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Original Research

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1,25-dihydroxyvitamin D_3 affects thapsigargin-induced endoplasmic reticulum stress in 3T3-L1 adipocytes

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ABSTRACT

BACKGROUND/OBJECTIVES: Endoplasmic reticulum (ER) stress in adipose tissue causes an inflammatory response and leads to metabolic diseases. However, the association between vitamin D and adipose ER stress remains poorly understood. In this study, we investigated whether 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) alleviates ER stress in adipocytes.
MATERIALS/METHODS: 3T3-L1 cells were treated with different concentrations (i.e., 10–100 nM) of $1,25(OH)_2D_3$ after or during differentiation (i.e., on day 0–7, 3–7, or 7). They were then incubated with thapsigargin (TG, 500 nM) for an additional 24 h to induce ER stress. Next, we measured the mRNA and protein levels of genes involved in unfold protein response (UPR) and adipogenesis using real-time polymerase chain reaction and western blotting and quantified the secreted protein levels of pro-inflammatory cytokines. Finally, the mRNA levels of UPR pathway genes were measured in adipocytes transfected with siRNA-targeting *Vdr*.
RESULTS: Treatment with $1,25(OH)_2D_3$ during various stages of adipocyte differentiation significantly inhibited ER stress induced by TG. In fully differentiated 3T3-L1 adipocytes, $1,25(OH)_2D_3$ treatment suppressed mRNA levels of *Ddit3*, *sXbp1*, and *Atf4* and decreased the secretion of monocyte chemoattractant protein-1, interleukin-6, and tumor necrosis factor-α. However, downregulation of the mRNA levels of *Ddit3*, *sXbp1*, and *Atf4* following $1,25(OH)_2D_3$ administration was not observed in *Vdr*-knockdown adipocytes. In addition, exposure of 3T3-L1 preadipocytes to $1,25(OH)_2D_3$ inhibited transcription of *Ddit3*, *sXbp1*, *Atf4*, *Bip*, and *Atf6* and reduced the p-alpha subunit of translation initiation factor 2 (eIF2α)/eIF2α and p-protein kinase RNA-like ER kinase (PERK)/PERK protein ratios. Furthermore, $1,25(OH)_2D_3$ treatment before adipocyte differentiation reduced adipogenesis and the mRNA levels of adipogenic genes.
CONCLUSIONS: Our data suggest that $1,25(OH)_2D_3$ prevents TG-induced ER stress and inflammatory responses in mature adipocytes by downregulating UPR signaling via binding with *Vdr*. In addition, the inhibition of adipogenesis by vitamin D may contribute to the reduction of ER stress in adipocytes.

Keywords: 1,25-dihydroxyvitamin D_3; endoplasmic reticulum stress; 3T3-L1 cells; adipocytes; thapsigargin; adipogenesis

Conflict of Interest
The authors declare no potential conflicts of interests.

Author Contributions
Conceptualization: Park CY; Data curation: Park CY, Wi D; Formal analysis: Park CY, Wi D; Writing - original draft: Park CY, Wi D; Writing - review & editing: Park CY, Wi D.

INTRODUCTION

Adipose tissue is a metabolically dynamic organ primarily composed of adipocytes. In obesity, adipose tissue dysfunction is characterized by adipocyte hypertrophy and chronic inflammation induced by macrophage infiltration, leading to lipid accumulation in non-adipose tissue, known as lipotoxicity, and the development of insulin resistance and type 2 diabetes [1,2]. While the mechanisms responsible for chronic inflammation and insulin-resistance in obese adipose tissue are not fully understood, endoplasmic reticulum (ER) stress has been implicated in adipose tissue inflammation and in obesity-induced insulin resistance in adipocytes [3-5].

The ER is a crucial organelle responsible for integrating multiple metabolic signals and maintaining cellular homeostasis, and also plays a role in lipid droplet formation [6]. ER stress occurs when unfolded protein responses (UPRs) are activated in response to various cellular stress conditions, including lipid accumulation, reactive oxidative stress, inhibition of protein glycosylation, imbalance of ER calcium, and the increased or misfolded protein synthesis [7]. UPRs are regulated by 3 ER membrane proteins: activating transcription factor (ATF) 6, inositol-requiring enzyme (IRE) 1, and protein kinase RNA-like ER kinase (PERK). These proteins become activated when UPRs accumulate in the ER lumen. In obesity, ER stress is induced in adipose tissue via increased PERK activation and phosphorylation of the alpha subunit of translation initiation factor 2 (eIF2α) [4,8]. Previous studies have shown that high-fat diet-induced obese mice and genetically obese (ob/ob) mice both exhibit increased UPR markers in their liver and adipose tissue [9]. ER stress leads to suppression of insulin receptor signaling (IRS) via hyperactivation of c-Jun N-terminal kinase (JNK) and IRS-1, resulting in insulin resistance and type 2 diabetes [9,10]. In adipocytes, ER stress induced by free fatty acid causes insulin resistance and inflammation mediated by reduced inhibitor of nuclear factor kappa-B (NF-κB) kinase subunit beta and JNK phosphorylation [11]. In addition, ER stress in 3T3-L1 adipocytes has been found to lead to upregulation of inflammatory cytokine gene expression [8,12]. Therefore, reducing ER stress in adipocytes may contribute to alleviating inflammatory responses in adipose tissue and insulin-resistant states [13].

The role of vitamin D in adipose tissue has been investigated due to the presence of the vitamin D receptor (VDR) and vitamin D metabolizing enzymes in adipocytes, as well as their associations with adipogenesis [14-16]. Vitamin D is a fat-soluble vitamin that can be stored in adipose tissue as cholecalciferol (i.e., vitamin D_3) and can regulate adipocyte differentiation and inflammatory responses in its active form, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$). Numerous studies have reported that $1,25(OH)_2D_3$ regulates adipocyte cellular activity by binding to the nuclear VDR with high affinity and thereafter modulating the transcription of genes involved in adipogenesis and inflammation [17]. Moreover, treatment with $1,25(OH)_2D_3$ has been shown to inhibit the production of interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, and IL-1β production and inactivate NF-κB by inducing IκBα in human adipocytes and 3T3-L1 cells. However, conflicting results have been observed regarding increased inflammatory cytokine levels in adipocytes treated with $1,25(OH)_2D_3$ [18-21]. Furthermore, vitamin D deficiency is associated with obesity and other metabolic diseases; for example, observational studies have reported an increased risk of obesity, type 2 diabetes, and impaired glucose metabolism in individuals with vitamin D deficiency [22-26]. Therefore, in addition to regulating inflammatory cytokines, vitamin D may play a pivotal role in mediating metabolic diseases, including those related to adipose tissue.

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Although the effects of vitamin D on ER stress have been investigated in epithelial cancer cells and macrophages [27,28], its impact on adipocyte ER stress remains unclear, despite the crucial role of ER stress in mediating metabolic diseases associated with obesity. Therefore, this study aimed to investigate whether $1,25(OH)_2D_3$ can inhibit an ER stress response induced by thapsigargin (TG) in 3T3-L1 adipocytes. To test this, we measured the expression levels of UPR response markers, adipogenesis, and inflammatory cytokine levels in response to vitamin D treatment during or after adipocyte differentiation prior to inducing ER stress.

MATERIALS AND METHODS

Cell culture and vitamin D treatment

3T3-L1 cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Waltham, MA, USA) containing 10% bovine calf serum (Gibco and 1% antibiotic-antimycotic [Gibco]) in a 95% air and 5% CO_2 incubator at 37°C. 3T3-L1 cells were then seeded in 6-well plates at a density of 3×10^4 cells and grown to reach 100% confluence. Once preadipocytes reached confluence (i.e., "day 0"), cells were differentiated by adding 0.5 μM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone, and 1 μg/mL insulin in DMEM containing 10% fetal bovine serum (Biowest, Nuaillé, France) for 3 days to induce differentiation. On day 3, insulin (1 μg/mL) alone was added for 2 more days. On day 5, the medium was replaced with DMEM (10% FBS) for 2 days, resulting in a total of 7 days of differentiation. On day 7, adipocytes were treated with a vehicle control (i.e., filtered 99.5% ethanol) or $1,25(OH)_2D_3$ (Sigma, St. Louis, MO, USA) dissolved in ethanol at various concentrations (i.e., 10, 50, or 100 nM). To examine the effect of $1,25(OH)_2D_3$ on ER stress when treated at different stages of adipocyte differentiation, the vehicle control (filtered 99.5% ethanol) or $1,25(OH)_2D_3$ was administered on day 0 (from day 0 to 7), day 3 (from day 3 to 7), or day 7 of adipocyte differentiation. In addition, fully differentiated adipocytes were incubated in serum-free media for 24 h with vehicle (control) or TG (500 nM; Sigma) to induce ER stress.

For each *in vitro* study, 3 different cell cultures per treatment were used, generating 3 technical replicate populations of 3T3-L1 preadipocytes. Cells and media were then collected for further analysis.

RNA interference of *Vdr*

3T3-LI adipocytes were first seeded into 6-well plates and fully differentiated. The expression of *Vdr* was then inhibited using siRNA oligonucleotides. To do so, fully differentiated adipocytes were transfected with siRNAs targeting *Vdr* (si*Vdr*; AccuTarget™ Genome-wide Predesigned siRNA, No. 22337-1, Bioneer, Daejeon, Korea) or with a non-targeting siRNA (AccuTargetTM Negative Control siRNA, SN-1001-CFG, Bioneer) using Lipofectamine RNAiMAX reagent (Invitrogen, Waltham, MA, USA), with all procedures performed according to the manufacturer's instructions. After 24 h, cells were treated with vehicle or $1,25(OH)_2D_3$ for 24 h, followed by TG for additional 24 h. The efficiency of the gene knockdown was determined via quantitative polymerase chain reaction (qPCR).

Total RNA extraction and real-time reverse transcription polymerase chain reaction

Total RNA was isolated from differentiated adipocytes using RNAiso Plus (Takara, Shiga, Japan), with all protocols following the manufacturer's instructions. The purity and

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Table 1. Primer sequences used for real-time qPCR analyses

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
Mouse *Ddit3*	ccaccacacctgaaagcagaa	aggtgaaaggcagggactca
Mouse *sXbp1*	tggaagaagagaaccacaaact	cattcccaagcgtgttcttaac
Mouse *Atf4*	caagaatgtaaaggggcaac	aatggatgacctggaaacca
Mouse *Atf6*	aagcatccgttctcatcacc	ggcagtgtggtctttcctgt
Mouse *Bip*	gacaagaaggaggatgtggg	gcatcgccaatcagacgctc
Mouse *Vdr*	gggatgatgggtaggttgtg	ggaagagggtagagggcaga
Mouse *Pparγ*	cagcaggttgtcttggatgtc	agccctttggtgactttctgg
Mouse *Cebpα*	cgcaagagccgagataaagc	gtcaactccagcaccttctgttg
Mouse *Fabp*	aagtgggagtgggctttgc	tggtgaccaaatccccattt
Mouse *Srebp1*	gtctccaccacttcgggttt	cgactacatccgcttcttgc
Mouse *Serca2b*	aaccaagccaaaacgaaaga	acacaaagaccgtggaggag
Mouse *Calnexin*	tcacataggcaccaccacat	agcttccaggggataaagga
Mouse *Pdia3*	gtggcatccatcttggctat	tctgaacccatcccagagtc
Mouse *Gapdh*	ggagaaacctgccaagta	aagagtgggagttgctgttg

qPCR, quantitative polymerase chain reaction.

concentration of RNA were evaluated using a Microvolume Spectrophotometer (DeNovix, Wilmington, DE, USA). Total RNA samples were reverse transcribed into cDNA using PrimeScript™II First strand cDNA synthesis kit (Takara). RT-qPCR was performed using a Roche Lightcycler 96 system (Roche, Basal, Switzerland) and TB green Premix Ex Taq (Takara). The relative mRNA expression levels of target genes were normalized to those of *Gapdh*. The primer sequences used to amplify *Ddit3*, *sXbp1*, *Atf4*, *Atf6*, *Bip*, *Vdr*, *Pparγ*, *Cebpα*, *Fabp*, *Srebp1*, *Serca2b, Calnexin*, *Pdia3*, and *Gapdh* are listed in **Table 1**.

Assessment of pro-inflammatory cytokine secretion by enzyme-linked immunosorbent assay (ELISA)

Next, we collected the medium from differentiated adipocytes to evaluate the secretion of pro-inflammatory cytokines. MCP-1, IL-6, and tumor necrosis factor (TNF)-α levels of the supernatants were determined using mouse ELISA kits (BD Bioscience, San Diego, CA, USA) with all procedures following the manufacturer's instructions. The results were normalized by total cell protein content and presented as cytokine protein amount (pg)/total protein (μg). Total cell protein contents were measured by bicinchoninic acid assay analysis. Briefly, 96-well plates were incubated with antibody overnight, then blocked with 200 μL Assay Diluent for 1 h. Standards and samples were then added and incubated for 2 h, followed by the addition of 100 μL of Working Detector. Finally, 100 μL of the Substrate and Stop Solutions were added to each well. The absorbance of each well was measured at 450 nm using a microplate spectrophotometer (Epoch, BioTek Instruments, Winooski, VT, USA).

Western blotting

Total proteins were extracted from 3T3-L1 cells using RIPA buffer (Biomax, Seoul, Korea) and a protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific, Waltham, MA, USA). The cell extract proteins were then separated on sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and transferred to polyvinylidene difluoride membranes. After blocking with 5% skim milk, the membrane was incubated overnight at 4°C with one of the following primary antibodies: anti-ATF6 (1:1,000, AB37149, Abcam, Cambridge, UK), anti-phospho-PERK (1:1,000, SAB5700521, Sigma), anti-PERK (1:1,000, C33E10, Cell Signaling, Danvers, MA, USA), anti-phospho-eIF2α (1:1,000, 9721, Cell Signaling), anti-eIF2α (1:1,000, 9722, Cell Signaling), or anti-β-actin (1:2,000, D6A8, Cell Signaling). Subsequently, each membrane was washed with TBS with 0.05% Tween-20 and incubated at room temperature with a horseradish peroxidase-linked rabbit secondary

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antibody (Cell Signaling, 7074) for 1 h. After incubation, membranes were stripped using a stripping buffer (Biomax). Fluorescent signals were visualized using West Glow FEMTO chemiluminescent substrate (Biomax) and were quantified using ImageJ software.

Oil red O staining

Differentiated 3T3-L1 cells were first washed with cold PBS and fixed in 10% formaldehyde for 15 min. After removing the cold PBS, the cells were washed with PBS 3 times and incubated with 0.1% Oil red O solution (Sigma) for 20 min at room temperature. The cells were then immediately washed with distilled water to remove the Oil red O solution. Stained cells were visualized by microscopy (Olympus, Ibaraki, Japan). To quantify lipid accumulation, the Oil red O in the cells was extracted using isopropanol for 1 min at room temperature to quantify lipid accumulation. The absorbance was measured at 510 nm using a microplate spectrophotometer (Epoch, BioTek Instruments).

Statistical analyses

All data are represented as mean ± SE. Statistical differences among experimental groups were determined by one-way analysis of variance followed by Least Significant Difference *post hoc* tests. Student's *t*-tests were used to comparing 2 groups to determine the effect of a specific treatment. For all statistical tests, $P < 0.05$ was used as the threshold of statistical significance. All statistical analyses were performed using SPSS version 26.0 (IBM SPSS Inc., Chicago, IL, USA).

RESULTS

Effect of $1,25(OH)_2D_3$ on mRNA expression of UPR-related genes in differentiated 3T3-L1 adipocytes

We first investigated whether $1,25(OH)_2D_3$ can alleviate the upregulation of UPR-related genes induced by TG in differentiated 3T3-L1 adipocytes (**Fig. 1**). To do so, fully differentiated 3T3-L1 adipocytes were treated with $1,25(OH)_2D_3$ (i.e., 0, 10, or 50 nM) for 24 h, followed by treatment with 500 nM TG for an additional 24 h to induce ER stress. $1,25(OH)_2D_3$ ranging from 10 to 100 nM have demonstrated no inhibitory effect on cell viability in either preadipocytes or mature adipocytes and is consistent with the physiological levels observed in humans [29,30]. TG significantly increased the mRNA expression levels of UPR-related genes by 2 to 35-fold, including for *Ddit3*, *sXbp1*, *Atf4*, *Atf6*, and *Bip*. We found that pretreatment with $1,25(OH)_2D_3$ prior to inducing ER stress significantly ameliorated the mRNA expression levels of *Ddit3*, *sXbp1*, and *Atf4,* when compared to only TG-treated adipocytes. For example, treatment with 10 nM $1,25(OH)_2D_3$ significantly lowered *Ddit3* mRNA expression ($P < 0.05$) and 50 nM of $1,25(OH)_2D_3$ significantly reduced the mRNA expression levels of *Ddit3*, *sXbp1*, and *Atf4* (all $P < 0.05$, vs TG-treated adipocytes). However, the mRNA expression levels of *Atf6* and *Bip* were not significantly affected by $1,25(OH)_2D_3$ pretreatment. Finally, we found that TG downregulated the mRNA levels of *Vdr* by 30% ($P < 0.05$, *t*-test), but pretreatment with $1,25(OH)_2D_3$ increased *Vdr* expression by 20 to 58-fold ($P < 0.01$).

Effect of $1,25(OH)_2D_3$ on pro-inflammatory cytokines secretion in differentiated 3T3-L1 adipocytes

Next, we investigated the effect of $1,25(OH)_2D_3$ treatment prior to ER stress induction on inflammatory cytokine secretion by differentiated 3T3-L1 adipocytes by measuring the protein levels of MCP-1, IL-6, and TNF-α in media (**Fig. 2**). We found that changes in

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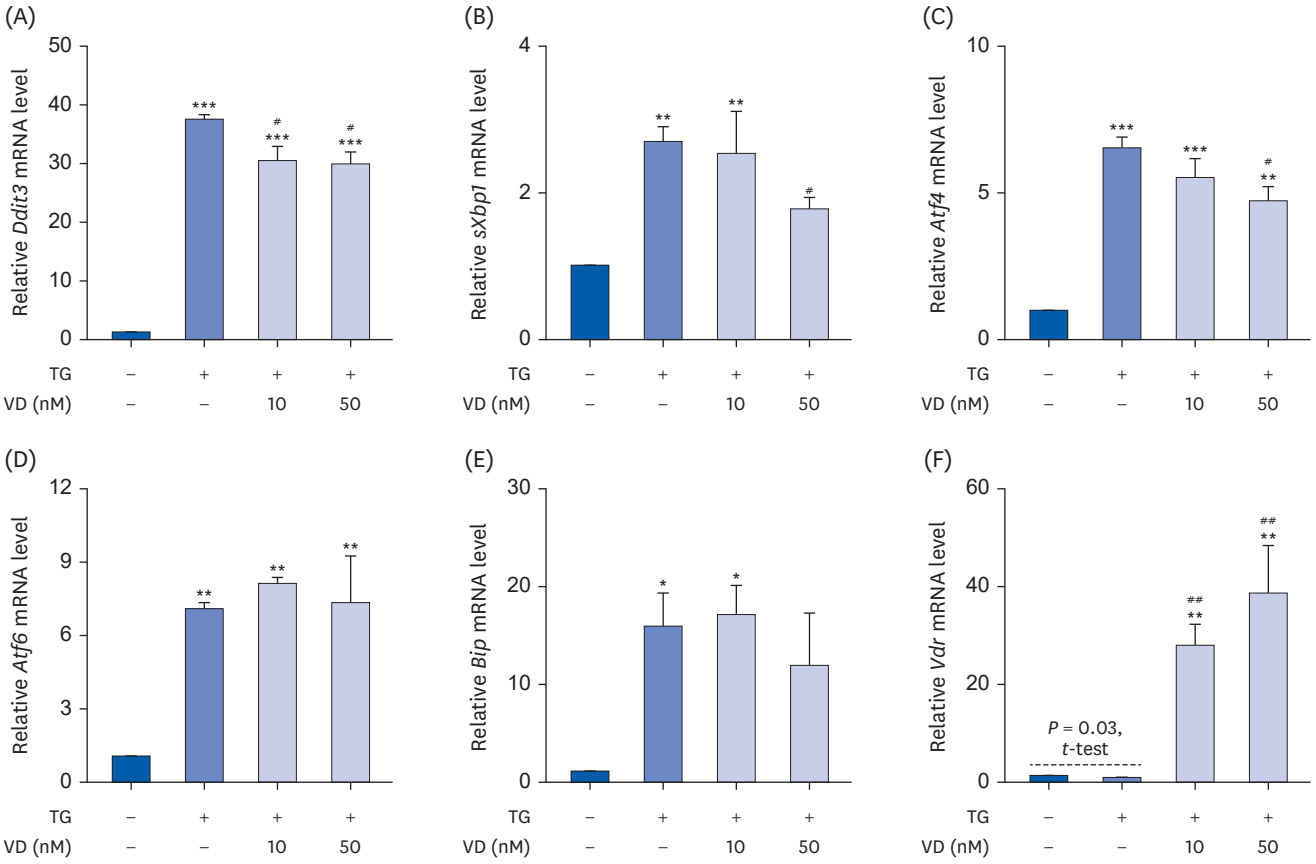

Fig. 1. Effects of VD on UPR-related genes in differentiated 3T3-L1 adipocytes. Fully differentiated 3T3-L1 adipocytes were treated with 10 or 50 nM VD for 24 h before being treated with 500 nM TG for another 24 h (3 technical replicates). Shown are the relative mRNA levels of the UPR-related genes (A) *Ddit3*, (B) *sXbp1*, (C) *Atf4*, (D) *Atf6*, (E) *Bip*, and (F) *Vdr* as measured by qPCR.
TG, thapsigargin; VD, 1,25-dihydroxyvitamin D_3; UPR, unfold protein response; qPCR, quantitative polymerase chain reaction.
$^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs. TG-treated.

pro-inflammatory cytokine levels induced by ER stressors or vitamin D in adipocytes. TG significantly upregulated secreted protein levels of MCP-1 ($P < 0.05$), IL-6 ($P < 0.001$), and TNF-α ($P < 0.001$). Treatment with 10 or 50 nM of $1,25(OH)_2D_3$ significantly downregulated the secreted protein levels of MCP-1 ($P < 0.01$, vs TG-treated adipocytes) and 10 nM of $1,25(OH)_2D_3$ significantly decreased the secreted protein levels of IL-6 and TNF-α (both $P < 0.05$, vs TG-treated adipocytes) in 3T3-L1 cells. Additionally, 50 nM of $1,25(OH)_2D_3$ slightly tended to reduce the protein level of IL-6 ($P = 0.09$).

Suppression of TG-induced ER stress by $1,25(OH)_2D_3$ is mediated by *Vdr* in differentiated 3T3-L1 adipocytes

The above results indicate that exposure of differentiated 3T3-L1 adipocytes to vitamin D alleviates the activation of ER stress. To identify whether the specific mechanism by which vitamin D acts in UPR signaling also involves VDR signaling, we used siRNA oligonucleotides targeting *Vdr* (**Fig. 3**). Transfection of differentiated 3T3-L1 adipocytes with *Vdr*-siRNA (si*Vdr*-cells) decreased the expression of the target gene by more than 60% compared to transfection with non-targeting siRNA (siCon-cells) (**Fig. 3A**). In si*Vdr*-cells, TG induced the upregulation of *Ddit3, Atf4*, *sXbp1, Atf6,* and *Bip* mRNA, but these levels were not affected by $1,25(OH)_2D_3$ treatment. In contrast, siCon-cells showed a significant 20–25% downregulation of *Ddit3, Atf4,* and *sXbp1* expression following $1,25(OH)_2D_3$ treatment (*Ddit3* and *Atf4: P* <

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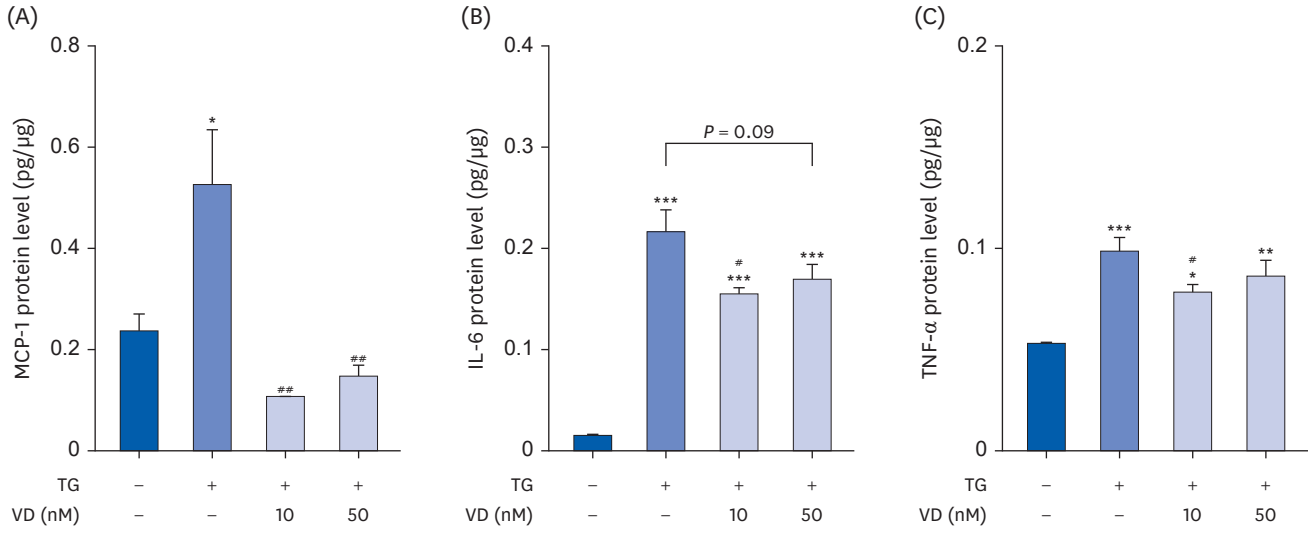

Fig. 2. Effects of VD on pro-inflammatory cytokines secretion in differentiated 3T3-L1 adipocytes. Fully differentiated 3T3-L1 adipocytes were treated with 10 or 50 nM VD for 24 h before being treated with 500 nM TG for another 24 h (3 technical replicates). The protein levels of (A) MCP-1, (B) IL-6, and (C) TNF-α in the cell supernatant were determined using enzyme-linked immunosorbent assay and normalized by the total cell protein content.
MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TG, thapsigargin; VD, 1,25-dihydroxyvitamin D_3.
$^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs. TG-treated.

0.01, *sXbp1:* $P < 0.05$, vs TG-treated siCon-cells, **Fig. 3B-D**). The mRNA levels of *Atf6* and *Bip* remained unaffected by $1,25(OH)_2D_3$ treatment both in siCon and si*Vdr* cells (**Fig. 3E and F**).

Effect of treatment with $1,25(OH)_2D_3$ at each stage of 3T3-L1 adipocytes differentiation on the UPR pathway

Next, we determined whether vitamin D treatment at each stage of adipocyte differentiation exhibited an inhibitory effect on UPR markers. To do so, 10 or 100 nM of $1,25(OH)_2D_3$ was used to treat preadipocytes (i.e., on day 0: from day 0 to 7), during differentiation (day 3: from day 3 to 7), or after differentiation (day 7) prior to inducing ER stress with TG (**Figs. 4** and **5**). Interestingly, treatment with 10 nM of $1,25(OH)_2D_3$ at the preadipocyte stage (day 0) significantly and clearly downregulated TG-induced *Ddit3*, *sXbp1*, *Atf4*, *Bip*, and *Atf6* mRNA expression by 30%–58%, when compared to only TG-treated adipocytes (**Fig. 4**). In addition to the mRNA levels of these UPR markers, we also found that the phosphorylation ratio of PERK and eIF2α and the protein level of p-PERK, p-eIF2α, and ATF6 were also significantly reduced by the 10 nM $1,25(OH)_2D_3$ treatment of preadipocytes (**Fig. 5**). When 10 nM of $1,25(OH)_2D_3$ was pretreated at day 3, the mRNA expression of *Ddit3*, *sXbp1*, *Atf4*, *Bip*, and *Atf6* was significantly reduced compared to adipocytes that were only treated with TG (**Fig. 4**). In addition, the protein level of ATF6 was significantly inhibited ($P < 0.001$) and ratio of p-PERK/PERK was tended to be decreased ($P = 0.051$) by $1,25(OH)_2D_3$ treatment on day 3 (**Fig. 5A and C**). Treatment with 10 nM of $1,25(OH)_2D_3$ after adipocyte differentiation only inhibited the mRNA levels of *Ddit3*($P < 0.001$), *sXbp1* ($P < 0.05$) and *Atf4* ($P < 0.05$) (**Fig. 4A-C**) and the phosphorylation ratio of eIF-2α ($P < 0.05$) (**Fig. 5B**). Phosphorylation of PERK and the protein level of ATF6 were not significantly decreased in response to $1,25(OH)_2D_3$ treatment in differentiated adipocytes (**Fig. 5A and C**).

Alteration of chaperone-related gene expression by $1,25(OH)_2D_3$ in ER stress-induced 3T3 adipocytes

Pdia3 and calnexin are chaperones that modulate the folding of newly synthesized proteins under ER stress and are also involved in the regulation of calcium homeostasis through their interaction with calcium pump *Serca2b* [31,32]. We investigated whether treatment with

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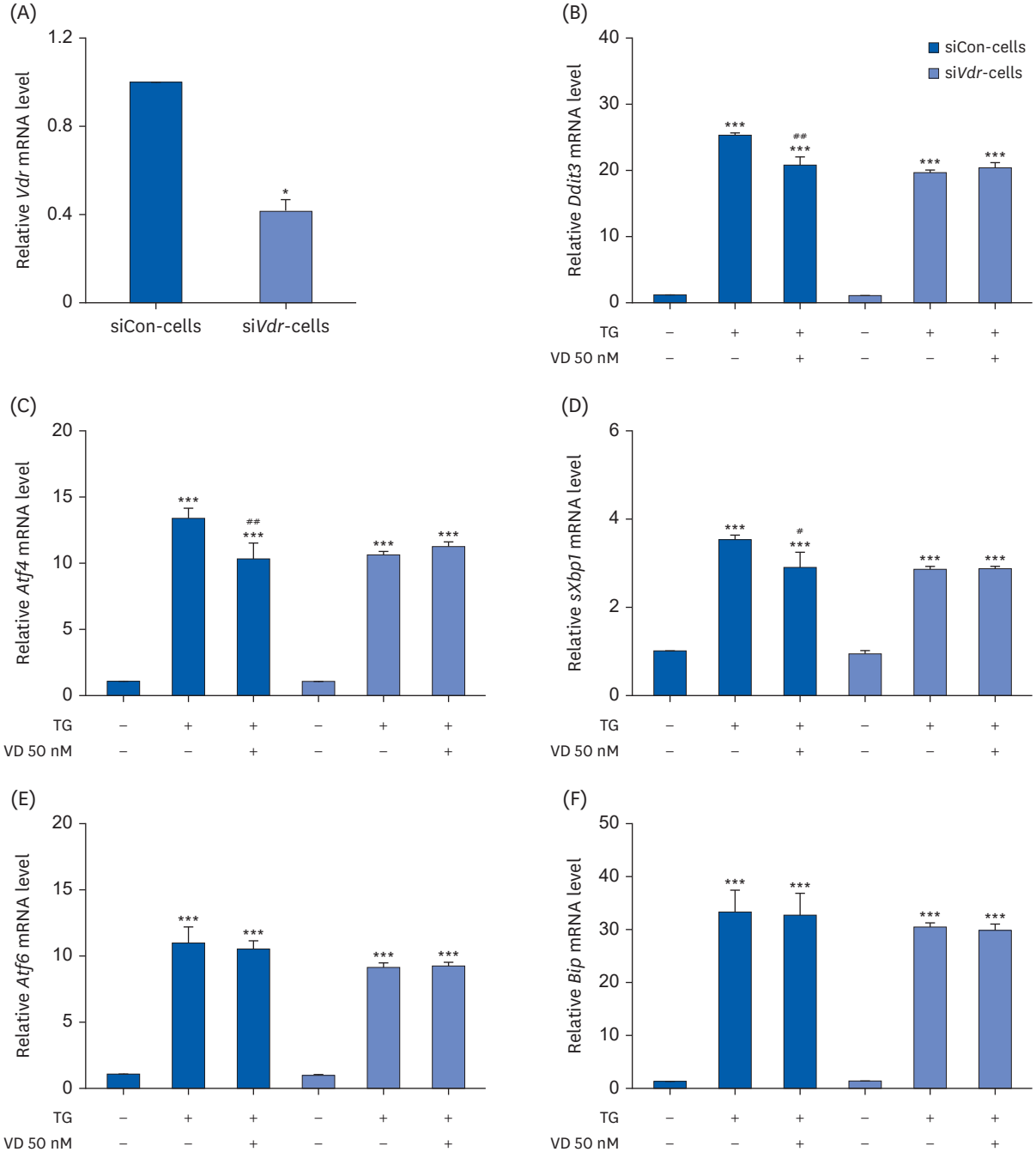

Fig. 3. Effects of VD on UPR-related genes in *Vdr*-knockdown 3T3-L1 adipocytes. Fully differentiated 3T3-L1 adipocytes were transfected with si*Vdr* or a siCon followed by treatment with vehicle or VD for 24 h (3 technical replicates). Shown are the relative mRNA levels of (A) *Vdr* and UPR-related genes: (B) *Ddit3*, (C) *Atf4*, (D) *sXbp1*, (E) *Atf6*, and (F) *Bip* as determined by qPCR analysis.
TG, thapsigargin; VD, 1,25-dihydroxyvitamin D_3; si*Vdr*-cells, siRNAs targeting *Vdr*; siCon-cells, non-targeting control siRNA; UPR, unfold protein response; qPCR, quantitative polymerase chain reaction.
$^{*}P < 0.05$ or $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$ or $^{\#\#}P < 0.01$ vs. TG-treated.

$1,25(OH)_2D_3$ prior to inducing ER stress had an impact on the mRNA expression of *Pdia3*, *Calnexin*, and *Serca2b* in 3T3-L1 adipocytes during (i.e., day 0: from day 0 to 7, day 3: day 3 to 7) and after (day 7) differentiation process (**Fig. 6**). TG consistently increased the mRNA levels of *Pdia3*, *Calnexin*, and *Serca2b* expression by 5 to 13-fold ($P < 0.001$).

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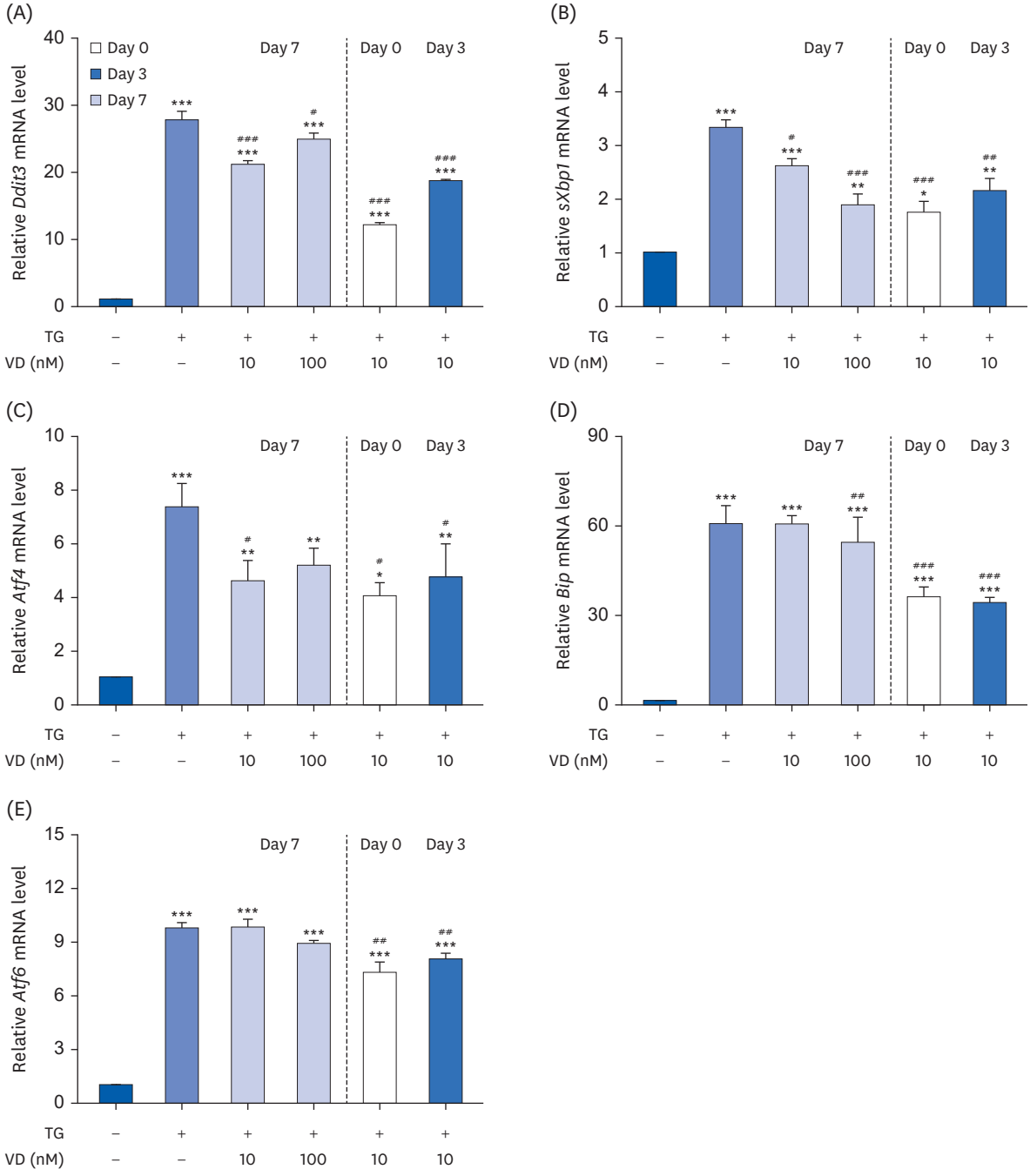

Fig. 4. Effect of VD during 3T3-L1 adipocyte differentiation on the mRNA levels of UPR-related genes. We used 10 or 100 nM of $1,25(OH)_2D$ to treat preadipocytes (day 0: from day 0 to 7), adipocytes during differentiation (day 3: from day 3 to 7), and adipocytes after differentiation (day 7) prior to treatment with 500 nM TG for 24 h (3 technical replicates). Shown are the relative mRNA levels of the UPR markers (A) *Ddit3*, (B) *sXbp1*, (C) *Atf4*, (D) *Bip*, and (E) *Atf6* as measured by qPCR. TG, thapsigargin; VD, 1,25-dihydroxyvitamin D_3; UPR, unfold protein response; qPCR, quantitative polymerase chain reaction.
$^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, or $^{\#\#\#}P < 0.001$ vs. TG-treated.

Treatment with $1,25(OH)_2D_3$ during (day 3) or after (day 7) adipocyte differentiation significantly reduced the mRNA levels of *Pdia3* (vs TG-treated adipocytes). Furthermore, $1,25(OH)_2D_3$ downregulated *Serca2b* mRNA expression only when it was administered before (day 0) or during (day 3) adipocyte differentiation (both $P < 0.01$, vs TG-treated adipocytes). However, the upregulation of calnexin mRNA expression by TG was not affected by $1,25(OH)_2D_3$.

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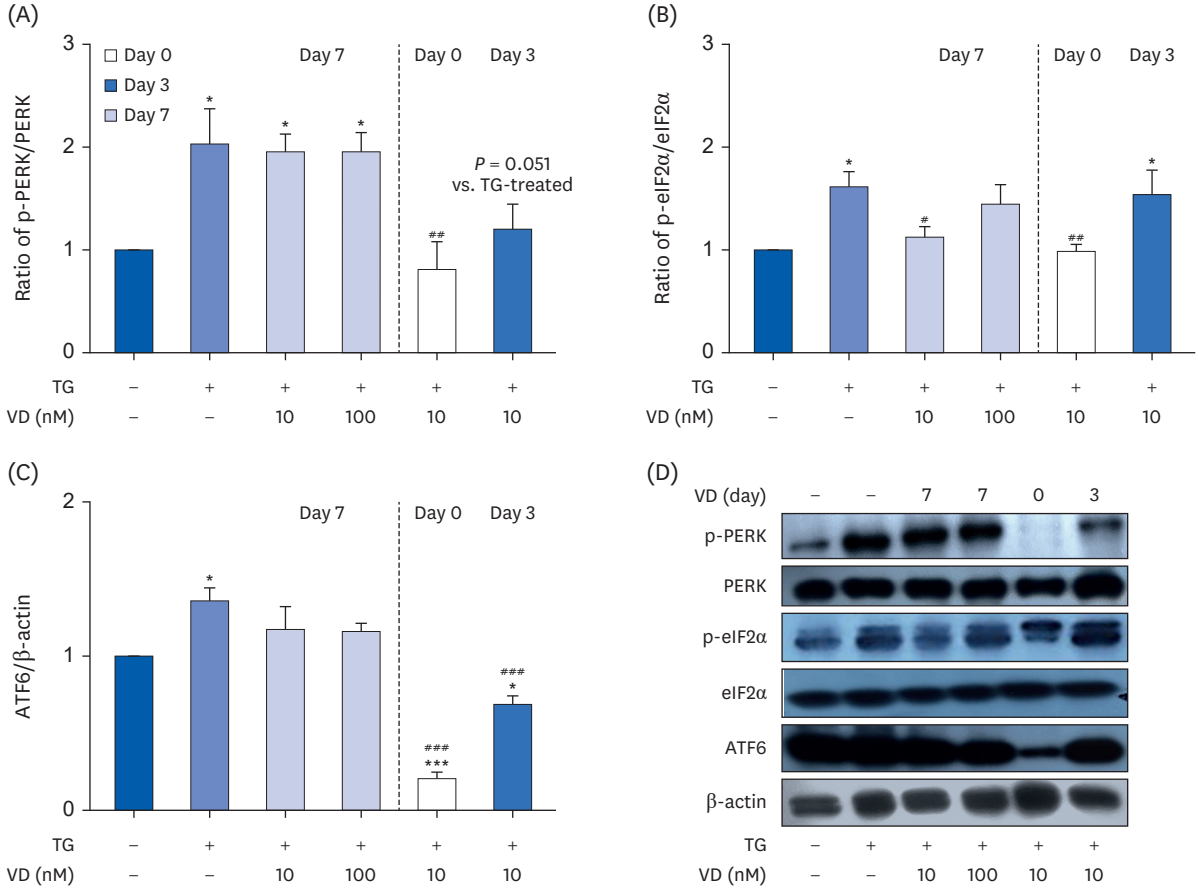

Fig. 5. Effect of VD during 3T3-L1 adipocyte differentiation on protein levels involved in the UPR pathway. We used 10 or 100 nM of $1,25(OH)_2D$ to treat preadipocytes (day 0: from day 0 to 7), adipocytes during differentiation (day 3: from day 3 to 7), and adipocytes after differentiation (day 7) prior to treatment with 500 nM TG for 24 h (3 technical replicates). Shown are the phosphorylation ratio of (A) PERK and (B) eIF2α and protein level of (C) ATF6 as estimated by western blot analysis. β-actin was used a loading control. Also shown are (D) Densitometry results of p-PERK, PERK, p-eIF2α, eIF2α, ATF6, and β-actin.
TG, thapsigargin; VD, 1,25-dihydroxyvitamin D_3; PERK, protein kinase RNA-like ER kinase; eIF2α, alpha subunit of translation initiation factor 2; ATF6, activating transcription factor 6; UPR, unfold protein response.
$^{*}P < 0.05$ or $^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, or $^{\#\#\#}P < 0.001$ vs. TG-treated.

Effect of treatment with $1,25(OH)_2D_3$ at each stage of 3T3-L1 adipocytes differentiation on adipogenesis in ER stress-induced 3T3-L1 adipocytes

Next, to examine whether the inhibitory effect of UPR markers by vitamin D is associated with the regulation of adipogenesis and lipogenesis, we measured lipid accumulation and the mRNA expression of genes involved in adipogenesis (**Fig. 7**). 3T3-L1 adipocytes were treated with $1,25(OH)_2D_3$ at each stage of adipocyte differentiation (i.e., day 0: day 0–7, day 3: day 3–7, or day 7) prior to the addition of TG. TG treatment of differentiated adipocytes was found to decrease lipid accumulation by 5% ($P < 0.05$, **Fig. 7A and B**) and cause the downregulation of *Pparγ*, *Cebpα*, *Fabp*, and *Srebp1* mRNA levels (**Fig. 7C-F**).

The effect of $1,25(OH)_2D_3$ on adipogenesis differed depending on the time point of its treatment during differentiation. Treatment of preadipocytes (day 0) with $1,25(OH)_2D_3$ not only clearly inhibited 80% of lipid accumulation (**Fig. 7A and B**) but also significantly reduced *Pparγ*, *Cebpα*, and *Fabp* mRNA levels (all $P < 0.001$, vs TG-treated adipocytes, **Fig. 7C-E**). However, the 10 nM $1,25(OH)_2D_3$ treatment on day 3 or day 7 did not significantly alter lipid accumulation (**Fig. 7A**). The mRNA levels of *Cebpα* and *Fabp* were significantly downregulated by $1,25(OH)_2D_3$ treatment on both day 3 and day 7 (vs TG-treated adipocytes).

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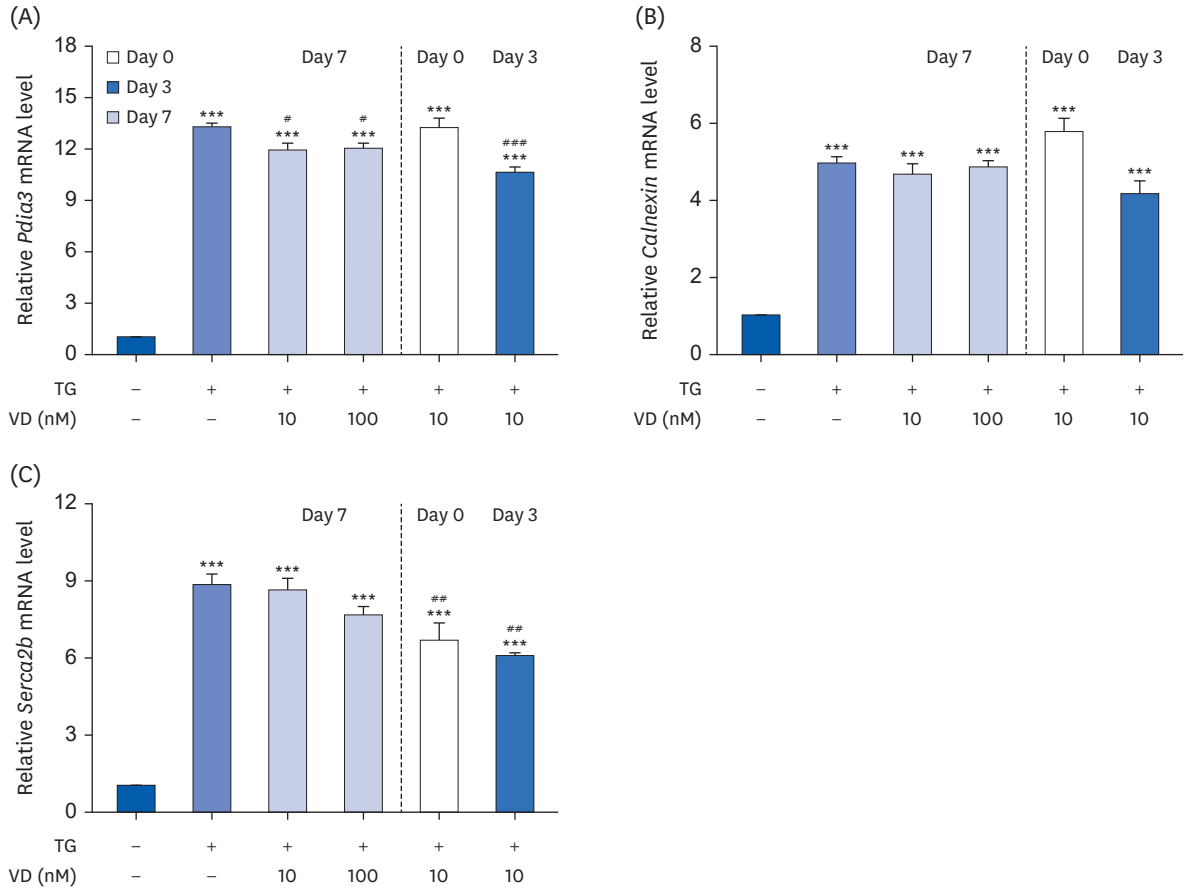

Fig. 6. Effect of VD during 3T3-L1 adipocyte differentiation on chaperon-related gene expression. We used 10 or 100 nM of 1,25(OH)$_2$D to treat preadipocytes (day 0: from day 0 to 7), adipocytes during differentiation (day 3: from day 3 to 7), and adipocytes after differentiation (day 7) prior to treatment with 500 nM TG for 24 h (3 technical replicates). Shown are the relative mRNA levels of the (A) *Pdia3*, (B) *Calnexin*, and (C) *Serca2b* as measured by qPCR.
TG, thapsigargin; VD, 1,25-dihydroxyvitamin D$_3$; qPCR, quantitative polymerase chain reaction.
$^{***}P < 0.001$ vs. Control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, or $^{\#\#\#}P < 0.001$ vs. TG-treated.

DISCUSSION

In this study, we demonstrate first that pretreatment with vitamin D can alleviate TG-induced ER stress via *Vdr* signaling in 3T3-L1 adipocytes, leading to reduced inflammatory responses (**Fig. 8**). Vitamin D treatment before adipocyte differentiation further reduced activation and expression of UPR-related proteins and *Pdia3.* ER stress occurring in adipocytes is associated with adipose tissue inflammation that is related to the development of insulin resistance and metabolic diseases. This study also showed that vitamin D treatment of adipocytes prior to inducing ER stress can ameliorate the inflammatory responses by reducing MCP-1, IL-6, and TNF-α levels and can inhibit adipogenesis by downregulating *Cebpα*, *Pparγ*, and *Fabp* expression.

TG is the non-competitive inhibitor of sarcoplasmic/ER Ca^{2+} ATPase (SERCA) that causes ER stress by depleting ER calcium stores and activating UPR. Several *in vitro* studies using 3T3-L1 cells have used TG to induce ER stress to mimic the effect of physiological cell stimuli related to calcium, including cell injury, and cellular aging [33-37]. In this study, TG induced ER stress by significantly upregulating proteins associated with the UPR pathway via 3 transmembrane receptors in the ER, consistent with previous findings that have shown ER stress induction in adipocytes using TG concentration exceeding 500 nM [38-41]. Nevertheless, the use of TG at a

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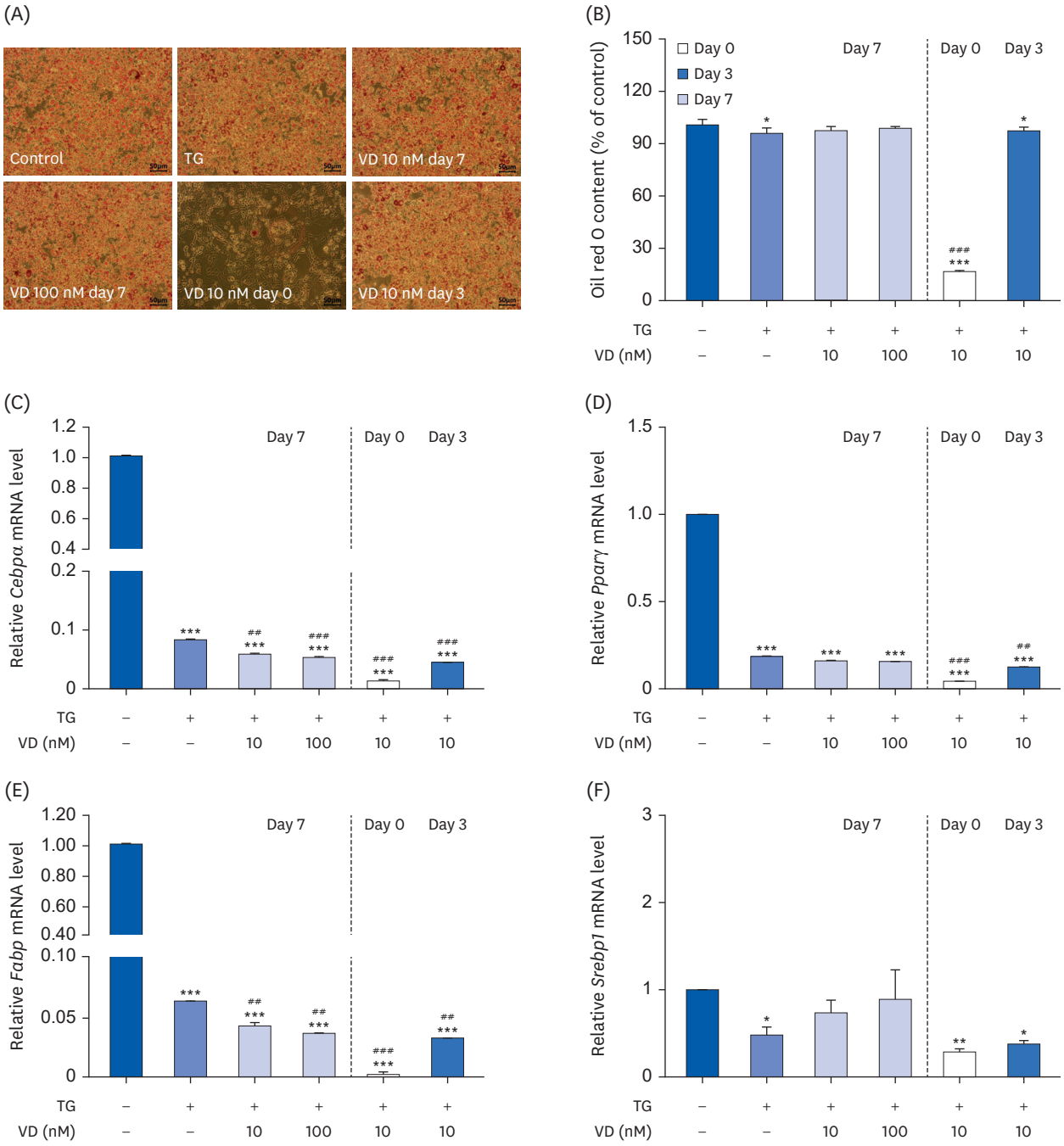

Fig. 7. Effect of VD during adipocyte differentiation on adipogenesis in ER stress-induced 3T3 adipocytes. We used 10 or 100 nM of $1,25(OH)_2D$ to treat preadipocytes (day 0: from day 0 to 7), adipocytes during differentiation (day 3: from day 3 to 7), and adipocytes after differentiation (day 7) prior to treatment with 500 nM TG for 24 h (3 technical replicates). Lipid accumulation was determined by oil red O staining. Microscopic images of oil red O staining were then (A) visualized and (B) quantified. Also shown (C)-(F) are the relative mRNA levels of the adipogenesis markers *Cebpα*, *Pparγ*, *Fabp*, and *Srebp1* as measured by qPCR.
TG, thapsigargin; VD, 1,25-dihydroxyvitamin D_3; ER, endoplasmic reticulum; qPCR, quantitative polymerase chain reaction.
$^{*}P < 0.05$, $^{**}P < 0.01$, or $^{***}P < 0.001$ vs. Control; $^{\#\#}P < 0.01$ or $^{\#\#\#}P < 0.001$ vs. TG-treated.

concentration of 500 nM in this study surpasses the physiological range, potentially resulting in a decline in cell viability. However, a prior study examining the influence of vitamin D on TG-induced ER stress in epithelial cells indicated that although ER stress-driven apoptosis led to a moderate reduction in cell viability, the substantial escalation in ER stress helped

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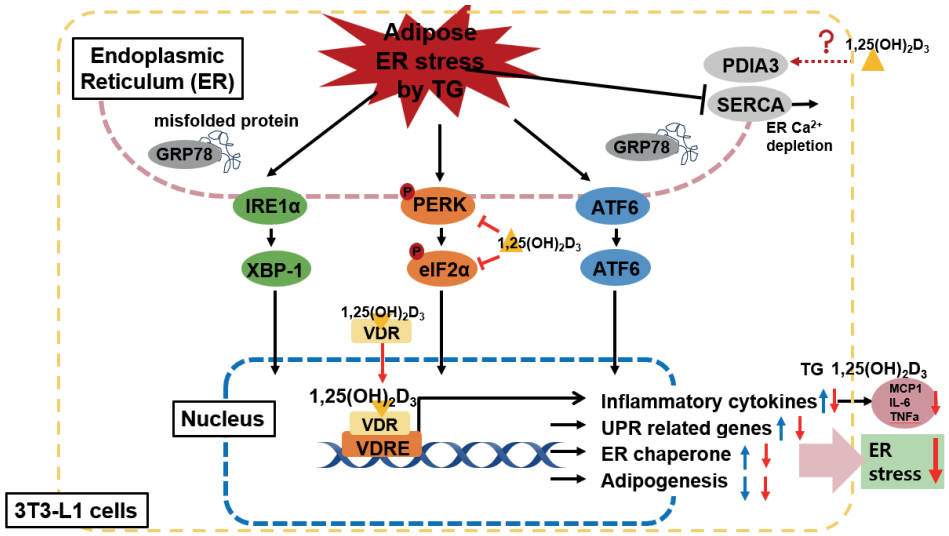

Fig. 8. 1,25(OH)$_2$D$_3$ may prevent TG-induced ER stress in adipocytes. The red arrow indicates regulation by 1,25(OH)$_2$D$_3$ treatment, while the blue arrow indicates regulation by TG.
ER, endoplasmic reticulum; TG, thapsigargin; GRP78, glucose-regulated protein 78; IRE1α, inositol-requiring enzyme 1α; XBP-1, X-box binding protein-1; 1,25(OH)$_2$D$_3$, 1,25-dihydroxyvitamin D$_3$; VDR, vitamin D receptor; VDRE, vitamin D response element; PERK, protein kinase RNA-like ER kinase; eIF2α, alpha subunit of translation initiation factor 2; ATF6, activating transcription factor 6; PDIA3, protein disulfide isomerase A3; SERCA, sarcoplasmic/ER Ca^{2+} ATPase; UPR, unfold protein response; MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

counterbalance any potential biases resulting from the decline in cell viability effects [27]. In the current study, treatment with TG in differentiated adipocytes notably increased the mRNA expression levels of *Ddit3*, *sXbp1*, *Atf4*, *Atf6*, and *Bip* by 2 to 35-fold, along with a rise in the protein levels of p-PERK, p-eIF2*α,* and ATF6 by 1 to 1.8-fold.

Pretreatment of differentiated adipocytes with 1,25(OH)$_2$D$_3$ inhibited ER stress by reducing the mRNA expression levels of *Ddit3*, *sXbp1*, and *Atf4* and suppressing the protein ratio of p-eIF2α/eIF2α. These results indicate that vitamin D alleviates ER stress by regulating the PERK/eIF2α/ATF4/C/EBP homologous protein (CHOP, another name of DDIT3) signaling pathway. Previous studies using epithelial cells, macrophages, and pancreatic beta cells have investigated the inhibitory effect of vitamin D on ER stress, and 1,25(OH)$_2$D$_3$ has been previously found to inhibit ER stress by suppressing UPR activation which is consistent with results in this study [27,28,42,43]. In human mammary epithelial MCF-7 cells, 100 nM 1,25(OH)$_2$D$_3$ treatments have been found to inhibit TG-induced mRNA levels of *Atf4*, *Ddit3, PERK,* and *sXbp1* [27]. In monocytes and macrophages isolated from type 2 diabetic patients, vitamin D has also been found to suppress ER stress by downregulating the phosphorylation of PERK and IRE1α and the protein level of CHOP [28]. Moreover, Hu *et al.* [42] reported that 1,25(OH)$_2$D$_3$ can protect pancreatic beta cells from H_2O_2-induced ER stress via inhibition of the PERK/ATF4/CHOP pathway. Prolonged ER stress has also been found to induce apoptosis and inflammatory responses via CHOP through the activation of the PERK. Furthermore, this has been implicated in many chronic diseases including cardiovascular and neurogenerative disorders [8,44-46]. In addition, chronic ER stress in adipose tissue can be induced by increased demand for protein synthesis under nutrient excess and has been linked to inflammatory responses and insulin resistance [8,47]. We also found that protein levels of MCP-1, IL-6, and TNF-α were significantly inhibited by 1,25(OH)$_2$D$_3$ pretreatment prior to inducing ER stress. In adipocytes or macrophages in microenvironments mimicking obese adipose tissue, vitamin

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D has been reported to reduce the levels of pro-inflammatory cytokines, including MCP-1, IL-6, and IL-1β by regulating toll-like receptors and mitogen-activated protein kinase (MAPK) signaling [18,48-50]. Further studies are required to investigate the role of vitamin D in the mechanism of inflammatory cytokine secretion during ER stress.

Furthermore, using 3T3-L1 cells transfected with *Vdr*-siRNA, we proved that suppression of *Ddit3*, *sXbp1*, and *Atf4* mRNA levels by vitamin D against TG occurs via *Vdr*. In *Vdr*-knockdown differentiated adipocytes, $1,25(OH)_2D_3$ did not inhibit UPR signaling induced by TG. Consistent with these results, measuring secreted alkaline phosphatase activity in HUVEC cells showed that the suppression of VDR expression blocks the ER stress inhibition effect caused by vitamin D [43]. In another study, macrophages transfected with *Vdr*-siRNA showed upregulated protein levels of p-PERK, CHOP, and IRE1α relative to macrophages transfected with control siRNA [28]. Although our results did not show a significant difference in UPR-related gene expression between *Vdr*-siRNA-treated and Con-siRNA-treated 3T3-L1 adipocytes, the ER stress preventive effect caused by vitamin D present in Con-siRNA adipocytes was not observed in *Vdr*-knockdown adipocytes. In addition, we found that TG-induced ER stress can downregulate 30% of 3T3-L1 *Vdr* mRNA expression, which was increased by $1,25(OH)_2D_3$ pretreatment prior to inducing ER stress. This suggests that increased $1,25(OH)_2D_3$-VDR interactions can regulate UPR signaling. However, *Vdr* mRNA expression did not differ between 10 or 50 nM of $1,25(OH)_2D_3$ pretreatment. This might contribute to the absence of a dose-response effect on several gene expression following vitamin D pretreatment.

ER stress was regulated not only by genomic pathway through $1,25(OH)_2D_3$-*Vdr* interactions but also partially by nongenomic pathway involving protein disulfide isomerase A3 (PDIA3). PDIA3, located on the cell membrane, mediates $1,25(OH)_2D_3$-dependent membrane signaling cascade, exerting nongenomic action. Through these mechanisms, they can modulate MAPK pathways and the activity of transcription factors such as NF-κB, STAT3, and p53, which are key players in inflammatory pathways [31]. Furthermore, PDIA3, calnexin, and calreticulin are chaperones that facilitate protein folding in response to excessive ER stress [51]. In this study, we observed that the elevated *Pdia3* mRNA expression induced by TG was mitigated by pretreatment with $1,25(OH)_2D_3$. In this manner, vitamin D seems to indirectly contribute to the suppression of ER stress and inflammatory responses.

Vitamin D treatment of preadipocytes (from day 0 to 7) showed a strong preventive effect against ER stress by downregulating all 3 UPR protein branches. In addition to the mRNA expression levels of *Ddit3, sXbp1,* and *Atf4* and the protein levels of the p-eIF2α/eIF2α and p-PERK/PERK ratios, we also found that vitamin D pretreatment on preadipocytes or in the early phase of adipocyte differentiation suppressed *Bip* mRNA expression and ATF6 protein and mRNA levels, which were not altered by $1,25(OH)_2D_3$ treatment on differentiated adipocytes. In addition, vitamin D treatment of preadipocytes prior to inducing ER stress blocked adipogenesis by blocking lipid accumulation and downregulation of mRNA expression levels of *Pparγ, Cebpα,* and *Fabp*. Peroxisome proliferator-activated receptor-gamma (PPARγ) and C/EBPα are 2 essential adipogenic transcription factors that promote and sustain the differentiation status of adipose cells [33,52]. $1,25(OH)_2D_3$ has been reported to regulate adipocyte differentiation via binding with VDR, resulting in the inhibition of adipogenesis and lipid droplet formation [53-55]. Moreover, $1,25(OH)_2D_3$ blocks adipogenesis especially the early stage of adipocyte differentiation by blocking C/EBPα and PPARγ, and this inhibition was less effective 48 h after the initiation of differentiation because mRNA

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level of *Vdr* increased to a maximum in early phase of differentiation [14,53]. Previous studies have demonstrated adipogenesis of 3T3-L1 cell can upregulate ER stress [12,35]. Therefore, the suppression of adipogenesis when preadipocytes were treated with $1,25(OH)_2D_3$ might be associated with ER stress inhibition via vitamin D-VDR pathway.

In vitro adipocyte differentiation is accompanied by the induction of ER stress, and obesity leads to chronic ER stress [4,12,35]. However, TG treatment of differentiated adipocytes is associated with a slight reduction in lipid accumulation (≤ 5%) and downregulation of *Pparγ*, *Cebpα*, *Fabp*, and *Srebp1* expression. Acute ER stress exceeding physiological levels inhibits adipocyte differentiation and negatively affects the functioning of adipose tissue; this promotes the development of type 2 diabetes and lipotoxicity [56]. Exogenous ER stressor have been reported to attenuate adipogenesis through signaling via CHOP, although this may be different from signaling pathway activated by vitamin D-VDR. Forced production of CHOP inhibited adipogenesis in 3T3-L1 cells and mice with deletion of *Chop*$^{-/-}$ gained more fat mass than wild-type mice on HFD indicating the role of CHOP in adipogenesis [35]. Therefore, while the attenuation of endogenous ER stress by vitamin D might have counteracted the effect of TG on the inhibition of adipocyte differentiation, the mechanism underlying the early stages of adipogenesis inhibition by vitamin D-VDR could plausibly operate independently of the pathway through which TG suppresses adipocyte differentiation. Further studies are needed to understand the association between the downregulation of adipogenesis and suppression of ER stress both induced by vitamin D during obesity and during the development of metabolic diseases.

This study is the first to confirm the suppression of TG-induced ER stress by vitamin D in adipocytes. ER stress and UPR signaling in adipocytes are closely linked to obesity-related metabolic diseases, including type 2 diabetes [4,9]. Therefore, vitamin D deficiency in diabetes and the association between adiposity and vitamin D status that has been observed by many clinical studies may be related to effects exerted by vitamin D as it regulates ER stress in adipocytes [22-24,26]. However, we only have demonstrated TG to induce ER stress in adipocytes, making it challenging to generalize that vitamin D can inhibit all forms of ER stress in adipocytes. ER stress induced by TG is associated with a calcium imbalance between the cytosol and ER via inhibition of the Ca^{2+} pump SERCA2b. The uptake of calcium by the ER via SERCA2b is regulated by PDIA3 and vitamin D [57]. Our study showed a significant increase in *Serca2b* mRNA expression in response to TG while vitamin D treatment led to its downregulation, despite TG's inhibitory role of SERCA2b protein. This observation suggests a potential compensatory mechanism aimed at maintaining calcium homeostasis in ER. Further research is required to elucidate the intricate relationship between calcium metabolism, ER stress, and vitamin D.

Taken together, our results suggest that $1,25(OH)_2D_3$ treatment can alleviate TG-induced ER stress by downregulating UPR markers in differentiated 3T3-L1 adipocytes, and that this effect is mediated by $1,25(OH)_2D_3$-VDR signaling. In addition, the inhibitory effect of $1,25(OH)_2D_3$ on UPR markers is associated with the suppression of inflammatory cytokine production, along with the modulation of *Pdia3* and adipogenesis in ER stress-induced adipocytes, but this depends on the stage of adipocyte differentiation at which the treatment is administered.

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REFERENCES

1. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011;11:85-97. PUBMED | CROSSREF
2. Choe SS, Huh JY, Hwang IJ, Kim JI, Kim JB. Adipose tissue remodeling: its role in energy metabolism and metabolic disorders. Front Endocrinol (Lausanne) 2016;7:30. PUBMED | CROSSREF
3. Bogdanovic E, Kraus N, Patsouris D, Diao L, Wang V, Abdullahi A, Jeschke MG. Endoplasmic reticulum stress in adipose tissue augments lipolysis. J Cell Mol Med 2015;19:82-91. PUBMED | CROSSREF
4. Cnop M, Foufelle F, Velloso LA. Endoplasmic reticulum stress, obesity and diabetes. Trends Mol Med 2012;18:59-68. PUBMED | CROSSREF
5. Ghemrawi R, Battaglia-Hsu SF, Arnold C. Endoplasmic reticulum stress in metabolic disorders. Cells 2018;7:63. PUBMED | CROSSREF
6. Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. Nat Rev Mol Cell Biol 2019;20:137-55. PUBMED | CROSSREF
7. Flamment M, Hajduch E, Ferré P, Foufelle F. New insights into ER stress-induced insulin resistance. Trends Endocrinol Metab 2012;23:381-90. PUBMED | CROSSREF
8. Kawasaki N, Asada R, Saito A, Kanemoto S, Imaizumi K. Obesity-induced endoplasmic reticulum stress causes chronic inflammation in adipose tissue. Sci Rep 2012;2:799. PUBMED | CROSSREF
9. Ozcan U, Cao Q, Yilmaz E, Lee AH, Iwakoshi NN, Ozdelen E, Tuncman G, Görgün C, Glimcher LH, Hotamisligil GS. Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. Science 2004;306:457-61. PUBMED | CROSSREF
10. Liang L, Chen J, Zhan L, Lu X, Sun X, Sui H, Zheng L, Xiang H, Zhang F. Endoplasmic reticulum stress impairs insulin receptor signaling in the brains of obese rats. PLoS One 2015;10:e0126384. PUBMED | CROSSREF
11. Jiao P, Ma J, Feng B, Zhang H, Diehl JA, Chin YE, Yan W, Xu H. FFA-induced adipocyte inflammation and insulin resistance: involvement of ER stress and IKKβ pathways. Obesity (Silver Spring) 2011;19:483-91. PUBMED | CROSSREF
12. Longo M, Spinelli R, D'Esposito V, Zatterale F, Fiory F, Nigro C, Raciti GA, Miele C, Formisano P, Beguinot F, et al. Pathologic endoplasmic reticulum stress induced by glucotoxic insults inhibits adipocyte differentiation and induces an inflammatory phenotype. Biochim Biophys Acta 2016;1863:1146-56. PUBMED | CROSSREF
13. Basseri S, Lhoták S, Sharma AM, Austin RC. The chemical chaperone 4-phenylbutyrate inhibits adipogenesis by modulating the unfolded protein response. J Lipid Res 2009;50:2486-501. PUBMED | CROSSREF
14. Wood RJ. Vitamin D and adipogenesis: new molecular insights. Nutr Rev 2008;66:40-6. PUBMED | CROSSREF
15. Abbas MA. Physiological functions of vitamin D in adipose tissue. J Steroid Biochem Mol Biol 2017;165:369-81. PUBMED | CROSSREF
16. Wamberg L, Christiansen T, Paulsen SK, Fisker S, Rask P, Rejnmark L, Richelsen B, Pedersen SB. Expression of vitamin D-metabolizing enzymes in human adipose tissue -- the effect of obesity and diet-induced weight loss. Int J Obes (Lond) 2013;37:651-7. PUBMED | CROSSREF
17. Park CY, Han SN. The role of vitamin D in adipose tissue biology: adipocyte differentiation, energy metabolism, and inflammation. J Lipid Atheroscler 2021;10:130-44. PUBMED | CROSSREF
18. Marcotorchino J, Gouranton E, Romier B, Tourniaire F, Astier J, Malezet C, Amiot MJ, Landrier JF. Vitamin D reduces the inflammatory response and restores glucose uptake in adipocytes. Mol Nutr Food Res 2012;56:1771-82. PUBMED | CROSSREF
19. Sun X, Zemel MB. Calcitriol and calcium regulate cytokine production and adipocyte-macrophage cross-talk. J Nutr Biochem 2008;19:392-9. PUBMED | CROSSREF
20. Sun X, Zemel MB. Calcium and 1,25-dihydroxyvitamin D3 regulation of adipokine expression. Obesity (Silver Spring) 2007;15:340-8. PUBMED | CROSSREF
21. Karkeni E, Marcotorchino J, Tourniaire F, Astier J, Peiretti F, Darmon P, Landrier JF. Vitamin D limits chemokine expression in adipocytes and macrophage migration in vitro and in male mice. Endocrinology 2015;156:1782-93. PUBMED | CROSSREF
22. Cheng S, Massaro JM, Fox CS, Larson MG, Keyes MJ, McCabe EL, Robins SJ, O'Donnell CJ, Hoffmann U, Jacques PF, et al. Adiposity, cardiometabolic risk, and vitamin D status: the Framingham Heart Study. Diabetes 2010;59:242-8. PUBMED | CROSSREF
23. Mai XM, Chen Y, Camargo CA Jr, Langhammer A. Cross-sectional and prospective cohort study of serum 25-hydroxyvitamin D level and obesity in adults: the HUNT study. Am J Epidemiol 2012;175:1029-36. PUBMED | CROSSREF

24. Pereira-Santos M, Costa PR, Assis AM, Santos CA, Santos DB. Obesity and vitamin D deficiency: a systematic review and meta-analysis. Obes Rev 2015;16:341-9. PUBMED | CROSSREF

25. Roth CL, Elfers CT, Figlewicz DP, Melhorn SJ, Morton GJ, Hoofnagle A, Yeh MM, Nelson JE, Kowdley KV. Vitamin D deficiency in obese rats exacerbates nonalcoholic fatty liver disease and increases hepatic resistin and Toll-like receptor activation. Hepatology 2012;55:1103-11. PUBMED | CROSSREF

26. Mezza T, Muscogiuri G, Sorice GP, Prioletta A, Salomone E, Pontecorvi A, Giaccari A. Vitamin D deficiency: a new risk factor for type 2 diabetes? Ann Nutr Metab 2012;61:337-48. PUBMED | CROSSREF

27. Wen G, Eder K, Ringseis R. 1,25-hydroxyvitamin D3 decreases endoplasmic reticulum stress-induced inflammatory response in mammary epithelial cells. PLoS One 2020;15:e0228945. PUBMED | CROSSREF

28. Riek AE, Oh J, Sprague JE, Timpson A, de las Fuentes L, Bernal-Mizrachi L, Schechtman KB, Bernal-Mizrachi C. Vitamin D suppression of endoplasmic reticulum stress promotes an antiatherogenic monocyte/macrophage phenotype in type 2 diabetic patients. J Biol Chem 2012;287:38482-94. PUBMED | CROSSREF

29. Rayalam S, Della-Fera MA, Ambati S, Yang JY, Park HJ, Baile CA. Enhanced effects of 1,25(OH)(2)D(3) plus genistein on adipogenesis and apoptosis in 3T3-L1 adipocytes. Obesity (Silver Spring) 2008;16:539-46. PUBMED | CROSSREF

30. Chang E, Kim Y. Vitamin D decreases adipocyte lipid storage and increases NAD-SIRT1 pathway in 3T3-L1 adipocytes. Nutrition 2016;32:702-8. PUBMED | CROSSREF

31. Żmijewski MA. Nongenomic activities of vitamin D. Nutrients 2022;14:5104. PUBMED | CROSSREF

32. Dai N, Groenendyk J, Michalak M. Binding proteins | Ca2+ binding/buffering proteins: ER luminal proteins. In: Jez J, editor. Encyclopedia of Biological Chemistry III (Third Edition). Oxford: Elsevier; 2021. p. 534-46.

33. Sha H, He Y, Chen H, Wang C, Zenno A, Shi H, Yang X, Zhang X, Qi L. The IRE1alpha-XBP1 pathway of the unfolded protein response is required for adipogenesis. Cell Metab 2009;9:556-64. PUBMED | CROSSREF

34. Kamiya T, Hara H, Adachi T. Effect of endoplasmic reticulum (ER) stress inducer thapsigargin on the expression of extracellular-superoxide dismutase in mouse 3T3-L1 adipocytes. J Clin Biochem Nutr 2013;52:101-5. PUBMED | CROSSREF

35. Han J, Murthy R, Wood B, Song B, Wang S, Sun B, Malhi H, Kaufman RJ. ER stress signalling through eIF2α and CHOP, but not IRE1α, attenuates adipogenesis in mice. Diabetologia 2013;56:911-24. PUBMED | CROSSREF

36. Trump BF, Berezesky IK. The role of cytosolic Ca2+ in cell injury, necrosis and apoptosis. Curr Opin Cell Biol 1992;4:227-32. PUBMED | CROSSREF

37. Ntambi JM, Takova T. Role of Ca2+ in the early stages of murine adipocyte differentiation as evidenced by calcium mobilizing agents. Differentiation 1996;60:151-8. PUBMED | CROSSREF

38. Deng J, Liu S, Zou L, Xu C, Geng B, Xu G. Lipolysis response to endoplasmic reticulum stress in adipose cells. J Biol Chem 2012;287:6240-9. PUBMED | CROSSREF

39. Bettaieb A, Matsuo K, Matsuo I, Wang S, Melhem R, Koromilas AE, Haj FG. Protein tyrosine phosphatase 1B deficiency potentiates PERK/eIF2α signaling in brown adipocytes. PLoS One 2012;7:e34412. PUBMED | CROSSREF

40. Park CY, Kim D, Seo MK, Kim J, Choe H, Kim JH, Hong JP, Lee YJ, Heo Y, Kim HJ, et al. Dysregulation of lipid droplet protein expression in adipose tissues and association with metabolic risk factors in adult females with obesity and type 2 diabetes. J Nutr 2023;153:691-702. PUBMED | CROSSREF

41. Lim J, Park HS, Kim J, Jang YJ, Kim JH, Lee Y, Heo Y. Depot-specific UCP1 expression in human white adipose tissue and its association with obesity-related markers. Int J Obes 2020;44:697-706. PUBMED | CROSSREF

42. Hu X, Hu C, Liu J, Wu Z, Duan T, Cao Z. 1,25-(OH)2D3 protects pancreatic beta cells against H2O2-induced apoptosis through inhibiting the PERK-ATF4-CHOP pathway. Acta Biochim Biophys Sin (Shanghai) 2021;53:46-53. PUBMED | CROSSREF

43. Haas MJ, Jafri M, Wehmeier KR, Onstead-Haas LM, Mooradian AD. Inhibition of endoplasmic reticulum stress and oxidative stress by vitamin D in endothelial cells. Free Radic Biol Med 2016;99:1-10. PUBMED | CROSSREF

44. Rozpędek-Kamińska W, Siwecka N, Wawrzynkiewicz A, Wojtczak R, Pytel D, Diehl JA, Majsterek I. The PERK-dependent molecular mechanisms as a novel therapeutic target for neurodegenerative diseases. Int J Mol Sci 2020;21:2108. PUBMED | CROSSREF

45. Wang X, Xu L, Gillette TG, Jiang X, Wang ZV. The unfolded protein response in ischemic heart disease. J Mol Cell Cardiol 2018;117:19-25. PUBMED | CROSSREF

46. Oyadomari S, Mori M. Roles of CHOP/GADD153 in endoplasmic reticulum stress. Cell Death Differ 2004;11:381-9. PUBMED | CROSSREF

47. Gregor MF, Hotamisligil GS. Thematic review series: adipocyte biology. Adipocyte stress: the endoplasmic reticulum and metabolic disease. J Lipid Res 2007;48:1905-14. PUBMED | CROSSREF

48. Park CY, Kim TY, Yoo JS, Seo Y, Pae M, Han SN. Effects of 1,25-dihydroxyvitamin D3 on the inflammatory responses of stromal vascular cells and adipocytes from lean and obese mice. Nutrients 2020;12:364. PUBMED | CROSSREF

49. Ding C, Wilding JP, Bing C. 1,25-dihydroxyvitamin D3 protects against macrophage-induced activation of NFκB and MAPK signalling and chemokine release in human adipocytes. PLoS One 2013;8:e61707. PUBMED | CROSSREF

50. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, Goleva E. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol 2012;188:2127-35. PUBMED | CROSSREF

51. Antoniotti V, Bellone S, Gonçalves Correia FP, Peri C, Tini S, Ricotti R, Mancioppi V, Gagliardi M, Spadaccini D, Caputo M, et al. Calreticulin and PDIA3, two markers of endoplasmic reticulum stress, are associated with metabolic alterations and insulin resistance in pediatric obesity: a pilot study. Front Endocrinol (Lausanne) 2022;13:1003919. PUBMED | CROSSREF

52. Farmer SR. Transcriptional control of adipocyte formation. Cell Metab 2006;4:263-73. PUBMED | CROSSREF

53. Kong J, Li YC. Molecular mechanism of 1,25-dihydroxyvitamin D3 inhibition of adipogenesis in 3T3-L1 cells. Am J Physiol Endocrinol Metab 2006;290:E916-24. PUBMED | CROSSREF

54. Lee H, Bae S, Yoon Y. Anti-adipogenic effects of 1,25-dihydroxyvitamin D3 are mediated by the maintenance of the wingless-type MMTV integration site/β-catenin pathway. Int J Mol Med 2012;30:1219-24. PUBMED | CROSSREF

55. Ji S, Doumit ME, Hill RA. Regulation of adipogenesis and key adipogenic gene expression by 1, 25-dihydroxyvitamin D in 3T3-L1 cells. PLoS One 2015;10:e0126142. PUBMED | CROSSREF

56. Turishcheva E, Vildanova M, Onishchenko G, Smirnova E. The role of endoplasmic reticulum stress in differentiation of cells of mesenchymal origin. Biochemistry (Mosc) 2022;87:916-31. PUBMED | CROSSREF

57. Li Y, Camacho P. Ca2+-dependent redox modulation of SERCA 2b by ERp57. J Cell Biol 2004;164:35-46. PUBMED | CROSSREF