Vitamin D, Vitamin D Receptor, and Adipose Tissue

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Vitamin D, Vitamin D Receptor, and Adipose Tissue: Focus on Cellular Mechanisms

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OUTLINE

ADIPOSE TISSUE: STORAGE DEPOT AND TARGET FOR VITAMIN D

Overview

Adipocytes function in storage of energy reserves, secretion of adipokines that regulate appetite, and control of thermogenesis. The major form of adipose tissue is termed white adipose tissue (WAT) and is located in subcutaneous and visceral depots. Development and maintenance of WAT is dependent on the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), which drives adipogenesis (generation of new adipocytes from mesenchymal precursors) and regulates fatty acid storage and glucose metabolism. PPARγ regulates multiple target genes essential for adipocyte differentiation, lipid metabolism, and glucose homeostasis, as well as the expression of adipokines and cytokines secreted from adipose tissue. Brown adipose tissue (BAT) is a unique type of adipose tissue present in mammals that functions in body temperature regulation. Activated BAT uniquely expresses an "uncoupling protein" (UCP1) within the inner mitochondrial membrane, which uncouples substrate oxidation from ATP synthesis, leading to increased fuel oxidation and thermogenesis. The vitamin D receptor (VDR) and vitamin D-metabolizing enzymes (CYP24A1, CYP27B1) are expressed in adipose tissue, and vitamin D signaling has been shown to alter the differentiation and phenotype of both WAT and BAT adipocytes and to modulate adiposity and energy metabolism in vivo. Examples of recently uncovered mechanisms that contribute to the effects

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of vitamin D on adipose tissue include $1,25(OH)_2D$ modulation of PPARγ and WNT signaling during adipogenesis in WAT and direct repression of UCP1 by VDR in BAT. These and other effects of vitamin D steroids on adipose tissue are reviewed in this chapter.

Storage of Vitamin D Metabolites in Adipose Tissue

In addition to serving as a target tissue for $1,25(OH)_2D$ actions, adipose tissue contains a large proportion of the body's vitamin D pool. Early studies demonstrated that the vast majority of an orally administered dose of radiolabeled vitamin D_3 given to vitamin D-deficient rats was found in adipose tissue, with ~50% present as unmodified vitamin D_3 [1]. The kinetics of tissue uptake and clearance was studied in vitamin D-replete rats intracardially administered radiolabeled vitamin D_3 [2]. Tissues with high early concentrations of radioactivity were liver (70% of dose within 20 mins) and serum; tissues with slower accumulation followed by decline included intestinal mucosa, kidney, bone, and muscle. In contrast, adipose tissue radioactivity rose slowly and showed no decline with 10% of the dose recovered in adipose tissue after 1 week. Metabolism was also tissue specific because 80% of the radioactivity in liver, serum, and mucosa after 1 week was present as more polar metabolites, but in kidney, adipose tissue, and muscle 70% of the recovered radioactivity was present as vitamin D_3. These studies clearly identified adipose tissue as the major site of uptake of unmodified vitamin D_3 from the circulation. Similar studies with human adipose tissue obtained from autopsy and surgical sources confirmed uptake and storage of vitamin D_3 in fat [3]. Lawson et al. [4] developed an high-performance liquid chromatography method sensitive enough to detect tissue vitamin D content and reported substantial (50–100 ng/g) amounts of vitamin D_3 in human perirenal, pericardial, cervical, and axillary adipose tissue samples obtained at autopsy. It should be noted that per-renal fat has been characterized as a brown fat depot in humans [5], suggesting that vitamin D likely also accumulates in BAT. Lawson's study did not identify any obvious differences in vitamin D_3 concentration in adipose tissue according to sex, age, or time of year at which sample was collected.

A few studies have addressed whether the concentration of vitamin D in adipose tissue is affected by supplementation or UV exposure. Using vitamin D-deficient rats, Lawson's group formally demonstrated that vitamin D_3 accumulates in adipose tissue and (to a lesser extent) muscle in response to chronic UV exposure [6]. This study provided evidence that the pool of vitamin D_3 in adipose tissue is released during vitamin D deficiency with an estimated half-life of 12 days. Similar chronic dietary and UV exposure studies in minipigs confirmed that both epidermis-derived and orally administered vitamin D_3 are stored in adipose tissue and slowly released over time [7]. More recent data in orally replete minipigs kept indoors [8] indicated that ~75% of the total body vitamin D_3 pool (and 35% of the $25(OH)D_3$ pool) is present in fat tissue.

Collectively, these studies demonstrate that regardless of source (oral vs. cutaneous), the majority of vitamin D in the body is in adipose tissue where it is predominantly stored in the form of vitamin D_3. An exciting new approach (time-of-flight secondary ion mass spectrometry) has even localized vitamin D_3 within adipocyte lipid droplets [9]. During states of severe deficiency (at least in animal models), vitamin D_3 levels in adipose tissue become undetectable. Although 25(OH)D is also present in adipose tissue, this metabolite is more evenly distributed throughout other body tissues (such as liver and muscle) than in vitamin D_3 itself [8]. Most studies have reported that adipose tissue does not contain significant amounts of $1,25(OH)_2D$, although it can be detected at low levels with very sensitive methods [9].

Given the demonstrated role of adipose tissue in storage of vitamin D, it has been of interest to determine whether obesity modifies uptake or release of the vitamin from fat. A recent metaanalysis of 23 published studies [10] has confirmed a significantly elevated prevalence of vitamin D deficiency in obese (35% higher) and overweight (24% higher) subjects compared with normal subjects irrespective of age, latitude, or the specific cut-off used to define vitamin D deficiency. The etiology of low serum 25(OH)D in obese individuals is not fully understood and is likely multifactorial. Underlying mechanisms that have been proposed include low vitamin D intake and/or cutaneous synthesis, "sequestration" or volume dilution of vitamin D and 25(OH)D in the larger tissue mass (both adipose and muscle tissue may contribute), and other factors such as genetic variation in vitamin D metabolism or transport [11].

With respect to the impact of obesity on vitamin D stores in adipose tissue, Malmberg [9] utilized fat biopsy material to compare the concentrations of D_3, $25(OH)D_3$, and $1,25(OH)_2D_3$ in subcutaneous WAT (sWAT) of lean and obese individuals. Contrary to what would be predicted if adipose tissue "sequesters" 25(OH)D, both vitamin D_3 and $25(OH)D_3$ concentrations in sWAT were significantly lower in obese samples relative to control samples despite similar serum 25OHD between groups in this study. In a study aimed at determining whether weight loss alters adipose stores of vitamin D, Piccolo et al. [12] used liquid chromatography/mass–spectrometry to measure $25(OH)D_3$ concentration in sWAT biopsies of overweight and obese subjects (body mass index (BMI) between 28 and 37 kg/m^2) enrolled in a 12 week controlled diet restriction study. They found that sWAT and serum $25(OH)D_3$ concentrations were significantly correlated at baseline and after 12 weeks of diet restriction. However, there were no significant changes in either sWAT or serum $25(OH)D_3$ after diet restriction despite an average13% reduction in body fat in the subjects. These two studies suggest that adipose tissue does not concentrate 25(OH)D at the expense of serum, and at least in overweight and obese females, $25(OH)D_3$ is not released from subcutaneous adipose tissue stores during moderate weight loss.

Several studies have addressed the effects of obesity on the response to vitamin D supplementation or UV exposure. Wortsman et al. [13] conducted two studies with normal and obese subjects: one examined the rise in serum vitamin D_3 before and 24 h after exposure to whole-body UV-B radiation;

the other examined the rise in vitamin D_2 before and 6, 10, and 24 h after a bolus dose of 50,000 IU vitamin D_2. In both studies, the rise in serum vitamin D (D_2 for oral supplementation and D_3 for UV exposure) was attenuated in obese subjects relative to nonobese controls. With the oral challenge, achieved serum 25(OH)D was also lower, and parathyroid hormone was higher, in the obese subjects. When analyzed in relation to BMI, two significant inverse correlations were found: BMI versus peak serum vitamin D_2 after oral supplementation, and BMI versus peak serum vitamin D_3 after UV exposure. This group also examined the cutaneous synthesis of vitamin D_3 after UV-B exposure of skin biopsies from normal and obese subjects, however, no differences in conversion of 7-dehydrocholesterol to vitamin D_3 were observed. These data demonstrate that for the same level of UV exposure/cutaneous synthesis or intake, the level of circulating vitamin D achieved decreases as BMI increases. These findings with vitamin D_2 supplementation were confirmed for vitamin D_3 intake by Didriksen et al. [11], who pooled data from three randomized controlled trials of vitamin D_3 supplementation (40,000 IU/week for 6 months) and found that subjects with higher BMI had the lowest baseline 25(OH)D levels and also the smallest increase in 25(OH)D after supplementation. Similar conclusions were made based on analysis of a large dataset of self-reported vitamin D_3 supplementation in relation to serum 25(OH)D [14]. From this population data, it was calculated that obese individuals require >2.5 times higher doses of oral vitamin D than normal-weight individuals to achieve the same circulating level of 25(OH)D. In a randomized controlled study of vitamin D-deficient women given seven doses of vitamin D_3 ranging from 400 to 4800 IU/day, it was found that women with low BMI (<25 kg/m^2) developed higher levels of serum 25(OH)D after supplementation compared with women with high BMI [15]. The authors argue that the differences between lean and obese individuals result from volume dilution of vitamin D_3 in the larger mass of fat and muscle as BMI increases, rather than concentration of the vitamin in fat tissue.

Only a handful of studies have measured the concentration of vitamin D metabolites in fat after supplementation. Didriksen et al. [16] studied 29 adults with impaired glucose tolerance in a randomized controlled trial of vitamin D supplementation (20,000 IU/week for 3–5 years) versus placebo. In addition to serum 25(OH)D_3, vitamin D_3 and 25(OH)D_3 were measured in sWAT obtained by needle biopsy, and body fat was measured with dual-energy X-ray absorptiometry. In the 18 subjects supplemented with vitamin D_3, the median concentrations of serum 25(OH)D_3, sWAT D_3, and sWAT 25(OH)D_3 were markedly increased [99 nmol/l, 209 ng/g, and 3.8 ng/g, respectively] relative to the 11 subjects in the placebo group [62 nmol/l, 32 ng/g, and 2.5 ng/g]. Based on total body fat mass, the investigators calculated average body stores of 6.6 mg vitamin D_3 and 0.12 mg 25(OH)D_3 in the subjects supplemented with vitamin D_3. These data clearly indicate that adipose tissue has the capacity to store large quantities of vitamin D steroids in response to chronic oral loading. However, because the mechanisms that control uptake and release of vitamin D from adipose tissue (or other body pools) have yet to be defined, the degree to which such reserves can be utilized, and under what conditions, are unclear. Identification of genes and pathways that regulate vitamin D storage in various tissues is a research priority. Understanding the underlying mechanisms will facilitate studies focused on whether genetics or physiological states (such as rapid growth, pregnancy, or aging) alter adipose pools of vitamin D leading to differences in serum 25(OH)D. Given the growing incidence of metabolic disturbances in the population, it will be also be critical to determine how pathological conditions such as obesity, diabetes, and metabolic syndrome alter the body's ability to access these reserves.

EFFECT OF VITAMIN D ON ADIPOGENESIS AT THE CELLULAR LEVEL

Model Systems for the Study of Adipogenesis

Adipocytes originate from multipotent mesenchymal stem cells, which can also differentiate into bone, cartilage, muscle, marrow stromal cells, and other connective tissue lineages. For all of these lineages, the process begins with proliferation and proceeds through commitment, lineage progression, differentiation, and maturation stages. Distinct triggers have been identified that promote commitment to each lineage in culture. For the adipocyte differentiation program, a "cocktail" of insulin, dexamethasone, and 3-isobutyl-1-methylxanthine (IBMX, a phosphodiesterase inhibitor) in media supplemented with 10% fetal bovine serum is sufficient to trigger adipogenesis in both established and primary culture models, including multipotent mesenchymal stromal cells derived from adipose tissue, bone, bone marrow, and muscle. Other media additions commonly used to stimulate adipogenesis in these model systems include PPARγ agonists (rosiglitazone, troglitazone), transferrin, indomethacin, and triiodothyronine [17]. The sources and concentrations of these reagents as well as the degree and kinetics of differentiation achieved vary considerably with the lineage, stage, tissue origin, and species of the cell population used. Heterogeneity is more pronounced in primary cell model systems because of the presence of cells at variable stages of commitment in these preparations. The impact of vitamin D signaling has been studied in both primary and established models of adipogenesis, and considerable inconsistencies have been reported, with 1,25$(OH)_2$D both enhancing and inhibiting adipocyte differentiation as described below. Sources of variability include not only the inherent heterogeneity of the model systems but also differences in methodology. The effects of 1,25$(OH)_2$D have been studied using a wide variety of protocols (i.e., with or without adipogenic triggers, short- versus long-term exposure, physiologic, and nonphysiologic concentrations, etc.) and with respect to many different outcomes (proliferation, lipid accumulation, gene expression, cytokine secretion, etc.). Although these data clearly indicate that 1,25$(OH)_2$D impacts both preadipocytes and differentiated adipocytes, the data are not

entirely consistent, particularly with respect to lipid accumulation. Highlights of the published data are provided below.

Effects of 1,25(OH)$_2$D Treatment and Vitamin D Receptor on Differentiation in 3T3-L1 Cells

The L1 subclone of 3T3 Swiss albino mouse embryonic fibroblasts (3T3-L1, CL-173 from ATCC) is the most commonly used cell model of adipogenesis because of its reproducible and rapid (within 7 days) adipose differentiation program in response to insulin, dexamethasone, and IBMX. 3T3-L1 cells are committed preadipocytes, which undergo mitotic expansion to a confluent state prior to the onset of lipid accumulation. An early clue to involvement of the vitamin D pathway in adipogenesis was the observation that VDR gene expression is dynamically and transiently upregulated early in 3T3-L1 adipogenic differentiation [18], resulting in marked increases in VDR protein abundance within 4 h of switch to adipogenic media (Fig. 34.1A), an effect that has been attributed to the IBMX component of the differentiation mix [19]. As differentiation proceeds, VDR expression decreases to almost undetectable levels in mature adipocytes [19–21]. The exact role of VDR in this model remains to be fully clarified because both knockdown of endogenous mouse VDR [19] and forced expression of human VDR [22] inhibit adipogenesis, suggesting that the transient nature of the VDR upregulation may be critical. It is worth noting that 3T3-L1 cells represent committed preadipocytes and cannot be induced to differentiate into the osteoblast lineage. Thus, this model is probably not appropriate to study whether VDR signaling is involved in control of the earliest events in mesenchymal stem cell lineage determination.

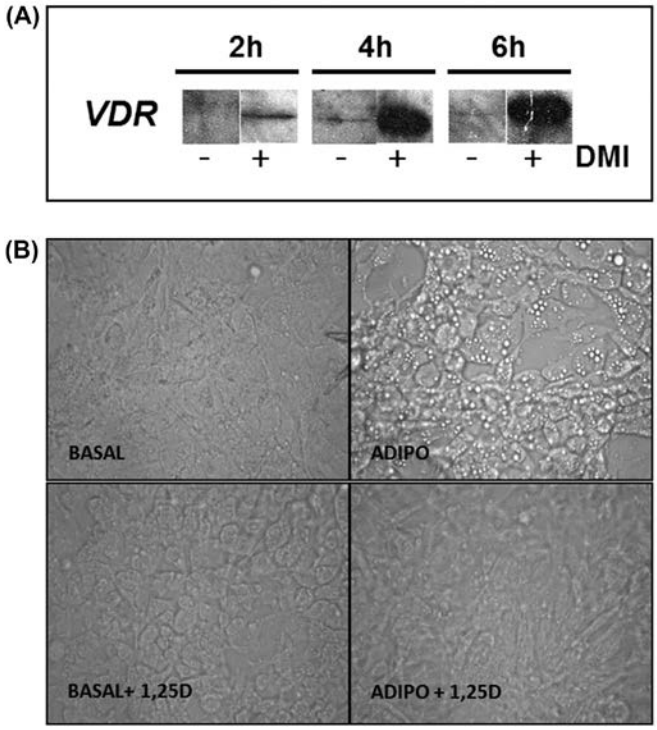

FIGURE 34.1 Vitamin D pathway in 3T3-L1 adipogenesis. (A) vitamin D receptor (VDR) protein expression in 3T3-L1 cells 2, 4, or 6 h after switch to adipogenic media containing dexamethasone, methylxanthine, and insulin (DMI). (B) Lipid accumulation in 3T3-L1 cells incubated in basal media or adipogenic media (ADIPO) in the presence or absence of 100 nM 1,25(OH)$_2$D (1,25D).

Of particular interest with respect to vitamin D signaling, undifferentiated (but not differentiated) 3T3-L1 cells express the endocytic receptor megalin, which facilitates uptake of 25(OH)D [23]. Furthermore, 3T3-L1 preadipocytes express CYP27B1 and synthesize 1,25(OH)$_2$D when exposed to physiological concentrations of 25(OH)D [24], supporting a role for VDR ligands in control of preadipocyte differentiation. Inclusion of 1,25(OH)$_2$D in the adipogenic media at concentrations of 1 nM or above is strongly inhibitory to the differentiation process in 3T3-L1 cells, with reduction in lipid accumulation (Fig. 34.1B) and suppression of key adipogenic transcription factors including PPARγ and C/EBPα [19,21,22,25]. 1,25(OH)$_2$D also decreases the endogenous formation of PPARγ ligands by 3T3-L1 cells, consistent with the finding that the PPARγ agonist troglitazone can partially overcome the inhibitory effects of 1,25(OH)$_2$D on lipid accumulation [19,22]. Addition of 1,25(OH)$_2$D within 48 h of the switch to adipogenic media is necessary for inhibition of differentiation. Although 1,25(OH)$_2$D is antiproliferative in many cell lines, it does not inhibit the mitotic expansion step of adipogenesis in 3T3-L1 cells [22]. The inhibitory effects of 1,25(OH)$_2$D appear to be related, at least in part, to its ability to stabilize VDR expression, supporting the contention that sustained VDR signaling blocks the differentiation program [19,22]. Indeed, Kong and Li [22] demonstrated that 1,25(OH)$_2$D inhibited PPARγ expression in mouse embryo fibroblasts from wild-type but not VDR knockout (VDRKO) mice. Through comparative study of synthetic vitamin D analogs, Thomson et al. demonstrated [26] that ligand binding to the VDR is necessary for inhibition of 3T3-L1 cell adipogenesis. Similarly, earlier studies profiled a series of endogenous vitamin D metabolites and reported that inhibition of adipogenesis correlated with binding to VDR [21].

Inhibition of adipogenesis in 3T3-L1 cells by 1,25(OH)$_2$D is typically measured as reduced lipid droplet formation, which correlates with decreases in expression of lipogenic genes such as fatty acid-binding protein 4 (FABP4) and lipoprotein lipase (LPL). Other data implicate the WNT/β-catenin pathway as a 1,25(OH)$_2$D target in 3T3-L1 cells. Components of the WNT/β-catenin pathway are substantially downregulated during adipogenesis, but 1,25(OH)$_2$D treatment was found to increase the expression of WNT10B, DVL2, phosphorylated GSK3β, and nuclear β-catenin [27]. Given that β-catenin inhibits PPARγ signaling, these data support a model whereby the antiadipogenic effects of 1,25(OH)$_2$D promote WNT/β-catenin signaling which in turn suppresses PPARγ activity.

In addition to the inhibitory effects of vitamin D signaling on adipogenesis, 1,25(OH)$_2$D has been shown to alter the phenotype of mature 3T3-L1 cells. Thus, even though VDR expression decreases during adipocyte differentiation, sufficient VDR is present in fully differentiated 3T3-L1 adipocytes for functional responses. Reported effects of 1,25(OH)$_2$D include modulation of FGF23, leptin and C/EBPβ expression [28], cytokine synthesis and secretion [29], basal and

TNFα stimulated proinflammatory responses (Il-6, MCP-1, IL-1β), and glucose uptake [30]. In addition, $1,25(OH)_2D$ alters mitochondrial membrane potential, ATP production, UCP1 expression, and generation of reactive oxygen species in differentiated 3T3-L1 cells, suggesting effects on energy expenditure [31,32]. A detailed study by Chang and Kim [33] reported that 24 h treatment of mature 3T3-L1 cells enhanced basal and isoproterenol-stimulated lipolysis in associated with changes in gene expression (FABP4, CEBPα, FAS, PPARγ, and SCD-1 were decreased whereas CPT1α, PGC1α, PPARα, and UCP1 were increased). $1,25(OH)_2D$ also increased activity of the SIRT1 deacetylase and enhanced cellular NAD-to-NADH ratio, suggesting that vitamin D status might promote fat mobilization concurrently with activation of the NAD–SIRT1 pathway (a major regulator of systemic metabolism and aging). These data indicate that $1,25(OH)_2D$ and VDR have the potential to influence paracrine signaling between mature adipocytes and immune cells as well as more distant communications between adipose depots and peripheral tissues.

Effects of $1,25(OH)_2D$ on Additional Established Rodent Cell Line Models of Adipogenesis

The effects of $1,25(OH)_2D$ have also been characterized in the Ob17 preadipocyte cell line, which was derived from epididymal fat tissue of a genetically obese mouse (ob/ob) on the C57/BL6J genetic background [34]. VDR expression is also transiently increased during adipogenesis in Ob17 cells, but in contrast to 3T3-L1 cells, $1,25(OH)_2D$ at low (0.25 nM) concentrations promotes, whereas higher concentrations inhibit, adipogenesis [34–36]. The discordant response of Ob17 cells to $1,25(OH)_2D$ (compared with 3T3-L1 cells) might be related to the underlying defect in ob/ob mice, which is a loss of function mutation in the adipokine leptin. Leptin secreted from adipocytes is an important regulator of appetite, but adipocytes also have leptin receptors, and treatment of 3T3-L1 cells with exogenous leptin inhibits adipogenesis [37]. $1,25(OH)_2D$ has been shown to directly regulate leptin transcription and secretion [38,39], and leptin can regulate renal vitamin D hydroxylases [40]. Further studies to define the interactions between leptin and $1,25(OH)_2D$ in the context of cellular adipogenesis are warranted.

Similar to the Ob17 cell studies, a bimodal dose response to $1,25(OH)_2D$ was reported by Ryan et al. [41] in C2C12 cells, an immortal myoblast line derived from a C3H mouse, which has the capacity to transdifferentiate into adipocytes or osteoblasts in response to different triggers. In this system, $1,25(OH)_2D$ at low concentrations (10^{-13} to 10^{-11} M) promoted adipocyte conversion (lipid accumulation and upregulation of PPARγ2 and FABP4) in the presence of insulin, dexamethasone, triiodothyronine, and rosiglitazone, whereas higher concentrations ($\geq 10^{-9}$ M) were inhibitory. In yet another model system (BMS2 cells, a multipotent mesenchymal cell line derived from bone marrow), inhibition of adipogenesis by $1,25(OH)_2D$ was reported at all concentrations tested [42]. As reported for 3T3-L1 cells, $1,25(OH)_2D$ was less effective in blocking adipogenesis of BMS2 cells in response to PPARγ agonists than that induced by insulin, dexamethasone, and IBMX. This group also generated several clones from parental BMS2 cells and interestingly found at least one example of a clone in which adipogenesis was stimulated by $1,25(OH)_2D$. Thus, it is clear that heterogeneity is present even in established cell lines, contributing to differential sensitivity to $1,25(OH)_2D$ and its phenotypic effects with respect to adipogenesis.

Vitamin D Signaling in Primary Cultures of Mesenchymal Cells

Several groups have utilized primary cultures of multipotent mesenchymal cells from various animal tissues for study of $1,25(OH)_2D$ modulation of adipogenesis. Fetal calvaria-derived mesenchymal cell cultures contain cells committed to either the osteoblast or adipocyte lineages as well as a low frequency of bipotential progenitors capable of both adipogenic and osteogenic differentiation. In these heterogeneous cultures, $1,25(OH)_2D$ dose dependently (from 0.1 to 100 nM) stimulates adipocyte foci development while inhibiting osteoblastic differentiation [43]. Follow-up studies indicated that $1,25(OH)_2D$ significantly altered the distribution of mesenchymal progenitors in the population, decreasing the percentage of osteogenic precursors while increasing the percentage of osteogenic/adipogenic bipotent cells [44]. Despite initial bipotency, exposure to $1,25(OH)_2D$ triggered commitment of these cells to adipogenesis because adipogenic differentiation was maintained after removal of $1,25(OH)_2D$. The effects of $1,25(OH)_2D$ on adipogenesis were additive with dexamethasone at low concentrations and synergistic at higher concentrations of either compound. Through generation of individual clones from fetal rat calvarial preparations, Bellows and Heersche [45] demonstrated that the $1,25(OH)_2D$-responsive adipocyte progenitors were distinct from the dexamethasone-responsive adipocyte progenitors. These data support the concept that $1,25(OH)_2D$ modulates lineage determination in primary cultures of mesenchymal progenitor cells, leading to survival and/or outgrowth of precursors capable of commitment to adipogenic differentiation.

Using procedures similar to those for fetal rat calvarial cell preparations, the effects of $1,25(OH)_2D$ on lineage determination of mesenchymal precursor cells isolated from bone marrow have been studied, again with conflicting results. Data from Kelly and Gimble [42] in murine femoral-derived bone marrow precursors are consistent with an antiadipogenic effect of $1,25(OH)_2D$ at nanomolar concentrations (10^{-12}–10^{-8} M). Other groups, however, reported that $1,25(OH)_2D$ enhanced lipid accumulation in bone marrow precursors derived from rats [46] and pigs [47].

Role of the Vitamin D Receptor in Control of Adipogenesis

To determine the specific role of the VDR in adipogenic differentiation of murine cells, Narvaez et al. [48] established mesenchymal cultures from bone marrow of 6-month-old

wild-type and VDRKO mice for ex vivo studies. Similar to 3T3-L1 cells [18,19,22], VDR was induced in murine mesenchymal cells within 4h of transfer to adipogenic media (insulin, dexamethasone, IBMX, indomethecin), remained high for 24h, and then decreased. In this model, peak lipogenesis was observed after 10 days in adipogenic media, and inclusion of 10nM $1,25(OH)_2D$ enhanced lipid droplet formation. Inclusion of $1,25(OH)_2D$ delayed the decrease in VDR expression and enhanced expression of PPARγ (see also Chapter 14). Cultures from VDRKO mice demonstrated impaired lipogenesis in both the presence and absence of $1,25(OH)_2D$, which was rescued by stable expression of human VDR. These data support the concept that adipogenesis per se is not defective in VDRKO cells because ectopic expression of VDR promoted both basal and $1,25(OH)_2D$ stimulated adipogenesis. Consistent with these data, Kong and Li [38] demonstrated that $1,25(OH)_2D$ stimulated leptin synthesis and secretion in adipose tissue cultures from adult (3–4 month old) wild-type, but not VDRKO, mice. Furthermore, basal levels of leptin synthesis and secretion were lower in cultures from VDRKO mice compared with that from their wild-type littermates.

In similar studies with bone marrow–derived mesenchymal precursors from neonatal 18-day-old wild-type and VDRKO mice, Cianferotti and Demay [49] reported opposite results. When cultured under adipogenic conditions, cultures from VDRKO mice accumulated more lipid than wild-type cultures and exhibited higher expression of PPARγ and other markers of adipogenic differentiation relative to those isolated from wild-type mice. These effects were correlated with upregulation of DKK1 and SFRP2 (inhibitors of the WNT pathway) in VDRKO cells, and suppression of these genes in wild-type cells treated with $1,25(OH)_2D$. The underlying basis for the opposing results in this study compared with those of Narvaez et al. [48] and Kong and Li [38] cited above is unclear, but it is worth pointing out that this group utilized cells from neonatal rather than adult mice and omitted insulin from the adipogenic differentiation media.

Taken together, these studies in both established and primary cultures indicate that $1,25(OH)_2D$ and VDR exert substantial effects on lineage determination in mesenchymal progenitor cells, although the specific effects vary with model system. The divergent responses to $1,25(OH)_2D$ in distinct in vitro model systems may be related to culture conditions (i.e., Ob17 cells differentiate in response to insulin and triiodothyronine rather than insulin, dexamethasone, and IBMX, and some studies fail to fully describe media additives), cell line origin (embryonic, fetal, neonatal, or adult tissue), and species/genetic background (rat, pig, different strains of inbred mice). With respect to the latter, comparison of bone marrow preparations from five strains of inbred mice has indeed demonstrated differences in media requirements for growth as well as differentiation potential and surface epitopes [50], therefore it is conceivable that responses to $1,25(OH)_2D$ could differ with genetic background. Another variable is that some cells (i.e., 3T3-L1) endogenously produce PPARγ ligands on treatment with adipogenic media, whereas others require exogenous supplementation with synthetic PPARγ ligands for differentiation. These uncertainties make it difficult to dissect the interactions between PPARγ and VDR activity. Furthermore, even in clonal cell lines, adipogenesis is a highly heterogeneous process, with only a subset of cells progressing to the characteristic phenotype of lipid droplet formation. Single-cell profiling techniques have demonstrated that changes in adipogenic marker genes do not strictly correlate with lipid droplet accumulation, and that the cell-to-cell variability in lipid droplet formation is highly dependent on the activity of the insulin signaling pathway [51]. For primary cultures an additional factor may be donor age because changes in adiposity are observed in VDRKO mice that become more pronounced with age (see Impact of Vitamin D Signaling on Adiposity in Mouse Models section). Thus, the differences in ages of donors (18 days–6 months) in the studies with VDRKO mice [38,48,49] may in part explain the contradictory results.

Effect of $1,25(OH)_2D$ on Differentiation of Human Mesenchymal Precursors

Given the heterogeneity and discrepancies observed in animal cell models of adipogenesis, several groups have instead focused on defining the effects of $1,25(OH)_2D$ on human mesenchymal progenitor cells. As described for murine primary cultures, human mesenchymal progenitor cells can be obtained from various sources, with the most commonly utilized being adipose-derived. The commercial vendor Zen-Bio (http://www.zen-bio.com/) provides primary cultures of human adipose-derived mesenchymal cells with deidentified data on tissue source of cells (subcutaneous vs. visceral) as well as age, gender, and BMI of donors. Narvaez et al. [48] assessed the effect of $1,25(OH)_2D$ on Zen-Bio sourced mesenchymal progenitor cells derived from subcutaneous adipose tissue of adult females with healthy BMI. Cultures from multiple donors were incubated in basal media and under adipogenic conditions in the presence and absence of 10nM $1,25(OH)_2D$ for 14 days. Although $1,25(OH)_2D$ did not enhance lipid accumulation in basal media, cells cultured in adipogenic media plus $1,25(OH)_2D$ exhibited enhanced lipid accumulation (Fig. 34.2) associated with upregulation of adipogenic markers such as PPARγ and FABP4. Of note, the effects of $1,25(OH)_2D$ were not dependent on the presence of the PPARγ ligands troglitizone or ciglitazone, and cotreatment with VDR and PPARγ ligands did not exert additive or synergistic effects on lipid accumulation in these cultures. These data are consistent with those of Nimitphonga et al. [52] who studied the effects of $1,25(OH)_2D$ on cultures of human preadipocytes isolated from subcutaneous and omental adipose tissue of patients with high mean BMI (33–42 kg/m^2). Cells treated with 10nM $1,25(OH)_2D$ in the presence of adipogenic triggers exhibited increased expression of PPARγ, LPL and FABP4 genes, and triglyceride accumulation, which was more pronounced in the absence of PPARγ agonists. This study also demonstrated that both preadipocytes and differentiated adipocyte cultures expressed CYP27B1 and, consistent with functional enzyme, adipogenesis was enhanced in response to physiologic concentrations of 25(OH)D.

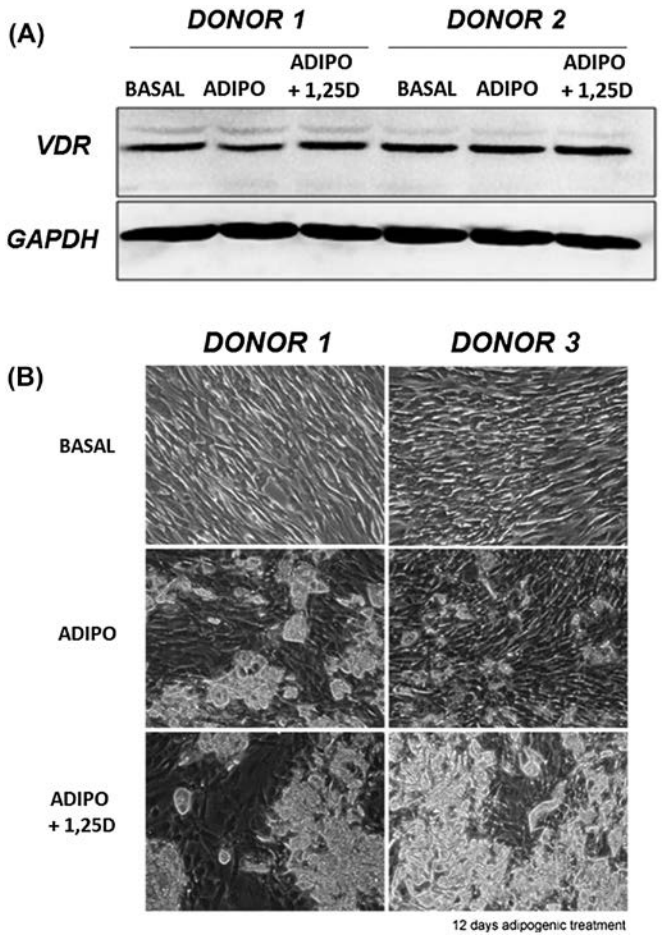

FIGURE 34.2 Vitamin D pathway in primary cultures of human adipose-derived pluripotent mesenchymal cells. (A) Vitamin D receptor (VDR) protein expression in adipose-derived mesenchymal cell cultures from two female donors of normal body mass index grown in basal media, adipogenic (ADIPO) media, or adipogenic media plus 10 nM $1,25(OH)_2D$ (ADIPO + 1,25D). (B) Phase contrast images depicting lipid accumulation in mesenchymal cell cultures from two female donors of normal BMI. Cells were incubated in basal media, adipogenic media (ADIPO), or adipogenic media plus 10 nM $1,25(OH)_2D$ (ADIPO + 1,25D).

In the paper by Narvaez [48], the induction of adipogenesis and its promotion by $1,25(OH)_2D$ was most pronounced in early passage primary cultures, suggesting that adipogenic precursors in these mixed cultures are short-lived. Indeed, $1,25(OH)_2D$ has been shown to delay senescence while maintaining differentiation capacity in human bone–derived mesenchymal stem cell cultures [53], suggesting that cellular aging may be an important factor in the ability of precursors to undergo adipogenic differentiation as well as its modulation by endogenous factors such as $1,25(OH)_2D$. This suggestion is consistent with the previously discussed data demonstrating regulation of the antiaging pathway governed by NAD-SIRT1 by $1,25(OH)_2D$ in mature 3T3-L1 cells and the age-related decline in differentiation potency of cells from VDRKO mice.

Low-passage primary cultures of human adipose-derived progenitor cells from female donors with normal BMI were utilized for genomic profiling of $1,25(OH)_2D$ actions [48]. Microarray analysis was conducted in cells treated with vehicle or 10 nM $1,25(OH)_2D$ under both basal and adipogenic conditions to identify genes that were selectively altered in cells differentiated in the presence of $1,25(OH)_2D$ [48]. Cells grown in basal media were less responsive to $1,25(OH)_2D$ than those grown in adipogenic media. There was limited overlap in $1,25(OH)_2D$-regulated gene expression under these two conditions, with only four genes (TGFβ2, Lectin 9C, Lectin 9B, and Serine C2) other than CYP24A1 regulated by $1,25(OH)_2D$ under both conditions. A cohort of 86 genes (26 upregulated, 60 downregulated) was identified that was significantly altered by $1,25(OH)_2D$ treatment only in the presence of adipogenic media. Many of the genes that were downregulated by $1,25(OH)_2D$ under adipogenic conditions (i.e., TGFBI, CD9, TPM1, THBD) are associated with bone, cartilage, and muscle formation suggesting that their downregulation by $1,25(OH)_2D$ may serve to inhibit differentiation into these lineages while promoting adipogenic differentiation. A notable exception is JAG1, which was upregulated by $1,25(OH)_2D$ under adipogenic conditions. JAG1 encodes the Jagged-1 ligand in the Notch signaling pathway, which is essential for human osteoblastogenesis, but has been shown to both promote and inhibit adipogenesis depending on the model system [54,55].

This array analysis identified STEAP4 as a VDR-modulated gene with strong potential to modulate adipogenesis as this was the gene that was most highly induced by $1,25(OH)_2D$ in adipogenic media. STEAP4 encodes a metalloreductase that has been linked to obesity, insulin sensitivity, metabolic homeostasis, and inflammation. During $1,25(OH)_2D$ stimulation of adipogenesis in cultures from different donors and clones, there was good correlation between STEAP4 induction and the magnitude of lipid accumulation. Human studies have demonstrated that STEAP4 is highly expressed in healthy adipose tissue but reduced in adipose tissue from obese and insulin-resistant subjects [56–58]. Furthermore, STEAP4 expression in adipose tissue inversely correlates with obesity-associated metabolic disturbances such as body fat, blood pressure, and fasting glucose [57]. Mechanistically, because STEAP4 enhances insulin actions in adipocytes [59–63], and insulin is a required component of adipogenic media, it is possible that promotion of adipogenesis by $1,25(OH)_2D$ is mediated via STEAP4 stimulation of insulin signaling. Consistent with this concept, $1,25(OH)_2D$ was shown to enhance insulin sensitivity in human adipocytes (Marcotorchino et al. [30]). It is also worth noting that STEAP4 is induced in human adipocytes by cytokines and the adipokine leptin [62] and that $1,25(OH)_2D$ exerts antiinflammatory effects in adipocytes [30,64]. These data suggest that STEAP4 may mediate many of the pleiotropic effects of $1,25(OH)_2D$ on adipocyte biology. In addition to STEAP4, $1,25(OH)_2D$ selectively altered expression of ADAMTS5 [65], HIG2 [66], and IGF2 [67–69], all of which have been associated with lipogenesis. Clearly, further studies are warranted to identify how these genes and their regulation by $1,25(OH)_2D$ contribute to the process of adipogenesis both in vitro and in vivo.

IMPACT OF VITAMIN D SIGNALING ON ADIPOSITY IN MOUSE MODELS

Genetically Engineered Mouse Models

Incidental observations on VDRKO mice initially suggested defects in maintenance of adipose tissue surrounding the prostate and mammary glands [70–72]. To specifically assess the impact of VDR ablation on adiposity and diet-induced obesity, Narvaez et al. [73] monitored body weights, food intake, metabolic factors, and gene expression in VDRKO mice on two distinct genetic backgrounds from weaning to 6 months of age. To maintain normocalcemia, wild-type and VDRKO mice were reared on the high-calcium rescue diet with normal or high fat content. These data demonstrated that VDRKO mice on either the inbred C57Bl6 or outbred CD1 genetic backgrounds exhibited reduced adiposity on both low- and high-fat rescue diets compared with wild-type mice. Interestingly, VDR ablation does not prevent adipose deposition during early development because young VDRKO mice have normal fat stores [73]. The lean phenotype of VDRKO mice begins in young adulthood and is characterized by reduced body and adipose tissue weights, decreased adipocyte size, and hypoleptinemia. Importantly, mice lacking CYP27B1, the 1α-hydroxylase enzyme that generates $1,25(OH)_2D$, displayed a lean phenotype similar to that of VDRKO mice, implicating liganded VDR in the observed effects.

The mechanisms underlying the inability of VDRKO and CYP27B1KO mice to accumulate weight with age or high-fat feeding are likely complex and not fully understood. Several groups have confirmed that VDR ablation is associated with hyperphagia [73,74], indicating that the chronic hypoleptinemia in these animals appropriately stimulates appetite and argues against an effect of VDRKO at the level of the hypothalamus. However, studies to assess the sensitivity of VDRKO mice to exogenous leptin administration would be necessary to confirm this suggestion. Intestinal lipid transport is not impaired in VDRKO mice [75], indicating that malabsorption is an unlikely cause of the lean phenotype. Direct effects of VDR signaling on adipocytes are suggested by the presence of VDR in both WAT and BAT [73] and by in vitro data indicating that $1,25(OH)_2D$ alters lipid accumulation, β-oxidation of fatty acids, and gene expression in ex vivo cultures derived from bone marrow or adipose tissue of wild-type but not VDRKO mice [38,48,75]. This suggestion is supported by data from Wong et al. [76] who demonstrated that ectopic expression of human VDR in wild-type CD1 mice (using the FABP4 promoter, which targets expression to mature adipocytes) reduced fatty acid β-oxidation and increased fat mass relative to control mice [76].

To date, one group has used cre-lox technology to delete VDR specifically in mature adipocytes [77]. In this study, Vdr-flox mice were crossed with Fabp4-cre mice to generate mice with adipose-specific VDR deletion (termed mouse line with adipose specific VDR deletion (CVF) mice). CVF mice and Fabp4-cre control mice (termed control mouse line (CN1) mice) were reared on high-calcium "rescue" diets (for comparison to global VDRKO mice) or on high-fat diets (to stimulate adiposity). VDR expression was significantly reduced in adipose tissue of CVF mice compared with CN1 mice. The phenotype of mice with adipose-specific VDR ablation did not mimic that of mice with global VDR deletion. Contrary to what would be expected if VDR functioned in WAT to maintain adiposity, female adipose-specific VDRKO mice exhibited higher growth rates after puberty and increased visceral fat pad weight compared with control mice on both rescue and high-fat diets. The impact of adipose-specific VDR ablation on body weight and visceral adiposity was gender specific and was not observed in males. Expression of UCP1 and PPARγ was elevated in WAT of female CVF mice, supporting these genes as targets of repression by VDR in mature adipocytes. Adipose-specific VDR deletion did not impair glucose tolerance or alter the weight of BAT, liver, pancreas, or bone in either male or female mice in response to high-fat feeding. These data indicate that VDR functions in mature adipocytes to suppress visceral adiposity, possibly through interaction with female reproductive hormones such as estrogens. Although these data may seem incongruent with results from global VDRKO mice, it should be noted that the FABP4 promoter used to drive VDR deletion by Matthews et al. [77] is not active in preadipocytes or in mesenchymal stem cells, thus VDR deletion is restricted to mature adipocytes that have already undergone adipogenic differentiation. Because available data indicate that VDR expression is quite low in mature adipocytes, the lack of a significant phenotype in male mice with adipose-specific VDR deletion suggests that if the lean phenotype of global VDRKO mice is directly mediated in adipose tissue, it is likely secondary to loss of VDR function in mesenchymal precursors or preadipocytes rather than in mature adipocytes. The presence of a phenotype in female mice with adipose-specific VDR deletion may indicate that estrogen or other female reproductive hormones upregulate VDR or its activity in mature adipocytes to promote lipolysis. Interactions between estrogen and vitamin D signaling have been well documented, and estrogen replacement therapy is known to prevent central adiposity associated with menopause [78]. Clearly, more studies are needed to dissect the actions of VDR in mature adipocytes versus less differentiated precursors in both males and females.

In addition to potential direct effects of $1,25(OH)_2D$ on adipocytes, VDR deficiency may exert other, more global effects that impact overall energy balance. Data have accumulated for altered energy metabolism as a major contributor to the lean phenotype of VDRKO mice. Narvaez et al. [73] reported that UCP1 expression in adipose tissue of VDRKO mice was 25-fold higher than that of wild-type mice. As noted earlier, UCP1 mediates nonshivering thermogenesis via uncoupling of mitochondrial respiration and its expression is normally limited to BAT. UCP1 and its homologs UCP2 and UCP3 are upregulated in both WAT and BAT of VDRKO mice [73,75]. The marked elevation of UCPs in VDRKO mice is associated with enhanced energy expenditure [71,75], which is likely of sufficient magnitude to prevent weight gain despite hyperphagia and high fat intake. In direct support of this concept, transgenic expression

of human VDR in adipose tissue resulted in decreased expression of UCPs in WAT and BAT, lower energy expenditure, and enhanced weight gain relative to control mice [76]. Cellular studies have demonstrated that UCP1, UCP2, and UCP3 are directly repressed by VDR [31,75,79,80], indicating that upregulation of UCPs in VDRKO tissue results from derepression. More recently, brown adipocyte differentiation in vitro was shown to be suppressed by either $1,25(OH)_2D$ treatment or VDR overexpression [81]. It is important to note that VDR suppression of UCPs may extend to tissues other than WAT and BAT, including skeletal muscle. Ectopic upregulation of UCP1 in muscle has been shown to have dramatic effects on whole-body energy expenditure, resulting in a phenotype similar to that of VDRKO mice [82]. Thus, enhanced energy expenditure and atrophy of adipose tissue in VDRKO mice is multifactorial, reflecting VDR actions in multiple tissues and cell types.

Another newly recognized VDR target gene, Elov13, codes for a fatty acid elongase that is critical for lipid accumulation in BAT [83]. Ji et al. [84] profiled the lipid composition of WAT from wild-type and VDRKO mice and identified a class of saturated and monounsaturated fatty acids (C18–C24) that were specifically elevated in the sWAT of VDRKO mice compared to wild-type controls. The altered fatty acid composition in VDRKO tissue was attributed to lack of repression of Elovl3 because a negative VDR response element was identified in the proximal promoter of the gene. Because Elovl3 is dramatically upregulated in BAT during cold exposure [83], it is tempting to speculate that the upregulation of Elovl3 in VDRKO tissue, and resulting alteration in fatty acid availability is causally related to the enhanced β-oxidation and UCP-mediated thermogenesis.

Additional metabolic abnormalities may contribute to the phenotype of VDRKO mice, including disturbed glucose metabolism [85–89]. Vitamin D and the VDR have long been implicated in control of insulin secretion from pancreatic beta cells [90], and insulin secretion in response to glucose challenge was impaired in a distinct strain of VDR mutant mice [87]. VDRKO mice on the C57Bl6 genetic background had normal circulating insulin and glucose, but VDRKO mice did not exhibit the characteristic hyperglycemia and hyperinsulinemia when bred onto the obesity-prone CD1 background [73]. The possibility that VDR acts in multiple tissues to control overall body metabolism is supported by data from mice with liver or endothelial cell-specific VDR deletion, which exhibited alterations in systemic lipid and glucose metabolism [89,91]. Further characterization of insulin secretion, tissue sensitivity to insulin, glucose tolerance, and lipid metabolism during VDR ablation as a function of age and diet will be an important future direction to clarify the complex phenotype of these mice.

Effect of Vitamin D Deficiency and Supplementation on Adiposity and Metabolism in Animal Models

Numerous studies have examined whether manipulation of dietary vitamin D or treatment with natural or synthetic VDR ligands alters adiposity and metabolism in animal models, and again conflicting results have been reported. Based on the lean phenotype and resistance to diet-induced obesity in normocalcemic mice lacking VDR or CYP27B1 [73–75], one would predict that vitamin D deficiency would reduce weight gain and, conversely, that vitamin D supplementation or treatment with VDR ligands might enhance indices of adiposity, particularly in response to high-fat diets. Consistent with the changes in leptin observed in VDRKO and VDR transgenic mice discussed above, Kong et al. [38] demonstrated that treatment of mice with a vitamin D analog for 1 week elevated serum leptin and increased leptin gene expression in adipose tissue. In a model of diet-induced obesity based on the "Western-style" diet, which is high in fat but low in methyl donors, calcium, and vitamin D, Bastie et al. [92] demonstrated that restoring calcium and vitamin D to the Western diet promoted more rapid weight gain, increased fat utilization, and impaired glucose tolerance. Consistent with these data, dietary vitamin D deficiency in conjunction with high-fat feeding was associated with reduced weight gain, decreased visceral adiposity, lower serum leptin, and elevated UCPs in WAT of Sprague–Dawley rats [93] and improved metabolic parameters, including insulin sensitivity, in ICR mice [94]. It should be noted, however, that dietary vitamin D deficiency studies—particularly in young growing animals—are often confounded by adverse effects on calcium homeostasis. In fact, in the rat study [93], the effects of vitamin D deficiency on adiposity and metabolism were associated with severe hypocalcemia and were reversed by the addition of calcium alone to the vitamin D-deficient diet. Because calcium may independently affect body weight [95], further studies to dissect out the contributions of each individual nutrient to control of adiposity in vivo are clearly needed.

In direct contrast to the predictions based on findings in VDRKO mice and the data cited above, the majority of studies have demonstrated that vitamin D supplementation attenuates diet-induced obesity, and that suboptimal vitamin D status (rather than overt vitamin D deficiency) promotes weight gain. Marcotorchino [96] reported that 10-week supplementation of mice fed high-fat diets with vitamin D_3 (15,000 IU/kg diet) limited weight gain, improved glucose homeostasis, increased lipid oxidation, and enhanced energy expenditure relative to mice consuming high-fat diets with the standard vitamin D content of 1500 IU/kg diet. Similar results were reported by Fan [97] who chronically treated mice with vitamin D_3 by gavage and reported decreases in body weight associated with upregulation of UCP3 in muscle. In another study, gradual weight reduction, lower levels of C-peptide and insulin, and attenuation of fatty liver was observed in high-fat diet–fed mice treated with $1,25(OH)_2D$ compared with control animals [98,99]. Consistent with these data on vitamin D supplementation attenuating weight gain, several studies have demonstrated that suboptimal vitamin D status exerts the opposite effects, i.e., increases in body weight and worsening of metabolic syndrome. In the goto-kakizaki rat model of type 2 diabetes, chronic vitamin D restriction (25 IU/kg diet for 8 weeks) resulted in elevated insulin resistance

and dysregulation of glucose metabolism in association with decreased adipose PPAR-γ expression and deterioration in β-cell function and mass relative to control rats fed 1000 IU vitamin D/kg diet [100].

A recent study has implicated vitamin D actions in neural control of body weight and feeding behavior. Trinko et al. [101] monitored body weights of mice fed a high-fat diet with normal (1100 IU/kg diet) or reduced (110 IU/kg diet) vitamin D_3 for over 100 days. Interestingly, no differences in body weight between groups was observed for the first 50 days, but for the remainder of the study the mice fed low vitamin D exhibited increased weight gain and elevated food intake compared with vitamin D-sufficient mice. Investigators focused on whether vitamin D status might alter feeding behavior and body weight through VDR activity in brain areas linked to dopamine signaling. In support of this concept, VDR expression was detected in dopamine-producing, as well as in dopamine-receiving, neurons. Furthermore, acute treatment with $1,25(OH)_2D$ selectively altered gene expression and enhanced dopamine response. Consistent with regulation of dopamine by $1,25(OH)_2D$, mice fed reduced levels of dietary vitamin D_3 showed blunted dopaminergic responses. These data support an intriguing and novel mechanistic link between VDR actions in brain and changes in weight and feeding behavior. It is worth noting that in this study $1,25(OH)_2D$ treatment and vitamin D deficiency had similar effects on amphetamine consumption, another behavior regulated by dopamine circuits. Thus, the effects of vitamin D status on feeding behavior and body weight may represent one of several actions regulated by VDR via neural dopamine activity.

To summarize, the majority of the animal feeding studies suggest that supplementation with vitamin D above the recommended amounts traditionally present in rodent chow, or treatment with $1,25(OH)_2D$, attenuates both body weight gain and metabolic disturbances associated with high-fat diets. In agreement with these data, several studies have demonstrated that chronic suboptimal vitamin D deficiency exacerbates the effects of high-fat feeding. "Suboptimal" vitamin D status should be considered conceptually distinct from severe vitamin D deficiency, which leads to weight loss, hypocalcemia, and rickets. However, inconsistencies in study protocols, diet composition (definition of high fat, amount of vitamin D and calcium, other nutrients), genetic strains of mice and rats, which are differentially sensitive to weight gain, and choice of end points make it difficult to reconcile the published data. In particular, the kinetics of changes in serum 25(OH)D during chronic dietary vitamin D restriction have not been reported, and no studies have assessed vitamin D concentrations in adipose tissue. Most of these dietary studies have been conducted in young growing animals, and thus may not be applicable to older animals or those fed normal or distinct obesogenic diets. Thus, Mallya et al. [102] fed adult mice diets containing 50, 250, 500, or 1500 IU vitamin D_3/kg diet for 4 months and reported no effects on body weight despite significant differences in vitamin D status as measured by 25(OH)D. Thus, in the absence of high-fat feeding, moderate vitamin D deficiency in adulthood may have minimal or no effect on body weight.

TRANSLATIONAL CONSIDERATIONS

Vitamin D Status and Body Weight: Insight Into Clinical Trials

Several randomized clinical trials of vitamin D supplementation in relation to weight loss have been conducted, with the majority indicating that increasing vitamin D status, as measured by serum 25(OH)D, has little effect on body weight in overweight or obese people [103]. A few newer studies are cited below as examples, but a thorough discussion is outside of the scope of this chapter. Readers are referred to recent published reviews [104–108], which discuss not only the role of vitamin D in weight loss but also the potential effects of vitamin D supplementation on metabolic consequences of obesity such as insulin sensitivity and inflammation.

A major consideration of vitamin D supplementation trials is the baseline vitamin D status of the population under study. If suboptimal vitamin D status is associated with weight gain, then recruitment of subjects based on low serum 25(OH)D will likely be necessary to detect an effect of vitamin D supplementation on weight loss. This strategy was employed by Wamberg et al. [109] who randomized 52 obese men and women with plasma 25(OH)D < 50 nmol/L to 26 weeks of treatment with 7000 IU (175 μg) of vitamin D daily or placebo. End points included body composition and assessment of subcutaneous and visceral WAT depots, insulin resistance, plasma lipids, and inflammatory markers. Although vitamin D supplementation increased average serum 25OHD from 33 to 110 nmolar, there were no changes in body composition, WAT depots, or any other end points. Thus, increasing 25OHD levels by vitamin D treatment for 26 weeks had no effects on body weight or metabolic complications of obesity in adults with high BMI ($35\,kg/m^2$) and low baseline plasma 25OHD. Contrasting results were reported by Salehpour et al. [110] who conducted a double-blind trial of 77 overweight women randomized to 1000 IU vitamin D (25 μg) daily or placebo for 12 weeks. Although a lower dose of vitamin D was used in this study compared with Wamberg's, a significant increase in serum 25(OH)D was still observed (from 37 to 75 nmolar), and there were statistically significant decreases in body fat mass in the vitamin D group compared with the placebo group. In addition, an inverse correlation between change in serum 25(OH)D and body fat mass was found. It is surprising that the lower dose of vitamin D given for a shorter period of time in this study elicited beneficial effects on body fat, whereas a higher dose given for a longer period of time in Wamberg's study did not. One important factor could be baseline BMI, as the subjects in Wamberg's study had an average BMI of $35\,kg/m^2$ as opposed to $29\,kg/m^2$ in Salehpour's study. Given the multifactorial nature of obesity, it is tempting to speculate that individuals with long standing or severe obesity may be resistant to signals that promote weight loss, including vitamin D.

CONCLUSIONS AND FUTURE DIRECTIONS

In summary, adipose tissue has long been recognized as a major site of vitamin D storage, and new technologies have facilitated quantitation of the amount of vitamin D and 25(OH)D in adipose tissue depots. Although it appears likely that vitamin D storage and release is regulated (for example, during physiological states such as puberty, pregnancy, and aging), but virtually nothing is known about the mechanisms by which this might be achieved. Understanding regulatory signals and genetic determinants of vitamin D storage might provide insight into how pathological conditions such as obesity and metabolic syndrome alter the process, leading to lower serum 25(OH)D in such conditions. With respect to adipose tissue as a target for vitamin D, both mesenchymal precursors and mature adipocytes express the VDR and the major vitamin D-metabolizing enzymes [24,73,111]. Direct actions of 25(OH)D, 1,25$(OH)_2$D, and VDR on adipogenic differentiation and gene expression have been documented, although the effects differ with model system. Animal studies support a modulatory role for dietary vitamin D and VDR activity in control of body weight, but much contradictory data exist, and detailed mechanisms are lacking. It is likely that vitamin D has distinct effects on mesenchymal precursors, preadipocytes, and mature adipocytes, which could account for much of the variability derived from in vitro studies and differences in the phenotypes of genetically engineered mice. In addition to direct effects on adipose tissue, it is quite clear that vitamin D signaling integrates pathways in different tissues (including muscle, liver, bone, and possibly brain) that influence metabolism and energy expenditure and indirectly alter the balance of lipogenesis and lipolysis in adipose tissue. Although obesity in humans is consistently associated with low serum 25(OH)D, most studies have failed to detect significant effects of vitamin D supplementation on overall body weight, although some data support beneficial effects of supplementation on fat mass, waist circumference, insulin sensitivity, and/or inflammation. As recent studies have demonstrated that vitamin D supplementation alters adipose tissue gene expression [112], and VDR has been identified as a gene induced by insulin in "healthy" obesity [113], further mechanistic research into the role of vitamin D and its receptor in adipose tissue biology is critical to resolve the current inconsistencies in this field and to facilitate translational impact.

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