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Genetic Factors and Molecular Mechanisms of Vitamin D and Obesity Relationship

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Abstract
Vitamin D (vitD) deficiency is associated with a wide range of chronic diseases and conditions, including obesity, and with an increasing severity of metabolic dysregulation, such as insulin resistance, hyperlipidemia, liver disease, and hypertension, both in children and adults. However, the nature of the association between low vitD status and obesity remains unclear. This fact has motivated the scientific community to conduct genetic association analyses between 25-hydroxyvitamin D (25[OH]D)-related genes and obesity traits. In this line, the variation in the vitD receptor (*VDR*) gene represents the bulk of the findings. Specifically, polymorphisms in the *VDR* gene have been associated with obesity traits in some but not all, studies. Thus, results regarding this matter remain inconclusive. Other genes aside from *VDR* have also been investigated in relation to obesity-related traits. However, again, findings have been inconsistent. In general, results point to the fact that the *DBP/GC* gene could be an important protein-linking obesity and vitD status. On the other hand, several studies have attempted to determine the molecular mechanism of the relationship between 25(OH)-D levels and obesity. Some of these studies suggest that vitD, due to its fat-soluble characteristic, is retained by the adi-

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pose tissue and has the capacity to metabolize 25(OH)-D locally, and this can be altered during obesity. Additionally, vitD is capable of regulating the gene expression related to adipogenesis process, inflammation, oxidative stress, and metabolism in mature adipocytes. Therefore, the aim of the present review was to evaluate the association between obesity and vitD deficiency describing the main molecular mechanism of the relationship and the link with genetic factors. ***Key Messages:*** Low serum 25(OH)-D is positively associated with obesity or BMI in adults and children. Circulating vitD concentrations are, at least, partially determined by genetic factors. VitD plays an important role in the adipogenesis process and inflammation status in adipocytes and adipose tissue.

Introduction

An adequate vitamin D (vitD) concentration is essential for growth, development, and health. It plays a relevant role in the regulation of calcium levels and bone metabolism. In addition, recent studies indicate that hypovitaminosis D compromises long-term condition as a consequence of its non-calciotropic effects, such as the implication/regulation of the immune system, endocrine pancreas, liver, skeletal muscle, and adipocytes. In fact, vitD deficiency is associated with a wide range of chronic diseases and conditions, including obesity, and several types of cancers. Suboptimal vitD status has also been associated with increasing severity of metabolic dysregulation (insulin resistance, hyperlipidemia, liver disease, and hypertension) in children and adults [1].

Obesity is defined as an excess amount of body fat and constitutes a worldwide epidemiological problem. Currently, it is the fifth greatest risk factor for mortality [2]. The identification of associated factors and risk groups related to hypovitaminosis D is critical. Yet, the nature of the association between low vitD status and obesity remains unclear. Therefore, the aim of the present review was to evaluate the association between obesity and vitD deficiency describing the main molecular mechanism of the relationship and the link with genetic factors.

VitD Status and Obesity

An association between low vitD and obesity has consistently been shown by cross-sectional studies. VitD status is measured by means of the plasma levels of 25-hydroxyvitamin D (25[OH]-D) [3]. The Institute of Medicine proposed that serum 25(OH)-D concentrations below 50 nmol·L^{-1} or equal to 20 ng·mL^{-1} should be considered to represent a deficiency status of this nutrient [4]. The first meta-analysis quantifying the association between Body mass index (BMI) and vitD deficiency was published in 2015 revealing a positive association between both of them among the 23 articles included [3]. Independent of the age group, the prevalence of vitD deficiency was 35% higher in obese subjects compared to the eutrophic group and 24% higher than in the overweight group. Taking into account the age, up to 37% of obese children and adolescents were vitD deficient, while in obese adults and elderly individuals, this prevalence was 33%. The results of this meta-analysis emphasized the prevalence of vitD deficiency in obese and overweight individuals.

At the end of 2015, another meta-analysis from different populations was published considering 15 studies, which included 3,867 subjects with obesity and 9,342 healthy subjects [5]. As in the first meta-analysis, the results showed that the prevalence of vitD deficiency was different between the obesity and the control groups, and the pooled OR (95% CI) was 3.43 (2.33–5.06). The prevalence of vitD deficiency was associated with obesity in Asians and European-Americans; OR (95% CI) were 3.70 (1.98–6.90) and 3.09 (1.89–5.04) among Asians and European-Americans respectively. Nevertheless, the impact of several confounding factors, such as diet intake, physical activity, educational level, season of the year, and presence of secondary hyperparathyroidism, were not included in any of the meta-analyses due to methodological divergences in the studies analyzed and the absence of information on these factors.

The worldwide rise in the obesity rate alongside the progressive increase of vitD deficiency in children and adolescents is alarming. Although literature on children and adolescents is scarce compared to that on adults, studies show that obese children and adolescents also have significantly low serum 25(OH)-D concentrations, like their adult counterparts. A large study, reported by Turer et al. [6] with a representative sample of American 6–8-year-old children and adolescents from the NHANES study (n = 12,292) found 49.0% prevalence of vitD deficiency in the severely obese group (n = 581) and 34.0% in the obese group (n = 1897). In this context, we characterized vitD status in 471 children and adolescents (2–18 years of age) and analyzed its correlation with gender, pubertal period, age, and BMI. An inverse lineal effect of BMI and age on 25(OH)-D concentrations was observed in children. Of note, a non-lineal regression mod-

el showed that 39.6% of 25(OH)-D levels variability was explained by BMI [1].

Most of the studies included in meta-analyses had cross-sectional designs, which make it more difficult to examine the relationship of causality between obesity and vitD deficiency. Therefore, longitudinal approaches are of great interest. Yet, prospective studies examining the association between serum 25(OH)-D and subsequent body weight change have shown inconsistent results [7–9]. However, the results from a bidirectional Mendelian randomization study, based on 42,024 participants of European descent, suggested a causal relation for a higher BMI on reduced serum 25(OH)-D status, while a general effect of serum 25(OH)-D on BMI was likely to be absent or minimal [10]. A recent meta-analysis based on 10,898 individuals from the Danish Inter99, the 1958 British Birth Cohort, and the Northern Finland Birth Cohort 1966 found no evidence of an association between 25(OH)-D and changes in weight or waist circumference, suggesting that associations between 25(OH)-D and changes in measures of adiposity were absent or marginal [11].

Several randomized clinical trials have investigated the efficacy of vitD supplementation on weight, body composition, and other metabolic parameters (fat mass, blood pressure, lipids, glucose tolerance, and insulin resistance); however, their results are contradictory. Some positive effects of vitD supplementation on fat mass, triglycerides, high density lipoprotein cholesterol, and oral glucose tolerance, but adverse effects on low-density lipoprotein cholesterol and blood pressure have been described. However, because of the reduced number of studies and significant heterogeneity in the methodologies applied, the current evidence is insufficient to draw conclusions [12]. In a recent systematic review, the effect of vitD supplementation (with or without calcium) on inflammatory markers, glucose, and insulin sensitivity measures in overweight and obese adults was assessed in randomized controlled trials [13]. Overall, the results did not find a clearly established benefit of vitD supplementation on inflammatory and glycemic markers among overweight and obese adults. Most of the reported trial outcomes were nonsignificant and included considerable bias.

A recently published interesting meta-analysis addressed the association between vitD3 and the percentage of fat mass, pooling together observational studies and randomized clinical trials [14]. The results state that the 25(OH)-D level is inversely correlated with the percentage of fat mass, but the meta-analysis did not support the hypothesis that vitD supplementation augments body-fat loss.

Genetic Factors Linking VitD Status to Obesity Pathology

Genetic Mechanisms Influencing VitD Status

It is clear that genetic factors play an important role in determining serum 25(OH)-D concentrations. Several twin and family studies have reported the vitD heritability to be between 23 and 80% [15, 16]. Furthermore, genome-wide association studies and candidate gene approaches have reached a consensus in identifying reproducible associations and a replicated genetic architecture of vitD [17, 18].

Most of the reported associations involve genes related to the vitD metabolism, highlighting important control points in vitD molecular pathways. According to the latest conducted genome-wide association studies and candidate gene studies [19–22], some of the most remarkable associations include genes such as Hydroxyvitamin D-1-α hydroxylase (*CYP27B1*), vitD 25-hydroxylase (*CYP2R1*), vitD binding protein (*DBP/GC*), vitD receptor (*VDR*), vitD 24-hydroxylase (*CYP24A1*), 7-dehydrocholesterol reductase (*DHCR7*), retinoid X receptors (*RXR*), calcium-sensing receptor (*CASR*), *NPY*, *FOXA2*, *SSTR4,* and *IVL*. Several review articles illustrating and detailing all these findings have been published by Jolliffe et al. [18] and by Bahrami et al. [17]. Specifically, we recommend that Jolliffe's work, which presents a meticulous and detailed table of all the reported associations, be looked into. Generally, all associated genes can be categorized into (1) genes involved in the upstream production of 25(OH)-D; (2) genes involved in the downstream activation of vitD to the active ligand 25(OH)-D; (3) carrier proteins (which bind to the vitD molecule and the active ligand 25(OH)-D; (4) receptors and related co-activating proteins (affecting executive ability of the ligand-receptor); and (5) other second-order processes that affect the regulatory pathways such as calcium concentrations. On the other hand, few null/negative associations have also been reported [23, 24].

According to these data, though clinical usage is not yet applicable, it seems certain that circulating 25(OH)-D concentrations is, at least, partially determined by genetic factors.

VitD-Related Genetic Markers and Obesity

Despite the huge evidence of aberrations in the vitD-endocrine system of people with obesity, the direction of the association between vitD and the obese phenotype remains poorly understood. This fact has motivated the scientific community to conduct genetic analyses in order to

test whether vitD-related genes, besides serum 25(OH)-D concentrations, are also associated with obesity traits. The advantage of studying the genetic determinants of 25(OH)-D levels and obesity is that genetic factors precede obesity and do not change with obesity status, providing the opportunity to determine which proteins link these 2 conditions at the molecular level, as well as facilitating the identification of population subgroups with high risk of vitD deficiency.

While mutations in *DBP/GC, CYP2R1,* and *DHCR7* have been the most widely investigated source of variation in circulating concentrations of 25(OH)-D [17, 18], when we refer to obesity, variation in the *VDR* gene represents the bulk of the findings. The fact that limited overlap between genetic determinants of vitD status and genetic determinants is associated with obesity is surprising and may suggest 2 different things. On the one hand, it might suggest a lack of studies investigating the influence of variation in genes other than *VDR* on obesity. On the other hand, it might have a biological significance, suggesting that variation in *VDR* is a more important determinant of obesity phenotype than circulating 25(OH)-D concentrations. This last hypothesis makes even more sense, since the VDR protein is present in adipose tissue (AT) and may contribute to the action of vitD and its analogs in adipocytes. In this way, obesity reported that associations with *VDR* polymorphisms could be related to either a direct effect of vitD in adipocyte differentiation and metabolism, or an indirect effect by modulation of insulin secretion.

VDR Genetic Variants

The *VDR* gene spans 63.49 kb on the 12q12-q14 in the human genome. *VDR* has a considerable noncoding region including exons 1F–1C and an additional region of 8 exons (2–9) that codify the VDR protein [25]. The minor allele of the *VDR* SNP rs10735810 (also known as FokI polymorphism) leads to a VDR protein that is 3 amino acids longer by directly introducing a new translation start codon. It influences the activity of VDR protein, leads to a fewer transcriptional activator effectiveness, and alters the functional properties of the receptor [17].

Mainly 4 *VDR* polymorphisms, including thers10735810 FoKI SNP and 3 additional ones (the rs7975232 ApaI, the rs1544410 BsmI, and the rs731236 TaqI), have been analyzed in relation to genetic predisposition to obesity; however, findings are contradictory.

Among the studies with statistically significant results, we highlight a few conducted in a variety of human ethnicities, including Caucasian, American, and Asian populations. The first study to report an association between *VDR* polymorphisms and obesity was conducted by Ye et al. [26]; they found that the *VDR* TaqI_T allele was associated with obesity in French Caucasian individuals with early onset of type 2 diabetes. Since then, many studies have reported both similar and contradictory results. Bienertová-Vašků et al. [27] conducted a *VDR*-genetic analysis in a central-European population and found that genetic variability in the *VDR* region (including FokI, ApaI, and EcoRV polymorphisms) may be an important factor influencing anthropometric characteristics associated with obesity. In concordance with these results, the *VDR* TaqI_T allele was associated with a higher risk of obesity in Greek individuals (contributing to an elevated BMI of 3 kg/m^2 per risk allele) in comparison to the alternative G allele [28]. Regarding American populations, in a recent study conducted on 1,773 healthy women aged 35–80 from Western New York State (USA), the *VDR* rs3782905 polymorphism was positively associated with adiposity markers [29]. In relation to children, allelic variation in *VDR* was found to be associated with obesity in a population of children and adolescents from Brazil [30]. Contrarily, a study conducted in a Saudi cohort identified that the *VDR* TaqI_G and Bsm-I_T minor allele polymorphisms were significantly more frequent in obese individuals [31]. Similar results were found in another study conducted by the same research group, again in Saudi population [32].

The TaqI, BsmI, FokI, ApaI, and other *VDR* polymorphisms have also been associated with obesity-related phenotypes in 6 additional studies conducted on Asian and European populations [33–38]. All reported associations for *VDR* SNPs are summarized in Table 1 and Table 2. Although the pathophysiological mechanisms underlying these associations remain unexplained, they could be related either to a direct effect of vitD on adipocyte differentiation and metabolism or to an indirect effect by modulation of insulin secretion. On the other hand, null associations between *VDR* SNPs and obesity have also been reported [39–44].

Other VitD-Related Genes (DHCR7, CYP2R1, DBP|GC, CYP27B1, CYP27A1, CYP24A1, and RXRG)

Studies have also reported associations between vitD-pathway genes other than *VDR* (such as *DHCR7*, *CYP2R1*, *DBP/GC*, *CYP27B1*, *CYP27A1*, *CYP24A1,* and *RXRG*) and many obesity-related traits. However, again, the findings have been inconsistent.

Table 1. Summary of positive associations between *VDR* polymorphism and obesity-related traits

SNP ID	Alternative name	Major (minor) alleles	Reported effect for minor allele or variant
rs10735810	FoKI	C (T)	Increased sum of skinfold thickness and total % of body fat in central-European population [27]. Increased fat free mass in a cohort of older Caucasian men [38].
rs7975232	ApaI	A (C)	Associated with reduced waist circumference fat in central-European population [27]. Lower risk of obesity and overweight in postmenopausal Vietnamese women [33].
rs731236	TaqI	A (G)	Associated with lower risk of obesity in French Caucasian individuals with early onset of T2D [26]. Significantly more frequent in Saudi obese individuals [31, 32]. Lower risk of obesity in Greek individuals [28]. Lower risk of obesity and overweight in postmenopausal Vietnamese women [33].
rs1544410	BsmI	C (T)	Significantly more frequent in Saudi obese individuals [31, 32]. Positive correlations with height and with lean body mass in Brazilian obese children [30]. Higher BMI in postmenopausal Vietnamese women [33]. Lower BMI and waist circumference in European ancestry men [35]. Lower fat mass in Swedish women [37].
rs3782905	–	G (C)	Larger waist circumference in female adults recruited from western New York [29].
rs11568820	CDX2	C (T)	Associated with larger BMI in Asian individuals [35].
rs4516035	EcoRV	T (C)	Associated with reduced sum of skin fold thickness in central-European population [27].

VDR, vitamin D receptor; T2D, type 2 diabetes; BMI, body mass index.

Most positive associations have been identified between obesity and the *DBP/GC* gene in individuals of European ancestry [45–47]. Instead, genes such as hydroxylases or *DHCR7* concentrate the majority of null association results [41, 42]. Of note is also the study of Vimaleswaran et al. [41], who looked for associations between 100 tagging polymorphisms from 8 genes (*DHCR7*, *CYP2R1*, *DBP/GC*, *CYP27B1*, *CYP27A1*, *CYP24A1*, *VDR,* and *RXRG*) and obesity traits in 5,224 participants of European ancestry. Their findings, despite the very large sample, suggested that the vitD pathway genes are unlikely to have a major role in obesity-related traits. Similar results were obtained from another study conducted in 6,922 Chinese women, where the association between 198 SNPs from *CYP27A1*, *CYP27B1*, *CYP24A1*, *DBP/GC,* and *VDR* genes and obesity has been investigated [42]. Although in comparison with the studies conducted on the *VDR* gene these studies have stronger and more powerful population samples, these are rather scarce and further analyses are required in order to make firm assertions. Therefore, the listed studies point to the fact that the *DBP/GC* gene could be an important protein linking obesity and vitD status, while the rest of vitD metabolism genes do not seem to play an important role in the etiology of this disease.

As a general conclusion, although genetic markers have been reported to be important factors for the development of vitD deficiency and its associated phenotypes, we can see how vitD-pathway genes remain poorly understood in the context of obesity and seem to be unessential factors for the development of the disease. Using a bidirectional genetic approach, the study concluded that a higher BMI (and the genes that predispose to obesity) decreases serum 25(OH)-D levels, whereas lower 25(OH)-D levels or the genes that are associated with reduced serum concentration of 25(OH)-D have, at most, very small effects on obesity.

Molecular Mechanism of VitD Related to Obesity

The AT is the main storage organ for vitD and its release, and it also expresses VDR and enzymes involved in vitD metabolism. Indeed, adipocytes respond to 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) exposure, and the depots of vitD in AT are proportionate to its plasma concentration and release it at a much lower rate that is proportionate to its concentration in AT. VitD, $1,25(OH)_2D$, is active in adipocytes and interacts with membrane receptors, adaptor molecules, phosphatases, and nuclear co-regulator proteins, participating in the control of gene expression and cell signaling [48].

VitD and Adipogenesis

The effects of $1,25(OH)_2$-D are still unclear in the adipogenesis process. Adipogenesis is the process of cell differentiation by which preadipocytes become mature

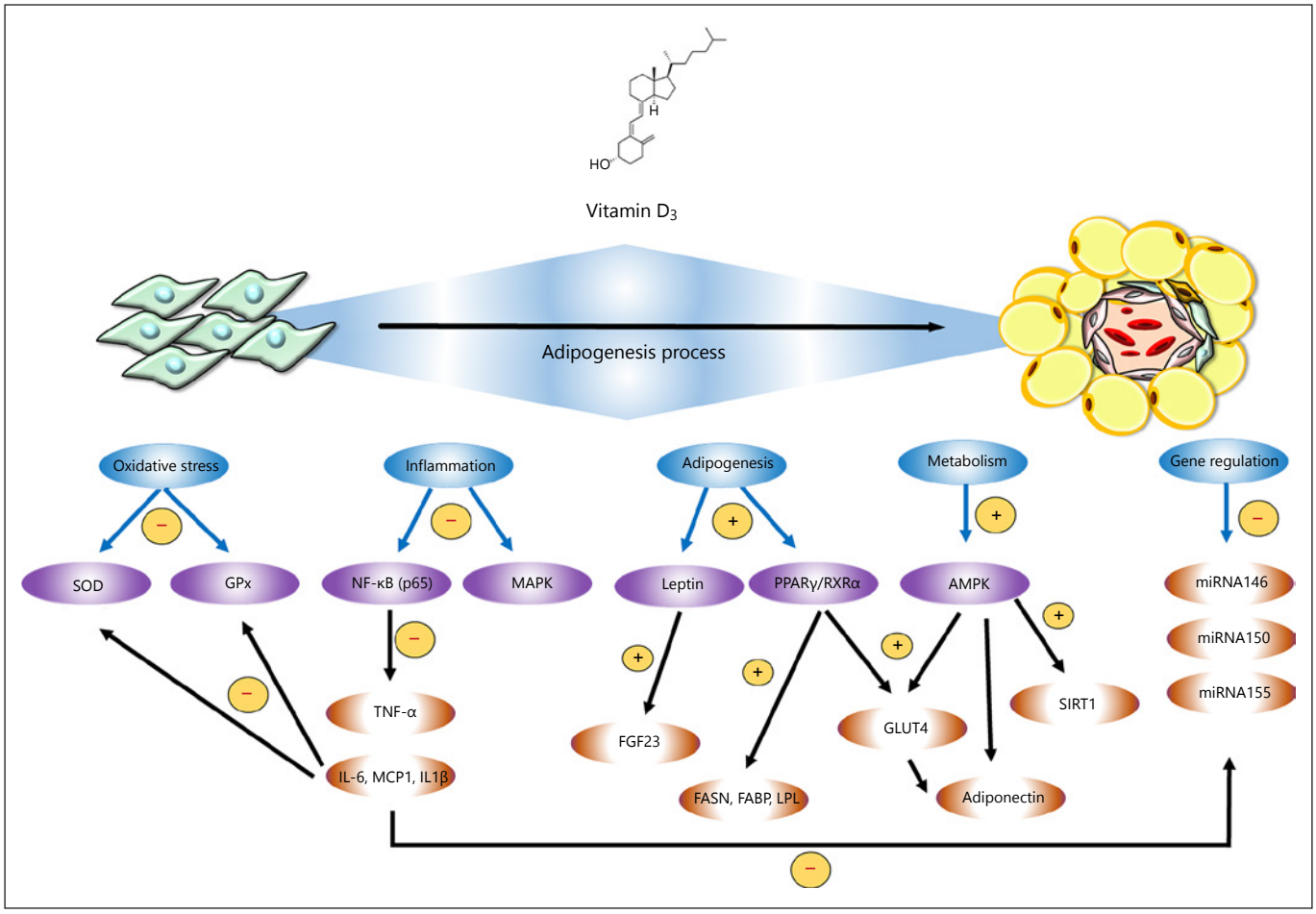
Table 2. Details of referenced studies showing positive associations between *VDR* polymorphism and obesity-related traits

Reference number	Sample size and population origin	Study design	Study variables
[26]	309 French subjects with T2D and 143 French controls	Case control	Disease status, age at diagnosis, duration, arterial hypertension, and lipid profile
[27]	882 Central European Caucasian individuals of Czech origin (232 males and 650 females)	Cohort (population-based study)	Anthropometry
[28]	184 individuals (82 with obesity and 102 controls), from Northern and Central Greece	Case control	Anthropometric (waist and hip circumference) and biochemical parameters (HDL, LDL, cholesterol, and triglycerides)
[29]	1,773 healthy female adults recruited from western New York	Cohort (population-based study)	Anthropometric measurements (BMI, waist circumference, and abdominal height) as well as age, education, total energy intake, smoking status, alcohol intake, and menopausal status
[30]	319 Brazilian obese children and adolescents (7–16 years of age)	Intervention study (20-week weight-loss program)	Anthropometric measurements, glucose tolerance test, and HOMA index
[31]	402 Saudi obese subjects and 489 non-obese Saudi controls	Case control	Anthropometry, cytokines and plasma LPS concentrations
[32]	570 Saudi individuals (285 subjects with metabolic syndrome and 285 controls)	Case control	Anthropometry and biochemical analysis
[33]	140 healthy postmenopausal Vietnamese women with rural origin	Cohort (population-based study)	Anthropometry, ethnicity, educational level, occupation, medical and reproductive history, dietary, smoking and drinking history, as well as physical activity and dietary intake questionnaires
[35]	176 unrelated randomly selected European ancestry men aged 25–65 years old	Cohort (population-based study)	Anthropometric (waist and hip circumference) and biochemical parameters (glucose, insulin, HDL, LDL, total cholesterol, and triglycerides)
[37]	175 healthy European Ancestry women aged 20–39 years old	Cohort (population-based study)	Anthropometric measurements (fat mass, lean mass, body weight, and body mass index) and muscle strength (quadriceps, hamstring, and grip strength)
[38]	302 older European Caucasian men	Cohort (population-based study)	Anthropometry, physical activity and dietary intake questionnaires

VDR, vitamin D receptor; T2D, type 2 diabetes; BMI, body mass index; LPS, lipopolysaccharide; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

adipocytes. In human cells, $1,25(OH)_2$-D stimulates adipogenesis by the upregulation of gene expression enzymes of the lipogenesis process such as fatty acid synthase (*FASN*), fatty acid binding protein (*FABP*), and peroxisome proliferator activator receptor (*PPAR*)-*γ*, which is the main transcription factor involved in the adipogenic differentiation [49]. However, $1,25(OH)_2$-D inhibits this process in mice 3T3–L1 pre-adipocytes, by downregulating the transcription factors *C/EBPα*, *C/EBPβ*, and *PPAR-γ*, and sequestering the nuclear receptor retinoic X receptor (RXR), a member of the nuclear receptor superfamily. On the contrary, $1,25(OH)_2$-D up-regulates *FASN* and lipoprotein lipase (*LPL*) in human subcutaneous preadipocytes [50], a process that might be mediated by an increased expression of *PPAR-γ* [49].

Generally, vitD, both 25(OH)-D and $1,25(OH)_2$-D, are capable of promoting adipogenic differentiation into mature adipocytes due to the presence of 1α-hydroxylase in mature adipocytes. Additionally, $1,25(OH)_2$-D stimulates the translocation of the glucose transporter 4 (*GLUT4*) into the membrane, promotes adiponectin secretion [50, 51] and the expression of typical adipocyte genes, such as leptin, and inhibits the expression of uncoupling proteins in vitro [52]. Thus, VDR directly inhibits the expression of the uncoupling protein-1 (*UCP1*), the critical protein for uncoupling fatty acid oxidation in brown AT (BAT). As a matter of fact, this process occurs cell autonomously and is independent of the physiologic VDR hormone ligand, $1,25(OH)_2$-D [53]. On the other hand, leptin is capable of increasing the secretion of fibroblast growth factor 23, which is a negative regulator of renal 1α-hydroxylase,



Color version available online

Fig. 1. Vitamin D effects on adipogenesis and inflammation. AMPK, adenosine monophosphate kinase; FABP4, fatty acid-binding protein 4; FASN, fatty acid synthase; FGF23, fibroblast growth factor 23; GLUT4, glucose transporter 4; GPX, gluthathion peroxidase; IL1β, interleukin 1-beta; IL6, interleukin 6; LPL, lipoprotein lipase; MAPK, mitogen-activated protein kinase; MCP1, monocyte chemotactic protein 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PPARγ, peroxisome proliferator-activator receptors gamma; RXRα, retinoid X receptor; SIRT1, sirtuin 1; SOD, superoxide dismutase; TNFα, tumor necrosis factor alpha.

thereby closing a negative feedback loop. However, an inhibitory effect of $1,25(OH)_2$-D on leptin secretion by human adipocytes has been observed in vitro. Indeed, the effect of vitD supplementation on leptin levels in humans remains poorly investigated and should be further addressed [52, 54]. Figure 1 summarizes the main vitD effects on adipogenesis in human adipocytes.

VitD regulates the adipokine secretion in adipocytes, such as adiponectin, leptin, and resistin [55]. Adiponectin is an anti-inflammatory and insulin-sensitizing hormone, which is the major adipokine secreted by adipocytes [56]. VitD is associated with low levels of adiponectin in children with obesity, and vitD supplementation ameliorates systemic inflammatory biomarkers, including adiponectin, in the subjects with type 2 diabetes [57]. However, no effect of $1,25(OH)_2$-D on adiponectin expression in human adipocyte culture has been observed [58].

VitD and Inflammation

Obesity is strongly associated with low-grade inflammation, and with the production and secretion of proinflammatory markers. Consequently, the immune cells play an important role in AT and immune homeostasis. A decreased vitD status is associated with obesity, and recent in vitro studies have described that $1,25(OH)_2$D exerts anti-inflammatory action on adipocytes [48, 59]. VitD promotes a lower chemokine and cytokine release by adipocytes and the chemotaxis of monocytes. In AT explants, vitD lowered the cytokine release from visceral

AT (VAT) but not from SAT [48, 55]. In VAT in people with obesity, there is a reduced adenosine monophosphate-activated protein kinase (AMPK) and it is closely associated with AT inflammation. In addition, as AMPK enhances sirtuin 1 by increasing NAD/NADH ratio and decreases AT macrophage infiltration and inflammation, both have been proposed as key regulators to prevent obesity and obesity-related metabolic dysfunction. A recent study conducted by Chang et al. [59] described that vitD deficiency significantly decreased mRNA levels of oxidation-related genes. Moreover, significant decrements of sirtuin 1 and AMPK activity were noted in obese rats fed with a vitD-insufficient diet. The observed deleterious effects of vitD deficiency on AT expansion, immune cell infiltration, and inflammatory status suggest that vitD plays a beneficial role in adipocyte metabolic metabolism and obesity progression [59].

Recently, it has been established that vitD modulates the expression of miRs in adipocytes in vitro. Three miRs (miR-146a, miR-150, and miR-155) have been identified, which are positively regulated by the tumor necrosis factor (TNF)-α in human adipocytes and its expression was strongly prevented by $1,25(OH)_2$-D preincubation. Additionally, the 3 miRs were increased following high-fat diet in epididymal white AT and reduced mice fed with high-fat diet supplemented with vitD. Thus, the involvement of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling in the induction of these miRs was confirmed both in vitro and in vivo using aP2-p65 transgenic mice. The ability of vitD to deactivate NF-κB signaling, via p65 and IκB phosphorylation inhibition in murine adipocytes, was observed and could constitute a driving molecular mechanism (Fig. 1) [60].

Besides the observed effects, vitD decreases proinflammatory cytokines and chemokines in mice after an injection of lipopolysaccharide and in diet-induced obese mice, which caused a metabolic inflammation [61]. In humans, a negative association was found between serum 25(OH)-D concentration and plasma interleukin (IL)-6 and TNF-α levels in normal-weight subjects. Nevertheless, vitD had no effect on inflammatory biomarkers in serum or AT in subjects with obesity [48, 62].

VitD exerts anti-inflammatory effects mediated by the inhibition of the NF-κB and mitogen activated protein kinase signaling pathways [60]. Additionally, vitD reduced toll-like receptor expression, which is increased in both immune cells and AT from people with obesity [63–65]. This is important, since the toll-like receptor is a transmembrane protein that pledges classical signaling cascades leading to the activation of NF-κB and cytokine production such as TNF-α [66]. In human adipocytes, $1,25(OH)_2$-D incubation decreases the levels of inflammatory markers such as IL-6, monocyte chemotactic protein-1, and IL-1β (mRNA and protein level). Moreover, this treatment downregulates the expression of the TNF-α-mediated pro-inflammatory marker. A similar effect has been observed in adipocyte-macrophage co-culture systems in which $1,25(OH)_2$-D decreased the proinflammatory marker expression under basal and TNF-α-stimulated conditions. The involvement of VDR and NF-κB was confirmed in this experimental condition. Finally, $1,25(OH)_2$-D treatment also provokes the dephosphorylating of p38, which is linked to the transcriptional induction of several Dusp family members. Consequently, a higher glucose uptake and AKT phosphorylation were observed leading to the fact that low-grade inflammation could be linked to vitD deficiency [67]. Another study has evaluated the effects of vitD administrations on markers of inflammation and oxidative stress in AT of high-fat diet induced obese rats. Thus, vitD treatment led to a significant reduction in AT TNF-α concentrations in both normal diet and high-fat diet obese rats. Monocyte chemotactic protein-1 concentration was also reduced in AT, and among markers of oxidative stress in AT, superoxide dismutase, and gluthathione peroxidase concentrations significantly increased in AT of high-fat diet supplemented with vitD (Fig. 1). Hence, vitD improved AT oxidative stress and inflammatory parameters in obese rats [68].

In summary, vitD plays an important role in the inflammation status in adipocytes and AT due to, principally, its capability for reducing the phosphorylation and translocation of NF-κB p65 into the nucleus, and, consequently, decreasing the proinflammatory and oxidative stress markers. Further studies are needed to elucidate the underlying mechanisms.

Conclusion and Future Recommendations

Based on the extensive scientific bibliography, a positive association between different levels of BMI and vitD deficiency both in adults and children has been clearly demonstrated. However, future prospective studies are necessary to evaluate the potential causal relationship between serum concentrations of vitD and obesity. There is less available information concerning vitD supplementation; it remains unclear whether it benefits the adverse metabolic profile in obesity. In order to further elucidate the role of vitD supplementation on obesity-related biomarkers, future randomized controlled trials should fo-

cus on improving the quality of the study design, particularly of trials assessing vitD, as most of the trials had 2 or more trial interventions (i.e., weight loss, resistance or exercise training interventions, with oral vitD supplementation). Second, the trials had a relatively small sample size and a variable vitD dosage and duration. Longer intervention periods of clinically safe higher doses should maybe be considered in future studies.

It seems certain that circulating 25(OH)-D concentrations are, at least, partially determined by genetic factors. In addition, the analysis of vitD-related genes in the context of obesity is a very interesting approach. In this sense, *VDR* genetic variants have been associated with obesity in some but not all studies. Moreover, unanimity in the findings across studies has not been reported for any particular population. Overall, the presence of so many studies with negative results as well as the presence of contradictory findings for certain *VDR* SNPs suggest that *VDR* genetic variants are unlikely to play a major role in obesity-related phenotypes and require further evidence for its association. Finally, it is worth mentioning that since most of the presented studies with null results failed to obtain significant P-values after correction for multiple testing, it might be helpful to perform studies with larger and more homogenous populations. Perhaps in this way, studies would come to consensus in identifying reproducible associations and would succeed in supporting the "plausible" hypothesis of *VDR* as an important gene in obesity.

Finally, a possible molecular mechanism of the relationship between obesity and vitD deficiency could be the capacity of vitD to regulate the gene expression related to the adipogenesis process, inflammation, oxidative stress, and metabolism in mature adipocytes as summarized in Figure 1. However, further studies are needed to elucidate the underlying mechanisms.

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Disclosure Statement

The authors declare no conflicts of interest related to the present article.

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