Involvement of the vitamin D receptor in energy metabolism: regulation of uncoupling proteins

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The vitamin D receptor (VDR) is a member of the nuclear receptor superfamily that mediates the actions of 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3], the hormonal form of vitamin D (22). The classical function of 1,25(OH)2D3 is to regulate calcium homeostasis (12, 24). However, VDR is expressed in cells not involved in calcium metabolism, and 1,25(OH)2D3 is involved in the regulation of many noncalcemic functions (36). Previous studies have shown that 1,25(OH)2D3 suppresses adipocyte differentiation in 3T3-L1 preadipocytes (6, 26); however, the in vivo role of 1,25(OH)2D3 and VDR in adipogenesis and metabolism remains unknown. In the present study, we used VDR-null mutant mice as a model to address this question. Our data provide evidence that VDR plays a role in energy metabolism, at least in part, through regulation of the UCPs.

MATERIALS AND METHODS

Animals and treatment. One-month-old C57BL/6 wild-type (WT) and VDR-deficient [VDR(−/−)] mice were weaned on a high lactose-high calcium diet (HCA, TD.96348, Harlan Teklad), which was able to normalize the plasma calcium level in VDR(−/−) mice as reported previously (28). For high-fat-diet feeding, mice were maintained on the HCA diet for 4 mo and then switched to a high-fat diet (HF, containing 42% fat, TD.88137; Harlan Teklad) and high-calcium water (containing 2 mM CaCl2) for 5 wk. The mice were weighed weekly to monitor their body weight. At the end of the treatment, the animals were killed, and plasma, WAT, and BAT were harvested. To measure intestinal triglyceride absorption, mice were gavaged with 250 μl olive oil after 6 h of fasting. Blood triglyceride levels were determined at different time points following the gavage. Before the gavage, the mice were injected intraperitoneally with 3.33 ml/kg body wt of 15% Triton [a lipoprotein lipase (LPL) inhibitor] to block the uptake of lipoproteins by tissues (7). For indirect calorimetric measurement, male WT and VDR(−/−) mice were individually placed in the respiratory chambers of a LabMaster System (TSE Systems, Midland, MI). After they were acclimated to the chambers for 2-3 days, oxygen consumption, CO2 production, energy expenditure, food and water intake, and locomotive movement were recorded for the following 3 days. The animal studies were approved by the Institutional Animal Care and Use Committee at the University of Chicago.

Plasma parameters. Total plasma cholesterol and triglyceride levels were determined using the Infinity cholesterol and triglyceride reagents from Thermo Scientific (Waltham, MA). Total free fatty acid levels were determined using a NEFA C test kit from Wako (Richmond, VA). Plasma calcium levels were determined using the Calcium Liquicolor Kit (Stanbio, Boerne, TX). Plasma leptin levels were measured using a commercial ELISA kit (Diagnostic Systems Lab, IL 60067; e-mail: cyan@medicine.bsd.uchicago.edu).
Webster, TX). Serum thyroid-stimulating hormone (TSH) was measured using a sensitive, heterologous, disequilibrium double-antibody precipitation RIA (38). Serum total thyroxine (T₄) and triiodothyronine (T₃) concentrations were measured by a solid-phase RIA (Coat-a-Count; Diagnostic Products, Los Angeles, CA) as described previously (1).

**Histology.** WAT and BAT were fixed in 10% formalin (pH 7.4), processed, and paraffin embedded. Sections (5 μm) were cut with a Leica microtome 2030 and stained with hematoxylin and eosin.

**BAT culture.** Brown fat pads were dissected immediately after mice were killed and placed in cold PBS (pH 7.4) kept on ice. The fat pads were rinsed with cold PBS, transferred to cold DMEM containing 2% FBS and 1 μg/ml insulin, and cut with sterile scissors into small pieces. The fat pieces were cultured in six-well plates at 37°C and 5% CO₂ in the presence or absence of 10⁻⁸ M 1,25(OH)₂D₃, and total RNAs were extracted after 24 h. The RNAs were subject to Northern blot analyses with ³²P-labeled cDNA probes of UCPs.

**White adipocyte culture.** Primary white adipocytes were cultured as previously described (25). Briefly, epididymal fat pads were dissected immediately after mice were killed and placed in cold PBS, transferred to cold DMEM containing 2% FBS, 5 mM glucose, and 1 mg/ml type II collagenase (Sigma). The isolated primary adipocytes were cultured in 24-well plates in DMEM supplemented with 2% FBS and 1 μg/ml insulin.

**Fatty acid β-oxidation assay.** Cultured primary adipocytes were incubated with 500 μM [³H]palmitate and 500 μM carnitine for 1 h. Cell lysates were precipitated two times with tricarboxylic acid and then neutralized with 6 N NaOH. The resulting solutions were applied to a DOWEX-1x2–400 Ion Exchange column and eluted with water into scintillation vials. The amount of tritiated water was measured with a scintillation counter.

**Northern blot.** Total cellular RNA was extracted using TRIzol reagents (Invitrogen, Grand Island, NY). Northern blot analysis was carried out as described previously (29). Briefly, total RNAs (10–20 μg/lane) were separated on 1% agarose gels containing 0.6 M formaldehyde and transferred to Nylon membranes that were cross-linked in an ultraviolet cross-linker. Hybridization was carried out at 63°C in the hybridization buffer described by Church and Gilbert (9) with a cDNA probe labeled with [³²P]dATP using the Prime-a-Gene Labeling System (Promega, Madison, WI). After being washed, the membranes were exposed to X-ray films at −80°C for autoradiography. Ethidium bromide-stained 18S RNA was used as an internal loading control.

**RT-PCR.** First-strand cDNAs were synthesized from 2 μg of total RNAs in 20 μl reaction using Moloney murine reverse transcriptase reverse transcriptase (Invitrogen, Carlsbad, CA) and hexanucleotide random primers. The first-strand cDNAs were served as the template for the PCR, which was carried out using a Bio-Rad DNA Engine. Glyceraldehyde-3-phosphate dehydrogenase served as the internal control.

**Statistical analysis.** Data values were presented as means ± SE. Statistical comparisons were made using Student’s t-test, with *P < 0.05 being considered significant.

**RESULTS**

We used genetic mutant mice lacking the VDR as a model to investigate the role of VDR in energy metabolism. As shown in Fig. 1, both male (Fig. 1A) and female (Fig. 1B) VDR(−/−) mice weighed significantly less than the WT counterparts on the HCa diet. When the mice were fed the HF diet, the body weight difference between WT and VDR(−/−) mice dramatically increased (Fig. 1, A and B), as both male and female VDR(−/−) gained weight at a slower rate than WT mice (Fig. 1, C and D). The weight difference was bigger in male mice.
than in female mice on either diet. Because VDR(−/−) mice had lower body mass before HF feeding, their slower growth cannot be completely explained by the resistance to the HF diet. In contrast to hypocalcemia seen in VDR(−/−) mice on the regular rodent chow, the HCa diet normalized the plasma calcium levels in VDR(−/−) mice (Fig. 1E). During the 5-wk HF diet feeding period, the high-calcium water was able to maintain the plasma calcium level at the normal range in VDR(−/−) mice (Fig. 1E). Therefore, the difference in weight gain in WT and VDR(−/−) mice was unlikely because of a difference in plasma calcium.

We then determined the effect of VDR inactivation on body fat depots. As shown in Fig. 2, VDR transcript was readily detected by RT-PCR in both WAT (Fig. 2E) and BAT (Fig. 2F) of WT animals but was absent from VDR(−/−) mice, indicating that the fat is the direct target organ of vitamin D. On the HCa diet, only male VDR(−/−) mice displayed significantly less body fat than WT mice. There was no difference in the body fat percentages of female mice (Fig. 2, A and B). On the HF diet, both male and female VDR(−/−) mice exhibited significantly lower body fat compared with WT mice (Fig. 2, A and B). Histological examination of the gonadal WAT showed that VDR(−/−) mice on the HF diet appeared to have smaller adipocytes compared with WT mice (Fig. 2C). Although VDR(−/−) mice had less BAT compared with WT mice regardless of the diets, the BAT in VDR(−/−) mice on HF diet accumulates less lipids and preserved much better cell morphology characteristic of brown fat than WT mice (Fig. 2D).

We further determined the WAT depot distribution in the body as subcutaneous, gonadal, and perirenal fat and the BAT in these mice. As shown in Table 1, on the HCa diet, male VDR(−/−) mice tended to have less fat in each depot; however, only the gonadal fat was significantly lower compared with WT mice. Female VDR(−/−) mice on the HCa diet had significantly less BAT but did not display significant differences in WAT depots, consistent with the observation that female VDR(−/−) mice did not display significantly lower body fat. On the HF diet, VDR(−/−) mice displayed significantly less adipose tissue in all four depots regardless of genders, but the difference between male WT and VDR(−/−) mice was more dramatic than between female mice (Table 1). Therefore, unless specifically indicated, only male mice were used in the following investigations.

Fatty acid synthase (FAS), the rate-limiting enzyme involved in de novo fatty acid synthesis (4), was higher in VDR(−/−) mice than in WT mice on the HCa; however, the difference was decreased when the mice were placed on the HF diet (Fig. 3A). However, the expression of malonyl-CoA dehydrogenase and stearoyl CoA-desaturase-1, two other enzymes involved in fatty acid metabolism, was not significantly different between WT and VDR(−/−) mice (data not shown).
LPL, which is responsible for the breakdown of circulating triglycerides (35), was similar in VDR(+/−) and WT mice on the HCa diet but lower in VDR(−/−) mice when placed on the HF diet (Fig. 3B). Peroxisome proliferator-activated receptor (PPAR)-γ, the gene responsible for the expression of a number of adipocytic genes, seemed to be expressed at slightly higher levels in VDR(−/−) mice on the HCa diet; however, the difference disappeared when the mice were placed on the HF diet (Fig. 3C).

We also assessed the levels of adipokines. On the HCa diet, circulating leptin levels in VDR(−/−) mice tended to be lower than in WT mice, but the difference was not statistically significant; however, on the HF diet, the increase in plasma leptin levels was much less in VDR(−/−) mice compared with WT mice (Fig. 4A). This is consistent with the notion that plasma leptin levels reflect the fat mass in the body (32). Indeed, leptin mRNA expression in WAT was lower in VDR(−/−) mice on both diets (Fig. 4B). However, the decrease in leptin levels was not enough to influence the food intake in VDR(−/−) mice (Fig. 5C). Adiponectin is an antihyperglycemic adipokine whose levels are inversely correlated to body weight (2). On the HCa diet, adiponectin mRNA expression in the WAT was indistinguishable in WT and VDR(−/−) mice; the HF diet reduced adiponectin expression in WT mice but not in VDR(−/−) mice (Fig. 4C). There was no detectable difference in resistin expression between WT and VDR(−/−) mice on the HCa diet or HF diet, even though resistin levels were increased in response to high-fat feeding in both mice (Fig. 4D).

The difference observed in the fat depots between WT and VDR(−/−) mice prompted us to measure the plasma lipid levels. There was no significant difference in circulating triglyceride, free fatty acids, or cholesterol between female WT and VDR(−/−) mice fed either the HCa or HF diet (data not shown). There was also no significant difference in free fatty acid levels in male mice on either of the diets (data not shown). Male VDR(−/−) mice tended to have lower triglyceride levels on the HCa diet, but the difference was not significant (Fig. 5A); on the HF diet, however, the triglyceride levels were significantly lower in male VDR(−/−) mice compared with the WT counterparts (Fig. 5A). Interestingly, plasma cholesterol levels in male VDR(−/−) mice were significantly lower than in WT mice on both diets (Fig. 5B). Based on these observations, we focused on male mice in the following investigations into the mechanism underlying the phenotypic differences in WT and VDR(−/−) mice.

Because the plasma lipid levels of the VDR(−/−) mice were decreased compared with WT mice, we monitored mouse food intake. The VDR(−/−) mice ate the same amount of food as WT mice (Fig. 5C), so we investigated whether difference in intestinal lipid absorption accounted for the difference in their plasma lipid levels. To this end, fasted mice were subject to a gavage of olive oil, and blood triglyceride levels were measured at different time points afterward. Surprisingly, no difference in the rate of intestinal triglyceride absorption was seen between WT and VDR(−/−) mice (Fig. 5D).

The lower body fat mass and plasma lipid profile in VDR(−/−) mice suggested an increase in the basal body metabolism. Thyroid hormone is known to stimulate metabolism (11, 37), and we therefore determined TSH, T₃, and T₄ levels in the plasma. VDR(−/−) mice did not have increased circulating thyroid hormones compared with WT mice (data not shown). Therefore, the phenotypic difference in VDR(−/−) and WT mice was unlikely caused by thyroid hormone.

To explore the mechanism underlying the difference in plasma lipid profile, we measured fatty acid β-oxidation in primary white adipocytes isolated from WT and VDR(−/−)
mice. As shown in Fig. 6, in the presence of carnitine, β-oxidation occurred at a higher rate in the white adipocytes of VDR(+/−/−) mice compared with WT mice (Fig. 6A). Examination of key genes involved in β-oxidation revealed that carnitine palmitoyltransferase II (CPTII), the protein responsible for transporting fatty acids into the mitochondrial matrix, was increased in VDR(+/−/−) mice (Fig. 6B). The increased rate of β-oxidation in adipocytes may partially explain the decreased level of circulating lipids in VDR(+/−/−) mice.

The brown fat plays a key role in thermoregulation and metabolism in mice. BAT expresses three UCPs. UCP1 is involved in the regulation of nonshivering thermogenesis (14). Although the roles of UCP2 and UCP3 are less well understood, both exhibit uncoupling abilities when expressed in yeast (15, 17). Interestingly, the expression of the UCPs in the BAT of VDR(+/−/−) mice was markedly upregulated. On the HCa diet, UCP1 and UCP3 were expressed at a higher level in VDR(+/−/−) mice compared with WT mice (Fig. 7, A, C, and G), whereas UCP2 levels were similar in these mice (Fig. 7, B and G); when the mice were placed on the HF diet, the expression of UCP1, UCP2, and UCP3 was increased much more in VDR(+/−/−) mice than in WT mice (Fig. 7, D–F and H). These results indicate that energy uncoupling is taking place at a higher rate in the BAT of VDR(+/−/−) mice, suggesting a higher basal energy expenditure. These results explain, at least in part, the lower adipose mass and lower plasma lipid profile in the mutant mice.

UCPs are known to be under the direct regulation of β-adrenergic receptor 3 (Adrβ3) (17, 39). Adrβ3 expression was unchanged in the BAT of VDR(+/−/−) mice compared with WT expression, but both exhibit uncoupling abilities when expressed in yeast (15, 17). Interestingly, the expression of the UCPs in the BAT of VDR(+/−/−) mice was markedly upregulated. On the HCa diet, UCP1 and UCP3 were expressed at a higher level in VDR(+/−/−) mice compared with WT mice (Fig. 7, A, C, and G), whereas UCP2 levels were similar in these mice (Fig. 7, B and G); when the mice were placed on the HF diet, the expression of UCP1, UCP2, and UCP3 was increased much more in VDR(+/−/−) mice than in WT mice (Fig. 7, D–F and H). These results indicate that energy uncoupling is taking place at a higher rate in the BAT of VDR(+/−/−) mice, suggesting a higher basal energy expenditure. These results explain, at least in part, the lower adipose mass and lower plasma lipid profile in the mutant mice.

UCPs are known to be under the direct regulation of β-adrenergic receptor 3 (Adrβ3) (17, 39). Adrβ3 expression was unchanged in the BAT of VDR(+/−/−) mice compared with WT mice, suggesting that it is not involved in VDR regulation of UCP expression (data not shown). Interestingly, VDR levels in the BAT were decreased in WT mice on the HF diet (Fig. 8A), in reverse correlation with the UCP expression, and consistent with the notion that vitamin D directly downregulates UCP expression in BAT. To test this hypothesis, primary BAT was isolated from WT and VDR(+/−/−) mice and treated with 1,25(OH)2D3. As shown in Fig. 8, the expression of UCP1 and UCP3 was markedly decreased in WT BAT cells after 24 h of treatment (Fig. 8A), whereas UCP2 levels remained unchanged (Fig. 8A), indicating that the regulation is mediated by VDR. Therefore, 1,25(OH)2D3 appears to directly downregulate UCP1 and UCP3 expression in BAT; as a result, inactivation of
VDR leads to the increased expression of these genes in VDR(−/−) mice, leading to changes in energy metabolism.

Finally, we further assessed the role of VDR in energy metabolism by indirectly measuring calorimetric parameters of WT and VDR(−/−) mice. As shown in Fig. 9, total energy expenditure was markedly higher in VDR(−/−) mice than WT mice in either daytime and nighttime under the HCa diet condition (Fig. 9A); consistently, the oxygen consumption (Fig. 9B) and CO2 production (Fig. 9C) of VDR(−/−) mice were also higher than WT mice. When the mice were placed on the HF diet, the difference in energy expenditure (Fig. 9D), oxygen consumption (Fig. 9E), and CO2 production (Fig. 9F) was even larger between WT and VDR(−/−) mice, with VDR(−/−) mice being much higher in all of these parameters. However, there was no significant difference in locomotive movement (data not shown) and in food intake between WT and VDR(−/−) mice. These data confirm that VDR(−/−) mice have increased energy metabolism, and the higher energy expenditure is not attributable to increased food intake or physical movement.

DISCUSSION

1,25(OH)2D3 is a pluripotent hormone involved in the regulation of a variety of biological processes. Beyond its traditional actions in the regulation of calcium homeostasis, great progress has been made in recent years to understand the role of vitamin D in renal and cardiovascular functions, in immune regulation, and in cancer prevention (23). However, little is known about its role in energy metabolism and adipocyte biology in vivo. Adipocytes express VDR and thus are potential targets of vitamin D regulation. In the present study, we investigated the effect of VDR deficiency on energy metabolism by characterizing the metabolic phenotype of VDR-null mutant mice. The data demonstrate that vitamin D is involved in energy metabolism in animals at least in part through regulation of the UCPs.

Because of the function of vitamin D in regulation of calcium homeostasis, VDR(−/−) mice develop hypocalcemia on regular rodent chow (30). To avoid the interference of the calcemic abnormality, in this study, we maintained the normocalcemic status of VDR(−/−) mice with the HCa diet or high-calcium water when treated with the HF diet. This ensured that the metabolic phenotypes seen in VDR(−/−) mice

Fig. 7. Expression of uncoupling protein (UCP) in BAT. A–C: total RNAs were isolated from BAT in male WT and VDR(−/−) mice fed the HCa diet, and UCP1 (A), UCP2 (B), and UCP3 (C) mRNA were determined by Northern blotting. D–F: BAT total RNAs were isolated from WT and VDR(−/−) mice on the HF diet, and UCP1 (D), UCP2 (E), and UCP3 (F) expression was determined by Northern blotting. G and H: PhosphorImaging quantitative data of the UCP mRNA levels from WT and VDR(−/−) mice on the HCa (G) or HF (H) diet. *P < 0.01 vs. WT.

Fig. 8. Suppression of UCP expression in primary brown fat tissues by 1α,25-dihydroxyvitamin D3 [1,25(OH)2D3]. A: RT-PCR determination of VDR expression in the BAT from WT and VDR(−/−) mice fed the HCa diet or the HF diet as indicated. B and C: primary BAT isolated from WT or VDR(−/−) mice were treated for 24 h with 1,25(OH)2D3 (10−8 M, VD), and UCP1 (B) and UCP3 (C) expression was analyzed by Northern blotting. All experiments were repeated at least 2–3 times with similar results, and shown are representative data.
were a direct result of VDR inactivation rather than secondary to abnormal calcium metabolism. The main finding of this study is that VDR(-/-) mice have less body fat mass and lower plasma levels of triglyceride and cholesterol than the WT counterparts. The phenotypic difference was widened between WT and VDR(-/-) mice when the mice were placed on the HF diet, as VDR(-/-) mice gained less weight and accumulated less fat mass than WT mice, even though the food intake and intestinal lipid absorption were the same as WT mice. Therefore, the phenotypic difference in these two genotypes is unlikely caused by the difference in energy intake, but rather by the difference in energy expenditure, which is markedly higher in VDR(-/-) mice (Fig. 9).

Studies of 3T3-L1 proadipocytes have previously shown that 1,25(OH)₂D₃ blocks adipocyte differentiation in vitro (6, 26), which predict an increase in adipogenesis in the absence of VDR. Therefore, it is somewhat surprising that mice lacking VDR actually have less fat mass. The discrepancy between in vitro and in vivo observations is unclear, but it is possible that, because VDR expression follows a specific temporal pattern during adipocyte differentiation (6, 26), the global VDR inactivation starting from the embryonic stages in VDR(-/-) mice may not recapitulate the blockade of vitamin D signaling seen in the in vitro 3T3-L1 adipogenesis. Because fat mass was reduced in all depots in VDR(-/-) mice, the defect in fat accumulation appears to be global. The decrease in adiposity can be attributed to a lower number of adipocytes, smaller size of adipocytes, or both. Whereas it is unclear whether there is a defect in adipogenesis in VDR(-/-) mice, the adipocytes in VDR(-/-) mice appeared to be smaller under the HF dietary stress, suggesting that VDR(-/-) mice stored less lipids in the fat tissues than WT mice. This is consistent with the fact that VDR(-/-) mice had lower plasma triglyceride and cholesterol concentration on both diets.

The low plasma lipid levels and adiposity seen in VDR(-/-) mice could be a consequence of reduced energy intake or increased energy expenditure. The observations that VDR(-/-) mice consumed as much food as WT mice and had normal lipid absorption in the intestine basically excluded the possibility of decreased energy intake as the underlying mechanism. However, evidence obtained from WAT and BAT studies and indirect calorimetric measurement demonstrates an increase in energy expenditure in VDR(-/-) mice.

In WAT, despite the reduction in fat mass, the expression of most genes and adipokines surveyed in the study was normal in VDR(-/-) mice. The expression of PPARγ, the master regulator of adipocyte differentiation, was not significantly different in WT and VDR(-/-) mice. On the HCa diet, VDR(-/-) mice displayed a slight increase in FAS expression, probably a compensatory effect secondary to the depressed plasma lipid levels. LPL is an enzyme that hydrolyzes triglyceride-rich lipoproteins (35), and the decreased LPL expression seen in VDR(-/-) mice on the HF diet may also reflect the depressed plasma lipid levels.

Adipokines that play a role in energy metabolism include leptin, resistin, and adiponectin. Leptin is the major energy sensor in the body, and leptin levels are known to be proportional to the fat mass (32). The fact that VDR(-/-) mice
exhibited a decreased level of circulating leptin reflects their low fat mass; thus, it is not surprising that the drastic differences in circulating leptin were not seen in leptin mRNA levels. Adiponectin has been implicated in increasing β-oxidation and decreasing circulating triglyceride levels (16, 44), and a high-fat dietary treatment is known to suppress adiponectin expression (5). Consistently, in WT mice, adiponectin expression was suppressed on the HF diet; however, VDR(−/−) mice still maintained the high-level adiponectin expression on this diet, which is consistent with the apparently higher β-oxidation in WAT of the mutant mice.

Fatty acid β-oxidation is a process taking place in mitochondria by which fatty acids are broken down to produce ATP (13, 31). Within white adipocytes, triglycerides are constantly converted into fatty acids and back into triglyceride in a futile cycle. In a fasted state, fatty acids are released by adipocytes to provide energy (42). In VDR(−/−) mice, the basal β-oxidation in WAT is significant higher, accompanied with increased mRNA expression of CPTII. These data suggest that, in the WAT of VDR(−/−) mice, instead of being converted back to triglyceride, a larger portion of the fatty acids derived from stored triglycerides is oxidized, leading to higher basal energy expenditure.

The more important finding that points to higher energy expenditure in VDR(−/−) mice is the elevated UCP expression and the metabolic data from indirect calorimetric measurement. UCP1 and UCP3 were markedly upregulated in the BAT of VDR(−/−) mice regardless of dietary conditions, and UCP2 was upregulated in VDR(−/−) placed on a HF diet. BAT is a main site of metabolic regulation in mice, and on the HF diet VDR(−/−) mice preserved the BAT morphology better than WT mice. UCPs are expressed in the mitochondria and serve to uncouple the electron transport chain from the production of ATP. UCP1 is expressed only in the BAT and is mainly responsible for adaptive thermogenesis (14). Oxidation of UCP1 in WAT caused a reduction in adipose stores in transgenic mice (27). The physiological roles of UCP2 and UCP3 remain unclear. UCP2 is widely expressed in the BAT, WAT, heart, and kidney (15). A positive association has been reported between UCP2, energy expenditure, and resting metabolic rate based in humans (8, 43); on the other hand, however, there is no significant weight difference between UCP2-null and WT mice on a normal or HF diet (3). UCP3 expression is restricted to skeletal muscle and brown fat and is thought to influence metabolic rate, since transgenic mice overexpressing UCP3 are resistant to diet-induced obesity (10). Increased UCP3 expression has also been linked to sleeping metabolic rates and increased energy expenditure in Pima Indians (40), providing evidence that the increased expression of UCP3 in the VDR(−/−) mice may contribute to their increased metabolic rate. However, UCP3 knockout mice displayed no change in weight or oxygen consumption compared with WT mice (18). Interestingly, both UCP2 and UCP3 are upregulated in the UCP1 knockout mice, suggesting compensatory roles of these proteins in the regulation of energy homeostasis (33). Because UCPs uncouple the production of ATP from the electron transport chain, VDR(−/−) mice would have to use more energy than WT mice to produce the same amount of ATP. This at least partly explains the low fat mass phenotype seen in VDR(−/−) mice and the accompanied high energy expenditure. Vitamin D regulation of UCPs was not via Adrβ3, and the thyroid hormone was not involved in the development of the phenotypes either. The experiment using primary brown fat culture confirmed that 1,25(OH)2D3 suppresses UCP expressions through direct gene regulation and that basal UCP expression is upregulated in the brown fat of VDR(−/−) mice. Consistently, a prior study has reported suppression of UCP2 by 1,25(OH)2D3 in human adipose tissues (41). Furthermore, we showed here that BAT VDR levels were decreased in WT mice on the HF diet, providing further evidence that the VDR is involved in UCP downregulation.

VDR(−/−) mice have higher energy expenditure compared with WT mice on either the HCa or the HF diet. The high energy expenditure explains, at least in part, the low adiposity and low plasma lipid profiles in VDR(−/−) mice. However, because VDR is ubiquitously expressed, the higher energy expenditure does not necessarily only result from the loss of VDR in the adipose tissues. Global VDR deficiency, which is the model used in this study, may likely alter body adiposity and energy expenditure in a number of ways. Future studies should be directed at the role of VDR in adipose and nonadipose tissues in the regulation of total body energy metabolism. In summary, this study demonstrates that the VDR-mediated action of vitamin D is involved in energy metabolism and adipocyte biology in vivo. In the absence of VDR, animals display low fat mass, resistance to high-fat-induced fat accumulation, and reduced plasma lipid levels, due to increased energy expenditure. The underlying mechanism for these metabolic abnormalities is that vitamin D is involved in the regulation of β-oxidation in WAT and directly suppresses the expression of UCP1 and UCP3 in BAT. These data unveil a novel aspect of vitamin D biology in regulation of energy metabolism.

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