Basic nutritional investigation

Vitamin D decreases adipocyte lipid storage and increases NAD-SIRT1 pathway in 3T3-L1 adipocytes

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Abstract

Objective: Previous studies suggest that low vitamin D status is associated with obesity characterized by excess lipid storage in adipocytes. The aim of the present study was to determine the effects of the most hormonally active form of vitamin D 1,25-dihydroxyvitamin D [1,25(OH)2D] on adipocyte fat storage and lipid metabolism in mature 3T3-L1 cells.

Methods: Differentiated 3T3-L1 cells were treated with various concentrations of 1,25(OH)2D. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell proliferation, intracellular lipid content, and basal and isoproterenol-stimulated lipolysis were measured to investigate the regulatory role of 1,25(OH)2D in adipocyte lipid metabolism. Reverse transcription polymerase chain reaction was performed to determine the effects of 1,25(OH)2D on adipogenesis-related markers, fatty acid oxidation-associated genes, and lipolytic enzymes. Sirtulin 1 (SIRT1) activity, nicotinamide adenine dinucleotide (NAD) and NADH were measured.

Results: 1,25(OH)2D treatment (24 h, 100 nmol/L) induced a decrease in intracellular fat accumulation and an increase of basal and isoproterenol-stimulated lipolysis without cell toxicity in adipocytes. Adipogenic gene levels were decreased. In contrast, mRNA levels of β-oxidation–related genes, lipolytic enzymes, and vitamin D responsive gene were elevated by 1,25(OH)2D. Additionally, significant incremental changes in NAD levels, the ratio of NAD to NADH, and SIRT1 expression and activity were noted in 1,25(OH)2D-treated 3T3-L1 adipocytes.

Conclusions: The observed potent inhibitory effect of 1,25(OH)2D on adipocyte fat storage in mature 3T3-L1 cells suggests that vitamin D might improve adipocyte metabolic function and protect against obesity. Increased NAD concentrations and SIRT1 activity may play a role in the mechanism of 1,25(OH)2D action.

Introduction

Obesity is recognized as a major risk factor for type 2 diabetes, dyslipidemia, hypertension, and heart disease [1,2]. It is characterized by a significant increase of adiposity, depending on hypertrophy of preexisting individual adipocytes and/or hyperplasia due to the formation of new adipocytes from precursor cells (adipogenesis) [3,4]. Additionally, the synthesis and release of adipocyte-derived factors regulating insulin sensitivity are altered, which, in turn, leads to systemic insulin resistance and type 2 diabetes [5]. Thus, an understanding of the molecular mechanisms of adipose tissue formation and alterations during the progression of obesity is required for the prevention and treatment of obesity.

Accumulating evidence shows that vitamin D deficiency is highly prevalent in obese people, indicating obesity might be an independent risk factor for vitamin D deficiency [6,7]. Indeed, body fat content is negatively correlated with 25-hydroxyvitamin D [25(OH)D] concentrations, a commonly accepted marker for vitamin D status [8–10]. In contrast, weight loss leads to a significant increase of 25(OH)D in obese children and adults [11,12]. Following human epidemiologic and clinical studies, numerous studies using in vitro adipocytes have demonstrated the regulation of 1,25-dihydroxyvitamin D [1,
25(OH)2D, calcitriol), the most active form of vitamin D, in adipocyte differentiation. In cultured 3T3-L1 adipocytes, 25(OH)2D treatment significantly inhibits adipogenesis [13–15] and decreases fat content by increasing the expression and activity of lipolysis by lipoprotein lipase (LPL), which is involved in adipocyte fat uptake and storage [16]. However, 1,25(OH)2D–reduced adipogenesis is not observed in primary mouse or human preadipocytes [17]. Given the high prevalence of vitamin D deficiency in obese individuals and the strong association between vitamin D and body fat mass, a better understanding of vitamin D action and regulation in adipocyte physiology and pathophysiology is critical for the prevention of obesity and its associated health problems. However, the mechanisms by which 1,25(OH)2D influences adipocyte differentiation, lipid metabolism, and the energy balance are largely unknown.

Sirtuin 1 (SIRT1), the mammalian homolog of yeast silent information regulator 2, is a nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylase and an important metabolic sensor in response to cellular energy status [18–20]. Fasting or caloric restriction induces an increase of intracellular NAD, which in turn stimulates SIRT1 activity, leading to hepatic glucose production and an increase of oxidative machinery activity [18,21,22]. SIRT1 promotes fat mobilization from adipose tissue to support lipid oxidation and inflammation [23]. Additionally, the genetic deletion of SIRT1 in adipose tissue induces an increase in adipocyte mass, inflammation, and metabolic disorders [24,25]. Thus, targeting SIRT1 activation has been proposed as a therapeutic tool for obesity and its associated metabolic dysfunction [26].

In the present study, we investigated the mechanisms by which active vitamin D affects adipocyte fat storage, lipolysis, and SIRT1 levels in 3T3-L1 cells. We hypothesized that 1,25(OH)2D 2D treatment inhibits intracellular fat accumulation and enhances the NAD-to-NADH ratio and SIRT1 activity, accompanied by changes in gene expression related to adipogenesis, fatty acid oxidation, and lipolytic enzymes in 3T3-L1 adipocytes.

Materials and methods

Cell culture

Murine 3T3-L1 cells obtained from the American Type Culture Collection (ATCC CL-173; Manassas, VA, USA) were maintained in high glucose (HG)-DMEM (Gibco, Grand Island, NY, USA) containing 10% bovine calf serum (Gibco), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Gibco) at 37°C in 95% air and 5% carbon dioxide. After the preadipocytes reached confluence (day 0), fibroblasts were incubated with 10% fetal bovine serum (FBS)-containing HG-DMEM in the presence of 0.5 mmol/L of 3-isobutyl-1-methylxanthine (Sigma, St. Louis, MO, USA), 1 μmol/L of dexamethasone (Sigma), and 5 μg/mL of bovine insulin (Sigma) for 2 d to induce differentiation (day 2), followed by incubation with insulin for an additional 2 d (day 4). The medium was replaced with DMEM containing 10% FBS every 2 d until >95% of the cells contained lipid droplets (day 6 or 7). Mature adipocytes were treated with vehicle control (0.1% ethanol) or 1,25(OH)2D (Sigma) dissolved in absolute ethanol at the given concentrations for the indicated time period.

**MTT assay**

Differentiated 3T3-L1 adipocytes were treated with different concentrations of 1,25(OH)2D in 10% FBS-containing medium for 24 or 48 h. To determine the relative viable levels, further incubation with fresh medium containing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) was executed for 1 h. After dissolving in DMSO, the absorbance was measured by a Varioskan plate reader (Thermo Electron, Waltham, MA, USA) at a wavelength of 595 nm. The results are expressed as the fold-change compared with the vehicle-treated cells.

**Oil Red O staining**

The intracellular lipid contents in differentiated adipocytes were measured by Oil Red O (ORO) staining. Briefly, the cells were washed with phosphate-buffered saline (PBS), fixed with 10% formaldehyde in PBS for 1 h, washed with distilled water, and completely dried. The cells were subsequently stained with ORO for 1 h at room temperature, washed with distilled water, and air-dried. ORO-stained adipocytes were observed under a microscope (Olympus, Tokyo, Japan) and digital images were captured at 40× magnification. For a quantitative analysis of intracellular lipid accumulation, ORO-stained lipids were eluted by adding 100% isopropanol and measured at a wavelength of 490 nm.

**Lipolysis**

The adipocytes were washed with PBS and incubated in the absence or presence of 10 μmol/L β-adrenergic agonist isoproterenol in 2% fatty acid free bovine serum albumin containing medium. Basal and isoproterenol-stimulated lipolysis was assessed by the release of glycerol in the culture medium using a free glycerol reagent (Sigma) as previously described [27].

**RNA analysis**

Total RNA was purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to manufacturer’s instruction. Reverse transcription (RT) of the total RNA was performed using MMLV reverse transcriptase (Bioneer, Daejeon, Korea) and the reaction was performed at 37°C for 60 min followed by incubation at 95°C for 5 min. The primers used for RT-polymerase chain reaction (PCR) are presented in Table 1. Real-time quantitative PCR was performed using the AccuPower 2× Greenstar qPCR Master Mix (Bioneer) and a fluorometric thermal

### Table 1

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<th>Forward sequence</th>
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AOC, acetyl-CoA oxidase; aP2, fatty acid-binding protein 2; CEBPβ, CCAAT/enhancer binding protein β; CPT1α, carnitine palmitoyltransferase 1α; CYP24, 1,25-dihydroxyvitamin D3 24-hydroxylase; FAS, fatty acid synthase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; PGC1α, peroxisome proliferative-activated receptor gamma coactivator 1α; PPARγ, peroxisome proliferator-activated receptor; SCD-1, stearoyl-CoA desaturase; SIRT1, sirtuin 1; UCP1, mitochondrial transcription factor A; UCP1, uncoupling protein 1
cycler (Rotor-GeneTM 2000; Corbett Research, Mortlake, NSW, Australia). The data were analyzed using the comparative Ct (ΔΔCT) method for relative quantification [28]. Each target gene was normalized to that of β-actin and expressed as a fold-change compared with controls.

SIRT1 activity assay

SIRT1 activity was measured using a SIRT1 activity assay kit (Abcam, Cambridge, MA, USA) according to the manufacturer’s instructions. In brief, nuclear fraction from differentiated 3T3-L1 adipocytes was extracted and purified. Next, SIRT1 activity was measured in the presence of NAD and fluorosubstrate peptides using a fluorometric microplate reader at 340 nm/460 nm wavelengths.

Measurement of the NAD-to-NADH ratio

A NAD/NADH assay kit was used to evaluate the levels of NAD, NADH, and their ratio (NADH/NAD) according to the manufacturer’s instructions. In brief, nuclear fraction from differentiated 3T3-L1 adipocytes was extracted and purified. Next, NAD/NADH activity was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). The NAD and NADH values were normalized to their respective protein concentrations.

Statistical analysis

The data are presented as the mean ± SEM of at least two independent triplicate experiments. Statistical analysis was performed using PASW Statistics 21 (SPSS Inc., Chicago, IL, USA). Differences were determined using Student’s t test. Statistical significance was defined as P < 0.05.

Results

1,25(OH)2D treatment (24 h) did not alter cell viability in mature 3T3-L1 adipocytes

The affect of 1,25(OH)2D on cell toxicity was examined by the MTT cell proliferation assay following treatment of differentiated adipocytes with vehicle or 1,25(OH)2D treatment. As shown in Figure 1A, 24-h incubation with 1,25(OH)2D up to 100 nmol/L had no effect on cell viability. In contrast, 48-h incubation with 1,25(OH)2D up to 100 nmol/L 1,25(OH)2D treatment (24 h) did not alter cell viability in mature 3T3-L1 adipocytes. As shown in Figure 1A, 24-h incubation with 1,25(OH)2D up to 100 nmol/L 1,25(OH)2D treatment did not alter cell viability in mature 3T3-L1 adipocytes. In contrast, 48-h incubation with 1,25(OH)2D up to 100 nmol/L 1,25(OH)2D treatment (24 h) did not alter cell viability in mature 3T3-L1 adipocytes.

1,25(OH)2D significantly decreases lipid accumulation in a dose-dependent manner

Next, we investigated the effect of 1,25(OH)2D on adipogenesis. Differentiated 3T3-L1 adipocytes were incubated with 1,25(OH)2D for 24 h in a dose-dependent manner on day 6 or 7 after adipocyte differentiation induction. Representative images of Oil Red O staining illustrate that 1,25(OH)2D inhibits lipid accumulation (Fig. 2B). As Figure 2A shows, 24 h of 1,25(OH)2D treatment inhibited adipogenesis in a dose-dependent manner with a 15% significant reduction evident at 100 nmol/L 1,25(OH)2D as measured by Oil Red O staining.

1,25(OH)2D promotes basal and isoproterenol-stimulated lipid utilization in 3T3-L1 adipocytes

To demonstrate whether the 1,25(OH)2D-decreased intracellular fat content is due to lipolysis, we measured glycerol levels in the culture medium after 24 h treatment. Figure 3 demonstrates that there were significant reductions of the basal lipidosis rates in 1,25(OH)2D-treated 3T3-L1 adipocytes. We next sought to determine whether 1,25(OH)2D leads to lipolysis in the isoproterenol-stimulated state. Incubation of 3T3-L1 adipocytes with β-adrenergic agonist (isoproterenol, 10 µmol/L) on day 7 induced a significant increase of 51% compared with the control in the basal state. 1,25(OH)2D-treated adipocytes stimulated with isoproterenol significantly promoted lipid mobilization. Thus, 1,25(OH)2D might increase fatty acid availability due to enhanced basal and isoproterenol-stimulated lipolysis from adipocyte lipid accumulation.

Effects of 1,25(OH)2D on gene expression involved in adipogenesis, fatty acid oxidation, lipolytic enzymes, vitamin D metabolism, and obesity-associated adipocytokine

We evaluated whether 1,25(OH)2D diminished lipid accumulation by regulating the gene involved in adipogenesis, fatty acid oxidation, and adipocyte-specific lipolytic enzymes. 1,25(OH)2D (24 h, 100 nmol/L) significantly decreased aP2, CEBPα, FAS, PPARγ, and SCD-1 adipogenic mRNA expression by 43.9%, 63.4%, 60.2%, 49.2%, and 62%, respectively (Fig. 4A). Additionally, mRNA levels of CPT1a, PGC1α, PPARα, and UCP1 involved in β-oxidation were significantly upregulated by 1.5-, 4.2-, 3.2-, and 1.9-fold in 1,25(OH)2D-treated cells compared with the vehicle control (Fig. 4B). Furthermore, 1,25(OH)2D increased lipolytic enzymes hormone-sensitive lipase (HSL) and lipoprotein lipase (LPL) mRNA levels by 76.5% and 95.4%, respectively, in mature adipocytes (P < 0.05). 1,25(OH)2D responsive gene, CYP24 was significantly increased, but leptin, an obesity-associated adipocytokine secreted by adipose tissue, was not significantly changed by 24 h incubation of 1,25(OH)2D.
1,25(OH)2D treatment significantly increases SIRT1 mRNA and activity

The effect of 1,25(OH)2D on SIRT1 involved in lipid metabolism through the regulation in fat mobilization has never been reported. Therefore, we examined the influence of 1,25(OH)2D on gene expression and activity of SIRT1 in 3T3-L1 adipocytes. 1,25(OH)2D-treated adipocytes had a 2-fold increase in SIRT1 mRNA expression compared with vehicle (100% ethanol)-treated control cells (Fig. 5A). Moreover, 1,25(OH)2D significantly increased SIRT1 activity in a dose-dependent manner, started at a dose of 1 nmol/L. The maximum SIRT1 activity was at a concentration of 100 nmol/L by 3.3-fold compared with vehicle (Fig. 5B).

1,25(OH)2D treatment increases NAD and the NAD-to-NADH ratio

Upon observing the results showing that 1,25(OH)2D-increased SIRT1 expression and activity, we investigated whether 1,25(OH)2D regulates adipocyte NAD biology by measuring NAD, NADH, and the NAD-to-NADH ratio in 3T3-L1 adipocytes. As shown in Figure 6, 100 nmol/L 1,25(OH)2D treatment significantly increased the NAD-to-NADH ratio but decreased NADH levels in differentiated 3T3-L1 cells.

Discussion

Associations between vitamin D status and obesity have been well documented in both epidemiologic and intervention studies. There is a high prevalence of vitamin D inadequacy in obese individuals. The present study demonstrates reduced fat accumulation and increased lipid mobilization with 1,25(OH)2D treatment in murine mature adipocytes. To the best of our knowledge, this study describes the first time that 1,25(OH)2D treatment significantly increases adipocyte SIRT1 activity and the NAD-to-NADH ratio. These results suggest that vitamin D might promote fat mobilization and hence decrease intracellular fat accumulation and increase lipolysis, concurrently with an increase of activity in the NAD-SIRT1 pathway.

Adipose tissue is now recognized as one of the most important tissues in obesity due to its dynamic changes during the progression of obesity: hyperplasia (an increase in cell number), hypertrophy (enlarged cell size), and adipocyte-derived peptides [3–5]. Targeting adipose development during the progression of obesity has been considered for the prevention and/or treatment of obesity and its associated metabolic disorders. First, we examined whether 1,25(OH)2D modulates adipocyte lipid metabolism. Adipocyte lipid accumulation was significantly decreased in 1,25(OH)2D-treated adipocytes without intracellular toxicity in the present study. A published paper showed that 1,25(OH)2D-induced apoptosis which, in turn, might result in the prevention and treatment of obesity [29]. Treatment of 1,25(OH)2D (12.5–400 nmol/L) was started at day 0, when differentiation was induced and 1,25(OH)2D was incubated for 1, 3, or 6 d. However, in the current study, mature adipocytes on day 6 or 7 was incubated with 1,25(OH)2D for 24 h (1–100 nmol/L). Despite this discrepancy, a study to investigate whether 1,25(OH)2D treatment induces apoptosis and its-associated decrease of lipid deposition in mature adipocyte is warranted. Additionally, our data illustrate that 1,25(OH)2D treatment increases fat mobilization by reducing intracellular fat contents and increasing both basal and isoproterenol-stimulated lipolysis. These results support human epidemiological and clinical studies demonstrating the negative association between adiposity/obesity and vitamin D status [8–10].
The inclusion of 1,25(OH)2D to a differentiation cocktail, significantly inhibited adipocyte differentiation by suppressing the expression of adipogenic genes such as CEBPa and PPARγ in murine 3T3-L1 and primary porcine cells [13,14,30]. Consistent with these studies, results from the present study also demonstrated that 1,25(OH)2D treatment significantly inhibited adipogenic transcription factors, despite the addition of 1,25(OH)2D in the mature status after differentiation (day 6 or 7) and only for 24 h. The lipogenic mRNA expression related to lipid accumulation was measured. ap2 indicates the extent of fat accumulation in adipose tissue [31]. Fatty acid synthase (FAS) induces de novo lipogenesis [32]. Additionally, stearoyl-coenzyme desaturase (SCD) converts saturated long-chain Fas (LCFAs) to monounsaturated fatty acids, which are the major components of triacylglycerol (TG) [33]. In the present study, 1,25(OH)2D significantly decreased these three lipogenic mRNA levels, suggesting the role of 1,25(OH)2D in the decrement of adipocyte fat storage capacity. Additionally, our results show that the gene expression involved in fatty acid oxidation was upregulated by 1,25(OH)2D treatment. Fatty acid oxidation is regulated by the CPT1-mediated entry of LCFAs into mitochondria [34] and a transcription factor, PGC1α. PGC1α promotes lipid catabolism by regulating mitochondrial biogenesis and metabolism in association with its nuclear receptors, PPARα and UCP1, and gene-encoding respiratory chain proteins involved in thermogenesis [35–37]. These data suggest that 1,25(OH)2D-increased

![Image 4](https://example.com/image4)

**Fig. 4.** The effects of 1,25(OH)2D on gene expression involved in adipogenesis, fatty acid oxidation, lipolytic enzymes, vitamin D metabolism and obesity-associated adipocytokine. 3T3-L1 adipocytes were incubated with 100 nmol/L 1,25(OH)2D for 24 h on day 6 or 7 after differentiation induction. Gene expression related to adipogenesis (A), fatty acid oxidation (B), and lipolytic enzymes (C) was analyzed by RT-PCR. CYP24 (D) and leptin (E) mRNA levels were also evaluated. mRNA gene expression was normalized with respect to the β-actin level. The results are presented as the mean ± SEM (n = 9/group). *P < 0.05; †P < 0.01 compared with the vehicle control.

![Image 5](https://example.com/image5)

**Fig. 5.** The effect of 1,25(OH)2D on the mRNA expression and activity of SIRT1. On day 6, 3T3-L1 adipocytes were incubated with 1,25(OH)2D for 24 h at the indicated concentrations. The mRNA levels of SIRT1 were determined by reverse transcription polymerase chain reaction (RT-PCR) (A). Quantitative RT-PCR was normalized for all samples to β-actin. (B) SIRT1 activity was analyzed by a fluorometric SIRT1 activity assay kit. The results are expressed as the mean ± SEM (n = 6–9/group). *P < 0.05; †P < 0.01 compared with the vehicle control.
lipolysis might be associated with increased fatty acid oxidation capacity. In the present study, lipolytic enzymes HSL and LPL were upregulated by 1,25(OH)2D, which is consistent with the findings of a cross-sectional study showing a positive correlation between vitamin D status and LPL [38], and in vitro studies demonstrating the increased expression of LPL in 1,25(OH)2D-treated 3T3-L1 and human adipocytes [16,17,39]. The reduction of adipogenic gene expression and the increase of β-oxidation and lipolytic mRNA expression suggest that these may play a role in the changes of 1,25(OH)2D-induced adipocyte fat storage and lipolysis. Moreover, vitamin D target gene, CYP24, is significantly induced in the current study, consistent with dose-responsive increments in 1,25(OH)2D-treated adipocytes [17,40]. However, further studies to investigate whether 1,25(OH)2D-induced changes in gene expression is dose-responsive and how 1,25(OH)2D affects the expression of genes and/or their enzyme activities are needed. Close association between obesity-induced production of leptin and vitamin D metabolism have been observed in both humans and in vitro systems [41,42]. However, in the present study, 1,25(OH)2D treatment did not change leptin gene expression. Therefore, further study to investigate whether not only 1,25(OH)2D but also different vitamin D metabolite, such as 25(OH)D affects production and secretion of leptin in adipocytes needs to be clarified. Additionally, 1,25(OH)2D-mediated adipocyte adipogenesis and adipocyte marker gene regulation are discrepant according to cell type/orign. In 3T3-L1 cells, 1,25(OH)2D treatment started at day 0 significantly inhibits adipogenesis and intracellular fat accumulation by down-regulating adipocyte markers such as CEBPa, CEBPβ, PPARY, or sterol regulatory element-binding protein 1 (SREBP1) [13,14]. In contrast, 1,25(OH)2D 2D-treated human subcutaneous preadipocytes demonstrated increased adipogenesis determined by increased gene expression of PPARY and TG accumulation. There is no distinct change in CEBPa and CEBPβ levels [17]. Therefore, it is necessary to investigate the influence of 1,25(OH)2D on fat utilization in human adipocytes.

Accumulating evidence demonstrates that SIRT1 activation plays an important role in metabolic functions by increasing the β-oxidation of free fatty acids and mitochondrial biogenesis in a NAD-dependent manner [18,21,22,43]. The major metabolic roles of NAD and NADH are associated with oxidative metabolism and energy homeostasis, which have been linked to protection against obesity [44,45]. In this study, we revealed that 1,25(OH)2D increased intracellular NAD levels and the NAD/NADH ratio in 3T3-L1 adipocytes. We also found that 1,25(OH)2D treatment increased both SIRT1 expression and activity. A NAD-dependent deacetylase, SIRT1, regulates adipose energy homeostasis and metabolism, which could be a potential therapeutic strategy to protect against obesity and obesity-associated metabolic disorders [46,47]. Additionally, SIRT1 inhibitors pre-adipocyte differentiation and increases the mobilization of free fatty acids from adipose tissues [23]. Furthermore, there is accumulating evidence showing an inverse association between SIRT1 levels and adipose tissue mass and inflammation [24,25,48]. Moreover, targeting SIRT1 activation by resveratrol supplementation shows the favorable effect on body composition in humans [26]. A recent research proposes SIRT1 activation is closely related to the activation of a steroid hormone receptor, vitamin D receptor (VDR), which mediates 1,25(OH)2D-induced genomic changes [49]. SIRT1 activator, resveratrol, significantly stimulates 1,25(OH)2D binding to VDR, the recruitment of its co-receptor, retinoid X receptor (RXR), and SIRT1 activation in human colorectal adenocarcinoma cells (Caco-2, HCT116), human embryonic kidney cells (HEK293), and mouse myoblast cells (C2 C12) cells [50]. Thus, because NAD and SIRT1 protect against adipose tissue mass and the derangement of metabolic functions that are known to be related to obesity, we speculate that 1,25(OH)2D-induced adipocyte fat storage and lipolysis might be related to a NAD-sirtulin pathway. Further research is necessary to delineate whether 1,25(OH)2D-elevated NAD levels lead to changes in PGC-1α deacetylation, mitochondria biogenesis, oxidative respiration, fatty acid oxidation, and energy homeostasis in a SIRT1-dependent manner and/or in a VDR-mediated manner in adipocytes and in vivo animal studies.

**Conclusion**

The present data demonstrated that the favorable effects of vitamin D, 1,25(OH)2D on adipocyte metabolism and function are associated with decreased adipocyte fat storage and increased lipolysis. 1,25(OH)2D treatment modulates adipogenic, fatty acid oxidation, or lipolytic related gene expression and increases the NAD-sirtulin pathway activity in 3T3-L1 adipocytes. Findings
from this study suggest that increasing vitamin D status might be an effective strategy for preventing obesity. Further research is warranted to explore the mechanisms of how vitamin D directly affects sirtuin-mediated metabolic regulation in adipocyte metabolism and obesity.

References