL (Kalen Biomedical, #770100, Germantown, MD), and chylomicron (BioVision, #7285-1000, Milipas, CA) were purchased. According to the manufacturer′s information, purchased VLDL contains 1.1 mg/ml protein, and chylomicron is composed of 98 % lipids and 2 % protein.

**Immunohistochemistry.** Whole-mounted epididymal adipose tissues (EATs) were incubated with primary antibodies against VLDLR (1:500 dilutions; Abcam, #92943, Cambridge, MA), ceramide (1:500 dilutions; Sigma-Aldrich, #C8104, St. Louis, MO), perilipin (1:1000; Fitzgerald, #20R-PP004, Stow, MA), CD11b (1:1000), and CD11c (1:1000; eBioscience, #E01079, #4290718, San Jose, CA). After incubation with fluorescently labeled secondary antibodies (Thermo Fisher Scientific, Waltham, MA) and 4',6-diamidino-2-phenylindole

(DAPI; Vector Laboratory, Burlingame, CA) staining, samples were examined under a Zeiss LSM 700-NLO confocal microscope.

**Lipid profiling.** Measurement of triglycerides was performed according to the manufacture`s protocol (INFINITY$^{TM}$, Thermo Scientific, Waltham, MA). For lipid profiling of macrophages, lipid metabolites were separated using an Agilent 1260 liquid chromatography system and a specific column (Thermo Scientific Accucore HILIC 100 × 2.1 mm; particle size 2.6 μm). Mobile phase A consisted of acetonitrile/water (95:5) with 10 mM ammonium acetate, pH 8.0, and mobile phase B consisted of acetonitrile/water (50:50) with 10 mM ammonium acetate, pH 8.0. For separation of mobile phases, a column was equilibrated with 100% mobile phase A, ramped to 20% mobile phase B in 5 min, and held for 5 min. The column was finally equilibrated with 100% mobile phase A for 5 min. Mass spectrometry analysis was performed using an Agilent 6430 triple quadruple mass spectrometer.

**Flow cytometry analysis.** After incubation with blocking antibodies, SVCs were stained with monoclonal antibodies against CD11b, F4/80, and CD11c (eBioscience, #E01079, #E08392-1636, #4290718, San Jose, CA). For sorting CD11c$^+$ (M1-like macrophages) and CD11c$^-$ (M2-like macrophages), FACS Aria II (BD Bioscience, San Jose, CA) was used.

**Western blot analysis.** Macrophages, 3T3-L1 adipocytes, and EATs were lysed with

radioimmunoprecipitation assay buffer. The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The blots were probed with following primary antibodies: anti-VLDLR, anti-lamin B (1:500 dilution; Abcam, #92943, #65986, Cambridge, MA), anti-β-actin (1:2000 dilution; Sigma-Aldrich, #A5441, St.Louis, MO), anti-p-GSK3β (Ser 9), anti-p-JNK (1:1000 dilution; Cell Signaling Technology, #D85E12, #85E11, Denver, MA), anti-GSK3β, anti-p-p38 (1:1000 dilution; Merck Millipore, #07-1413, #MABS64, Germany), anti-JNK, anti-p38 (1:1000 dilution; Santa Cruz Biotechnology, #sc-7345, #sc-81621, Dallas, TX).

**Statistical analysis.** The data are presented from multiple independent experiments, and represent the mean and standard deviation (SD). $P$-values were calculated by Student's $t$-test; $P < 0.05$ was regarded significantly.

**Table 1.** qRT-PCR primer sequences

| *Gene* | *5′ sequence* | *3′ sequence* |
|---|---|---|
| Adiponectin | GGCAGGAAAGGAGAGCCTGG | GCCTTGTCCTTCTTGAAGAG |
| Angptl4 | GGCTGGTGGTTTGGTACCTGT | CCGTTGCCGTGGGATAGA |
| ATGL | CTCCAGCGGCAGAGTATAGG | ACCATCACAGTGTCCCCATT |
| CD36 | GAGCAACTGGTGGATGGTTT | GCAGAATCAAGGGAGAGCAC |
| CPT1α | ACTCCTGGAAGAAGAAGTTC | GTATCTTTGACAGCTGGGAC |
| Cyclophilin | CAGACGCCACTGTCGCTTT | TGTCTTTGGAACTTTGTCTG |
| FAS | CGGTAGCTCTGGGTGTA | TGCTCCCAGCTGCAGGC |
| GLUT4 | GATTCTGCTGCCCTTCTGTC | ATTGGACGCTCTCTCTCCAA |
| IL-10 | GCTCTTACTGACTGGCATGAG | CGCAGCTCTAGGAGCATGTG |
| IL-1β | TGCAGAGTTCCCCAACTGGTACATC | GTGCTGCCTAATGTCCCCTTGAATC |
| iNOS | AATCTTGGAGCGAGTTGTGG | CAGGAAGTAGGTGAGGGCTT |
| LDLR | AGGCTGTGGGCTCCATAGG | TGCGGTCCAGGGTCATCT |
| LPL | GCCCAGCAACATTATCCAGT | GGTCAGACTTCCTGCTACGC |
| PAI1 | TCAGCCCTTGCTTGCCTCAT | GCATAGCCAGCACCGAGGA |
| PGC1α | CCTCCTCATAAAGCCAACCA | GGGCCGTTTAGTCTTCCTTT |
| PPARγ | GAACGTGAAGCCCATCGAGG | GTCCTTGTAGATCTCCTGGA |
| SAA | AGCGATGCCAGAGAGGCTGT | ACCCAGTAGTTGCTCCTCTT |
| TNFα | CGGAGTCCGGGCAGGT | GCTGGGTAGAGAATGGATCA |
| VLDLR | TGACGCAGACTGTTCAGACC | GCCGTGGATACAGCTACCAT |

# Results

**VLDLR is highly expressed in ATMs from obese adipose tissue**

VLDLR is abundantly expressed in adipose tissue (Takahashi et al., 2004; Takahashi et al., 2003). However, it is largely unknown whether VLDLR expression might be altered in obese adipose tissue. To address this, VLDLR expression was examined in EATs from lean and obese mice. Compared to lean EATs, the level of VLDLR mRNA was elevated in obese EATs (Fig. 4a and 4b). mRNA levels of other LDLR family members, including LDLR and apolipoprotein receptor (ApoER) 2, were not significantly changed in EATs of obese mice (Fig. 4a and 4b). As positive controls, the mRNA levels of TNF$\alpha$ and Acrp30 were examined. In accordance with a previous report (Kim et al., 2012), the level of VLDLR mRNA in human adipose tissue showed a positive correlation with body mass index (BMI) (Fig. 5a and 5b). To further characterize the expression patterns of adipose tissue VLDLR, EATs were fractionated into adipocytes and SVCs. Unlike in adipocytes, the level of VLDLR mRNA was elevated in SVCs from HFD-induced obese mice as compared to those from control animals (Fig. 6a). To verify high expression of VLDLR in SVCs of DIO, SVCs were further separated into F4/80 and CD11b double-positive ATMs by using fluorescence-activated cells sorting. Upon HFD, the level of VLDLR mRNA was clearly raised in F4/80$^+$ and CD11b$^+$ ATMs, while those of LDLR and ApoER 2 were not altered (Fig. 6b). Moreover, elevated
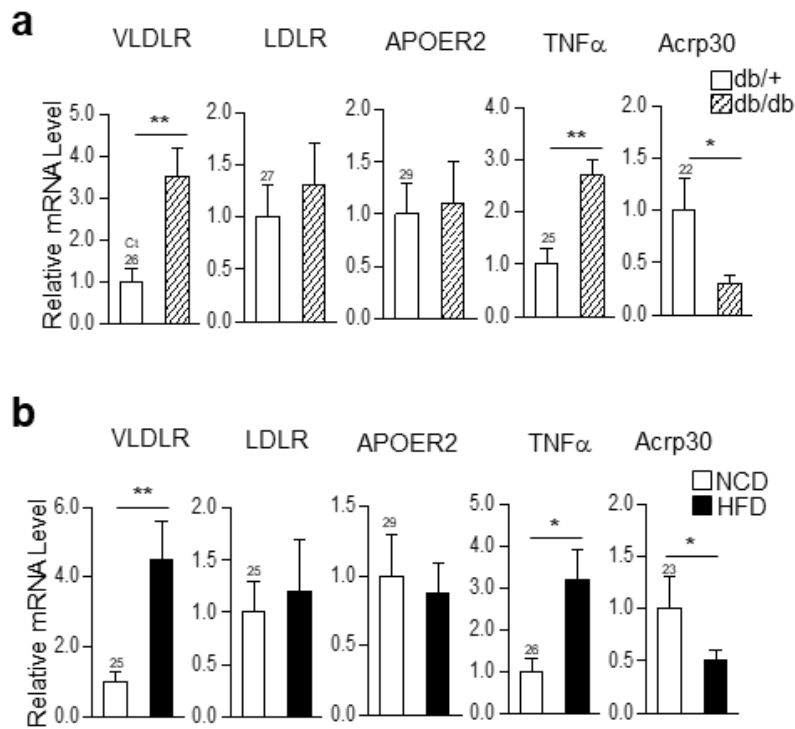
**Figure 4. VLDLR expression is elevated in WAT from obese mice.** (a, b) Relative mRNA level of VLDLR in adipose tissue of (a) lean (db/+) (n = 4) and obese (db/db) (n = 5) mice and (b) normal chow diet (NCD)-fed (n = 6) and high-fat diet (HFD)-fed (n = 7) mice as measured by RT-qPCR. Data represent mean ± SD. $^*$P<0.05, $^*$P<0.01, Student`s t-test.
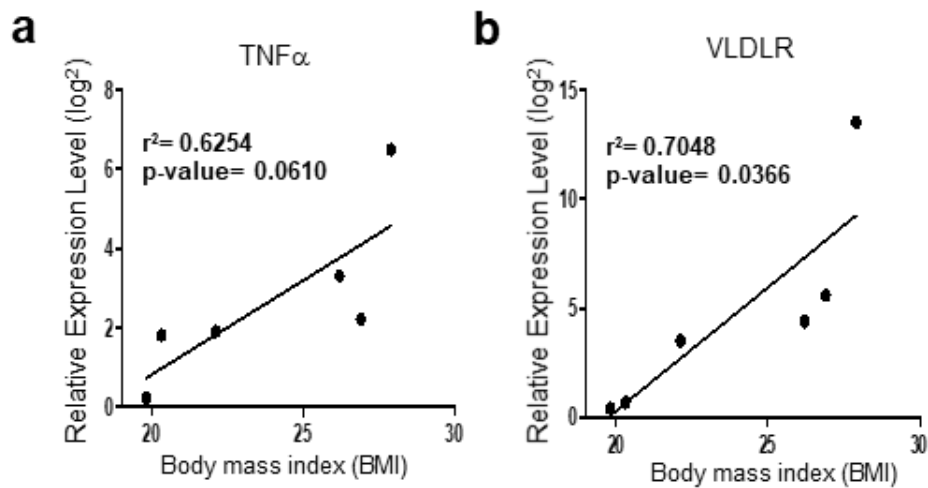
**Figure 5. The level of VLDLR mRNA shows a positive correlation with body mass index in the human fat tissue.** (a, b) Correlation between mRNA levels of both TNF$\alpha$ (a) and VLDLR (b) genes, and body mass index (BMI) in human adipose tissue. $r^2$ and p-values are indicated on the graph.
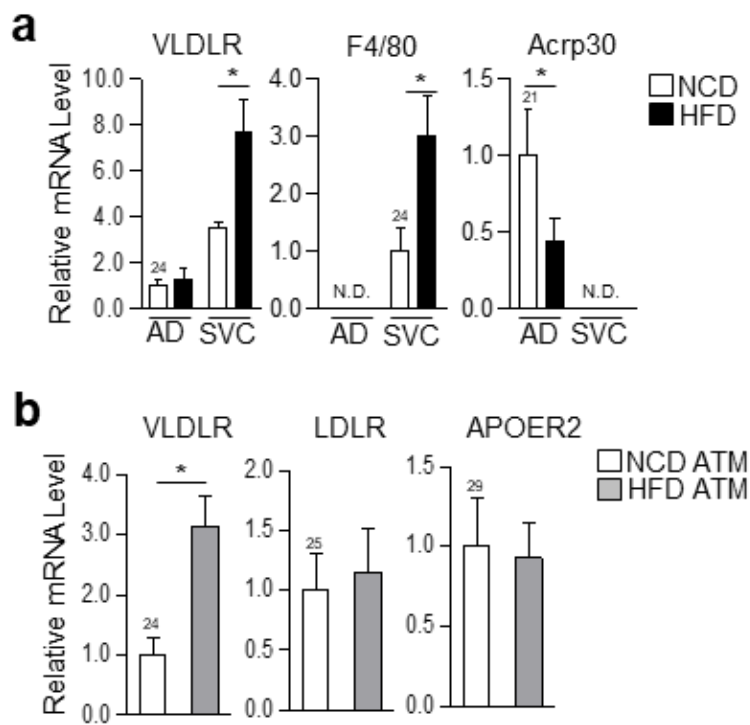
**Figure 6. VLDLR expression is elevated in obese ATMs.** (a) Relative mRNA level of VLDLR in adipocyte (AD) and stromal vascular cell (SVC) fractions from NCD-fed (n = 5) and HFD-fed (n = 5) mice. (b) Relative mRNA level of VLDLR in sorted ATMs (F4/80+, CD11b+) from NCD-fed (n = 12) and HFD-fed (n = 9) mice. Data represent mean ± SD. [*]P<0.05, Student`s t-test.

VLDLR protein was detected in CD11b$^+$ ATMs from obese adipose tissues (Fig. 7). In DIO, the levels of VLDLR mRNA were also elevated in peritoneal and liver macrophages (Fig. 8a and 8b). On the other hand, the level of VLDLR mRNA in liver macrophages was quite low (Fig. 8b). Together, these results indicated that VLDLR is highly expressed in obese adipose tissue, particularly in ATMs.

**VLDLR-overexpressed macrophages potentiate pro-inflammatory responses in the presence of VLDL**

To investigate whether macrophage VLDLR might contribute to storage of intracellular lipid metabolites, VLDLR was overexpressed in peritoneal macrophages (Fig. 9a and 9b). As shown in Fig. 10a and 10b, the level of intracellular triglycerides was increased in VLDLR-overexpressing macrophages in the presence of VLDL, while that of cholesterol was not altered. Given the high expression of VLDLR in obese ATMs, I next tested whether VLDLR in ATMs might be involved in pro-inflammatory responses. In VLDLR-overexpressing macrophages, the presence of VLDL stimulated the expression of pro-inflammatory marker genes including iNOS, TNFα, monocyte chemoattractant protein (MCP)-1, serum amyloid A (SAA), IL-1β, and interferon (IFN) γ (Fig. 11). These results suggested that elevation of macrophage VLDLR expression would stimulate pro-

29
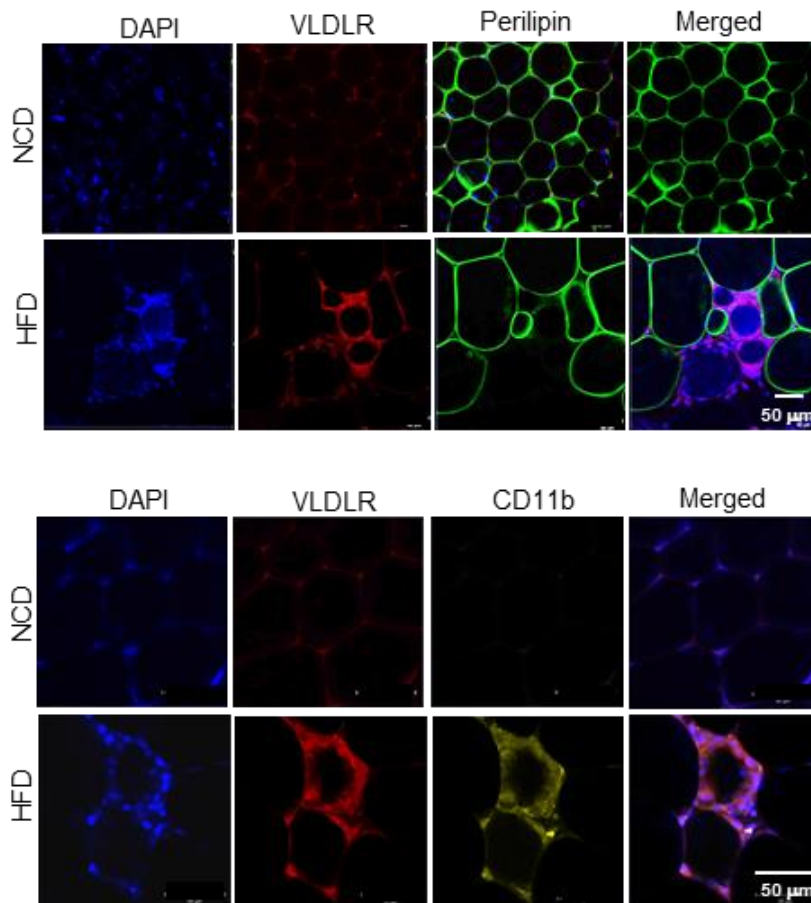
**Figure 7. The protein level of VLDLR is increased in obese ATMs.** VLDLR protein was monitored in ATMs from NCD-fed and HFD-fed mice. Whole-mount immunohistochemistry analysis of the nucleus (blue), VLDLR (red), perillipin (green), and CD11b (yellow) in EATs from NCD-fed and HFD-fed mice. Data represent mean ± SD. *P<0.05, *P<0.01, Student`s t-test.
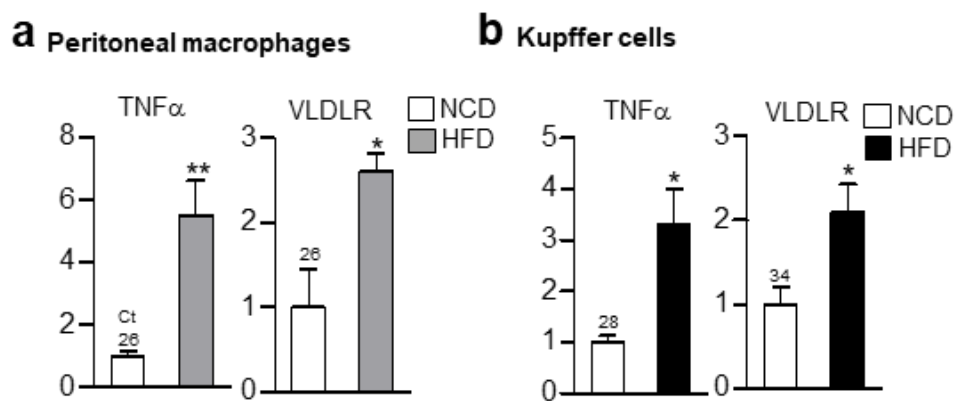
**Figure 8. The expression level of VLDLR is increased in obese macrophages.**
Peritoneal macrophages (a) and Kupffer cells (b) were isolated from C57BL6/J wild type
mice. Relative mRNA level of VLDLR in macrophages of normal chow diet (NCD)-fed (n
= 3) and high-fat diet (HFD)-fed (n = 3) mice as measured by RT-qPCR. Data represent
mean ± SD. $^{*}$P<0.05, $^{**}$P<0.01, Student`s t-test.
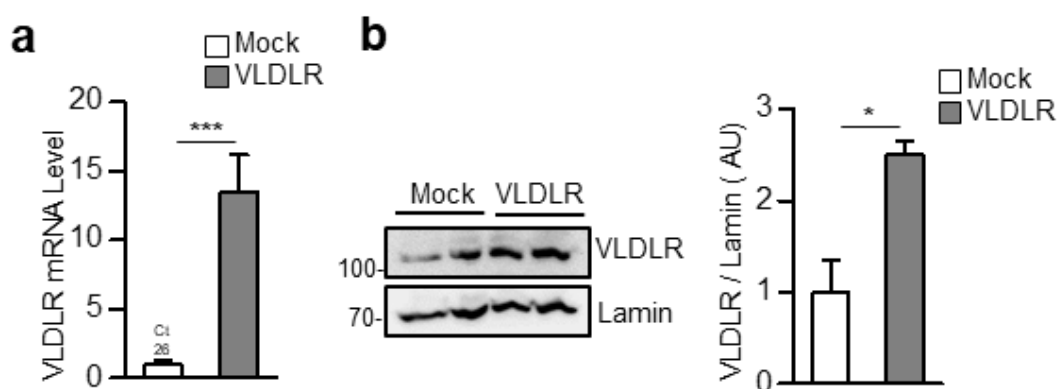
**Figure 9. VLDLR is overexpressed in macrophages.** Peritoneal macrophages were transfected with pcDNA3.1-mock (Mock) vector or pcDNA3.1-VLDLR (VLDLR) expression vector. Total RNA was isolated to analyze VLDLR mRNA expression (a) and whole cell lysates were extracted for western blot analysis (b). Data represent mean ± SD. $^{*}$P<0.05, $^{*}$P<0.001, Student`s t-test.
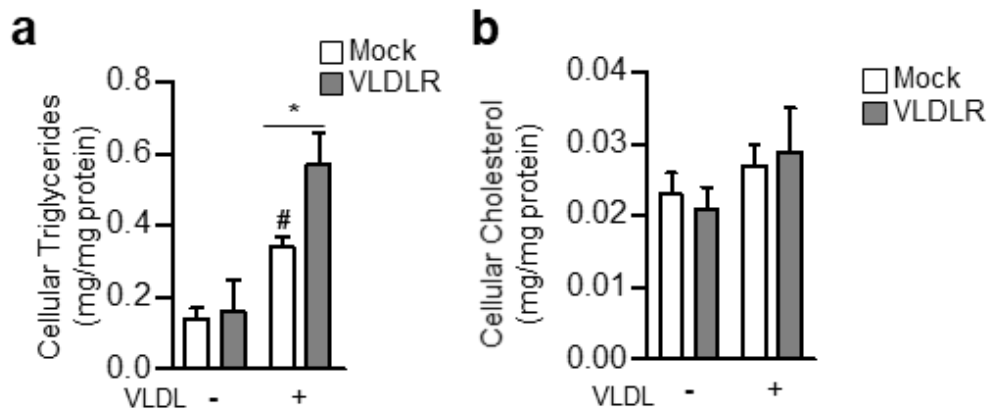
**Figure 10. Macrophages overexpressing VLDLR accumulate triglycerides.** Human VLDL (30 µg/ml) was treated to cultures of peritoneal macrophages that had been transfected or not with VLDLR overexpression vector. Intracellular triglyceride (a) and cholesterol (b) levels in peritoneal macrophages. Data represent mean ± SD. [#]P<0.05 vs VLDL, [*]P<0.05, Student`s t-test.
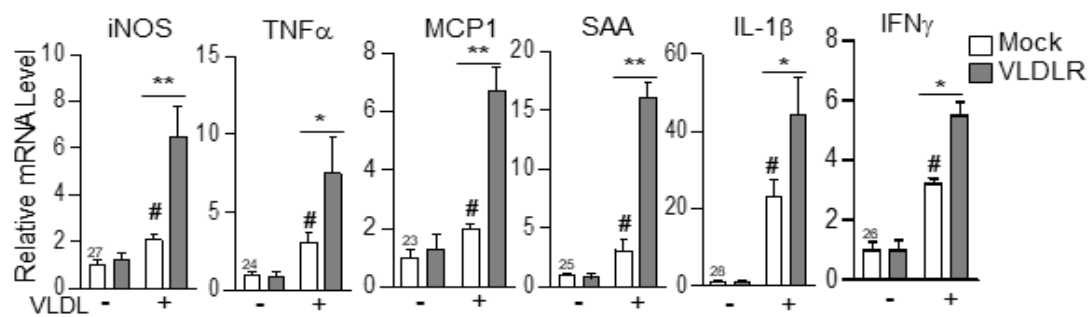
**Figure 11. Macrophages overexpressing VLDLR induce the expression levels of pro-inflammatory genes in the presence of VLDL**. mRNA levels of pro-inflammatory genes were analyzed with (+) or without (-) human VLDL challenges in peritoneal macrophages. Each mRNA level was normalized to cyclophilin mRNA. Data represent mean ± SD. [#]P<0.05 vs VLDL, *P<0.05, **P<0.01, Student`s t-test.

inflammatory responses in the presence of VLDL, simultaneously with intracellular

triglycerides accumulation.

**Macrophage VLDLR deficiency attenuates VLDL-induced pro-inflammatory responses**

As VLDLR-overexpressing macrophages had elevated intracellular triglycerides in

the presence of VLDL, I investigated whether macrophages could uptake VLDL via VLDLR.

To address this, peritoneal macrophages isolated from WT or VLDLR KO mice were

challenged with fluorescence-conjugated VLDL (VLDL-DiI). As illustrated in Fig. 12a and

12b, VLDLR KO macrophages hardly took up VLDL compared to WT macrophages. While

WT macrophages accumulated intracellular triglycerides with VLDL in a time-dependent

manner, VLDLR KO macrophages marginally increased intracellular triglycerides (Fig. 12c).

Intracellular cholesterol did not differ between WT and VLDLR KO macrophages with or

without VLDL (Fig. 12d). It has been reported that VLDL would be uptaken by receptor-

mediated endocytosis or lipoprotein lipase (LPL)-dependent lipolysis (Takahashi et al., 2004;

Takahashi et al., 2003). To test whether LPL might be involved in VLDL uptaking, I

investigated LPL expression and its enzymatic activity in WT and VLDLR KO macrophages.

As shown in Fig. 13, the levels of LPL mRNA and its enzymatic activity in macrophages

were not associated with VLDLR expression. Moreover, suppression of LPL via siRNA did
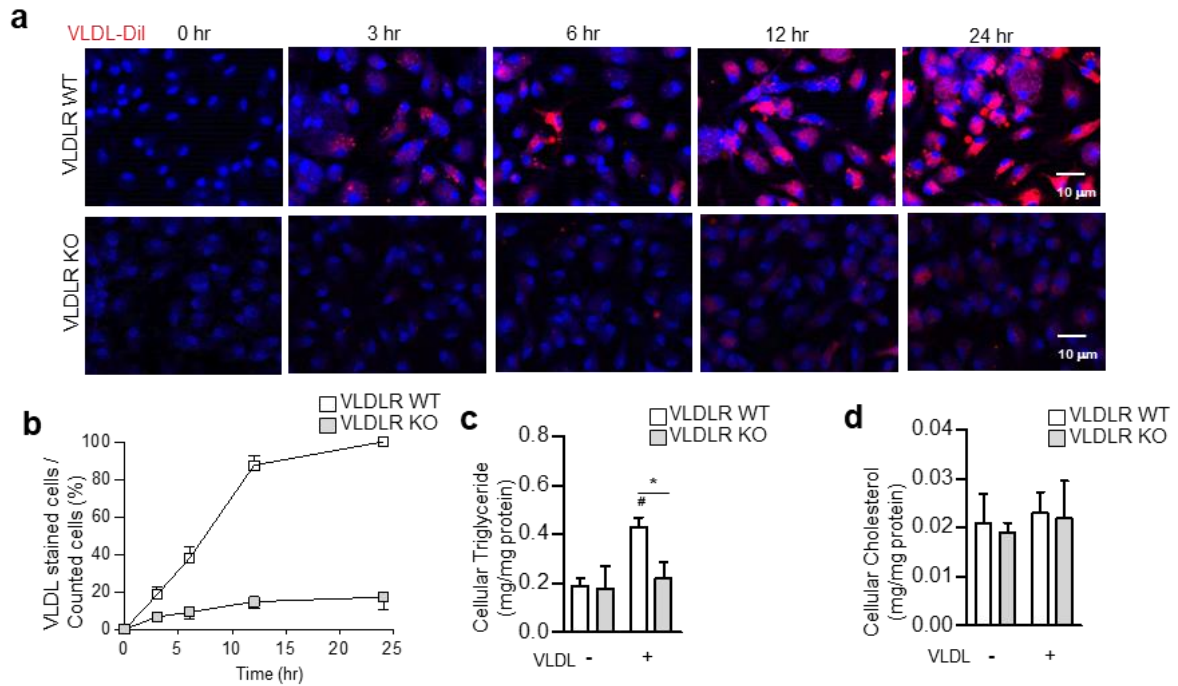
**Figure 12. Macrophages of VLDLR deficiency reduce intracellular triglyceride accumulation in the presence of VLDL.** (a) Peritoneal macrophages from WT or VLDLR KO mice were treated with VLDL-Dil (10 µg/ml) for the indicated periods. VLDL-Dil (red) was detected by fluorescence microscopy. (b) Quantification of accumulated VLDL-Dil fluorescence in WT and VLDLR KO macrophages during different incubation periods. (c, d) Intracellular triglyceride (c) and cholesterol (d) in WT or VLDLR KO macrophages with (+) or without (-) human VLDL (30 µg/ml) challenges. Data represent mean ± SD. [#]P<0.05 vs VLDL, *P<0.05, Student`s t-test.
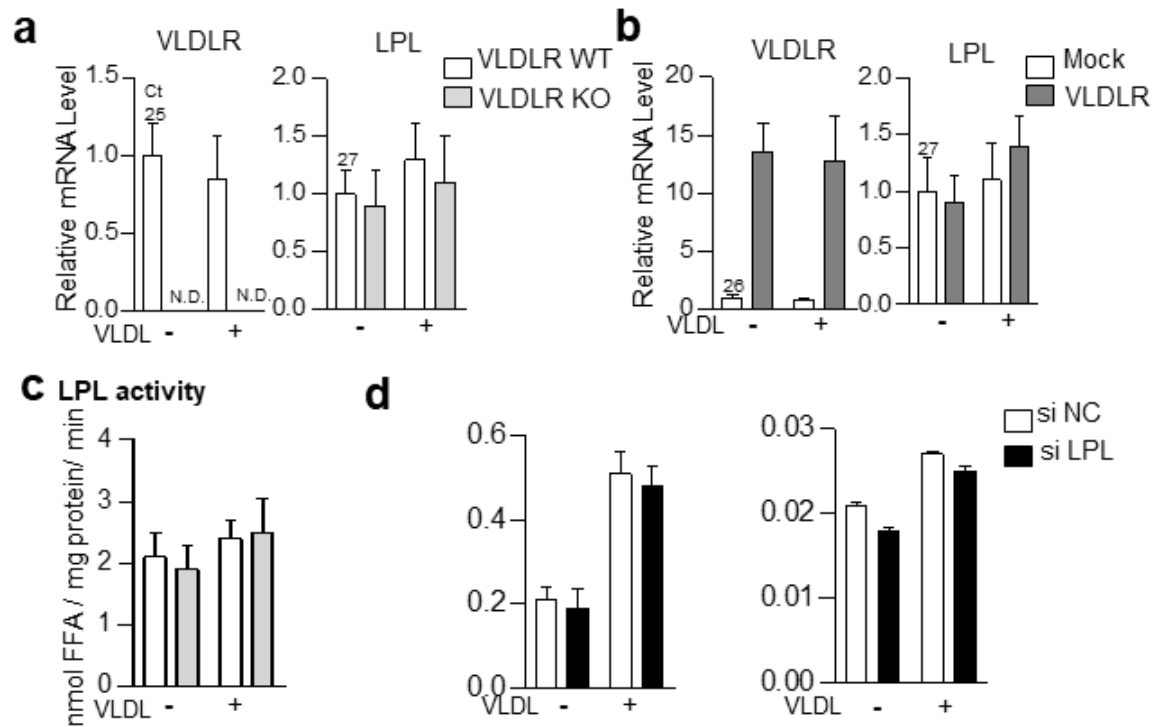
**Figure 13. Macrophage LPL is not crucial to modulate intracellular triglycerides contents with or without VLDL.** (a) Relative mRNA levels of VLDLR and LPL in WT and VLDLR KO BMDMs. (b) Relative mRNA levels of VLDLR and LPL in peritoneal macrophages overexpressing VLDLR. Each mRNA level was normalized to cyclophilin mRNA. (c) LPL enzymatic activity in WT and VLDLR KO BMDMs. (d) Intracellular triglycerides and cholesterol levels in peritoneal macrophages suppressing of LPL expression with siRNA. (a-d) All the experiments were performed with or without human VLDL (30 μg/ml).

not significantly alter cellular triglycerides and cholesterol contents in the absence or presence of VLDL. To validate the potential roles of macrophage VLDLR, peritoneal macrophages from WT or VLDLR KO mice were treated with or without VLDL and subjected to gene expression profiling. In the presence of VLDL, VLDLR KO macrophages did not have augmented expression of various pro-inflammatory marker genes such as iNOS, TNF$\alpha$, IL-6, IL-1$\beta$, MCP-1, and SAA, while WT macrophages did show stimulated expression of these pro-inflammatory genes (Fig. 14). These results imply that macrophages would uptake VLDL via VLDLR and potentiate inflammatory responses, concomitantly with intracellular triglycerides accumulation. It has been well established that cytokines produced from macrophages could impair insulin action in adipocytes (Berg et al., 2002; Yamauchi et al., 2001). Thus, I speculated that deficiency of macrophage VLDLR might affect insulin-induced glucose uptake and insulin signaling in adipocytes. To address this, conditioned media (CM) were collected from VLDL-treated peritoneal macrophages isolated from WT or VLDLR KO mice and were treated to differentiated 3T3-L1 adipocytes. Compared with CM from VLDL-treated WT macrophages, CM from VLDL-treated VLDLR KO macrophages slightly but substantially enhanced insulin-stimulated glucose uptake ability and increased the level of glucose transporter 4 (GLUT4) mRNA (Fig. 15a and 15b). Furthermore, the phosphorylation levels of AKT and GSK3$\beta$ were elevated in adipocytes treated with CM from VLDLR KO macrophages upon insulin (Fig. 15c). Together, these results indicated that
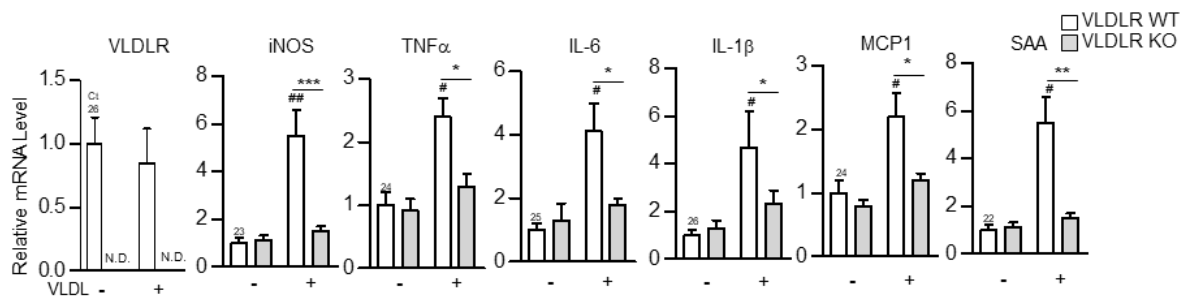
**Figure 14. Pro-inflammatory genes are less increased in VLDLR deficient macrophages upon VLDL.** Relative mRNA levels of VLDLR and pro-inflammatory genes in WT or VLDLR KO macrophages with (+) or without (-) human VLDL (30 mg/ml) challenge. Each mRNA level was normalized to cyclophilin mRNA. Data represent mean ± SD. [#]P<0.05 vs VLDL, [#]P<0.01 vs VLDL, *P<0.05, *P<0.05, **P<0.01, ***P<0.01, Student`s t-test.
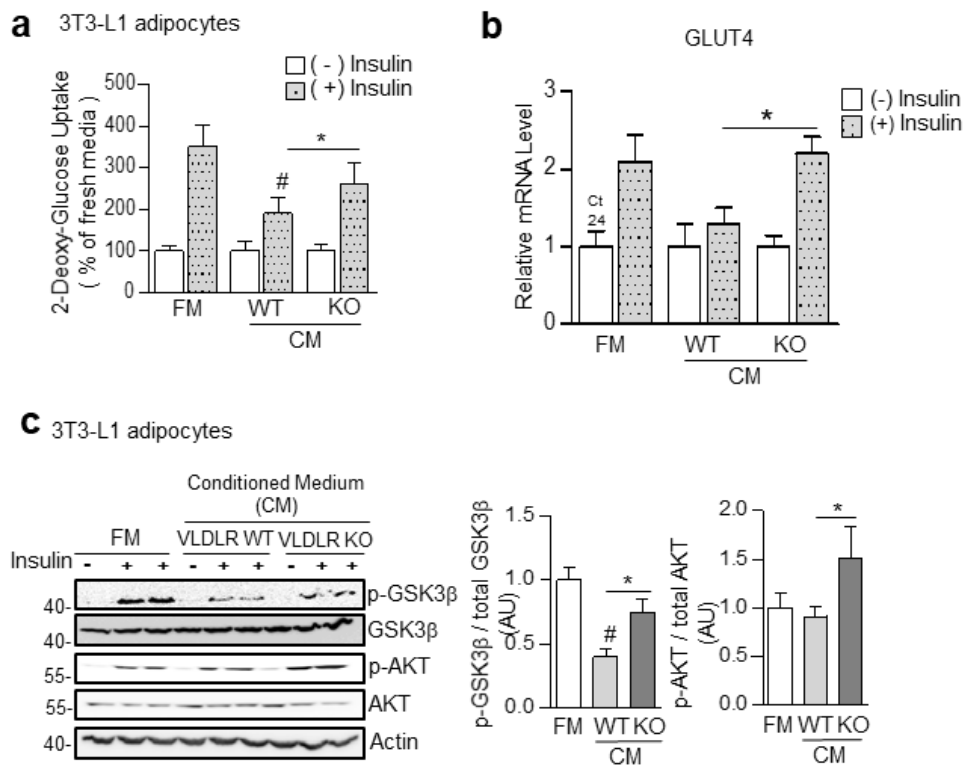
**Figure 15. Insulin sensitivity is enhanced by condition media from VLDLR deficient macrophage treatment.** Insulin-dependent glucose uptake ability (a) and mRNA level of GLUT4 (b), and insulin signaling cascades (c) were examined in 3T3-L1 adipocytes after conditioned media treatment from WT or VLDLR KO macrophages in the presence of human VLDL (30 $\mu$g/ml). FM and CM stand for fresh media and conditioned media, respectively. Data represent mean ± SD. [#]P<0.05 vs FM, *P<0.05 WT vs KO under CM treatment, Student`s t-test.

macrophage VLDLR could mediate pro-inflammatory responses in the presence of VLDL, which would aggravate insulin action in adipocytes.

**VLDLR accelerates M1-like macrophage polarization upon VLDL**

The findings that macrophage VLDLR expression was upregulated in obese adipose tissue and stimulated pro-inflammatory gene expression upon VLDL promoted us to test whether VLDLR might be abundantly expressed in either M1- or M2-like ATMs. ATMs were fractionated into M1-like ATMs (F4/80$^+$, CD11b$^+$ and CD11c$^+$) and M2-like ATMs (F4/80$^+$, CD11b$^+$ and CD11c$^-$). Compared with M2-like ATMs, M1-like ATMs more abundantly expressed VLDLR mRNA as well as TNF$\alpha$ and CD11c mRNAs (Fig. 16a). To gain further insights in the role of macrophage VLDLR, LPS and IFN$\gamma$ or IL-4, were added to cultured BMDMs to induce M1- or M2-like macrophage polarization, respectively. In BMDMs, VLDLR mRNA and protein were upregulated under M1-like macrophage-driving condition rather than under M2-like macrophage-driving condition (Fig. 16b and 16c). As positive controls, TNF$\alpha$ and arginase 1 (ARG1) expression were measured for M1-driving and M2-driving conditions, respectively. As VLDL was uptaken through macrophage VLDLR, VLDL-DiI was incubated in BMDMs during M1- or M2-like macrophage polarization. As shown in Fig. 16d, M1-derived BMDMs accumulated more VLDL-DiI than did M2-derived
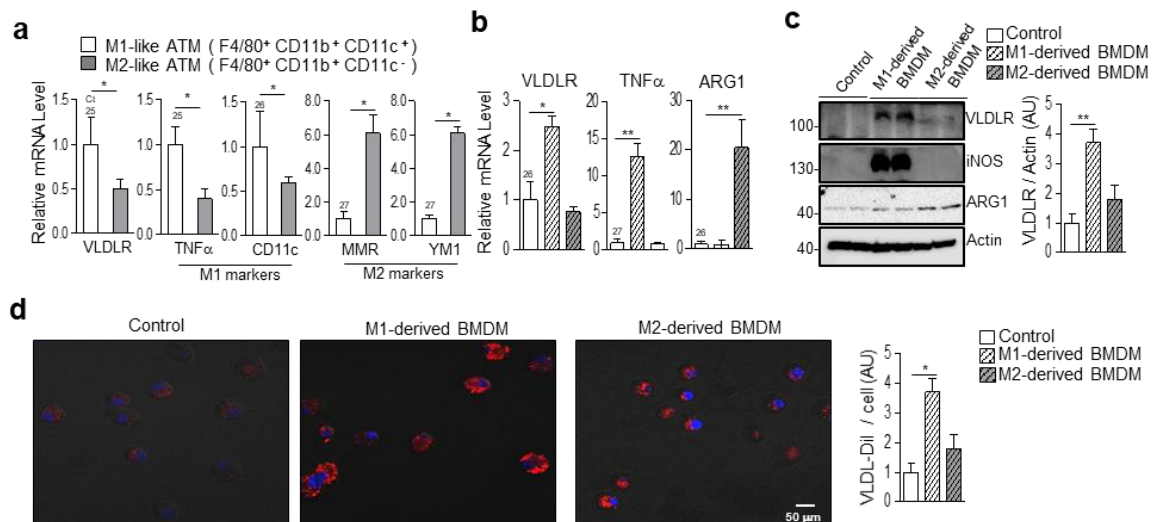
**Figure 16. M1-like macrophage polarization accelerates VLDL uptake in a VLDLR dependent manner.** (a) Relative mRNA level of VLDLR in sorted M1-like macrophages (F4/80+, CD11b+, CD11c+) and M2-like macrophages (F4/80+, CD11b+, CD11c–) from EATs (n = 9). Relative levels of VLDLR mRNA (b) and protein (c) in BMDMs cultured under M1-driving (LPS and IFN-g) or M2-driving (IL-4) condition for 24 h. (d) Intracellular accumulation of VLDL-Dil (red) in BMDMs after M1-driving or M2-driving condition. Data represent mean ± SD. *P<0.05, **P<0.01, Student`s t-test.

BMDMs, implying that M1-like macrophages would uptake and store more VLDL due to elevated VLDLR expression. Next, to validate whether VLDLR might contribute to promote M1-like macrophage polarization in the presence of VLDL, BMDMs from WT or VLDLR KO mice were induced to M1- or M2-like macrophage phenotype with or without VLDL incubation. In the presence of VLDL, M1-derived BMDMs from WT mice further increased the expression of M1 marker genes such as iNOS, TNFα, and IL-1β (Fig. 17a). On the contrary, in M2-derived BMDMs from either WT or VLDLR KO mice, the mRNA levels of M1 and M2 marker genes were not altered with or without VLDL (Fig.17b - 17d). These data suggested that macrophage VLDLR could potentiate M1-like macrophage polarization by uptaking VLDL.

**The level of C16:0 ceramides is elevated in VLDL-treated macrophages through VLDLR**

It has been reported that lipid metabolites of VLDL could be converted into various ceramides, and increased ceramides promote pro-inflammatory responses in macrophages (Aflaki et al., 2012; Chaurasia et al., 2016; Iqbal et al., 2015). To investigate whether the VLDL-VLDLR axis might affect ceramide metabolism in macrophages, the levels of intracellular ceramides were determined in WT or VLDLR KO macrophages with or without
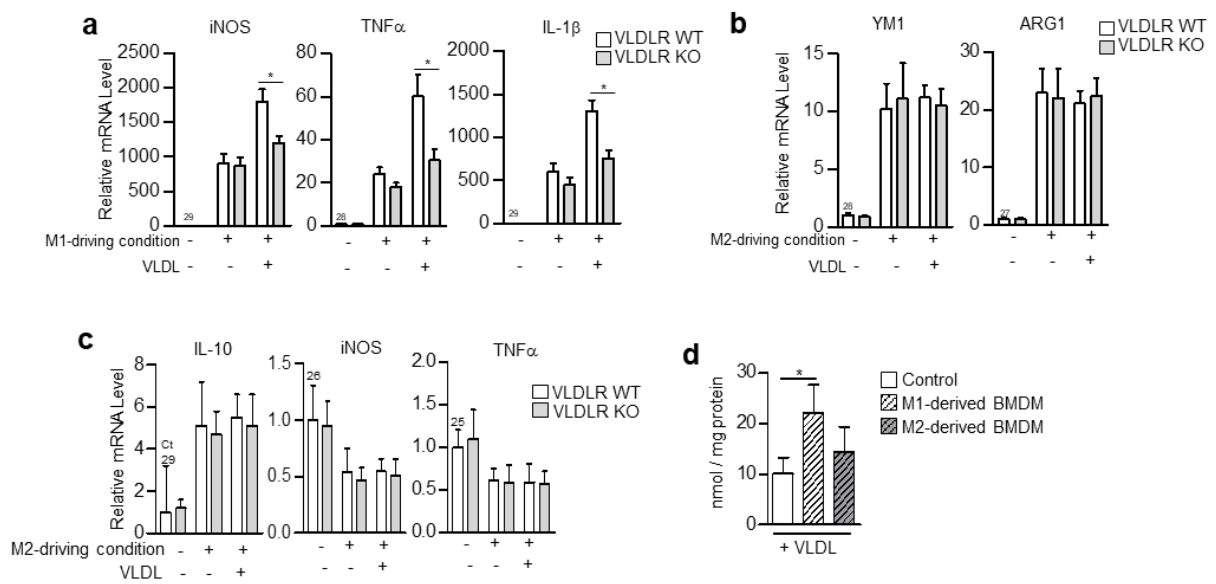
**Figure 17. VLDLR accelerates M1-like macrophage polarization with VLDL**. Relative mRNA levels of pro-inflammatory (a) or anti-inflammatory (b) genes in M1-derived or M2-derived BMDMs from WT and VLDLR KO mice with or without human VLDL (30 mg/ml). Each mRNA level was normalized to cyclophilin (c) mRNA. Relative mRNA levels of inflammatory genes in M2-derived BMDMs from WT and VLDLR KO mice with or without human VLDL (30 mg/ml). Each mRNA level was normalized to cyclophilin mRNA. (d) The levels of intracellular ceramides in M1- or M2-derived BMDMs with human VLDL (30 mg/ml). Data represent mean ± SD. *P < 0.05 ± VLDL, Student's t-test.

44

VLDL challenges. As shown in Fig. 18a, VLDLR KO macrophages accumulated less ceramides than did WT macrophages with VLDL incubation, which was positively correlated with the level of intracellular VLDL. Although it has been shown that elevated circulating ceramides would confer systemic insulin resistance (Holland et al., 2013; Holland et al., 2011), the levels of secreted ceramides were not different in CM from WT and VLDLR KO BMDMs (Fig. 18b). Next, to identify specific ceramide species in VLDL-incubated macrophages, lipidomic profiling was conducted using WT or VLDLR KO macrophages. In WT macrophages, the level of C16:0 ceramides was elevated by VLDL, whereas VLDLR KO macrophages did not increase C16:0 ceramides in the presence of VLDL (Fig. 19a). To further elucidate how VLDL-dependent C16:0 ceramides might be accumulated in WT macrophages, I measured the levels of C16:0 dihydroxy ceramides for the *de novo* pathway and sphingosine for the salvage pathway. In VLDL-incubated WT macrophages, elevated level of C16:0 dihydroxy ceramides was decreased by myriocin, which is a potent inhibitor of *de novo* synthesis of ceramides (Fig. 19b). However, the level of sphingosine was not altered by myriocin in WT or VLDLR KO macrophages (Fig. 19c). These data implied that macrophage VLDLR would increase intracellular C16:0 ceramide species in the presence of VLDL, at least probably, via *de novo* synthesis.
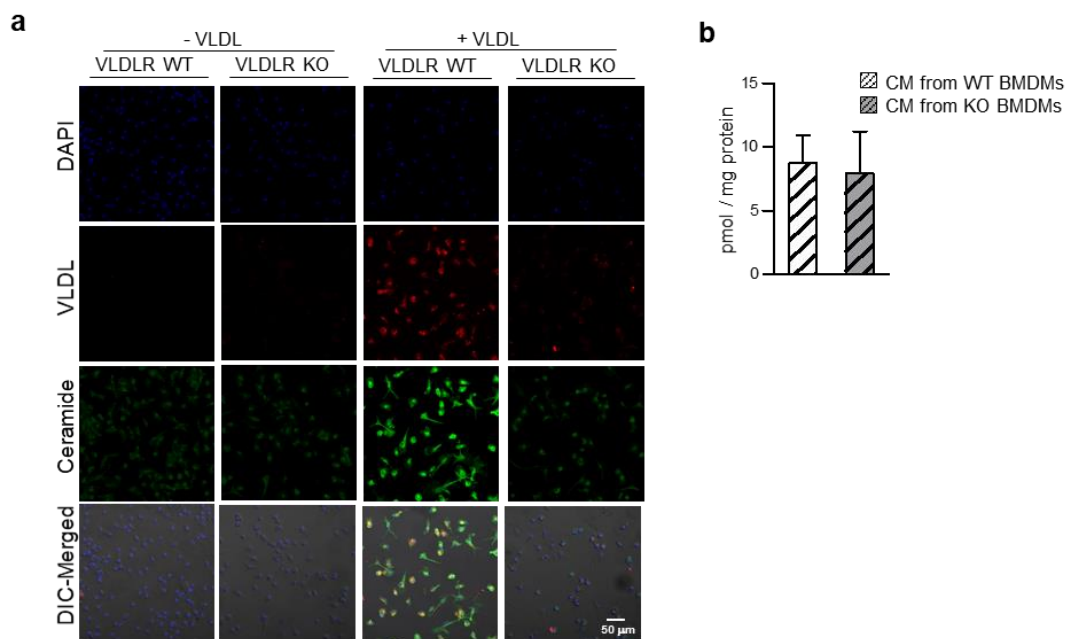
**Figure 18. The level of ceramides is elevated through VLDL-VLDLR axis in macrophages.** (a) Microscope imaging analysis of the nucleus (blue), VLDL-Dil (red), ceramides (green) and DIC-merged of WT or VLDLR KO BMDMs with or without VLDL (30 mg/ml). (b)The levels of secreted ceramides in CM from WT and VLDLR KO BMDMs.
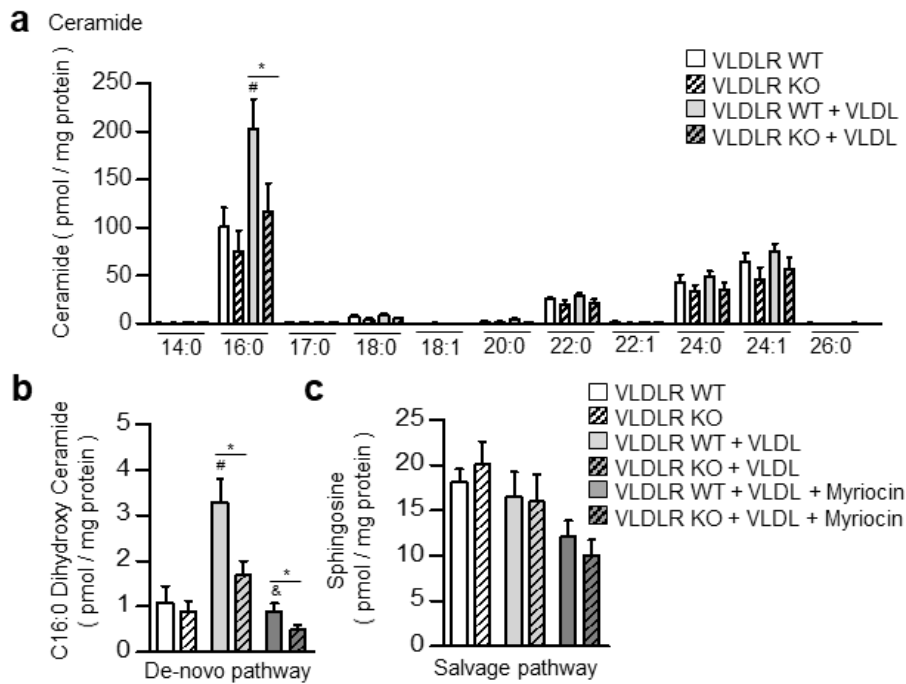
**Figure 19. The level of C16:0 ceramides is elevated through VLDL-VLDLR axis in macrophages.** (a) Lipid profile analysis was performed in BMDMs from WT or VLDLR KO mice with or without VLDL incubation. (b, c) The levels of C16:0 dihydroxy ceramides and sphingosines were measured in WT or VLDLR KO BMDMs with or without VLDL (30 μg/ml) or myriocin (10 μM). Data represent mean ± SD. [#]P<0.05 vs VLDL, *P<0.05 VLDLR WT vs VLDLR KO, Student`s t-test.

47

**Increased ceramides promote pro-inflammatory responses via VLDL-VLDLR axis in macrophages**

It has been demonstrated that accumulated VLDL stimulates pro-inflammatory gene expression in macrophages (den Hartigh et al., 2014; Saraswathi and Hasty, 2006). However, underlying mechanisms by which VLDL could induce pro-inflammatory responses are not fully understood in macrophages. To test whether VLDL-VLDLR axis-dependent ceramide accumulation might mediate pro-inflammatory responses, pro-inflammatory gene expression was examined in WT or VLDLR KO macrophages under various conditions. In the presence of VLDL, the levels of iNOS, TNFα, and IL-1β mRNA were less increased in VLDLR KO macrophages than WT macrophages (Fig. 20a). Moreover, myriocin downregulated the expression of pro-inflammatory genes in either WT or VLDLR KO macrophages treated with VLDL (Fig. 20a). Given that VLDLR would be involved in the clearance of triglycerides derived from VLDL and chylomicrons (Goudriaan et al., 2004), I investigated whether chylomicron might affect inflammatory responses and cellular ceramides in macrophages. On the other hand, it has been well established that mitogen activated protein kinase (MAPK) signaling cascades play a crucial role in the regulation of pro-inflammatory gene expression upon various cellular stresses (Hirosumi et al., 2002; Shoelson et al., 2006). Thus, to understand the molecular mechanisms by which macrophage VLDLR could upregulate pro-inflammatory gene expression with ceramides, several MAPK signaling pathways were

48

tested. As shown in Fig. 20b, the phosphorylation levels of c-Jun N-terminal kinase (JNK) and p38 MAPK were potentiated in VLDL-treated macrophages. However, myriocin attenuated their phosphorylation, even in the presence of VLDL (Fig. 20b). Given that nuclear factor (NF)-κB is one of the key transcription factors governing the expression of most pro-inflammatory genes, I tested its involvement in the regulation of pro-inflammatory genes in VLDL-treated macrophages. As indicated in Fig. 20c, BAY 11-7082, an inhibitor of IKKα, decreased the levels of VLDL-induced iNOS, TNFα, and IL-1β mRNA in VLDL-treated macrophages. These data suggested that the VLDL-VLDLR axis in macrophages would stimulate, at least partly, the MAPK and NF-κB pathways, leading to the promotion of pro-inflammatory responses upon increased cellular ceramides.

**Deficiency of hematopoietic VLDLR improves glucose intolerance and insulin intolerance in DIO**

Given that macrophages from VLDLR KO mice attenuated pro-inflammatory responses, even in the presence of VLDL, I asked whether deficiency of macrophage VLDLR might relieve adipose tissue inflammation in DIO. To address this, bone marrow transplant (BMT) experiments were performed via adoptive transfer of bone marrow cells from VLDLR KO mice or their WT littermates into lethally irradiated C57BL/6 WT mice. Then, both
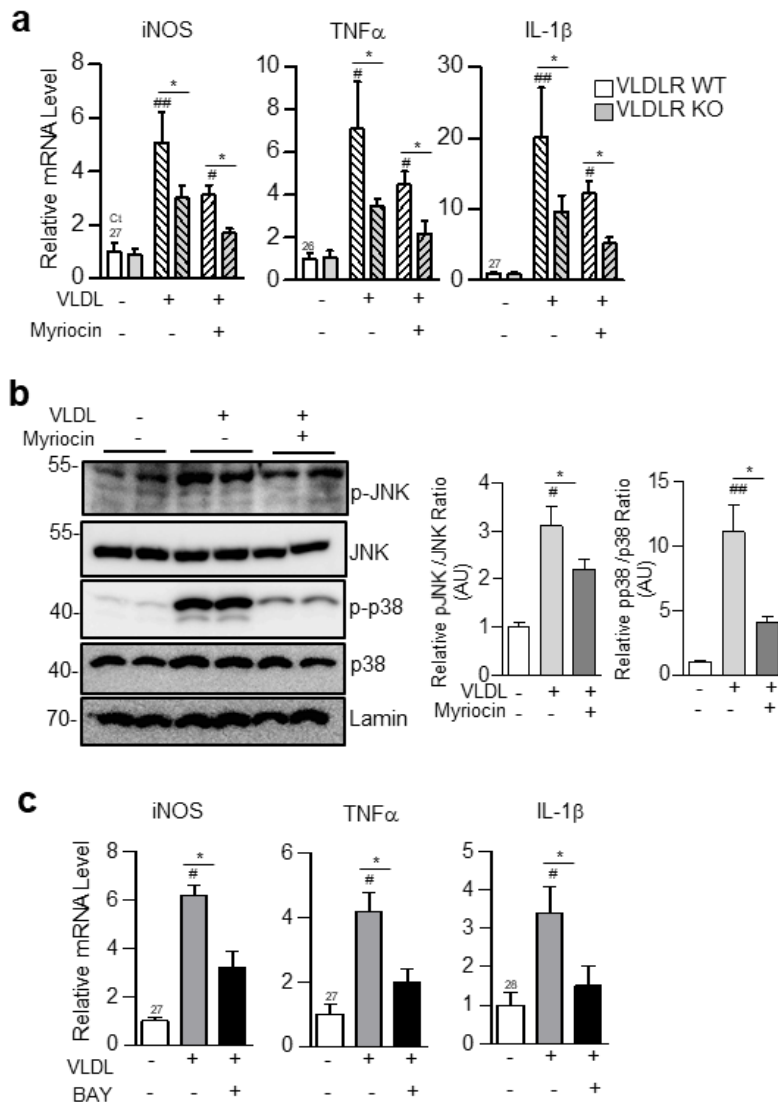
**Figure 20. MAPK pathway is involved in VLDL-induced inflammatory responses through ceramides.** (a) Relative mRNA levels of pro-inflammatory genes in BMDMs from WT and VLDLR KO mice with human VLDL (30 μg/ml) or myriocin (10 μM) treatment. (b) Western blots of MAPK (JNK and p38) phosphorylation in BMDMs treated with or without human VLDL (30 μg/ml) or myriocin (10 μM). (c) Relative mRNA level of pro-inflammatory genes in BMDMs cultured with or without human VLDL (30 μg/ml) or BAY 11-7082 (10 μM). Each mRNA level was normalized to cyclophilin mRNA. Data represent mean ± SD. [#]P<0.05 vs VLDL, *P<0.01 vs Myriocin or BAY, Student`s t-test.

groups were fed a HFD, and I compared the WT mice that had received bone marrow cells from VLDLR KO mice (KO BMT) with those that had received WT bone marrow (WT BMT). Genotyping analysis confirmed that bone marrow cells in KO BMT mice were successfully replaced with VLDLR KO bone marrow cells (Fig. 21a). Immunohistological analysis revealed that VLDLR was highly expressed in CD11b$^+$ ATMs of WT BMT mice, whereas it was hardly detected in KO BMT mice (Fig. 21b). Upon HFD, there were no significant differences in body weights and body weight gains between WT BMT and KO BMT mice (Fig. 22a and 22b). In addition, there were no changes in organ weight in brown, epididymal, subcutaneous, and perirenal adipose tissues, and other metabolic organs (Fig. 22c). Nonetheless, interestingly, fasting glucose and insulin were decreased in KO BMT mice as compared to WT BMT mice after HFD (Fig. 23a and 23b). However, the serum levels of triglycerides and cholesterols were not different between both groups (Fig. 23c and 23d). Moreover, HFD-fed KO BMT mice were more glucose-tolerant and insulin-sensitive than HFD-fed WT BMT mice (Fig. 23e and 23f). These data implied that the reconstitution of hematopoietic cells in WT mice with VLDLR KO bone marrow cells would ameliorate insulin resistance in DIO.

**Deficiency of macrophage VLDLR alleviates adipose tissue inflammation in DIO**
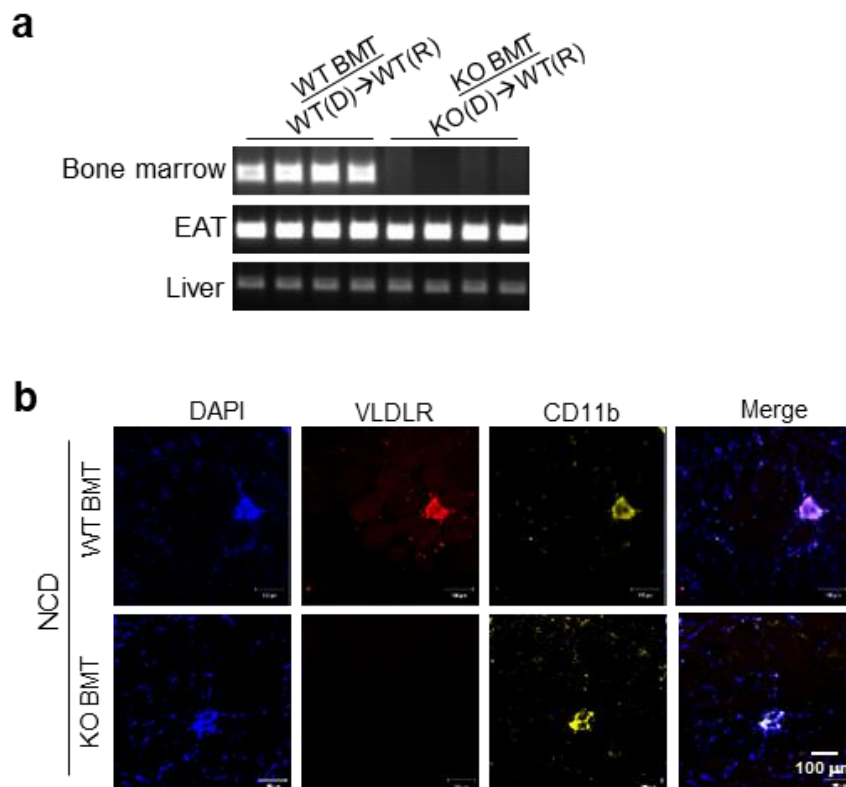
**Figure 21. Bone marrow transplantation is performed using WT and VLDLR deficient mice.** WT BMT [WT(D)→WT(R)] and KO BMT [KO(D)→WT(R)] mice (n = 6 per group) were fed NCD or HFD for 15 wk. (a) WT and KO BMT mice were genotyped. Genomic DNAs were isolated from bone marrow, EATs, and livers from WT and KO BMT mice. (b) Expression patterns of VLDLR protein in ATMs of WT and KO BMT mice. Whole-mount immunohistochemistry of the nucleus (blue), VLDLR (red), and CD11b (yellow) in NCD-fed EAT.
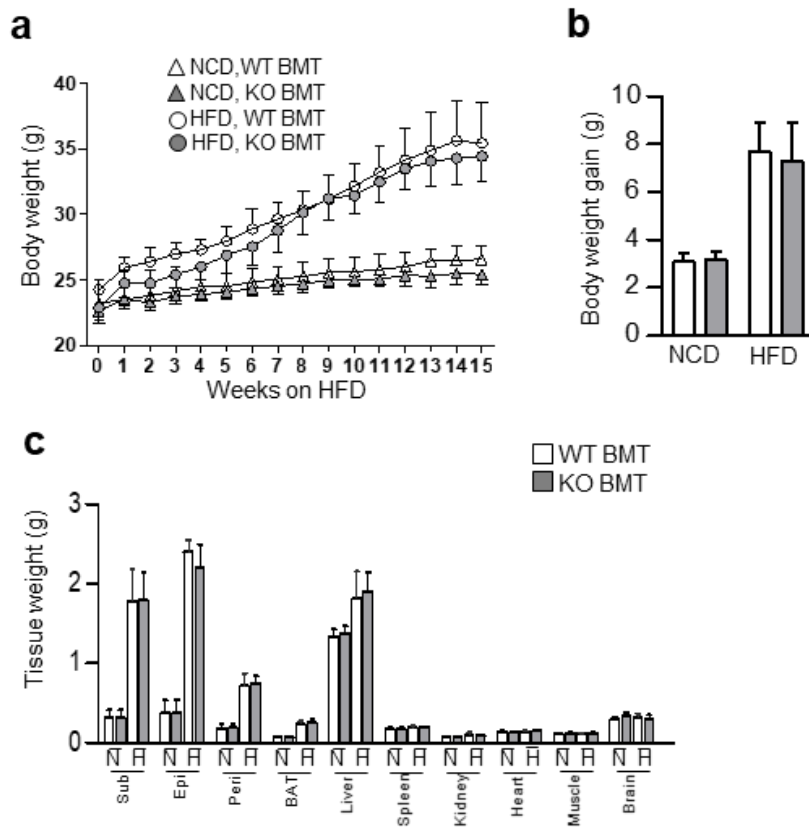
**Figure 22. Adoptive transfer of VLDLR-deficient bone marrows is not altered in body weight and metabolic organs weight upon HFD.** (a) Body weights throughout the experimental period. (b) Total body weight gain and (c) various tissue weights in WT and KO BMT mice after NCD (N)-fed or HFD (H)-fed 15 weeks.
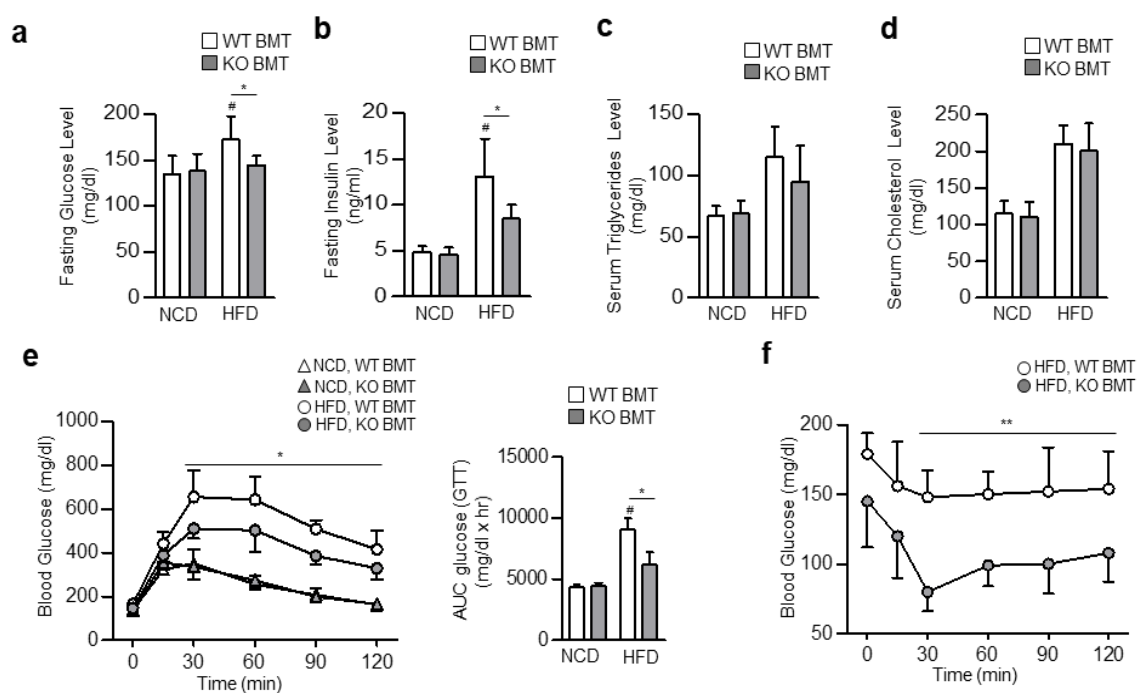
**Figure 23. HFD-fed KO BMT mice ameliorate insulin resistance.** Fasting serum glucose (a), insulin (b), triglyceride (c), and cholesterol (d) in WT and KO BMT. (e) Glucose tolerance test (GTT), area under the curve (AUC), and (f) insulin tolerance test (ITT) analysis of WT and KO BMT mice. Data represent mean ± SD. *P<0.05, **P<0.01, Student`s t-test.

To investigate whether improved glucose intolerance and insulin resistance in HFD-fed KO BMT mice might be associated with decreased adipose tissue inflammation, macrophage infiltration in adipose tissues of HFD-fed WT BMT and KO BMT mice was determined. Compared to HFD-fed WT BMT mice, the number of crown-like structures (CLSs) was decreased in HFD-fed KO BMT mice (Fig. 24a). Consistent with the decreased number of CLSs, HFD-fed KO BMT showed less accumulation of CD11c$^+$ pro-inflammatory macrophages than HFD-fed WT BMT mice (Fig. 24b). Furthermore, mRNA levels of macrophage marker genes such as F4/80, CD11b, and CD11c were lower in EATs of HFD-fed KO BMT mice as compared to those of HFD-fed WT BMT mice (Fig. 24c). Additionally, mRNA levels of pro-inflammatory genes such as TNFα, MCP-1, IL-1β, iNOS, and SAA were lower in EATs and subcutaneous adipose tissue of HFD-fed KO BMT mice (Fig. 24d). Together, these data indicated that macrophage VLDLR could potentiate adipose tissue inflammation in DIO.
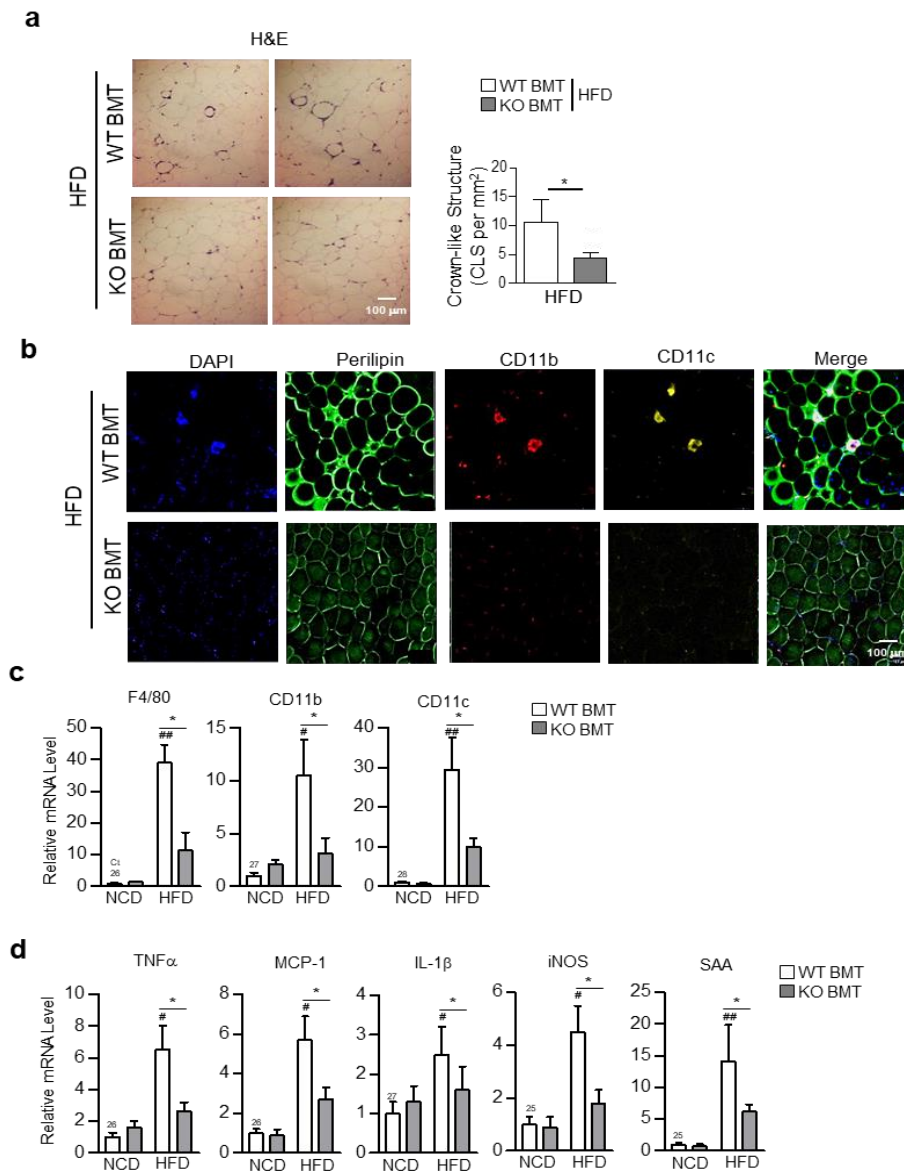
**Figure 24. Adipose tissue inflammation is reduced in HFD-fed KO BMT mice.** (a) Histological analysis of EAT from WT and KO BMT mice using H&E staining. (b) Whole-mount immunohistochemistry of the nucleus (blue), CD11b (red), and CD11c (yellow). Immune cell markers in EATs from WT and KO BMT mice. (c, d) Relative mRNA levels of macrophage (c) and pro-inflammatory (d) markers in EATs from WT and KO BMT mice. Data represent mean ± SD. *P<0.05, Student`s t-test.
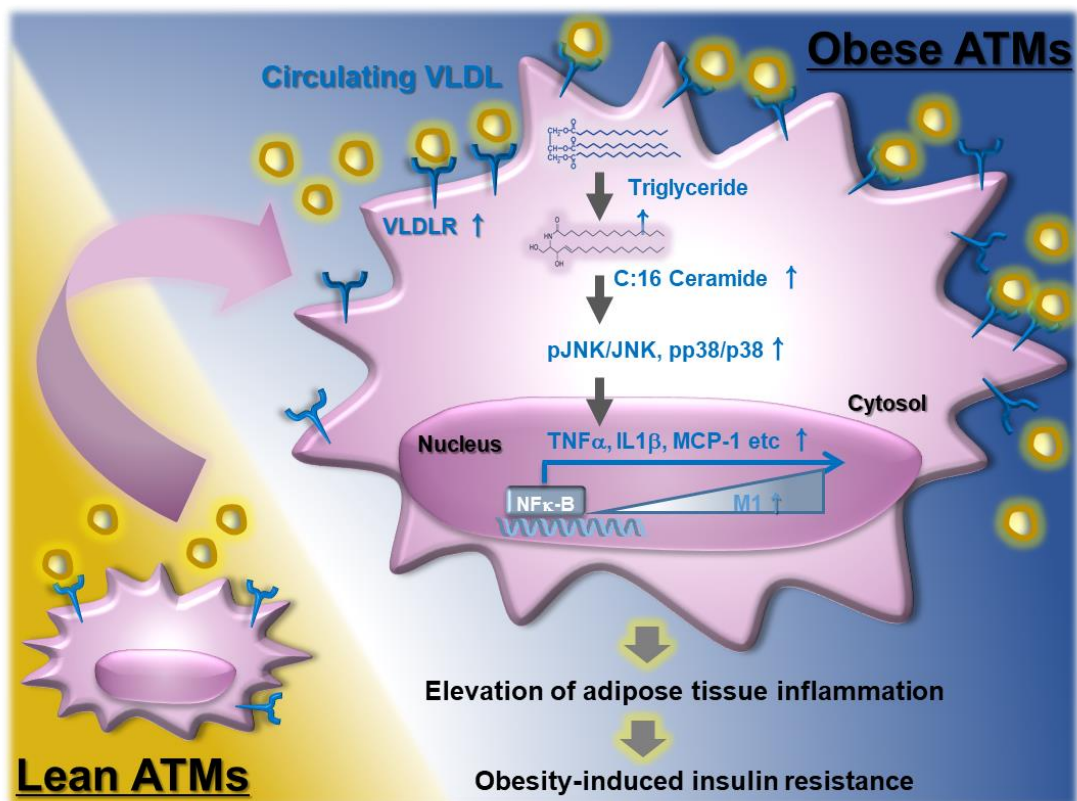
**Figure 25. Graphic abstract of chapter 1.** In obese ATMs, VLDL-VLDLR axis stimulates adipose tissue inflammation, accompanied with obesity-induced insulin resistance.

**DISCUSSION**

Elevated plasma triglyceride-rich lipoprotein such as VLDL is considered a risk factor for prevalence of obesity, type 2 diabetes mellitus, and atherosclerosis (Carr and Brunzell, 2004; Chahil and Ginsberg, 2006; Eckel, 2011). As peripheral VLDLR is involved in the clearance of circulating VLDL and/or chylomicron (Goudriaan et al., 2004; Takahashi et al., 2004; Takahashi et al., 2003), dysregulation of VLDLR has been implicated in metabolic complications (Boycott et al., 2005; Crawford et al., 2008; den Hartigh et al., 2014; Goudriaan et al., 2001; Nguyen et al., 2014; Saraswathi and Hasty, 2006). In accordance, VLDLR-deficient mice are protective from DIO and improve systemic insulin resistance (Goudriaan et al., 2001; Nguyen et al., 2014). Recently, Nguyen et al. reported that VLDLR KO mice attenuated adipocyte hypertrophy and that ER stress and pro-inflammatory responses were downregulated in adipose tissue upon HFD (Nguyen et al., 2014). However, it is largely unknown whether macrophage VLDLR might be crucial for obesity-induced insulin resistance through adipose tissue inflammation. Here, I have shown that elevated VLDLR in ATMs would be a key factor to provoking adipose tissue inflammation and insulin resistance in obesity.

Emerging evidence has indicated that macrophage polarization is important in the progress of metabolic disorders (Berg et al., 2002; Gordon and Taylor, 2005; Hirosumi et al.,

2002; Lumeng et al., 2007b; Shoelson et al., 2006; Sica and Mantovani, 2012; Yamauchi et al., 2001). For example, M1-like macrophage polarization contributes to metabolic impairment that is closely associated with obesity and diabetes (Hirosumi et al., 2002; Lumeng et al., 2007a; Lumeng et al., 2007b; Shoelson et al., 2006). Moreover, deletion of M1-like macrophages ameliorates insulin resistance in obese mice, whereas the reduction of M2-like macrophages predisposes lean mice to the development of insulin resistance (Charo, 2007; Patsouris et al., 2008). Thus, it is important to elucidate the molecular mechanisms by which macrophages could assume either M1- or M2-like character, which affects metabolic alterations in obesity. In this study, several lines of evidence supported the idea that macrophage VLDLR would exert regulatory roles in M1-like macrophages in obese adipose tissue. Firstly, M1-like macrophages highly expressed VLDLR as compared to M2-like macrophages in adipose tissue. Secondly, M1-derived BMDMs contained more ceramides than M2-derived BMDMs in the presence of VLDL. Thirdly, VLDL stimulated pro-inflammatory gene expression in VLDLR-overexpressing macrophages. Fourthly, VLDLR-deficient macrophages showed lower pro-inflammatory responses in the presence of VLDL, eventually leading to augmented insulin action in adipocytes. Lastly, VLDLR expression was elevated during M1-derived macrophage differentiation. In addition, macrophages from VLDLR KO mice had lower expression of pro-inflammatory genes during M1-derived macrophage differentiation in the presence of VLDL. These data imply that macrophage

59

VLDLR would be important for potentiating M1-like macrophage polarization in the presence of VLDL. Given that the balance shifting between M1- and M2-like macrophages is critical for the progression of inflammatory responses in obese adipose tissue, it is plausible that macrophage VLDLR elevation in obesity confers systemic insulin resistance through adipose tissue inflammation.

Ceramides are a member of the sphingolipid family and are essential components of the cell membrane (Chaurasia and Summers, 2015; Merrill, 1983; Summers, 2006). Recent studies have shown that increased ceramide level in obese adipose tissue coincides with development of insulin resistance (Chaurasia and Summers, 2015; Mitsutake et al., 2012; Summers, 2006; Xia et al., 2015). In addition, pharmacological inhibition or genetic ablation of ceramide synthesis protects from an increase in adiposity and improves systemic insulin sensitivity in obesity (Gosejacob et al., 2016; Holland and Summers, 2008; Raichur et al., 2014; Turpin et al., 2014). Thus, the regulation of ceramide production has been proposed as a potential therapeutic target against obesity-mediated metabolic diseases (Chaurasia and Summers, 2015; Gosejacob et al., 2016; Holland and Summers, 2008; Mitsutake et al., 2012; Raichur et al., 2014; Summers, 2006; Turpin et al., 2014; Xia et al., 2015). Since macrophage VLDLR plays an important role in the uptake of lipid metabolites in VLDL (Aflaki et al., 2012; Chaurasia et al., 2016), I assessed whether macrophage VLDLR deficiency might change certain lipid metabolites in the presence of VLDL. In macrophages, the level of

ceramides was increased, probably, via VLDL-VLDLR axis. Furthermore, analysis of the sphingolipid composition revealed that the levels of C16:0 ceramides and their precursors (C16:0 dihydroxy ceramides) were selectively reduced in VLDLR KO macrophages, whereas ceramide species with other chain lengths were not altered. *De novo* synthesis of C16:0 ceramides contributes to inflammasome activation in macrophages and is a key component that connects lipid oversupply and inflammatory pathways as well as insulin resistance in obesity (Gosejacob et al., 2016; Raichur et al., 2014; Turpin et al., 2014). In accordance with these findings, I observed that inhibition of ceramide synthesis could suppress inflammatory signaling pathways, which consequently downregulated the MAPK pathways and NF-κB

signaling in VLDL-treated macrophages. These data indicate that C16:0 ceramide synthesis via VLDL-VLDLR axis in macrophages would stimulate pro-inflammatory responses, at least partly, via MAPK pathways. As it has been reported that MAPK activation in macrophages would promote intracellular ceramide contents (Schilling et al., 2013), I tested whether MAPK pathways might affect the level of intracellular ceramides upon VLDL. The levels of cellular ceramides in VLDL-treated macrophages were not altered in the absence or presence of MAPK inhibitors. Although it needs to be elucidated how cellular VLDL could be converted into C16:0 ceramides in macrophages, our data suggest that macrophage VLDLR could participate in alteration of certain sphingolipids via VLDL uptaking, which

eventually leads to insulin resistance in obesity.

It has been reported that VLDLR is involved in inflammatory responses in immune cells (Eck et al., 2005; Goudriaan et al., 2001; Nguyen et al., 2014; Perman et al., 2011). For instance, monocyte-derived macrophages from VLDLR-deficient mice exhibit reduced secretion of inflammatory cytokines (den Hartigh et al., 2014; Nguyen et al., 2014; Saraswathi and Hasty, 2006). Furthermore, VLDLR deficiency impairs IL-1β induction upon activation of transcription factor activator protein (AP)-1, which is known to be regulated by MAPK p38 in macrophages (den Hartigh et al., 2014; Saraswathi and Hasty, 2006). In this study, I found that VLDLR-deficient macrophages showed downregulation of pro-inflammatory responses and M1-like polarization; this effect would eventually restore insulin signaling in adipocytes. Notably, I discovered that deletion of VLDLR in hematopoietic cells would be sufficient to alleviate systemic insulin resistance in DIO. Furthermore, hematopoietic VLDLR deficiency alleviated pro-inflammatory cytokine gene expression and decreased macrophage accumulation in adipose tissue upon HFD. Also, I observed that ceramide contents in ATMs of HFD-fed KO BMT mice were decreased. Together, these findings suggest that VLDLR in hematopoietic cells would play a major role in mediating inflammatory responses under nutrient-rich stress, such as HFD. In future, it remains to be investigated whether the reduced adipose tissue inflammation in HFD-fed KO BMT mice might be also attributable to other hematopoietic immune cells such as lymphocytes,

neutrophils, or eosinophils, as well as macrophages with VLDLR deficiency.

In conclusion, I have demonstrated that macrophage VLDLR plays important roles in mediating chronic inflammation and insulin resistance in DIO. In macrophages, ablation of VLDLR reduced cellular ceramides and relieved inflammatory responses in the presence of VLDL. Moreover, decreased pro-inflammatory signaling in VLDLR-ablated macrophages could prevent systemic insulin resistance in obesity. Collectively, our data suggest that regulation of macrophage VLDLR would be one of potential therapeutic targets in obesity-induced metabolic disorders.

# CHAPTER TWO:

# In brown adipose tissue, VLDLR mediates adaptive thermogenesis via facilitating PPAR delta signaling

## Abstract

In brown adipose tissue (BAT), dissipation of lipid metabolites to generate heat is an essential process for survival under low-ambient temperature. In plasma, lipid metabolites such as triglycerides are circulated in the form of lipoproteins, including very low density lipoprotein (VLDL). VLDLR receptor (VLDLR) is involved in the uptake and storage of circulating VLDL. However, it is largely unknown whether VLDL uptake via VLDLR predisposes thermogenic activity in BAT. In this study, I showed that VLDLR-mediated VLDL uptake plays an important role in brown adipocytes for adaptive thermogenesis under cold stimuli. By blocking VLDL-secretion, activation of BAT and thermogenic capacity by cold were downregulated. In addition, ability to produce heat was impaired in VLDLR knockout (KO) mice upon cold exposure. In brown adipocytes, VLDL treatment upregulated thermogenic gene expression in a VLDLR-dependent manner, simultaneously expedited mitochondrial oxygen consumption. In VLDLR KO mice, the expression levels of PPARδ target genes were downregulated in BAT, while administration of PPARδ agonist restored thermogenic abnormality and mitochondrial dysfunction. Moreover, VLDL-dependent thermogenic activation was attenuated in brown adipocyte-selective PPARδ KO mice. Collectively, these data suggest that the VLDL-VLDLR axis in brown adipocyte could mediate thermogenic regulation, probably, via PPARδ activation during cold acclimation.

# Introduction

Adipose tissue is largely divided into white adipose tissue (WAT) and brown adipose tissue (BAT) (Choe et al., 2016; Rosen and Spiegelman, 2014; Waki and Tontonoz, 2007). WAT is specialized in storing excess nutrients as lipid metabolites, whereas BAT is dedicated to generate heat by dissipating of stored energy sources (Cannon and Nedergaard, 2004; Choe et al., 2016). During cold exposure, BAT is activated when additional heat production is required to maintain core body temperature (Sun et al., 2011). The BAT has evolved as a specialized thermogenic organ, particularly, in nocturnal animals, hibernating mammals, and newborns (Himms-Hagen, 1990), (Jastroch et al., 2018). In BAT, heat production is stimulated by norepinephrine of sympathetic nervous system, leading to activation of β-adrenergic signaling and subsequent uncoupling of ATP production from mitochondrial respiration (Bronnikov et al., 1999; Chaudhry et al., 1992). In mitochondria, uncoupling protein (UCP) 1, which is selectively expressed in BAT, mediates adaptive thermogenesis under cold stimulation (Kozak et al., 1988; Nicholls et al., 1978; Rowlatt et al., 1971). Recently, emerging evidence has shown that adult human also has thermogenic BAT depot, which affects systemic energy homeostasis (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). Thereby, it seems that fundamental roles of BAT could be crucial for adaptive thermogenesis under cold and energy homeostasis.

Triglycerides are key metabolites of energy fuels, building blocks, as well as cellular mediators of signaling cascade. Since triglycerides are insoluble in water, they cannot be transported on their own in extracellular water, such as blood. Thus, triglycerides are surrounded by a hydrophilic external apo-proteins that function as transport vehicles, called lipoproteins. Very low-density lipoprotein (VLDL) and chylomicron are the major representative triglyceride-rich lipoproteins (TRLs) (Takahashi et al., 2003). It has been demonstrated that circulating VLDL can be absorbed by very low-density lipoprotein receptor (VLDLR) through receptor-mediated endocytosis or lipoprotein lipase (LPL)-dependent lipolysis (den Hartigh et al., 2014; Takahashi et al., 2004). VLDLR, a member of the low-density lipoprotein (LDL) receptor (LDLR) family, is abundantly expressed in adipose tissue, heart, kidney, and skeletal muscle (Takahashi et al., 1992; Yamamoto et al., 1993). Several studies have been reported that VLDLR-mediated VLDL uptake is closely associated with whole-body energy homeostasis (Goudriaan et al., 2001; Nguyen et al., 2014; Perman et al., 2011). For instance, it has been reported that the level of circulating VLDL was reduced in cold-stimulated plasma, whereas there were no significant differences in concentration of other lipoproteins between control and cold exposure (Radomski, 1966). Also, recent studies have shown that TRLs including VLDL shuttled from serum into BAT were detected upon cold exposure (Bartelt et al., 2011; Schreiber et al., 2017). However, it remains largely unknown whether VLDLR-mediated VLDL uptake in brown adipocytes is an

essential process of thermogenic regulation that allows whole-body to maintain homoeothermic status.

In this study, I demonstrate that VLDLR is elevated in cold-stimulated brown adipocytes, eventually promoting adaptive thermogenesis by facilitating PPARδ activation. VLDLR-deficient mice were intolerant upon cold exposure, whereas administration of synthetic PPARδ agonists led to improve not only thermogenic abnormality but also mitochondrial dysfunction. Moreover, VLDL-dependent thermogenic regulation was not activated in brown adipocyte-selective PPARδ knockout mice. Altogether, current data suggest that upregulated VLDLR of brown adipocytes would trigger thermogenic activity by enhancing PPARδ signaling pathways with modulation of lipid metabolism under cold condition.

## Materials and Methods

**Animals and treatment.** All animal experiments were approved by the Seoul National University Institutional Animal Care and Use Committee(SNUIACUC). C57BL6/J and VLDLR-deficient mice were obtained from The Jackson Laboratory (Bar Harbor, ME; strain 002529 B6;127S7-Vldlr <tm1Her>J). PPARδ BKO mice were generated by crossing UCP1-Cre mice with PPARδ-loxP mice (provided by Dr. Kang, Seoul National University, Korea). All mice were housed in colony cages and under 12-h light/12-h dark cycles. For the cold tolerance test, 7-week-old male mice were placed in a cold chamber at 6℃.

**Cell culture.** Stromal vascular cells(SVCs) were prepared as previously reported with minor modification. Briefly, BAT was dissected and wash with PBS, minced, and digested by collagenase I (Worthington Biochemical, Lakewood, NJ). For differentiation, attached SVCs were grown to confluence in DMEM supplemented with 10% FBS. After achieving confluent growth, the cells were stimulated with DMEM containing 10% FBS, dexamethasone (1 μmol/L), methylisobutylxanthine (520 μmol/L), insulin (850 nmol/L), and rosiglitazone (1 μmol/L) for 48 hours. The culture medium was replaced with DMEM containing 10% FBS, insulin (850 nmol/L), and rosiglitazone (1 μmol/L) for 6 days. To test effects of lipoproteins, human VLDL (Kalen Biomedical, #770100, Germantown, MD), and chylomicron (BioVision,

#7285-1000, Milipas, CA) were purchased. According to the manufacturer′s information, purchased VLDL contains 1.1 mg/ml protein, and chylomicron is composed of 98 % lipids and 2 % protein.

**Thermal imaging.** The surface temperature of the mice was imaged using an infrared camera (CX320 Thermal Imaging Camera; COX Co., Seoul, Korea).

**Cellular oxygen consumption.** Cellular oxygen consumption rate (OCR) of differentiated adipocytes were analyzed by Seahorse XFe24 extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA). According to the manufacturer`s instruction, all the experiments were performed. All the values were normalized to protein level in each plate.

**Western blot analysis.** Adipose tissue was lysed with radioimmunoprecipitation assay buffer. The proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The blots were probed with following primary antibodies: anti-VLDLR, anti-lamin B (1:500 dilution; Abcam, #92943, #65986, Cambridge, MA), anti-β-actin (1:2000 dilution; Sigma-Aldrich, #A5441, St.Louis, MO), UCP1(1:1000 dilution; Abcam, Cambridge, MA).

**Statistical analysis.** The data are presented from multiple independent experiments, and

represent the mean and standard deviation (SD). $P$-values were calculated by Student′s $t$-test;

$P < 0.05$ was regarded significantly.

**Data availability.** The data that support the findings of this study are available from the

corresponding author on reasonable request.

# Results

## Cold exposure stimulates VLDLR and concurrently accelerates VLDL uptake in BAT

Similar to WAT, VLDLR is abundantly expressed in BAT. However, it is largely unknown whether the expression level of VLDLR in adipose tissue might be altered upon cold exposure. To address this, wild type (WT) mice were placed at either room temperature (RT, 22°C) or cold temperature (CT, 6°C) for 6 hours. As shown in Fig. 26a and 26b, the mRNA and protein levels of VLDLR were elevated in BAT of cold exposed mice. However, mRNA levels of other LDLR family members, including LDLR and apolipoprotein receptor (AopER) 2, were not significantly altered by cold (Fig. 26a) As positive controls for cold exposure, mRNA levels of UCP1 and PGC1α were examined. In iWAT, the mRNA and protein levels of VLDLR were not altered upon cold exposure (Fig. 26c and 26d). Since it has been reported that VLDLR is involved in uptake of circulating VLDL, primary brown adipocytes from RT- or CT exposed mice were challenged with fluorescence-conjugated VLDL (VLDL-DiI, 10 μg/ml). To investigate whether brown adipocytes could absorb VLDL upon cold challenges, the level of VLDL-DiI was monitored by fluorescence microscopy. As indicated in Fig. 27, primary brown adipocytes from CT exposed mice accumulated higher degrees of VLDL-DiI than those from RT exposed mice. Next, to test whether VLDLR
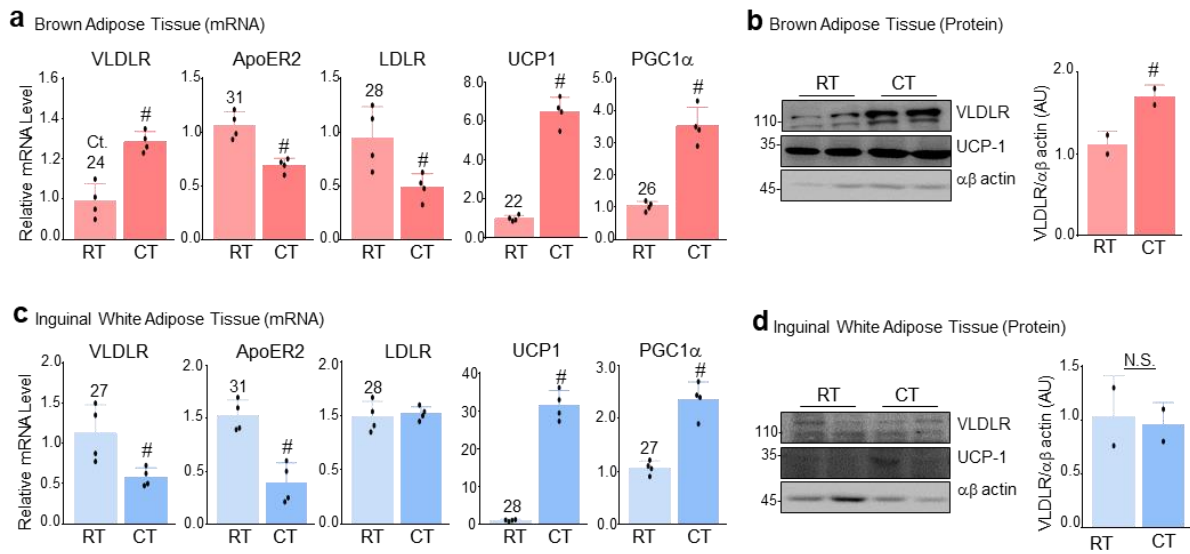
**Figure 26. The expression level of VLDLR is increased in cold-stimulated BAT.** (a-d) C57BL6/J wild type (WT) mice were exposed to room temperature (RT, 22°C) or cold temperature (CT, 6°C). After 6-hour exposure, brown adipose tissue (BAT) and inguinal white adipose tissue (iWAT) were prepped for analyses. The mRNA levels of LDLR family and thermogenic genes were examined in BAT (a) and iWAT (c). The protein level of VLDLR was examined in BAT (b, left panel) and quantified using ImageJ software (b, right panel). The protein level of VLDLR was assessed in iWAT (d, left panel) and quantified as described (d, right panel). Data represent mean ± SD. [#]P<0.05 RT vs CT, N.S. Non Significant, Student`s t-test.
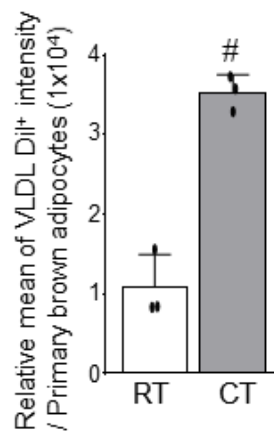
**Figure 27. The level of VLDL is elevated in cold-exposed primary brown adipocytes.** C57BL6/J WT mice were exposed to room temperature (RT, 22°C) or cold temperature (CT, 6°C). Then, primary brown adipocytes were isolated from BAT. These primary brown adipocytes were challenged with fluorescence VLDL (VLDL-DiI, 10 μg/ml) for 1 hour. Quantification of accumulated VLDL-DiI in primary brown adipocytes from RT or CT exposed mice using fluorescence-activated cell sorting (FACS) analysis. Data represent mean ± SD. [#]$P<0.05$ RT vs CT, Student`s t-test.

expression in brown adipocytes might be changed by β adrenergic stimulation, differentiated

brown adipocytes were treated with isoproterenol, one of the potent β adrenergic receptor

agonists. As shown in Fig. 28a and 28b, mRNA and protein levels of VLDLR were increased

in isoproterenol treated brown adipocytes. Similar to previous data, fluorescent microscopic

analysis revealed that uptake of VLDL-DiI in brown adipocytes appeared to be increased by

β adrenergic activation (Fig.28c). These data suggest that cold exposure would upregulate the

expression level of VLDLR and concurrently accelerates VLDL uptake in brown adipocytes.


**Circulating VLDL is involved in adaptive thermogenesis during cold exposure**

To investigate whether circulating VLDL might be involved in adaptive

thermogenesis under cold stimulation, WT mice were administrated either vehicle or

CP346086 as a chemical inhibitor of VLDL secretion. As shown in Fig 29, reduction in

VLDL secretion was confirmed by the level of ApoB protein which is a major constituent of

VLDL in plasma. To assess thermogenic activity, WT mice were exposed to either RT or CT

for 6 hours. When rectal temperature and infrared camera images were adopted to measure

body temperature, CP346086-treated mice exhibited lower body temperature upon cold

exposure (Fig. 30). To examine whether the level of circulating VLDL could affect BAT

activation during cold exposure, quantitative PCR analysis were accessed. As shown in Fig.

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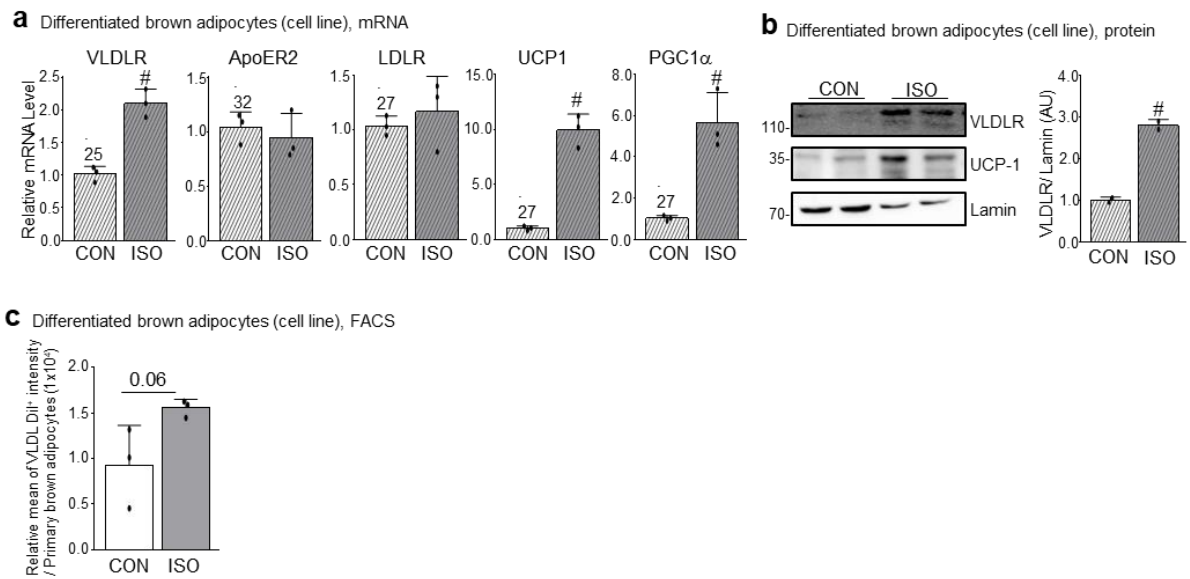
**Figure 28. β adrenergic activation induces the expression of VLDLR and uptake of VLDL in brown adipocytes.** (a-c) Differentiated brown adipocytes (cell line) were treated isoproterenol (ISO, 1 μM) for 6 hours. a. Relative mRNA level of LDLR family and thermogenic genes in brown adipocytes as described. b. The protein level of VLDLR in brown adipocytes (b, left panel) and quantification of protein bands (b, right panel). c. Brown adipocytes treated with fluorescence VLDL (VLDL-DiI, 1 μg/ml) for 1 hour. Quantification of accumulated VLDL-DiI in brown adipocytes using FACS analysis. Data represent mean ± SD. [#]P<0.05 CON vs ISO, Student`s t-test.
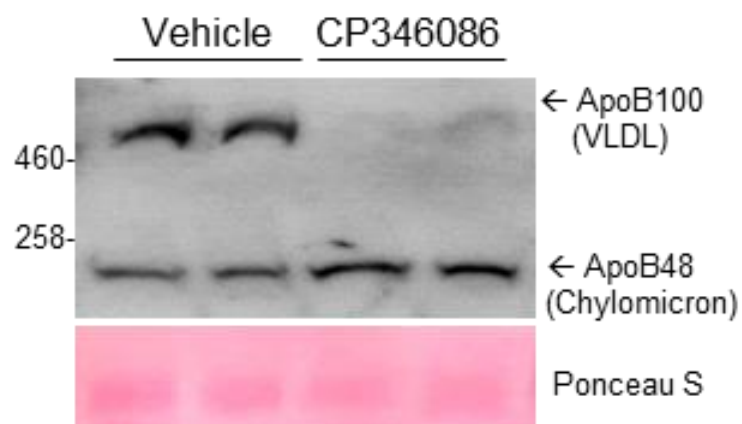
**Figure 29. Circulating VLDL is downregulated after VLDL-secretion blocking.**
C57BL6/J WT mice were administrated by vehicle or CP346086 (microsomal triglyceride transfer protein inhibitor, 1.3 mg/kg) for 6 hours. Western blotting analysis was examined in plasma from described mice using ApoB antibody. Ponceau S staining were examined for internal control of protein loading in western blots.

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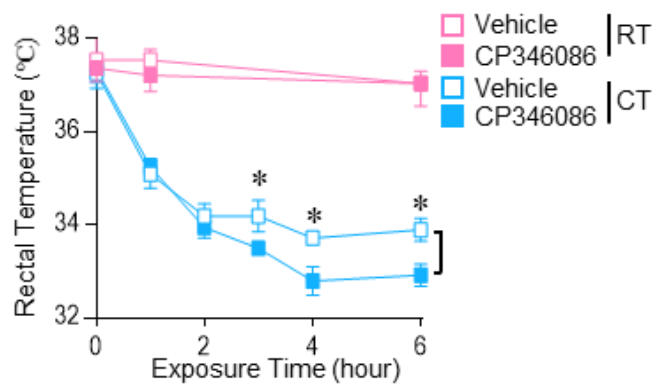
**Figure 30. Circulating VLDL is required for adaptive thermogenesis upon cold exposure.** C57BL6/J WT mice were administrated by vehicle or CP346086 (1.3 mg/kg), then these mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. Body temperature was assessed using a rectal probe during cold exposure. Data represent mean ± SD. *P<0.05 Vehicle vs CP346086 upon CT, Student`s t-test.

31, mRNA levels of thermogenic genes, such as UCP1, PGC1α, and DIO2, and protein level of UCP1 were less increased in CP346086-treated BAT, compared to DMSO-treated BAT after cold exposure. These data suggested that circulating VLDL would be involved in BAT activation upon cold, probably, by conferring adaptive thermogenesis.


**VLDLR KO mice were cold-intolerant compared to VLDLR WT mice**

The findings that the expression level of VLDLR was upregulated in cold-acclimated BAT and that circulating VLDL appeared to be affect thermogenic activity upon raised the question whether VLDLR might contribute to adaptive thermogenesis in response to cold challenge. To address this, VLDLR WT and VLDLR KO mice were exposed to RT or CT for 6 hours. As shown in Fig. 32, VLDLR KO mice exhibited lower body temperature during cold exposure, compared to VLDLR WT mice. Next, to address whether VLDLR might affect BAT activation by cold, mRNA level of thermogenic genes were examined in BAT. As indicated in Fig.33a, the expression levels of several thermogenic genes were significantly elevated in cold-challenged BAT of VLDLR WT mice, whereas those were marginally elevated in BAT of VLDLR KO mice. In addition, the protein level of UCP1 was less increased in cold-stimulated BAT of VLDLR KO mice, compared to control mice (Fig. 33b). It has been reported that thermogenic activity is highly associated with enhanced
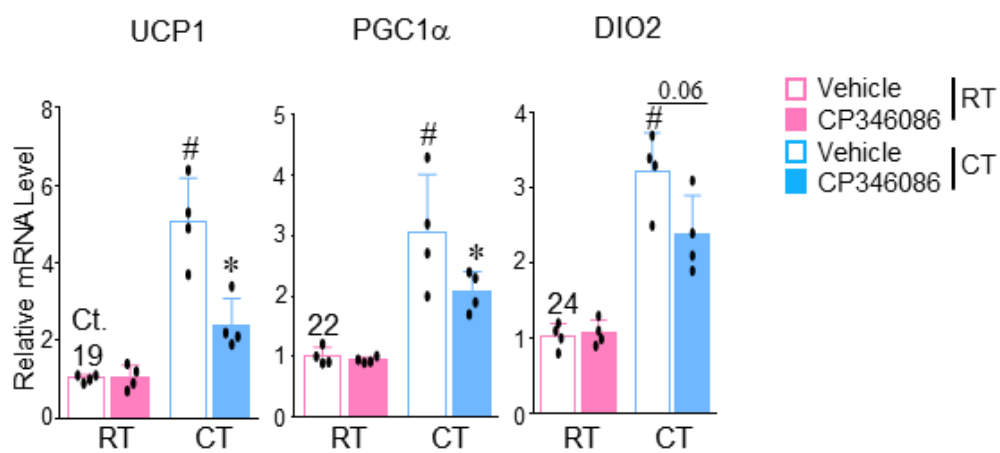
**Figure 31. In cold stimulated BAT, thermogenic genes are reduced after blocking of VLDL secretion.** C57BL6/J WT mice were administrated by vehicle or CP346086 (1.3 mg/kg), then these mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. Quantitative PCR analyses of thermogenic genes (UCP1, PGC1$\alpha$, DIO2) were performed in BAT from described mice. Data represent mean ± SD. [#]P<0.05 RT vs CT, *P<0.05 Vehicle vs CP346086 upon CT, Student`s t-test.
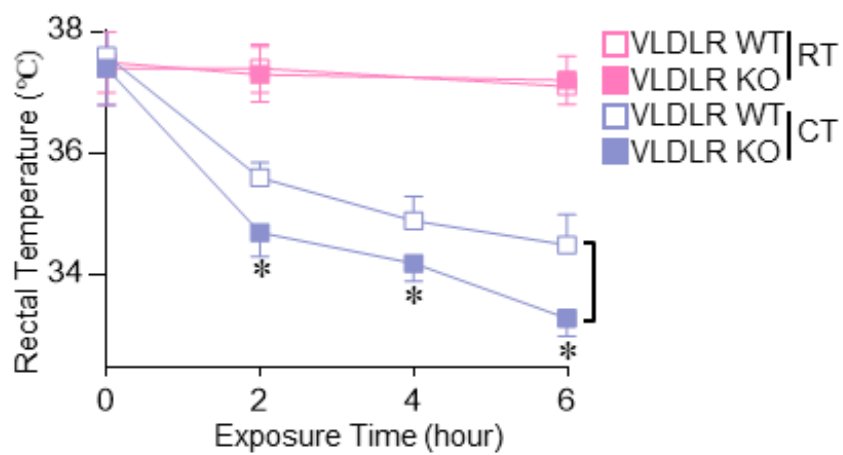
**Figure 32. VLDLR KO mice are impaired thermogenic activity under cold stimulation.**
VLDLR WT and VLDLR KO mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. Using rectal probes, body temperature was measured during cold exposure. Data represent mean ± SD. *P<0.05 VLDLR WT vs VLDLR KO Student`s t-test.

**a** Brown adipose tissue (mRNA)
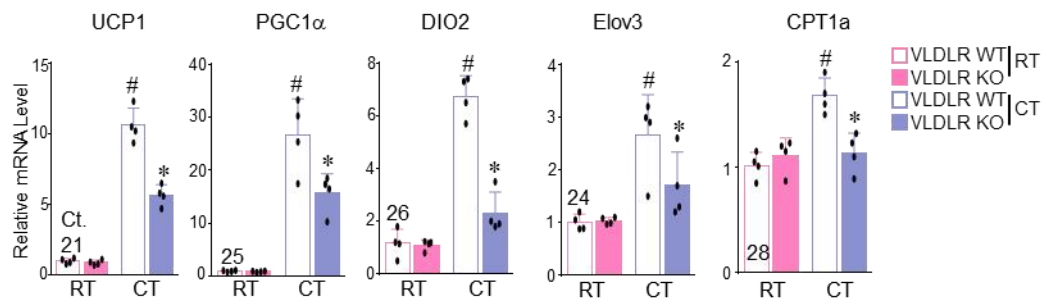
**b** Brown adipose tissue (Protein)
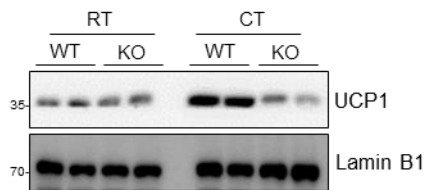
**Figure 33. The mRNA levels of thermogenic genes are reduced in BAT of cold-stimulated VLDLR KO.** VLDLR WT and VLDLR KO mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. The relative mRNA levels of thermogenic genes (a) and protein level of UCP1 (b) were examined in cold stimulated BAT. In western blot data, Lamin B1 was examined for internal control of protein loading. Data represent mean ± SD. [#]P<0.05 RT vs CT, *P<0.05 VLDLR WT vs VLDLR KO, Student`s t-test.

mitochondrial activity such as oxygen consumption rate (OCR) in brown adipocytes (Ma and

Foster, 1984; Shabalina et al., 2013). Thus, I examined OCR in BAT SVC-derived adipocytes

(BSAD) from VLDLR WT mice or VLDLR KO mice. Compared to VLDLR WT BSAD, the

degree of OCR during basal and maximal respiration was less increased in VLDLR KO

BSAD with VLDL and isoproterenol treatments (Fig. 34a). Furthermore, the mRNA levels of

thermogenic genes were less increased in VLDLR KO BSAD, compared to VLDLR WT

BSAD (Fig. 34b). Together, these data propose that VLDLR deficiency might lead to impair

adaptive thermogenesis upon cold exposure.

**In response to cold, PPARs signaling might be associated with VLDLR-dependent thermogenic activity**

To perform an unbiased approach of alterations in gene expression during cold exposure, I

analyzed published RNA transcriptome data (GSE119452) (Li et al., 2018). The analyses

using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways revealed that

PPARs pathway would be the most significantly changed in cold-exposed BAT (Fig. 35a).

Further analyses from RNA-seq (GSE119452) showed that the expression levels of both

thermogenesis-related genes and PPARs-related genes were seemed to upregulate in cold-

stimulated BAT (Fig. 35b). Since it has been reported that PPARs pathway are activated
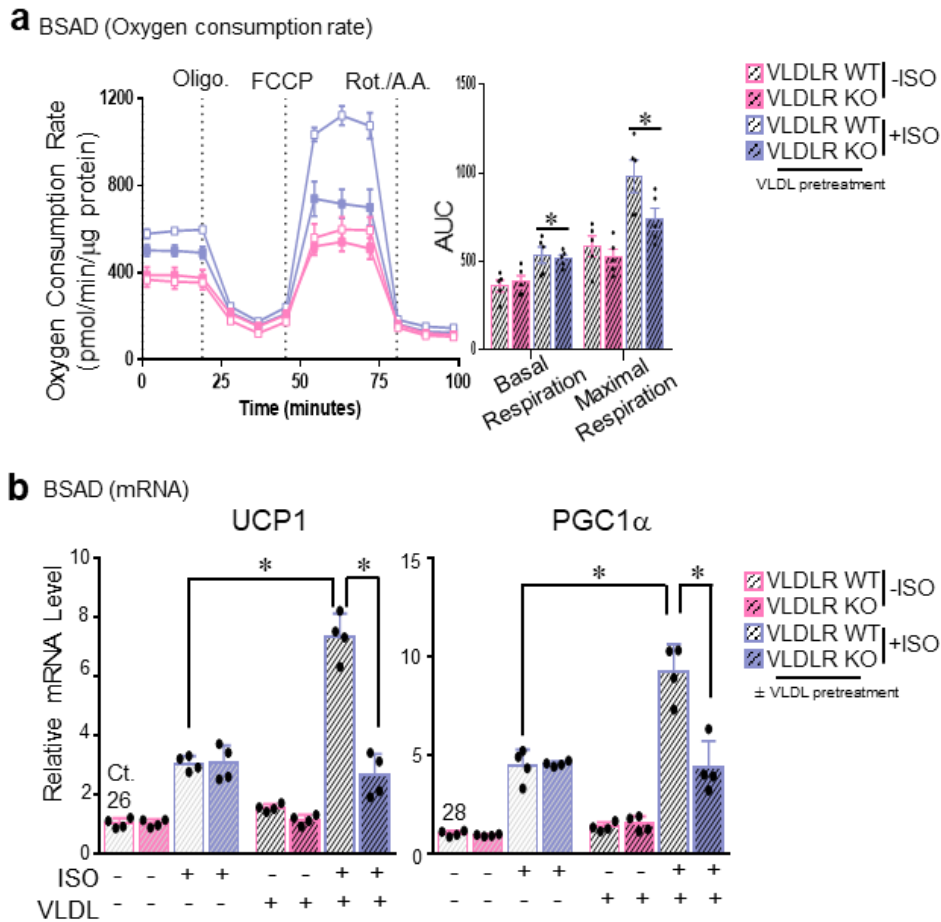
**Figure 34. In brown adipocytes, degree of mitochondrial oxygen consumption rate is downregulated by VLDLR deficiency.** (a,b) BAT SVC-derived adipocytes (BSAD) from VLDLR WT and VLDLR KO mice were analysed. These BSAD were pre-treated with VLDL (20 μg/ml) for 18 hours, then sequential treated with isoproterenol (0.5 μM) for 6 hours. a Degree of oxygen consumption rate (OCR) was measured in BSAD as described using Seahorse. b Quantitative PCR analyses of thermogenic genes were examined in BSAD with or without VLDL and isoproterenol as described.
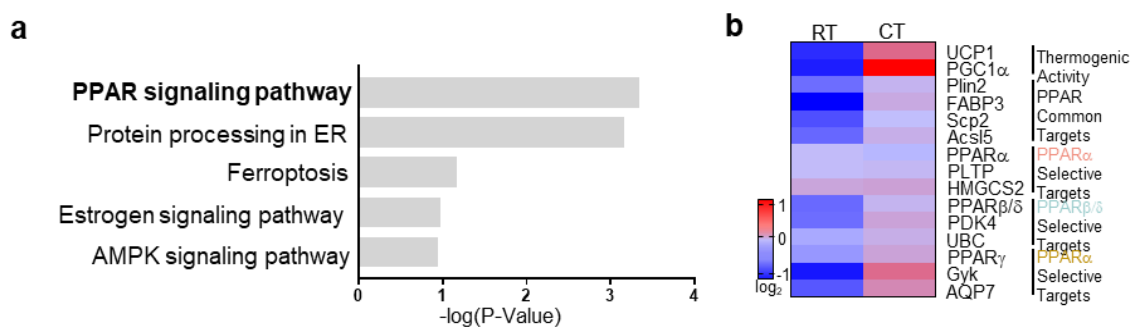
**Figure 35. PPARs signaling are associated with cold-stimulated BAT.** (a,b) Analyses were achieved using published RNA-sequencing data (GSE119452). RNA-sequencing was performed in BAT of C57BL6/J WT mice exposed to either RT (22°C) or CT (6°C) for 6 hours. a KEGG pathway analyses using Enrichr (http://amp.pharm. mssm.edu/Erichr) for up- and down-regulated genes in BAT from mice as described. b Heatmap image of detailed gene expressions related to thermogenesis and PPARs signaling from RNA-sequencing data (GSE119452).

by intracellular VLDL (Chawla et al., 2003, Lee et al., 2006, Brown et al., 2011), I speculated that VLDL-VLDLR axis might modulate PPARs signaling in cold-stimulated BAT (Fig. 36a). The expression levels of PPARδ target genes, including PDK4 and UBC, were downregulated in BAT of VLDLR KO mice, while those of PPARα or PPARγ target genes were not altered (Fig. 36a). Next, BSAD was subjected to conduct the *in vitro* experiments with or without VLDL and isoproterenol. In VLDLR WT BSAD, the mRNA level of PDK4, the target of PPARδ, was elevated in the presence of VLDL and isoproterenol, while it was not efficient in VLDLR KO BSAD (Fig. 36b). With VLDL and isoproterenol treatment, the expression levels of UCP1 and PDK4, which were lower in VLDLR KO BSAD were enhanced by GW501516, the potent PPARδ agonist (Fig. 37a). Furthermore, mitochondrial OCR of VLDLR KO BSAD was slightly but significantly elevated with PPARδ agonist, GW501516 (Fig. 37b). These findings propose that PPARδ pathway might be involved in VLDLR-mediated thermogenic activation in brown adipocytes, at least partly.

**PPARδ agonist restores the thermogenic capacity in VLDLR-deficient brown adipocytes**

The findings that PPARδ activation might be associated with VLDLR-dependent thermogenic activity led me to test whether activation with PPARδ agonist could restore thermogenic
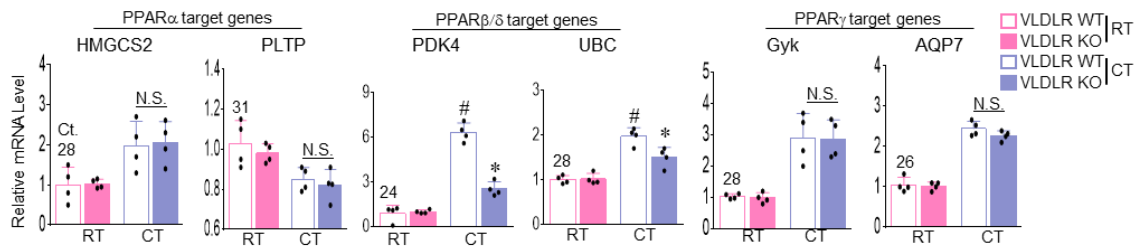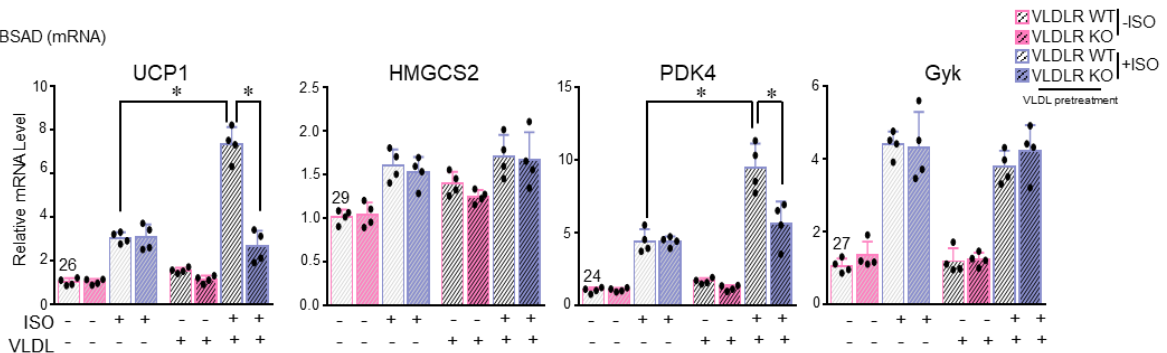
**Figure 36. In BAT, the mRNA levels of PPARδ target genes are downregulated by VLDLR deficiency.** a VLDLR WT and VLDLR KO mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. Then, quantitative PCR analyses of PPARs target genes were performed in cold stimulated BAT. Data represent mean ± SD. #P<0.05 RT vs CT, *P<0.05 VLDLR WT vs VLDLR KO, Student`s t-test. b BSADs from VLDLR WT and VLDLR KO mice were adopted. These BSADs were pre-treated with VLDL (20 μg/ml) for 18 hours, then sequential treated with isoproterenol (0.5 μM) for 6 hours. Relative mRNA levels of PPARs target genes were examined in BSADs with or without VLDL and isoproterenol as described.
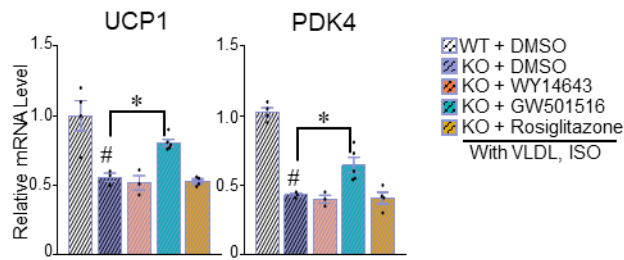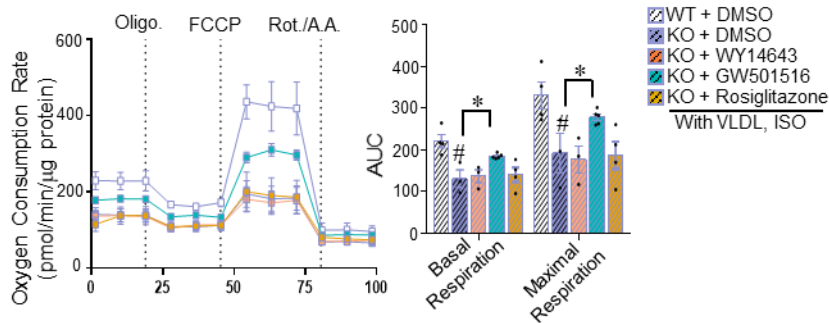
**Figure 37. In brown adipocytes, PPARδ is involved in VLDL/VLDLR-dependent thermogenic gene regulation and mitochondrial oxygen consumption under β adrenergic stimulation.** (a, b) BSADs from VLDLR WT and VLDLR KO mice were adopted. These BSADs were pre-treated with VLDL (20 μg/ml) and PPARs agonists (PPARα; WY14643, PPARβ/δ; GW501516, PPARγ; Rosiglitazone) for 18 hours, then sequential treated with isoproterenol (0.5 μM) for 6 hours. a In BSADs, quantitative PCR analyses of thermogenic genes were assessed with or without PPARs agonists in the presence of VLDL and isoproterenol as described. b OCR were measured in BSADs as described using Seahorse.

88

activity in VLDLR KO mice. To address this, I injected PPARδ agonists, GW501516, to

VLDLR WT and VLDLR KO. Upon GW501516 treatments, rectal temperature of cold-

exposed VLDLR KO mice was significantly elevated (Fig 38a). Similarly, the levels of key

thermogenic genes, such as UCP1, PGC1α, and DIO2, were rescued by GW501516 in cold-

stimulated VLDLR KO BAT. (Fig. 38b). These data suggest that the PPARδ signaling could

be mediated in VLDLR-dependent thermogenic activity under cold stimulation.


**In brown adipocytes, deficiency of PPARδ impairs thermogenic activity upon cold**

**exposure**

Above the data indicate that, in brown adipocyte, VLDLR could potentiate PPARδ

signaling upon cold exposure. However, it has not been elucidated whether brown adipocyte

PPARδ could contribute to thermogenic capacity under cold stimuli. To test this, I generated

brown adipocyte-selective PPARδ KO (PPARδ BKO) mice by crossing UCP1-Cre and

PPARδ-loxP mice (Fig. 39a). The level of PPARδ protein was greatly repressed in isolated

primary brown adipocytes from PPARδ BKO mice (Fig. 39b). In PPARδ BKO mice, there

were no significant changes in body weights and metabolic organs weights, such as BAT,

iWAT, eWAT, liver, and muscle (Fig. 39c). As it has been reported that PPARδ regulate
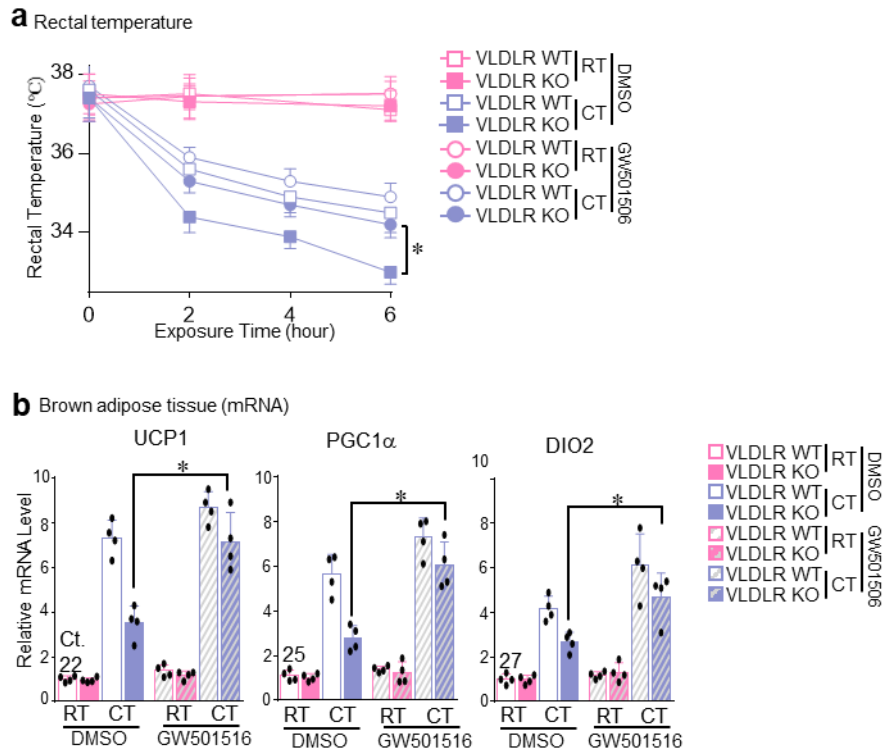
**Figure 38. GW501516 restores thermogenic activity in VLDLR KO mice.** (a, b) VLDLR WT and VLDLR KO mice were administrated by intraperitoneal injection of GW501516 (3 mg/kg) for 3 days. Dimethyl sulfoxide (DMSO) was used as negative control for GW501516. Then described mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. a Body temperature was measured in VLDLR WT and VLDLR KO mice after GW501516 injection using a rectal probe during cold exposure. b Quantitative PCR analyses of thermogenic genes (UCP1, PGC1$\alpha$, DIO2) were performed in BAT from VLDLR WT and VLDLR KO mice after cold exposure with GW501516 injection. Data represent mean ± SD. *P<0.05 Student`s t-test.
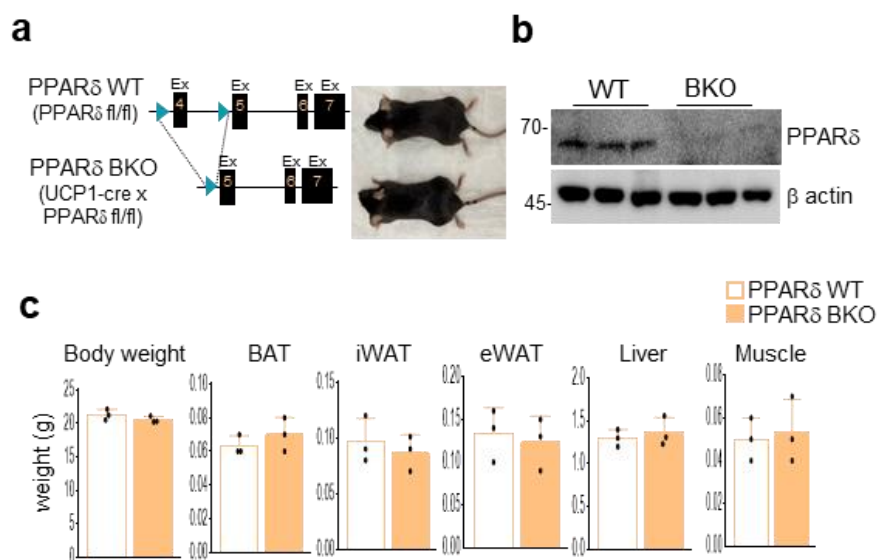
**Figure 39. In brown adipocyte, PPARδ is depleted using UCP1-Cre mice.** Brown adipocyte specific PPARδ knockout (PPARδ BKO) mice were generated by crossing UCP1-Cre and PPARδ-loxP mice (a). b The protein level of PPARδ was examined in primary brown adipocytes from PPARδ WT and PPARδ BKO mice. c Total body weight and various tissue weights were measured in PPARδ WT and PPARδ BKO.

expression of UCP1 (Villarroya et al., 2007), PPARδ WT mice and PPARδ BKO mice were subjected to examine their thermogenic activity under either RT or CT. As shown in Fig. 40, rectal temperature of PPARδ BKO mice was further downregulated during cold exposure. Next, I examined gene expression in BAT from PPARδ WT mice and PPARδ BKO mice upon cold. While the mRNA levels of thermogenic genes were elevated in cold-challenged BAT of PPARδ WT mice, those were downregulated in BAT of PPARδ BKO mice (Fig. 41). Together, these data suggest that brown adipocyte PPARδ could regulate thermogenic activation under cold acclimation.

**VLDL-induced thermogenic activity was diminished in PPARδ BKO mice**

Given that VLDL-VLDLR axis potentiated PPARδ activity and PPARδ modulated thermogeniesis in brown adipocytes, I asked a question whether VLDL might be required for PPARδ-dependent thermognic regulation in brown adipocytes. To address this, I injected VLDL into either PPARδ WT and PPARδ BKO mice, then exposed to RT or CT. As shown in Fig. 42, rectal temperature revealed that VLDL-induced thermogenic activity was attenuated in PPARδ BKO mice during cold exposure. These data propose that VLDL-induced thermogenic activity would be mediated by PPARδ in brown adipocytes.
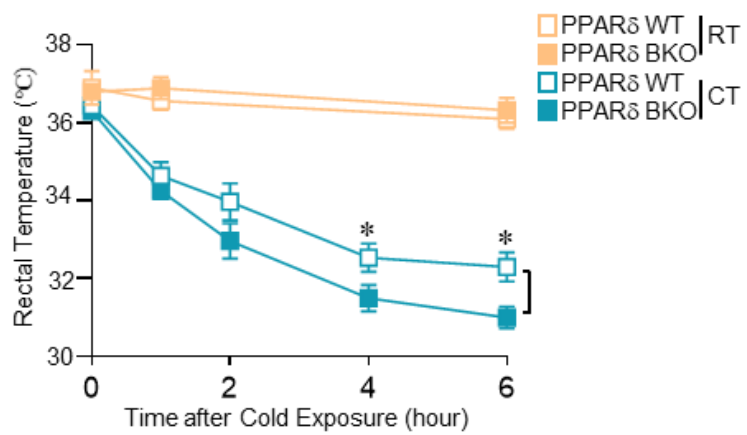
**Figure 40. PPARδ BKO mice are impaired thermogenic activity during cold exposure.** PPARδ WT and PPARδ BKO mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. Rectal temperature was examined during cold exposure. Data represent mean ± SD. *P<0.05 PPARδ WT vs PPARδ BKO Student`s t-test.
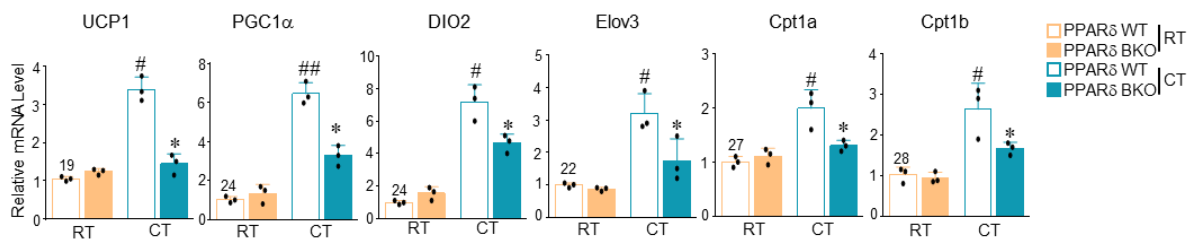
**Figure 41. Upon cold exposure, thermogenic activity is downregulated in BAT from PPARδ BKO mice.** PPARδ WT and PPARδ BKO mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. Quantitative PCR analyses of thermogenic genes were performed in BAT of mice as described. Data represent mean ± SD. [#]P<0.05 RT vs CT, *P<0.05 PPARδ WT vs PPARδ BKO upon CT, Student`s t-test.
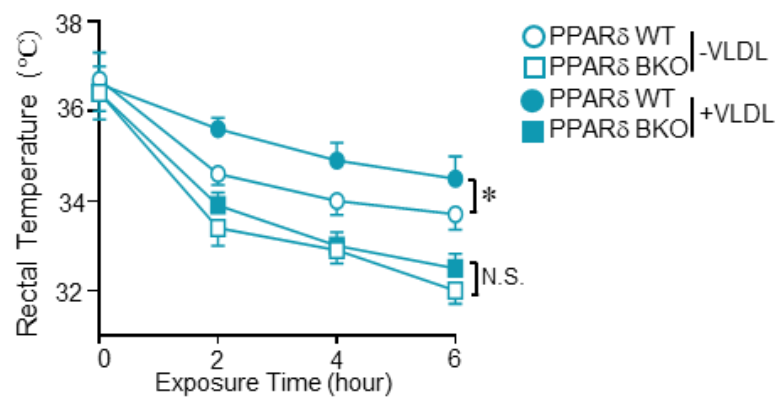
**Figure 42. VLDL-induced thermogenic activity is diminished in PPARδ BKO mice.**
PPARδ WT and PPARδ KO mice were administrated by human VLDL (20 mg/dl), then these mice were exposed to either RT (22°C) or CT (6°C) for 6 hours. During cold exposure, rectal temperature was examined using a rectal probe. Data represent mean ± SD. *P<0.05 PPARδ WT vs PPARδ BKO with or without VLDL administration, N.S. Non Significant, Student`s t-test.
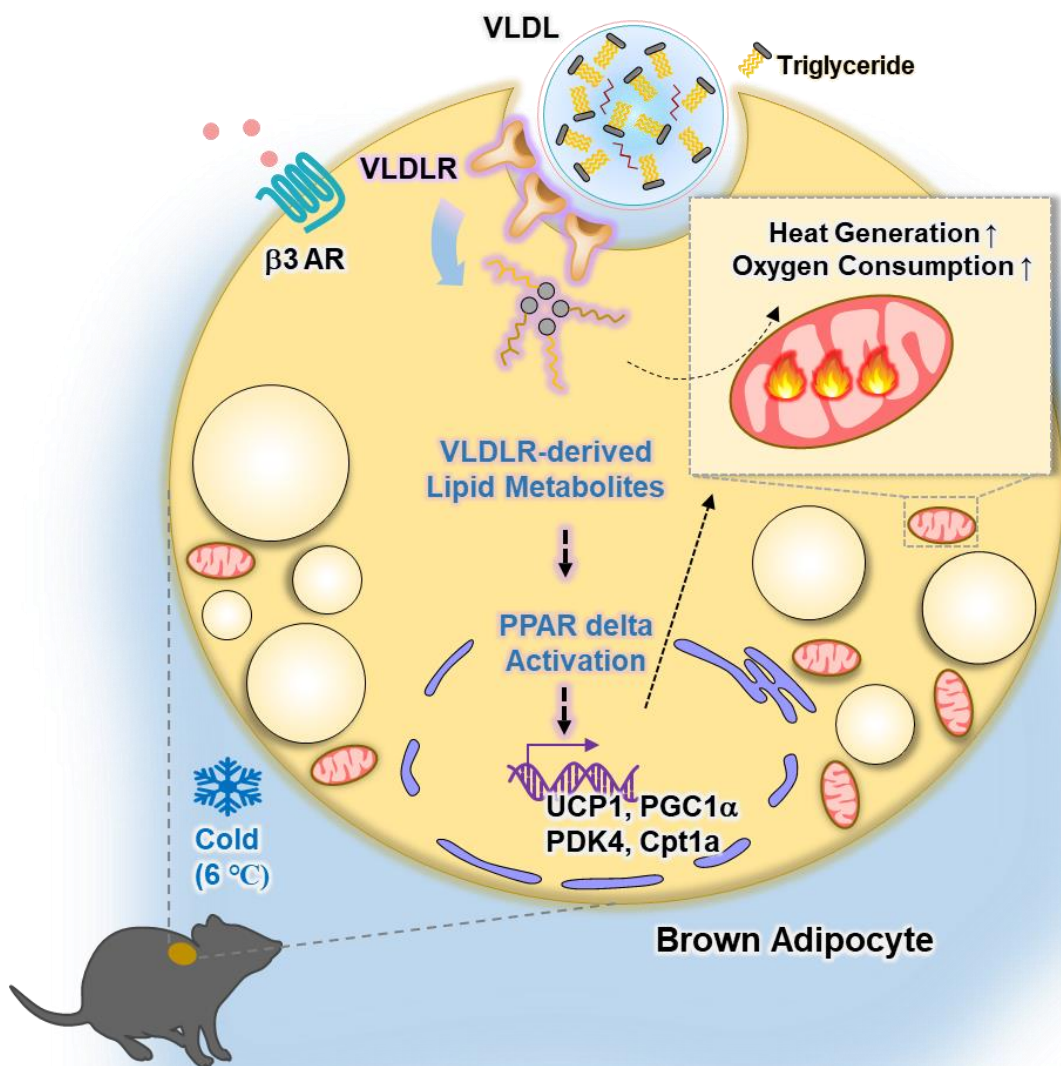
**Figure 43. Graphical abstract of chapter 2.** Brown adipocyte VLDL-VLDLR axis mediates adaptive thermogenesis via facilitating PPARδ signaling during cold exposure

# CONCLUSION

In adipose tissue, regulation of lipid metabolism is closely related to systemic energy homeostasis. VLDLR, which is responsible for absorption of triglyceride-rich lipid metabolites, has been reported to be abundantly expressed in adipose tissue (den Hartigh et al., 2014; Takahashi et al., 2004). However, there is a lack of knowledge available on whether adipose tissue VLDLR would regulate systemic energy homeostasis. Through this thesis, VLDLR has been investigated in WAT to aggravate pro-inflammatory responses during obesity as well as in BAT to facilitate adaptive thermogenesis under cold stimulation. Together, current findings suggest that adipose VLDLR would play key roles to modulate substrate utilization and to control systemic energy homeostasis under environmental alterations.

## 1. VLDLR as a stress responder

VLDLR has a pivotal role in uptake of circulating VLDL, major triglyceride-carrying lipoproteins (Takahashi et al., 1992; Yamamoto et al., 1993). In this notion, VLDLR KO mice would be a useful animal model to study of VLDLR *in vivo*. Without any stress, VLDLR KO mice appeared to be normal, at least, in the aspects of plasma lipoprotein levels similar to

wild type mice (Frykman et al., 1995). In this study, I found that the expression level of adipose tissue VLDLR was altered in various environmental conditions. In WAT, the expression level of VLDLR was increased under energy-excessive state, such as obesity, which eventually results in glucose intolerance, accompanied with adipose tissue inflammatory responses. In BAT, the expression level of VLDLR was enhanced by cold, which leads to adaptive thermogenesis via heat generation. Together, these data suggest that VLDLR in fat tissues acts as one of the key stress responders against changes upon environmental alterations, which might provide evolutionary advantages on survival and systemic energy homeostasis.

## 2. VLDLR-dependent lipid metabolites as cellular signaling messengers

Since lipid metabolites are primary energy sources and building blocks of new organelles and membranes, cells have a tendency to increased uptake of exogenous lipids via their own receptors and transporters (Jaworski et al., 2007). Emerging evidence has suggested that intracellular lipid metabolites are important signaling molecules responsible for the regulation of a diverse range of signaling pathways (Bieberich, 2008). For example, intracellular ceramides stimulate signal transduction pathways that are associated with cell death or cell growth inhibition (Chaurasia and Summers, 2015; Merrill, 1983; Summers,

2006). In addition, diacylglycerols activate isoforms of the protein kinase C (PKC) family, which are associated with cell growth and survival (Chaurasia and Summers, 2015; Gosejacob et al., 2016; Holland and Summers, 2008; Mitsutake et al., 2012; Raichur et al., 2014; Summers, 2006; Turpin et al., 2014; Xia et al., 2015) Nevertheless, it is largely unknown whether adipose VLDLR-derived lipid metabolites would serve as certain mediators of cellular signaling cascade. In this study, I found that lipid metabolites were absorbed into adipose tissue through VLDLR, leading to manipulation of cellular signaling cascade. In WAT, lipid metabolites through VLDLR were converted into C16:0 ceramide, which activates signaling cascade of intracellular inflammatory responses. In BAT, VLDLR-derived lipid metabolites contributed to activation of PPAR-signaling process, which eventually potentiates heat generation. These findings indicate that VLDL-VLDLR axis would contribute to various intracellular signaling, in fat tissues upon environmental conditions. Although it needs to be further validated how cellular VLDL could be converted into C16:0 ceramide or certain lipid metabolites for PPARδ activation in an adipose VLDLR dependent manner, data of this study suggests that adipose VLDLR could participate in lipid metabolites by VLDL uptake, eventually leading to regulate systemic energy homeostasis.

## 3. VLDLR as a therapeutic target

Emerging evidence has proposed that VLDLR might be a good candidate for gene therapy to control metabolic diseases (Chen et al., 2000; Yuan et al., 2011). It has been reported that VLDLR is highly associated with hyperlipidemia (Chen et al., 2000) (Yuan et al., 2011). Also, adenovirus-mediated overexpression of VLDLR in livers of LDLR-deficient mice resulted in marked decrease of plasma triglycerides by absorption of circulating VLDL (Chen et al., 2000). In addition, elevation of VLDLR expression in liver of ApoE-deficient mice resulted in a reduction of plasma VLDL, imply that gene therapy with VLDLR gene could be used downregulation of plasma VLDL levels in hyperlipidemic patients (Yuan et al., 2011). However, no studies have conducted with a therapeutic approach targeting VLDLR for physiological or pathological disorders other than hyperlipidemic disease. In this study, I found that VLDLR in WAT participated adipose tissue inflammation and insulin resistance in obesity. In particular, overexpression of VLDLR stimulated mRNA expression of pro-inflammatory cytokine genes including TNFα, IL-6, and IL-1β. Also, VLDLR-deficiency downregulated inflammatory signals and insulin resistance driven by cross-talk between adipocytes and macrophages. Thus, these data suggest that unwanted VLDLR activation in WAT activation would be one of the pro-inflammatory pathways in obese adipose tissue. Collectively, these data suggest that regulation of adipose tissue VLDLR would be one of the potential therapeutic targets against obesity-induced metabolic disorders.

# 국문 초록

　　체내 중성지방은 세포 성장 및 분열에 필요한 주요 대사산물이며, 전신 에너지항
상성에 필수적인 호르몬의 전구체로 사용된다. 혈장에서 중성지방과 같은 지질대사물은
VLDL (very low density lipoprotein)이라고 불리는 지질단백질의 형태로 순환한다.
VLDL 수용체(VLDLR)는 혈중 VLDL의 흡수 및 저장에 관여한다. VLDLR는 다른 조직
에 비해 지방조직에서 많은 량 발현하고 있다. VLDLR 결핍 생쥐는 대조군 생쥐와 비교
하여 지방조직 질량이 감소된 형태를 보였으며, 이는 지방조직 VLDLR가 지질대사에 밀
접하게 관여되어 있음을 시사한다. 현재까지 VLDLR는 VLDL 흡수 및 지방조직 질량 감
소와 관련됨이 보고되었지만, 지방조직 VLDLR가 다양한 환경상태에서 전신 에너지항상
성에 기여할 수 있는지 여부는 알려지지 않았다.

　　본 학위논문 1 장에서는 백색지방조직(WAT)의 VLDLR 발현 증가로 인해 비만
생쥐에서 지방조직 염증반응과 체내 인슐린 저항성을 촉진시킴을 규명했다. 더욱이,
VLDL 처리는 VLDLR 의존적으로 C16:0 세라마이드의 축적을 유도하였으며, 이는 백색
지방조직 내 전염증반응(pro-inflammatory responses) 및 M1 유사 대식세포 유도를 촉
진한다는 실험결과를 얻었다. 뿐만 아니라, VLDLR 결핍 골수를 정상 생쥐로 이식하였을
때, 고지방식이 의존적 비만 유도 염증반응이 완화되고 인슐린 저항성이 개선됨을 관찰
했다. 이를 통해 백색지방조직 VLDL-VLDLR 축이 비만에서 지방조직 염증반응 및 인
슐린 저항성의 악화를 야기하는 원인일 수 있음을 규명했다.

101

본 학위 논문 2 장에서는 저온자극 의존적 열생산 과정에 갈색지방조직 VLDLR가 매개될 수 있음을 밝히고자 했다. VLDLR의 혈중 리간드로 알려진 VLDL의 분비가 억제된 야생형 생쥐는 저온자극 동안 열생산 능력이 감소되어 있었다. 또한, VLDLR 결핍 생쥐에서 열생산 능력이 손상되어 있음을 관찰했다. 갈색지방세포에서 VLDL 처리는 열생산 유전자 발현을 향상시키는 동시에 VLDLR 의존적 미토콘드리아 산소소비를 촉진하는 한다는 사실도 발견했다. 또한, VLDLR 결핍 생쥐에서 PPARδ 표적 유전자의 발현 수준이 갈색지방조직에서 감소 되어있는 반면, PPARδ agonist의 투여는 열생산이상과 미토콘드리아 기능장애를 회복시킨다는 실험결과를 얻었다. 더욱이, VLDL 의존적 열생산능은 갈색지방세포 특이적 PPARδ 결핍 생쥐 (PPARδ BKO)에서 약화되어 있음을 관찰했다. 이러한 데이터는 갈색지방조직 VLDL-VLDLR 축이 저온노출 동안 PPARδ 활성화를 유도하며 열생산에 관여할 수 있음을 시사한다.

본 연구를 통하여, 지방조직 VLDLR가 VLDL 흡수를 매개하며 지질대사뿐만 아니라 지방조직 신호 전달과정을 조절할 수 있음을 규명했다. 이를 통한 지방조직 염증반응 및 열생산 조절은, 지방조직 VLDLR가 대사환경 변화에 대한 적응 및 전신 에너지항상성을 조절할 수 있는 새로운 인자일 수 있음을 제안한다.

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학번: 2011-20339

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