Targeted Expression of Human Vitamin D Receptor in Adipocytes Decreases Energy Expenditure and Induces Obesity in Mice

Kari E. Wong1, Juan Kong2, Wenshuo Zhang1, Frances L. Szeto1, Honggang Ye2, Dilip K. Deb2, Matthew J. Brady1,2, & Yan Chun Li1,2,3

1Committee on Molecular Metabolism and Nutrition, 2Department of Medicine, Division of Biological Sciences, The University of Chicago, Chicago, IL, USA

Running title: Role of VDR in Energy Metabolism
Key words: vitamin D, vitamin D receptor, adipocytes, energy metabolism, lipolysis

3 Corresponding author:
Yan Chun Li, Ph.D.
Department of Medicine, The University of Chicago,
900 E. 57th Street, KCBD 9-9110, Chicago, IL 60637
Tel: 773-702-2477; Fax: 773-702-5790, Email: cyan@medicine.bsd.uchicago.edu

Our previous studies demonstrated a high fat diet-resistant lean phenotype of vitamin D receptor (VDR)-null mutant mice mainly due to increased energy expenditure, suggesting an involvement of the VDR in energy metabolism. Here we took a transgenic approach to further define the role of VDR in adipocyte biology. We used the aP2 gene promoter to target the expression of the human (h) VDR in adipocytes in mice. In contrast to the VDR-null mice, the aP2-hVDR Tg mice developed obesity compared to the wild-type counterparts without changes in food intake. The increase in fat mass was mainly due to markedly reduced energy expenditure, which was correlated with decreased locomotive activity and reduced fatty acid β-oxidation and lipolysis in the adipose tissue in the transgenic mice. Consistently, the expression of genes involved in the regulation of fatty acid transport, thermogenesis and lipolysis were suppressed in the transgenic mice. Taken together these data confirm an important role of the VDR in the regulation of energy metabolism.

Introduction

The maintenance of body weight depends on the balance between energy intake and energy utilization. Obesity results when energy consumed exceeds energy utilized. Energy is acquired through diets and can be stored in adipose tissue or utilized by the body to maintain basic cellular functions and physical activities. Energy can also be used for adaptive thermogenesis in response to a cold environment (1). The adipose tissue is unique in that it represents both arms of energy balance. The white adipose tissue (WAT) has the ability to sense the energy state of the body. When energy availability is high, the WAT stores the excess energy as triglyceride in lipid droplets. When energy is needed, triglyceride is broken down to free fatty acids to release into the circulation. This process, known as lipolysis, is regulated by two enzymes, adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL). ATGL initiates the first, rate-limiting step of lipolysis by hydrolyzing triglyceride to diacylglyceride (2,3), which is further broken down to monoglyceride by HSL (4). Monoglyceride lipase cleaves the final ester bond of monoglyceride to release glycerol, and this step is not rate-limiting (5). Lipolysis is activated by catacholamines through the cAMP signaling pathway, leading to protein kinase A (PKA) activation. PKA phosphorylates HSL, which promotes HSL translocation to the lipid
droplet and access to triglyceride stores (6). HSL and ATGL activity is suppressed by insulin during feeding, as insulin increases the amount of perilipin around the lipid droplets to prevent their access to triglycerides (7).

The principal role of the brown adipose tissue (BAT) is to regulate adaptive thermogenesis through the expression of uncoupling proteins (UCPs). UCP1 separates oxidative phosphorylation from ATP production to release energy as heat (8-10). Studies have shown that increased expression of UCP1 in the BAT or its ectopic expression in the WAT results in increased metabolism and resistance to diet-induced obesity (11). The primary roles of UCP2 and UCP3 are less well characterized; however, both proteins have uncoupling capabilities (9,10).

The VDR is a member of the nuclear receptor superfamily (12). Its high affinity ligand is 1,25-dihydroxyvitamin D (1,25(OH)2D3), the hormonal form of vitamin D. In addition to its classic role in the regulation of calcium homeostasis, the vitamin D hormone has numerous non-calcemic activities (13), including regulation of adipocyte biology. 1,25(OH)2D3 inhibits adipocyte differentiation in the NIH3T3-L1 preadipocyte model (14,15). Previous work from this and other laboratories has implicated a role for VDR in the regulation of global energy metabolism in vivo (16,17). VDR-null mutant mice are lean and resistant to high fat diet-induced obesity, in part due to the up-regulation of UCPs in adipose tissues. Data obtained from primary BAT confirmed the suppression of UCP1 and UCP3 by 1,25(OH)2D3 (17). Consistently, VDR-null mice exhibited increased β-oxidation in the WAT. Given the broad range of regulatory activities of the VDR (13), however, global inactivation of the VDR in the VDR-null model affects the function of multiple tissues, which complicates the interpretation of the metabolic data. As such, the contribution of the VDR signaling in adipocytes to energy metabolism remains uncertain. To further define the role of the VDR in adipocyte biology, here we generated a transgenic (Tg) mouse model that specifically expresses the human (h) VDR in the adipose tissue. In contrast to the VDR-null mice, the Tg mice showed increased adipose mass and reduced energy metabolism compared to the wild-type (Wt) counterparts. Together these data provide further evidence confirming an important role of the adipocyte VDR in the regulation of energy metabolism.

Materials and Methods

Generation of aP2-hVDR transgenic mice. A 2 kb cDNA fragment containing the full-length coding sequence of hVDR was placed behind the 5.4 kb aP2 promoter/enhancer (18) in a plasmid vector (Fig. 1A). The aP2-hVDR-polyA cassette was released from the vector by NotI and SalI restriction digestion. The fragment was purified and microinjected into CD-1 mouse embryos following standard procedures (19). Potential founders were screened using primers specific to the hVDR cDNA: 5’-CGTGTGAATGATGGTGGAGGGAGCC-3’ (forward), and 5’-GTCTTGGTTGCCACAGGTCCAGGAC-3’ (reverse). Founder Tg lines were confirmed by Southern blotting. The expression of hVDR in the adipose tissues was confirmed by Northern and Western blottings, using a 32P-labeled hVDR cDNA probe or an anti-VDR antibody (Santa Cruz Biotechnology, Santa Cruz, CA), respectively.

Animal treatment. Wt mice in CD1 background and aP2-hVDR Tg mice overexpressing the hVDR in the adipose tissue were usually fed a standard chow (SC) diet (Teklad Global 18% Rodent Diet 2018; Harlan Teklad), and maintained in a standard 12 light/12 dark cycle. To induce obesity, the mice were weaned on the SC diet and then at two months of age were switched to a high-fat (HF) diet containing 42% fat (TD.88137; Harlan Teklad) for 5 weeks. Body weight was monitored weekly. Total body fat content was quantified using dual energy X-ray absorptiometry (DEXA).
scans (Lunar PIXIImus II, Madison, WI) under anesthesia. Fat mass in different parts of the body was quantified by directly weighing the dissected fat pads. Indirect calorimetric measurement was carried out using the LabMaster System (TSE Systems, Midland, MI), and oxygen consumption, CO$_2$ production, energy expenditure, food and water intake, and locomotive movement were recorded for 4 days following 3-day acclimation. At the end of the experiment, the animals were sacrificed, and plasma, WAT, BAT and skeletal muscle were harvested. All 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). Plasma non-esterified fatty acid (NEFA) levels were determined using NEFA C test kit from Wako (Richmond, VA). Plasma leptin levels were measured using a commercial ELISA kit (Diagnostic Systems Lab, Inc., Webster, TX).

**Glucose tolerance test.** Two-month old male mice were fasted 6 hours before receiving an i.p. injection of glucose (2 mg/kg body weight). Blood samples were taken from tail bleeding at 0, 15, 30, 60 and 120 min after the glucose injection and blood glucose was measured using a Contour glucometer (Bayer HealthCare LLC, Mishawaka, IN). Random blood glucose levels were measured in 3-month old mice in the morning on three nonconsecutive days with the Contour glucometer.

**Northern blot.** Total cellular RNAs were extracted using TRizol reagents (Invitrogen, Grand Island, NY). RNAs were isolated from the gonadal WAT depot. Northern blot analysis was carried out as described previously (20).

**Western Blot.** Gonadal WAT and BAT were homogenized in Laemmli buffer containing a protease inhibitor cocktail (Promega, Madison, WI). After centrifugation, the lower layer was collected and boiled for five minutes. Protein concentrations were determined using a BioRad Protein Assay kit (BioRad, Hercules, CA). Proteins were separated by SDS-PAGE and transferred onto Immobilon membranes. Western blotting was carried out as described previously (21).

**Quantitative real-time RT-PCR.** First-strand cDNAs were synthesized from 2 µg total RNAs in a 20 µl reaction using Moloney murine reverse transcriptase (Invitrogen, Carlsbad, CA) and hexanucleotide random primers. The first-strand cDNAs served as the template for PCR amplification, which was carried out using the LightCycler® 480 Real-Time PCR System (Roche Applies Science, Indianapolis, IN). Beta-2 microglobulin was used as an internal control. The primer sequences are available upon request.

**In vitro lipolysis assays.** Freshly dissected WAT (100 mg) was incubated in Krebs-Ringer bicarbonate-HEPES (KRHB) buffer supplemented with 3% fatty acid free BSA and 5mM glucose. The isolated white fat was then incubated at 37°C for one hour with gentle shaking and treated with or without isoproterenol (10 µM, Sigma, St Louis, MO). Following the incubation, the media was collected and glycerol content was assessed using a commercial kit (Sigma, St. Louis, MO) according to the manufacturer’s instruction.

**In vitro fatty acid β-oxidation assay.** The rate of fatty acid β-oxidation in BAT was measured in the presence of 500 µM $^3$H-palmitate and 500 µM carnitine as described previously (17).

**NIH3T3-L1 adipocytes.** Culture and differentiation of NIH3T3-L1 cells to mature adipocytes were performed as described previously (15). Differentiated cells were treated with ethanol or 20 nM 1,25(OH)$_2$D$_3$ for 24 hours and the mRNA level of ATGL and HSL was quantified by real time RT-PCR.

**Statistical analysis.** Data values were presented as means ± SEM. Statistical comparisons were made using Student's $t$-test, with $P \leq 0.05$ being considered significant.
Results

Increased fat mass in aP2-hVDR Tg mice. We used the aP2 promoter/enhancer, an adipocyte-specific gene promoter (18,22), to target hVDR expression specifically in the adipose tissue. We chose hVDR as the transgene to distinguish it from the endogenous mouse VDR in genotyping, as the function of hVDR and mouse VDR is exchangeable. We identified five positive Tg lines by Southern blot (not shown) and Northern blot analyses of the WAT (Fig. 1B). In this study we used Tg line 21. Unless specifically indicated, all data were obtained from male mice. Northern and Western blot analyses confirmed a high level expression of hVDR at mRNA and protein levels in both WAT and BAT in this Tg line (Fig. 1C and D). The expression of the hVDR transgene was undetectable in other tissues (not shown).

On the SC diet, the Tg mice exhibited significantly increased body weight at two and four months of age compared to the Wt mice (Fig. 2A). The body weight difference was even larger at 6 months of age between these two genotypes (not shown). The body fat content, estimated by weighing the sum of dissected subcutaneous, perirenal and gonadal fat pads, was significantly higher in the Tg mice than in the Wt mice at 4 months of age (Fig. 2B). We then used DEXA scan to assess more accurately the body fat content. DEXA scan showed a trend toward increased body fat percentage in the Tg mice at two months of age (p=0.065) (Fig. 2C); at three months of age, the increase in body fat percentage was significant in female Tg mice (Fig. 2C). When placed on the HF diet for 5 weeks, the Tg mice displayed markedly higher body weight and total fat content compared to the Wt counterparts, the latter assessed by DEXA scan (Fig. 2A and C). Thus the hVDR transgene increased body fat mass in both male and female mice. As expected, all mice on the HF diet showed higher body weight and total fat mass in both genotypes compared to mice on the SC diet (Fig. 2A and C). On both the SC and HF diets, various fat depots of the body, including subcutaneous, gonadal and perirenal fats, were significantly larger in the Tg mice (Fig. 2D and E). The Tg mice also had greater BAT mass, although the difference did not reach significance (Fig. 2D and E). The HF diet widened the difference in total body fat content and in the size of subcutaneous and gonadal fat pads between the Wt and Tg mice compared to the SC diet (Fig. 2C, D and E).

Altered adipokine levels in aP2-hVDR Tg mice. As aggregate adipose mass affects adipokine production, we examined the expression of leptin and adiponectin in the WAT. The level of leptin mRNA was slightly increased in the Tg mice compared to Wt mice; however this difference was not significant (Fig. 3A). In contrast, the level of adiponectin was significantly reduced (by 65%) in the Tg mice compared to the Wt mice (Fig. 3B). Serum leptin levels were not significantly different between the Wt and Tg mice on the SC diet; however, the levels were significantly elevated in the Tg mice on the HF diet (Fig. 3C), probably due to the greater increase in fat mass under this dietary condition.

Increased cholesterol levels in aP2-hVDR Tg mice. We next examined the circulating lipid levels. There was no difference in plasma triglyceride or NEFA levels between the Wt and Tg mice (Fig. 4A and B). Interestingly, the Tg mice exhibited an increase in the level of plasma cholesterol compared to the Wt mice (Fig. 4C). This is in contrast to the VDR-null mutant mice that had decreased plasma cholesterol levels relative to the Wt mice (17). Together these observations suggest a possible involvement of the adipocyte VDR in the regulation of plasma cholesterol levels.

Reduced global metabolism in aP2-hVDR Tg mice. We assessed mouse global metabolism by indirect calorimetry. Total energy expenditure, oxygen consumption (VO2) and carbon dioxide production (VCO2) (not shown) were markedly reduced in the Tg mice compared to the Wt mice on the SC diet (Fig. 5A and B);
however, there was no difference in the respiratory exchange rate between these two genotypes (Fig. 5C), indicating no change in fuel substrate selection in the Tg mice. Food intake was the same for the Wt and Tg mice during the light and dark cycle of the day (Fig. 5D), indicating that the difference in body weight or fat mass between these mice was not due to a difference in energy intake. Interestingly, the Tg mice moved less than the Wt mice during the active dark cycle, as measured by the number of beam breaks. During the light cycle, however, when all mice are presumably less active, there was no difference in the number of beam breaks between these mice (Fig. 5E). Less physical activity may contribute to the increase in fat mass seen in the Tg mice. It is unlikely, however, that the extra weight that the Tg mice are carrying physically prevents these mice from moving. Similar results, including the metabolic data, food intake and physical activity, were seen between Wt and Tg mice when they were fed the HF diet (Fig. 6A-E).

Lower β-oxidation and thermogenesis in aP2-hVDR Tg mice. The decrease in metabolism seen in the Tg mice suggests possible suppression of total fuel utilization. Since the respiratory exchange rate of these mice was not altered, it is likely that both carbohydrate and lipid oxidation are decreased. We thus quantified the expression of key genes involved in β-oxidation and glycolysis in the BAT, WAT and skeletal muscle. Skeletal muscle had no expression of the hVDR transgene. Carnitine palmitoyl transferase (CPT)1 and CPT2 are responsible for the transport of long chain fatty acids into the mitochondrial matrix for β-oxidation (23). In the Tg mice, the expression of CPT1 and CPT2 in both BAT and WAT was significantly suppressed compared to the Wt mice (Fig. 7A and B). CPT2, not CPT1, was also significantly lowered in the skeletal muscle of the Tg mice (Fig. 7C). Consistently, the rate of β-oxidation, measured in vitro using adipocytes isolated from the BAT, was reduced in the Tg mice compared to the Wt counterparts (Fig. 8).

Hexokinase (HK) and pyruvate kinase (PK) are regulatory enzymes involved in glycolysis. HK phosphorylates glucose to glucose-6-phosphate in the first step of glycolysis. PK catalyzes the production of pyruvate in the final step of glycolysis. In the Tg mice, both HK and PK mRNA levels were significantly reduced in the BAT (Fig. 7A), only HK was reduced in the WAT (Fig. 7B), but no changes in HK and PK were observed in the skeletal muscle (Fig. 7C).

The UCPs are involved in the regulation of adaptive thermogenesis. Consistently with the obese phenotype, the transcripts of UCP1, UCP2 and UCP3 were significantly suppressed in the BAT of the Tg mice compared to the Wt mice (Fig. 7A). Similarly, the mRNA levels of both UCP2 and UCP3 were lower in WAT of the Tg mice (Fig. 7B), but they were not significantly changed in the skeletal muscle (Fig. 7C). UCP1 is not normally expressed in WAT or skeletal muscle and thus was not examined.

Decreased lipolysis in aP2-hVDR Tg mice. Although the metabolism of the Tg mice was decreased compared to the Wt mice, they maintained similar levels of NEFAs, the major fuel source for the body. NEFAs are generated from WAT through the breakdown of triglycerides in the process of lipolysis, which is regulated by ATGL and HSL. Both ATGL and HSL transcript levels in WAT were significantly suppressed in the Tg mice compared to the Wt counterparts (Fig. 9A). We further determined lipolysis in the WAT isolated from these mice by measuring glycerol release into the media. Under the basal condition, the Tg WAT released less glycerol than the Wt WAT; when the WAT was stimulated with isoproteranol, a β-adrenergic receptor agonist known to increase lipolysis, the Tg WAT still released significantly less glycerol than the Wt counterpart (Fig. 9B). These data indicate that overexpression of the hVDR transgene suppresses lipolysis in adipose tissues. To confirm that ATGL and HSL are regulated by vitamin D, we treated differentiated NIH3T3-L1 adipocytes with ethanol or 1,25(OH)2D3, and
found that ATGL and HSL transcripts were significantly suppressed by 1,25(OH)\textsubscript{2}D\textsubscript{3} in these cells (Fig. 9C). Together these observations strongly suggest that vitamin D regulates lipolysis by directly targeting ATGL and HSL.

Glucose intolerance in aP2-hVDR Tg mice. Finally we examined the ability of the Tg mice to handle glucose. The non-fasting blood glucose level was higher in the Tg mice compared to the Wt mice (Fig. 10A). When these mice were subject to an intraperitoneal glucose tolerance test, the Tg mice were not able to clear glucose as effectively as the Wt mice, indicating glucose intolerance (Fig. 10B).

Discussion

In our previous studies of the VDR knockout mouse model we demonstrated a role of the VDR in the regulation of energy metabolism (17). These null mutant mice developed a lean phenotype that is resistant to high fat diet-induced obesity. These studies, however, have limitations because the expression of VDR was ablated from the entire animal, which cannot exclude the possibility that VDR inactivation in a number of tissues contributes to the metabolic phenotype. By overexpressing the hVDR specifically only in the adipose tissue we are able to correlate the role of the VDR in the adipose tissue to metabolic phenotypes. Furthermore, in the global knockout model, the VDR is missing from the adipocyte lineage throughout development; therefore, results might be confounded by developmental impairment. The aP2 gene is expressed late in adipocyte differentiation (18,24). Therefore, overexpressing the hVDR using the aP2 promoter enables us to evaluate the role of the VDR in mature adipocytes without interfering adipocyte differentiation.

In this study we showed that overexpression of the hVDR in the adipose tissue in transgenic mice leads to obesity with increased body weight and fat mass. Because there was no difference in energy intake between the Wt and Tg mice, the increase in body weight and fat mass seen in the Tg mice was likely due to a decrease in energy expenditure. This notion was confirmed in calorimetric studies, which showed reduced energy expenditure and oxygen consumption in the Tg mice. Unexpectedly, the Tg mice had significantly less locomotive activity than the Wt mice during the dark cycle, when the animals are most active. This reduction in movement may partially explain the increased fat mass in the Tg animals. It is not yet clear why the Tg mice move less than their Wt counterparts. It is possible that the adipose tissue somehow signals the brain to decrease movement, but there is no evidence to support such a speculation at this time.

Consistent with the reduced energy metabolism in Tg mice, hVDR overexpression in the adipocytes led to reduced fatty acid β-oxidation and lipolysis, accompanied by suppression of key genes involved in these processes. HK, an enzyme in regulation of glycolysis, and CPT1 and CPT2, regulators of long chain fatty acid transport into the mitochondrial matrix for oxidation, were down-regulated in both WAT and BAT in the Tg mice. Importantly, the rate-limiting enzymes involved in lipolysis, ATGL and HSL, were also suppressed in the WAT of the Tg mice. The suppression of β-oxidation and lipolysis by the VDR provides a critical explanation for the increased adipose mass seen in the Tg mice. These observations suggest a direct role of VDR in the regulation of β-oxidation and lipolysis in the adipose tissue. Further investigations are needed to understand the molecular mechanism whereby the vitamin D/VDR signaling regulates these regulatory proteins. Given that the VDR functions as a ligand activated transcription factor, it is not surprising that some of these metabolic regulators are directly regulated by the VDR in the adipose tissue. In this regard, the in vitro data that we obtained from the NIH3T3-L1 adipocytes support direct regulation of ATGL and HSL by the vitamin D hormone. The critical role of these two enzymes in lipolysis warrants further investigations to dissect the molecular
mechanism underlying these important regulations. In addition, we also examined the effect of 1,25(OH)_{2}D_{3} on the expression of CPT1, CPT2, HK and PK in 3T3-L1 adipocytes as well as in an immortalized brown fat cell line (25), but observed no direct regulation of these genes by vitamin D in both adipocyte cell lines (data not shown). These observations suggest that vitamin D influences fatty acid transport and glycolysis by indirectly regulating these genes.

Another factor that may contribute to the increased adipose mass is the suppression of UCP expression in the Tg mice. Previous studies have demonstrated increased expression of UCP1 and UCP3 in the WAT and BAT of VDR knockout mice, which contribute to the increased energy metabolism and a lean phenotype in these mutant mice (16,17). Our previous data from primary BAT culture and adipocyte cells indicated that UCP1 and UCP3 are under direct regulation by the vitamin D/VDR signaling (17). Consistently, in this study we showed that hVDR overexpression in the adipose tissue led to the suppression of UCP1, UCP2 and UCP3. Although the roles of the UCP proteins in the regulation of metabolism and body fat mass are controversial (26-29), these data are consistent with the metabolic phenotypes seen in the Tg mice.

The aP2-hVDR Tg mice showed glucose intolerance, which may be partly attributed to the decreased expression of adiponectin. Low levels of adiponectin are correlated with insulin resistance (30,31). Low adiponectin expression in the Tg mice is probably due to the increase in fat mass.

Epidemiological studies have reported a relationship between obesity and vitamin D status in humans. Generally, adiposity or body mass index (BMI) is inversely correlated to the levels of serum 1,25(OH)_{2}D_{3} (32) and 25-hydroxyvitamin D_{3} (33-35) in healthy adults; however, whether low vitamin D levels play any roles in the development of obesity is unknown. One theory to explain this observation is that in obesity more vitamin D, which is highly fat-soluble, is trapped in the body fat, leading to low serum vitamin D status (34). It is speculated that the trapped vitamin D would increase the local vitamin D concentration in the fat, and based on the finding from the present study, activation of the VDR in adipocytes negatively affects energy expenditure, further promoting obesity.

In summary, here we have provided further evidence that the VDR is able to regulate global metabolism by exerting its effects on the adipose tissue. As the energy sensing tissue of the body, the WAT is sensitive to changes in metabolic rate. Small changes in energy expenditure over time can have gross impacts on fat mass. This is probably the main reason for the Tg mice to develop obesity. Previous studies have shown that ablation of the VDR results in a lean phenotype (16,17), and this present work demonstrates that overexpression of the VDR specifically in the adipose tissue has an opposite effect. Together, these studies provide very strong evidence for a previously unrecognized role of the adipocyte VDR in energy metabolism. The underlying mechanism for VDR regulation of energy metabolism involves the regulation of UCPs and enzymes involved in β-oxidation and lipolysis. The exact molecular basis of VDR regulation in these pathways, however, remains to be established.

Acknowledgment

We thank Graeme Bell for assistance in the use of the DEXA scan, and Linda Degenstein for the production of transgenic mouse lines. The Diabetes Research and Training Center at the University of Chicago was supported by National Institutes of Health grant 5P60DK020595-34. The University of Chicago Transgenic Mouse Facility was supported in part by National Institutes of Health grant 5P30CA014599-36. This work was supported in part by National Institutes of Health grants T32DK07074 and R01HL085793.
References
1. Lowell, B. B., and Spiegelman, B. M. (2000) *Nature* **404**(6778), 652-660
2. Bezaire, V., Mairal, A., Ribet, C., Lefort, C., Girousse, A., Jocken, J., Laurencikiene, J., Anesia, R., Rodriguez, A. M., Ryden, M., Stenson, B. M., Dani, C., Ailhaud, G., Arner, P., and Langin, D. (2009) *J Biol Chem* **284**(27), 18282-18291
3. Schweiger, M., Schreiber, R., Haemmerle, G., Lass, A., Fledelius, C., Jacobsen, P., Tornqvist, H., Zechnier, R., and Zimmermann, R. (2006) *J Biol Chem* **281**(27), 18282-18291
4. Haemmerle, G., Zimmermann, R., Hayn, M., Theussl, C., Waeg, G., Wagner, E., Sattler, W., Magin, T. M., Wagner, E. F., and Zechnier, R. (2002) *J Biol Chem* **277**(2), 40236-40241
5. Schweiger, M., Schreiber, R., Haemmerle, G., Lass, A., Fledelius, C., Jacobsen, P., Tornqvist, H., Zechner, R., and Zimmermann, R. (2006) *J Biol Chem* **281**(52), 40236-40241
6. Bezaire, V., Mairal, A., Ribet, C., Lefort, C., Girousse, A., Jocken, J., Laurencikiene, J., Anesia, R., Rodriguez, A. M., Ryden, M., Stenson, B. M., Dani, C., Ailhaud, G., Arner, P., and Langin, D. (2009) *J Biol Chem* **284**(27), 18282-18291
7. Schweiger, M., Schreiber, R., Haemmerle, G., Lass, A., Fledelius, C., Jacobsen, P., Tornqvist, H., Zechnier, R., and Zimmermann, R. (2006) *J Biol Chem* **281**(52), 40236-40241
8. Haemmerle, G., Zimmermann, R., Hayn, M., Theussl, C., Waeg, G., Wagner, E., Sattler, W., Magin, T. M., Wagner, E. F., and Zechnier, R. (2002) *J Biol Chem* **277**(2), 40236-40241
9. Fredriksson, G., Tornqvist, H., and Belfrage, P. (1986) *Biochimica et biophysica acta* **876**(2), 288-293
10. Matthias, A., Ohlson, K. B., Fredriksson, J. M., Jacobsson, A., Nedergaard, J., and Cannon, B. (2000) *J Biol Chem* **275**(7), 5011-5015
11. Flessing, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997) *Nat Genet* **15**(3), 269-272
12. Kong, D. W., He, Y., Karas, M., and Reitman, M. (1997) *J Biol Chem* **272**(39), 24129-24132
13. Kong, J., Clarke, G., Enerback, S., Spiegelman, B., and Kozak, L. P. (1995) *J Clin Invest* **96**(6), 2914-2923
14. Haussler, M. R., Whitfield, G. K., Haussler, C. A., Hsieh, J. C., Thompson, P. D., Selznick, S. H., Dominguez, C. E., and Jurutka, P. W. (1998) *J Bone Miner Res* **13**(3), 325-349
15. Bouillon, R., Carmeliet, G., Verlinden, L., van Etten, E., Verstuyf, A., Luderer, H. F., Lieben, L., Mathieu, C., and Demay, M. (2008) *Endocrinology* **29**(6), 726-776
16. Blumberg, J. M., Tzameli, I., Astapova, I., Lam, F. S., Flier, J. S., and Hollenberg, A. N. (2006) *J Biol Chem* **281**(16), 11205-11213
17. Kong, J., and Li, Y. C. (2006) *Am J Physiol Endocrinol Metab* **290**(5), E916-924
18. Narvaez, C. J., Matthews, D., Broun, E., Chan, M., and Welsh, J. (2009) *Endocrinology* **150**(2), 651-661
19. Wong, K. E., Szeto, F. L., Zhang, W., Ye, H., Kong, J., Zhang, Z., Sun, X. J., and Li, Y. C. (2009) *American journal of physiology* **296**(4), E820-828
20. Ross, S. R., Graves, R. A., Greenstein, A., Platt, K. A., Shyu, H. L., Mellovitz, B., and Spiegelman, B. M. (1993) *Genes Dev* **7**(B), 1318-1324
21. Li, Y. C., Bolt, M. J., Cao, L. P., and Sitrin, M. D. (2001) *Am J Physiol Endocrinol Metab* **281**(3), E558-564
22. Soloveva, V., Graves, R. A., Rasenick, M. M., Spiegelman, B. M., and Ross, S. R. (1997) *Molecular endocrinology (Baltimore, Md)* **11**(1), 27-38
23. Eaton, S. (2002) *Prog Lipid Res* **41**(3), 197-239
24. Ross, S. R., Graves, R. A., and Spiegelman, B. M. (1993) *Genes Dev* **7**(B), 1318-1324
25. Fasshauer, M., Klein, J., Kriauciunas, K. M., Ueki, K., Benito, M., and Kahn, C. R. (2001) *Molecular and cellular biology* **21**(1), 319-329

26. Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Ricquier, D. (2000) *Nat Genet* **26**(4), 435-439

27. Vidal-Puig, A. J. (2000) *Nat Genet* **26**(4), 387-388

28. Vidal-Puig, A. J., Gruije, D., Zhang, C. Y., Hagen, T., Boss, O., Ido, Y., Szczepanik, A., Wade, J., Mootha, V., Cortright, R., Muoio, D. M., and Lowell, B. B. (2000) *J Biol Chem* **275**(21), 16258-16266

29. Enerback, S., Jacobsson, A., Simpson, E. M., Guerra, C., Yamashita, H., Harper, M. E., and Kozak, L. P. (1997) *Nature* **387**(6628), 90-94

30. Hotta, K., Funahashi, T., Bodkin, N. L., Ortmeyer, H. K., Arita, Y., Hansen, B. C., and Matsuzawa, Y. (2001) *Diabetes* **50**(5), 1126-1133

31. Yatagai, T., Nagasaka, S., Taniguchi, A., Fukushima, M., Nakamura, T., Kuroe, A., Nakai, Y., and Ishibashi, S. (2003) *Metabolism: clinical and experimental* **52**(10), 1274-1278

32. Parikh, S. J., Edelman, M., Uwaifo, G. I., Freedman, R. J., Semega-Janneh, M., Reynolds, J., and Yanovski, J. A. (2004) *J Clin Endocrinol Metab* **89**(3), 1196-1199

33. Snijder, M. B., van Dam, R. M., Visser, M., Deeg, D. J., Dekker, J. M., Bouter, L. M., Seidell, J. C., and Lips, P. (2005) *J Clin Endocrinol Metab* **90**(7), 4119-4123

34. Wortsman, J., Matsuoka, L. Y., Chen, T. C., Lu, Z., and Holick, M. F. (2000) *Am J Clin Nutr* **72**(3), 690-693

35. Arunabh, S., Pollack, S., Yeh, J., and Aloia, J. F. (2003) *J Clin Endocrinol Metab* **88**(1), 157-161

**Figure legends**

Figure 1. Generation of aP2-hVDR transgenic mice. (A) Illustration of the DNA construct used for transgenic mouse production. (B) Northern blot analysis of white adipose tissues from several transgenic (Tg) founder lines and wild-type (Wt) control, using hVDR cDNA probe. (C) Northern blot analysis of white (W) and brown (B) fat tissues from line 21 Tg mice and Wt control. (D) Western blot analyses of hVDR protein expression in the white and brown fat from line 21 Tg mice.

Figure 2. Body weight and fat mass. (A) Body weight of Wt and Tg male mice fed a standard chow (SC) diet at 2- or 4-months (m) of age or fed a high fat (HF) diet for 5 weeks starting at 2 months of age. (B) Dissected total body fat mass in 4-month old Wt and Tg mice on the SC diet. (C) Body fat percentage determined by DEXA scan in 2-month old male mice (2m), 3-month old female mice (3m/F) on the SC diet or male mice on the HF diet. (D and E) Fat mass from distinct adipose depots in 4-month old mice on the SC (D) or HF (E) diet. BF: brown fat; Sub: subcutaneous fat; Gon: gonadal fat; PR: peri-renal fat. *P ≤ 0.05 vs. Wt; n=4-11 per genotype.

Figure 3. Leptin and adiponectin levels. (A and B) Real time RT-PCR quantitation of leptin (A) and adiponectin (B) mRNA levels in the WAT from Wt and Tg mice on the SC diet. (C) Serum leptin levels determined by ELISA from Wt and Tg mice on the SC or HF diet. *P ≤ 0.05 vs. Wt; n=3-5 for each genotype.
Figure 4. Plasma lipid levels. Plasma levels of triglyceride (A), NEFA (B) and cholesterol (C) levels in 4-month old Wt and Tg mice. \( P \leq 0.05 \) vs. Wt; \( n=4-5 \) for each genotype.

Figure 5. Metabolic parameters of mice on the SC diet. Wt and Tg mice fed the SC diet were individually placed in metabolic cages. After 3-day acclimation, the metabolic parameters were measured for 4 days. (A) Energy expenditure (EE); (B) Oxygen consumption; (C) Respiratory exchange rate (RER); (D) Food intake in the light and dark cycle; and (E) Physical activity as measured by the number of beam breaks. \( P \leq 0.05 \) vs. Wt; \( n=5-6 \) in each genotype.

Figure 6. Metabolic parameters of mice on the HF diet. (A) Energy expenditure (EE); (B) Oxygen consumption; (C) Respiratory exchange rate (RER); (D) Food intake in the light and dark cycle; and (E) Physical activity. \( P \leq 0.05 \) vs. Wt; \( n=5-6 \) in each genotype.

Figure 7. Expression levels of regulatory genes involved in fuel utilization. (A) BAT; (B) WAT; and (C) skeletal muscle. Total RNAs were isolated from these tissues and levels of the indicated gene transcripts were determined by quantitative real-time RT-PCR. \( P \leq 0.05 \) vs. Wt; \( n=7-9 \) in each group.

Figure 8. Rate of fatty acid β-oxidation in BAT. The BAT obtained from Wt and Tg mice was incubated in the presence of \( ^3 \)H-palmitate and carnitine, and the production of \( ^3 \)H₂O was quantified by scintillation counting. \( P \leq 0.05 \) vs. WT; \( n=3-5 \) in each group.

Figure 9. Lipolysis in WAT. (A) Real time RT-PCR quantitation of rate limiting enzymes in lipolysis, adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL), in the WAT from Wt and Tg mice. \( n=7-9 \) per genotype. (B) In vitro lipolysis assay. WAT isolated from Wt or Tg mice was not treated (NT) or treated with isoproterenol (Iso) for one hour before determining the amount of glycerol released from WAT into the medium. \( P \leq 0.05 \) vs. Wt; \( n=3 \) per genotype. (C) Suppression of ATGL and HSL by 1,25-dihydroxyvitamin D (1,25VD) in NIH3T3-L1 adipocytes. Differentiated 3T3-L1 cells were treated with ethanol (EtOH) vehicle or 20 nM 1,25VD overnight, and ATGL and HSL mRNA levels were quantified by real time RT-PCR. \( P<0.05 \) vs. EtOH.

Figure 10. Glucose tolerance test. (A) Non-fasting blood glucose levels in Wt and Tg mice, taken on three separate days. \( P \leq 0.05 \) vs. Wt; \( n=3 \); (B) Intraperitoneal glucose tolerance test performed in Wt and Tg mice fasted for 6 hours. \( P \leq 0.05 \) vs. Wt; \( n=7-8 \) per genotype.
Figure 1
Figure 2

A

Body weight (g)

2m  4m  HF
SC  Wt  Tg

B

Body fat (g)

Wt  Tg

C

Body fat (%)

2m  3m/F  HF
SC  Wt  Tg

D

Fat weight (g)

BF  Sub  Gon  PR
Wt  Tg

E

Fat weight (g)

BF  Sub  Gon  PR
Wt  Tg
Figure 3
**Figure 4**

(A) Triglycerides (mg/dL)

(B) NEFA (mEq/L)

(C) Cholesterol (mg/dL)

- Wt: Wild Type
- Tg: Transgenic
Figure 5

A

EE (kcal/hr/kg)

B

VO2 (ml/hr/kg)

C

RER

D

Food intake (g/day)

E

No. Beam Breaks/12 h

Wt  Tg

Light  Dark

Wt  Tg

Light  Dark

*
Figure 7

A

B

C

Relative mRNA level

Wt  Tg

CPT1  CPT2  HK  PK  UCP1  UCP2  UCP3

Relative mRNA level

Wt  Tg

CPT1  CPT2  HK  PK  UCP2  UCP3

Relative mRNA level

Wt  Tg

CPT1  CPT2  HK  PK  UCP2  UCP3
Figure 8

![Bar chart showing the comparison of \( ^3\text{H}_2\text{O} \) (nmol/mg tissue/hr) between Wt and Tg groups. The chart illustrates a significant difference (*) between the two groups.](http://www.jbc.org/Downloaded from)
Figure 9

A

Relative mRNA level

ATGL   HSL

Wt  □ Tg

B

Glycerol release (μg/g tissue)

NT   Iso

Wt  □ Tg

C

Relative mRNA level

ATGL   HSL

EtOH □ 1,25VD

*
Figure 10

A

B

Glucose (mg/dL)

Time

0 15 30 45 60 90 120

Wt Tg

Glucose (mg/dL)

0 50 100 150 200 250 300 350

Wt Tg

* * * *
Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice
Kari E. Wong, Juan Kong, Wenshuo Zhang, Frances L. Szeto, Honggang Ye, Dilip K. Deb, Matthew J. Brady and Yan Chun Li

J. Biol. Chem. published online August 12, 2011

Access the most updated version of this article at doi: 10.1074/jbc.M111.257568

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts