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VITAMIN D METABOLISM OF LIPIDS

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Abstract
Though its historical significance in maintaining bone mineral homeostasis is widely recognized, new research indicates that vitamin D is also important for metabolic regulation. Using MS imaging, this study looked into potential connections between vitamin D deficiency [VDD] and disruptions in the metabolism of bioactive lipids. Across the world, vitamin D insufficiency is a prevalent health issue. Studies have started looking into its extra-skeletal benefits, or how it might prevent metabolic illnesses including obesity, hyperlipidemia, and diabetes mellitus, in addition to its well-known skeletal properties. These adverse metabolic effects of vitamin D deprivation have been studied, along with the underlying mechanisms. According to available data, a vitamin D shortage may affect the functioning of the pancreatic β-cell, which could lead to a compromise in the secretion of insulin. Furthermore, a lack of vitamin D may worsen oxidative stress, apoptosis, and inflammation in the pancreas and other organs, all of which contribute to insulin resistance. All of these will be involved in the disruption of glucose homeostasis. In order to determine how vitamin D may be used to prevent and treat metabolic illnesses, this review outlines the effects of vitamin D that have been documented on metabolism.on PCOS treatment using herbal remedies.

Keywords: Abdominal obesity, metabolic disorders, vitamin D supplementation, vitamin D intake.

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Introduction

Vitamin D (calciferol) is a prohormone traditionally associated with bone mineral homeostasis; however, recent research has implicated a role for vitamin D in additional physiological and cellular processes, including metabolic control, cell differentiation, oxidative stress, xenobiotic metabolism, neuro-development, and immune function (1). Vitamin D (calciferol) refers to a group of fat-soluble secosteroids that exists in two forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is derived from plant's ergosterol upon exposure to ultraviolet B (UVB) light, whereas vitamin D3 is derived from 7-dehydrocholesterol (7DHC) found in the human and animal skin following exposure also to UVB light (2). The main exogenous sources of vitamin D2 are plants, plankton, and fungi, whereas the main sources of vitamin D3 are dairy products, fish, meat, and poultry. Materials and Methods:

1.1. Chemicals and Reagents:

Fetal bovine serum (FBS; CAS No. F4135), Hanks' balanced salt solution (CAS No. H6648), phorbol-12-myristate-13-acetate (PMA; ≥99% TLC; CAS No. P8139), bovine serum albumin (BSA; CAS No. A9418),palmitic acid (≥99% GC; CAS No. P0500), oleic acid (≥99% GC; CAS No. O1008), standard of VD3 (calcitriol; ≥99% HPLC; CAS No. D1530-10UG), Trypan Blue (CAS No. T8154), hydrochloric acid (37%; CAS No. 320331), methanol (≥99.9% HPLC; CAS No. 34860), ethanol (EtOH; ≥99.8% HPLC; CAS No. 51976), pluronic
F127 (CAS No. P2443) and Nile Red (≥97%; HPLC CAS No. 19123) were obtained from Merck (Darmstadt, Germany). RPMI-1640 medium (CAS No. 21875091), sodium pyruvate (CAS No. 11360070), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; CASNo. 15630106), gentamicin (CAS No. 15710064), and trypsin–EDTA (CAS No. 25200056) were purchased from Life Technologies (Monza Brianza, Italy). Enzyme-linked immunosorbent assay (ELISA) kits related to PPAR- γ, C/EBP-β, CD36, CPT-1A, andABCA1 were purchased from MyBioSource (San Diego, CA, USA), while ultrapure water was from a Milli-Q apparatus (Millipore, Milford, MA, USA).

1.2. Preparation of Calcitriol Stock Solution:

Lyophilized VD3 (Figure 2) standard (10 μg) was dissolved in EtOH in order to prepare a stock solution.

The final concentration of EtOH in the medium for cell culture was 0.0025%. The VD3stock solution was aliquot and stored at −20 °C until use.

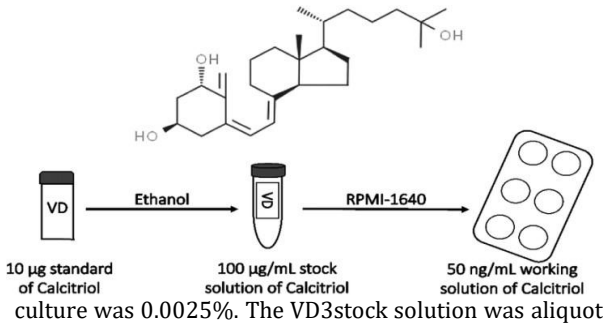

Figure 1. Preparation of calcitriol working solution and its chemical structure.

1.3. Preparation of Fatty Acid Solution and Its Control:

The FFA stock solution was prepared in EtOH by using oleic and palmitic acid in a 2:1 ratio with a final concentration of 0.2 M. The mix of FFA and their ratio were chosen based on previous experiments, where the cytotoxicity of palmitic acid (saturated fatty acid) is blunted in the mixture with oleic acid (mono-unsaturated fatty acid) in order to reproduce a more in vivo situation as oleic and palmitic acids are abundant in human plasma (3,4). The solution was stored in a dark tube at −20 °C.

1.4. THP-1 Cell Culture:

The THP-1 cell line (human monocytes leukemia) was obtained from the American Type Culture Collection (Manassas, VA, USA). The cell culture medium was RPMI-1640 supplemented with 10% of heat-inactivated FBS, 1% sodium pyruvate, 1% HEPES, and 0.1% gentamicin. Every third day during cell growth, the THP-1 cells were sub cultured by withdrawing a volume of medium containing cells from the culture flask and adding complete fresh medium to obtain the appropriate seeding density of 3 × 105 cells/mL. The THP-1 cell line maintains monocytic characteristics for over 14 months of continuous growth (5). In our study, a maximum period of 2 months was adopted for cell growth, which corresponds to a passage number between 5 and 15.

1.5. Viability Assay:

The Trepan Blue dye exclusion assay was used to assess the toxicity of the compounds tested on THP-1-derived macrophages. The cell count was performed by using a TC20TM automated cell counter and dual-chamber cell counting slides (BIORAD, Segrate, Milan, Italy). The cells were resuspended in fresh complete medium in order to reach 4 × 105 cells/mL as the final concentration, and 1 mL of cell suspension (4 × 105 cells) was addedinto each well of a 12-well plate. Each compound and concentration were assessed in triplicate over three independent experiments (Table 1). Triton X-100(0.1%) was used as positive control. The absolute viability is expressed as the percentage of viable cells out of all counted cells.

The cell lysate was centrifuged at 4500× g for 20 min at 4 ◦C. Aliquots of supernatants and cell lysate were stored at −80 ◦C until analysis.

1.6. Data Analysis:

The effect of VD3 supplementation on cell viability, the lipid accumulation process, and the protein levels of C/EBPβ, PPAR-γ1, CD36, CPT-1A, and ABCA1 was evaluated by one-wayANOVA using STATISTICA software (Statsoft Inc., Tulsa, OK, USA). Differences between treatments were identified by the least significant difference (LSD) test by setting the level of statistical significance at $p < 0.01$. Results are reported as means s ± standard deviation (SD).

2. Mechanism underlaying the Action of Vitamin D in Improving Impaired Metaboilsm:

2.1. Vitamin D Improves Pancreatic β-cell Function:

Functional, pancreatic β-cells play important role in maintaining the blood glucose homeostasis. These cells adapt to an excessive blood glucose level by increasing the insulin secretion, and the latter is further exaggerated in the state of insulin resistance. Compensatory hyperinsulinemia will result in β-cell hyperplasia and hypertrophy, which helps to maintain the blood glucose levels up to a point where β-cells could no longer produce sufficient insulinto keep pace with the demand. Chronic exposure to high glucose and free fatty acids (FFA) levels could cause progressive β-cell dysfunction, which may eventually lead to β-cell apoptosis in DM (6,7).

2.2. Vitamin D Improves Insulin Sensitivity:

There are several mechanisms by which vitamin D affects insulin sensitivity in insulin target tissues. When vitamin D is deficient, insulin sensitivity will begin to decline, thus setting the stage for the onset of DM and other DM-related illnesses (8). Firstly, vitamin D modulates the secretion of insulin-sensitizing hormones such as adiponectin and leptin and increases the expression of disulfide-bond A oxidoreductase-like (DsbA-L) protein, a key regulator for production.

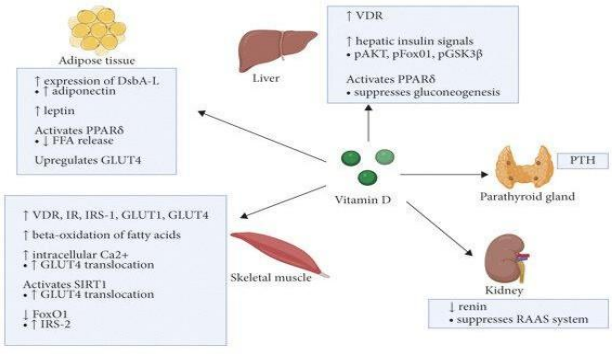

Figure 2: Schematic diagram summarizing the roles of vitamin D in maintaining insulin sensitivity.

2.3. Vitamin D Ameliorates Chronic Inflammation:

During the development of insulin resistance, chronic lowgrade inflammation occurs, which can cause impairment in adipose tissue function by causing mitochondrial dysfunction and triggering endoplasmic

reticulum (ER) stress—all of which would contribute towards insulin resistance. Although it is unclear whether insulin resistance or inflammatory response occurs first, it was suggested that inflammation in T2DM is the causative factor for insulin resistance(9). The onset of insulin resistance is believed to occur with the dysregulation of metabolic pathways in the adipose tissue. In obesity-related insulin resistance, poor blood flow in
hypertrophied adipose tissue leads to macrophages infiltration due to tissue hypoxia and subsequently inflammation.

In a study using diabetic male SD rats, oral supplementation of 0.03 μg/kg/day vitamin D for eight (8) weeks resulted in lower expression of pro-inflammatory cytokines as well as reduced hyperglycemia as reflected by a decrease in FPG levels and HOMA-IR. Moreover, oral supplementation of 150 IU/kg calcitriol per day for sixteen (16) consecutive weeks in high-fat diet (HFD)-induced diabetic C57BL/6J male mice lowered the concentrations of various inflammatory markers including TNF-α, C-reactive protein (CRP) and IL-6, and the levels of C-peptide and insulin (10).

2.4. Vitamin D Attenuates Oxidative Stress:

Oxidative stress is recognized as a key mechanism in insulin resistance. Among the endogenously produced oxidative stress agents are the reactive oxygen species (ROS), which include superoxide, hydrogen peroxide, and hydroxyl radicals. ROS possesses physiological significance even at low levels, especially to the signaling pathways. The main source for ROS is NADPH oxygenase (NOX) and malondialdehyde (MDA) (11). The oxidative processes are regulated by antioxidants such as superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), and catalase. Higher production of ROS and declining antioxidative capacity may lead to excessive lipids, proteins, and DNA oxidation products (12). Besides, vitamin D could help to diminish the ROS formation by downregulating NOX through the suppression of Noxgene expression. Studies supported the antioxidant properties of vitamin D where in vitamin D-deficient mice, the inhibition of oxidative stress could improve insulin resistance. In addition to this, a study on SD male weanling rats reported that vitamin D deficiency is linked to a decreased SOD and catalase enzymes in the rat skeletal muscles, and there were higher nitrate levels indicating nitrosative stress in the tissue.

2.5. Vitamin D Abrogates Apoptosis:

In the pancreas, unresolved inflammation in the insulin resistance state could enhance the immune cell infiltration, which leads to the dysfunction of insulin-secreting β-cells and ultimately cell death. Markedly increased caspase activation and adipocyte apoptosis have been observed in insulin-resistant adipose tissue (13). In the skeletal muscle, an increase in circulating saturated fatty acids along with poor fatty acid handling results in increased levels of ceramide, which acts as a second messenger in triggering an apoptotic response via the mitochondrial system. In the liver, insufficient unfolded protein response (UPR) to elevated ER stress leads to adverse effects such as hepatic fat buildup, inflammation, and cell death.A study on streptozotocin - induced type 1 diabetic FVB mice demonstrated enhanced C/EBP homologous protein (CHOP) and caspase-12 cleavage in response to ER stress in the liver, which resulted in hepatocyte apoptosis (14).

3. Quality Assessment:

Two review authors (NR, MZ) independently assessed the quality of meta-analyses according to the AMSTAR 2 tool. This tool contains 16 items, of which 7 are critical domains that can critically affect the validity of a review and its conclusion (15).

4. Data Synthesis and Statistical Analysis:

The reported ESs and CIs were used to estimate the overall ES. We pooled estimates of treatment effects where possible, using standard statistical techniques. The random-effects model was applied to conduct statistical analysis using the restricted maximum likelihood
method. Due to the natural differences between standardized mean difference (SMD) and weighted mean difference (WMD), the analysis was performed for each separately [16].

4.1. Effect of VD3 on Cell Viability:

The effect of the different VD3 concentrations (0.1–100 nM) tested for 24 h in the presence of FFA/BSA solution (500 μM) on the cellular viability measured by Trypan Blue exclusion assay. The control condition is represented by cells in their normal growth medium without VD3 and FFA. VD3 + FFA did not reduce the cell viability, which remained higher than 90% ($p > 0.05$), while, as expected, the addition of Triton X-100 (0.1%) induceda significant reduction (−77.9%, $p < 0.0001$).

4.2. Effect of VD3 on Lipid Accumulation in THP-1 Derived Macrophages:

Figure 3 depicts the effect of the supplementation with VD3 on lipid accumulation in THP-1- derived macrophages. Exposure to 500 μM of FFA significantly increased ($p < 0.01$) the lipid accumulation in macrophages compared to cells without treatment (No FFA). Treatment with
50 and 100 nM of VD3 significantly lowered ($p < 0.01$) lipid accumulation in macrophages compared to the positive control (FFA exposure only). In particular, the size of the effect was similar, −27% and −32%, respectively, for VD3 at 50 and 100 nM. Since the effect on lipid accumulation was comparable at 50 and 100 nM, the experiments on gene expression were performed by using the lowest concentration (17).

4.3. Effect of VD3 on PPAR-γ1 Protein Levels:

The results of the protein expression kinetic of PPAR-γ1 after the administration of VD3 at a concentration of 50

nM. A statistically significant increase ($p < 0.01$) in PPAR-γ1 protein levels was documented at 2 and 24 h following the incubation with FFA compared to NoFFA (+65.3% and +54.4%, respectively). The treatment with VD3 (VD3 + FFA) induced an increase in PPAR-γ1 levels at 2 h compared to the negative control (+57%; $p < 0.01$), while the exposure for 24 h determined a reduction in PPAR-γ1 protein level (−33.4%; $p < 0.01$). No difference was found when considering the negative control (No FFA) or the other time points analyzed.

4.4. Effect of VD3 on CD-36 Protein Levels:

The result of FFA and VD3 treatments on the expression of CD-36 in macrophages at different time points. There was a statistically significant increase of CD-36 protein expression after 1 h of FFA incubation compared to the negative control (+45.2%; $p < 0.01$), while, at the same time point, the treatment with VD3 in the presence of FFA was able to counteract the increase of CD-36 protein levels induced by FFA administration (−29.1%; $p < 0.01$). No statistically significant difference was observed between the negative control and the VD3 + FFA. No difference was documented for the other time points analyzed.

4.5. Effect of VD3 on C/EBPβ Protein Levels:

The protein levels of C/EBPβ at different time points after the treatment with VD3 (50 nM) and FFA (500 μM). A statistically significant increase in C/EBPβ protein levels was documented following cell incubation with FFA (positive control) compared to negative control (No FFA) at 2 h (+65.2%; $p < 0.01$) and 4 h (+23.9%; $p < 0.01$). The treatment with VD3 inhibited the FFA-induced increase in C/EBPβ protein level at both 2 h (−35.9%; $p < 0.01$) and 4 h (−16.1%; $p < 0.01$) compared to only FFA. No difference was documented with respect to No FFA.

4.6. Effect of VD3 on CPT-1A Protein Levels:

The earliest statistically significant modification occurred after 2 h of treatment with VD3, which was an increase of CPT-1A protein levels compared to both negative control and the FFA condition (+96.7% and +71.4%, respectively; $p < 0.01$). No significant difference was documented between the negative control and the positive control at the same time point. After 4 h, the incubation with FFA increased the CPT-1A protein levels, similar to the condition of VD3 + FFA (not statistically different) but significantly higher than the negative control (+48%; $p < 0.01$).

Conclusion

According to our findings, VD3 influences the synthesis of several proteins involved in the cellular absorption, transport, oxidation, and efflux of lipids in macrophages, which has a positive impact on lipid metabolism. Maintaining an appropriate dietary condition in terms of vitamin D may therefore be a useful tactic to lower the risk of CVDs. Because PPAR-γ1 interacts directly with other proteins, including C/EBsPβ, CD36, CPT-1A, and ABCA1, it appears to be a crucial regulator in the control of lipid metabolism. Further mechanistic studies are encouraged to corroborate the actual findings and investigate new potential metabolic pathways, including those related to oxidative stress and inflammation, in order to better elucidate the biological role of VD3. However, we cannot rule out the possibility that VD3 may also influence lipid metabolism through the up- or down-regulation of other potential target genes. clarify VD3's biological function more thoroughly. handle PCOS.

Author contributions

All authors are contributed equally.

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Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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