1,25-Dihydroxyvitamin D₃ upregulates leptin expression in mouse adipose tissue

Juan Kong^{1,2}, Yunzi Chen^{1,2}, Guojun Zhu¹, Qun Zhao³ and Yan Chun Li^{1,2}

¹Laboratory of Metabolic Disease Research and Drug Development, Shengjing Hospital, China Medical University, Shenyang, People's Republic of China

²Division of Biological Sciences, Department of Medicine, The University of Chicago, 900 East 57th Street, KCBD 9110, Chicago, Illinois 60637, USA

³Ministry of Health Key Laboratory of Congenital Malformation, Shengjing Hospital, China Medical University, 36 Sanhao Street, Heping District, Shenyang 110000, People's Republic of China

Correspondence should be addressed to Y C Li; Q Zhao

Emails

cyan@medicine.bsd.uchicago.edu; rezh2001@yahoo.com

Abstract

Leptin is an adipose tissue-derived hormone that plays a critical role in energy homeostasis. Vitamin D has been shown to regulate energy metabolism, but the relationship between vitamin D and leptin is unclear. Leptin expression and secretion was reduced in vitamin D receptor (VDR)-null mice and increased in transgenic (Tg) mice overexpressing the VDR in adipocytes; however, as leptin is mainly determined by fat mass, it is unclear whether the vitamin D hormone directly regulates leptin expression. To address this question, we determined the effect of vitamin D on leptin expression *in vivo* and *ex vivo*. One-week treatment of WT mice with the vitamin D analog RO-27-5646 led to a significant increase in adipose leptin mRNA transcript and serum leptin levels. Moreover, in adipose tissue cultures, 1,25-dihydroxyvitamin D markedly stimulated mRNA expression and secretion of leptin, but not resistin, in adipose tissues obtained from WT mice, but not from VDR-null mice, and leptin upregulation induced by 1,25-dihydroxyvitamin D was more robust in adipose tissues obtained from VDR Tg mice compared with WT mice. These data demonstrate that 1,25-dihydroxyvitamin D stimulates adipose leptin production in a VDR-dependent manner, suggesting that vitamin D may affect energy homeostasis through direct regulation of leptin expression.

Key Words

- ▶ Vitamin D
- ▶ Vitamin D receptor
- ▶ Leptin
- ▶ Adipocytes

Journal of Endocrinology
(2013) **216**, 265–271

Introduction

White adipose tissue not only serves the purpose of an energy store but also functions as an endocrine organ for the regulation of energy homeostasis. Adipocytes release an array of endocrine signals that are integrated into the metabolic regulatory circuit and influence energy intake, storage and expenditure, and insulin sensitivity (Fruhbeck *et al.* 2001, Tilg & Moschen 2006). One important hormone secreted by the white adipose tissue is leptin, a 16 kDa peptide that plays a central role in

energy homeostasis (Zhang *et al.* 1994). Leptin acts on its receptor in the hypothalamus to curb appetite and increase energy expenditure when body fat stores increase (Schwartz *et al.* 2000). As such, the level of plasma leptin is positively correlated with the body's adipose mass (Mantzoros 1999). High leptin in the circulation signals excessive energy storage to the CNS to suppress food intake and increase energy consumption. In addition, leptin is involved in the regulation of other functions

such as reproduction and immunity (Donato *et al.* 2011, Procaccini *et al.* 2012).

The vitamin D hormone is a pleiotropic hormone that has a broad range of physiological functions (Bouillon *et al.* 2008). The active form of vitamin D is 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), which activates the vitamin D receptor (VDR), a member of the nuclear receptor superfamily, to exert biological activities (Haussler *et al.* 1998). The VDR is widely expressed in the body, including tissues not involved in calcium and phosphate homeostasis, which explains why 1,25(OH)₂D₃ plays multiple physiological roles beyond mineral metabolism (Nagpal *et al.* 2005). Previous studies from this and other laboratories have implicated a role for the VDR in the regulation of adipogenesis and energy metabolism. 1,25(OH)₂D₃ suppresses 3T3-L1 preadipocyte differentiation into mature adipocytes (Blumberg *et al.* 2006, Kong & Li 2006). Global VDR-null mice exhibit a lean phenotype that is resistant to high-fat diet-induced obesity, suggesting an involvement of VDR in the regulation of energy expenditure (Narvaez *et al.* 2009, Wong *et al.* 2009). Our recent studies demonstrated that transgenic (Tg) mice with targeted expression of human (h) VDR in the adipose tissue developed obesity due to reduced energy expenditure (Wong *et al.* 2011). Interestingly, an early study reported that 1,25(OH)₂D₃ suppressed leptin secretion in human adipose tissue (Menendez *et al.* 2001). Indeed, given the important role of leptin in energy metabolism, the relationship between vitamin D and leptin is of great interest. In the VDR-null and hVDR Tg mice, however, adipocyte leptin transcript levels were correlated with fat mass in these mice (Wong *et al.* 2009, 2011); therefore, it remains unclear whether vitamin D directly regulates leptin expression in the adipose tissue *in vivo*. The main goal of this study was to address this question using *in vivo* and *ex vivo* approaches. Our data indicate that 1,25(OH)₂D₃ upregulates leptin production in mouse adipose tissue.

Materials and methods

Animal studies

Global VDR(−/−) mice were described previously (Li *et al.* 1997). These mice were raised on a high-calcium diet containing 2% calcium, 1.5% phosphorus, and 20% lactose (Harlan, Teklad, WI, USA) right after weaning to normalize serum calcium levels as described (Li *et al.* 1998). aP2-hVDR Tg mice that specifically overexpress hVDR in adipocytes were reported previously (Wong *et al.*

2011). Three- to four-month-old mice were used for studies as mice at this age had adequate fat tissue for experiments. Mice were killed by exsanguinations under anesthesia, serum were collected and frozen at −20 °C until use, and epididymal fat pads were harvested for adipose tissue culture or for RNA isolation. To assess the *in vivo* effect of vitamin D on leptin, WT mice were treated daily with vehicle (propylene glycol:ethanol=90:10) or vitamin D analog RO-27-5646 (dissolved in vehicle; provided by Dr Uskokovic) by i.p. injection for 1 week at 1.5 µg/kg body weight (Qiao *et al.* 2005). Mice were killed 16 h after the last injection and epididymal fat pads and serum were harvested as earlier. All animal study protocols were approved by the Institutional Animal Care and Use Committee of the University of Chicago.

Adipose tissue culture

Epididymal fat pads were dissected immediately after mice were killed and placed in cold PBS (pH 7.2) kept on ice. The fat tissue was rinsed twice with cold PBS, transferred into cold DMEM containing 20% fetal bovine serum, and cut with sterile scissors into small pieces (0.1–0.3 mm). The fat pieces were cultured in six-well plates at 37 °C and 5% CO₂ for 24 and 48 h in the presence or absence of 1,25(OH)₂D₃ (0.1–100 nM), and total RNAs were extracted at the end of treatment. The media were harvested for leptin assays.

Cloning of leptin and resistin cDNA probes

Leptin and resistin cDNA probes were cloned by RT-PCR. Total RNAs (5 µg) were reverse transcribed into cDNA using M-MLV reverse transcriptase (Invitrogen). PCR primers were designed according to mouse leptin and resistin cDNA sequence deposited in the GenBank database. Leptin primers are 5'-ATGTGCTGGA-GACCCCTGTG-3' (forward) and 5'-TCAGCATTCAGGGC-TAACATCC-3' (reverse), and resistin primers are 5'-CAACTCCCTGTTTCCAAATGC-3' (forward), and 5'-CTCAAGACTGCTGTGCCTTCT-3' (reverse). PCRs were carried out at the following condition: 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s for 30 cycles. The PCR products were cloned into pSK(+) plasmid. The identity of mouse leptin and resistin cDNAs was confirmed by DNA sequencing.

Northern blot

Total RNAs were extracted from freshly dissected epididymal fat tissues or from cultured adipose tissues using TRIzol reagents (Invitrogen). Total RNAs (15 µg/lane) were

separated on 1% formaldehyde-agarose gels and transferred onto nylon membranes. The membranes were hybridized with ^{32}P -labeled leptin and resistin cDNA probes as described previously (Kong & Li 2006). Transcripts were detected by autoradiography and quantified using a PhosphorImager (Molecular Dynamic, Sunnyvale, CA, USA). The membranes were striped and rehybridized with ^{32}P -labeled 36B4 or *GAPDH* cDNA probe for the internal loading control.

RT-PCR

First-strand cDNAs were reverse-transcribed from 5 μg total RNAs using M-MLV reverse transcriptase (Invitrogen) and hexanucleotide random primers. The VDR transcript was amplified by PCR using primers 5'-GCCACGGGCTTC-CACTTCAACG-3' (forward) and 5'-TACGTCTGCAC-GAATTGGAGGC-3' (reverse), using a Bio-Rad DNA Engine (Bio-Rad). Leptin transcript was quantified by real-time RT-PCR using a LightCycler 480 Real-Time PCR System (Roche Applies Science) and SYBR green PCR reagent kits (Clontech) as described (Wong *et al.* 2009, 2011). Leptin PCR primers were 5'-TGTGTCGGTTCCTGTGGCTTT-3' (forward) and 5'-CTGCGTGTGTGAAATGTCATTG-3' (reverse). Beta-2 microglobulin was used as an internal control for normalization, and its PCR primers were 5'-ACCGGCC-TGTATGCTATCCAGAAA-3' (forward) and 5'-ATTTCATGT-GAGGCGGGTGGAAC-3' (reverse). The relative amount of leptin transcripts was calculated using the $2^{-\Delta\Delta\text{Ct}}$ formula as described previously (Zhang *et al.* 2008).

Serum leptin measurement

Leptin concentrations in the serum and culture media were determined using a commercial mouse leptin ELISA kit (Diagnostic Systems Laboratories, Webster, TX, USA) according to the instruction provided by the manufacturer. The detection limit of this kit was 0.5 ng/ml.

Statistical analysis

Data were presented as the mean $\pm$ S.E.M. and analyzed with the Student's *t*-test to assess significance. *P* values of 0.05 or lower were considered statistically significant.

Results

We first examined the effects of adipose VDR status on leptin production in mice. Northern blot analysis showed that adipose leptin mRNA transcript in the epididymal fat was downregulated (by $69 \pm 17\%$) in *VDR(-/-)* mice

compared with WT mice (Fig. 1A and B), which was confirmed by real-time RT-PCR quantitation (Fig. 1C). Similarly, serum leptin levels were markedly reduced (by $86 \pm 5\%$) in the mutant mice (Fig. 1D). Resistin

Figure 1

Leptin expression in *VDR(-/-)* mice. (A and B) Northern blot analysis (A) and PhosphorImaging quantitation (B) of leptin and resistin mRNA levels in the epididymal fat of WT and *VDR(-/-)* mice. (C) Real-time RT-PCR quantitation of leptin mRNA transcript in these mice. *P < 0.05 vs WT; *n* = 4–6. (D) Serum leptin concentration in WT and *VDR(-/-)* mice determined by ELISA. ***P < 0.001 vs WT. *n* ≥ 10.

Figure 2

Leptin expression in adipocyte-specific hVDR Tg mice. (A and B) Northern blot analysis (A) and Phosphorimaging quantitation (B) of leptin and resistin mRNA levels in the epididymal fat of WT and hVDR Tg mice. (C) Real-time RT-PCR quantitation of leptin mRNA transcript in these mice. * $P < 0.05$ vs WT, $n = 5-7$. (D) Serum leptin concentration in WT and hVDR Tg mice determined by ELISA. * $P < 0.05$ vs WT. $n = 5-7$.

mRNA levels were increased (by $75 \pm 8\%$) in VDR(–/–) mice (Fig. 1A and B). On the other hand, in aP2-hVDR Tg mice that carry targeted hVDR overexpression in adipocytes, adipose leptin transcript was upregulated (by $52 \pm 32\%$) (Fig. 2A and B), and this observation was confirmed by real-time RT-PCR quantitation (Fig. 2C). Serum leptin levels were also elevated (by $47 \pm 6\%$) (Fig. 2D), compared

with WT mice. As VDR(–/–) mice were lean and aP2-hVDR Tg mice were obese (Wong *et al.* 2009, 2011), the changes in leptin production in these mice may just reflect the status of adiposity.

To address whether the vitamin D–VDR signaling affects leptin expression in the adipose tissue, we treated WT mice with a low-calcemic vitamin D analog RO-27-5646 for 1 week. Compared with vehicle-treated controls, RO-27-5646 treatment markedly upregulated adipose leptin transcript expression (by $290 \pm 84\%$) (Fig. 3A and B) in these mice, and this observation was confirmed by real-time RT-PCR quantitation (Fig. 3C). Serum leptin concentration in the treated mice was also markedly increased (by $210 \pm 128\%$) (Fig. 3D). This short-term treatment had no effects on body weight (data not shown). These results suggest that vitamin D signaling may directly upregulate adipose leptin expression.

We then used adipose tissue cultures to confirm the stimulatory effect of the vitamin D hormone on leptin expression. As shown in Fig. 4, VDR is expressed in white adipose tissue (Fig. 4A), providing a basis for $1,25(\text{OH})_2\text{D}_3$ signaling. Treatment of white adipose tissue with $1,25(\text{OH})_2\text{D}_3$ (10 nM) for 24 h significantly stimulated leptin secretion (by $41 \pm 19\%$) from the tissue (Fig. 4B). $1,25(\text{OH})_2\text{D}_3$ treatment dose dependently induced leptin mRNA transcript expression but had no effects on resistin expression (Fig. 4C and D). The latter indicates that the regulatory effect of $1,25(\text{OH})_2\text{D}_3$ on leptin is gene specific. Real-time RT-PCR quantitation confirmed the stimulation of leptin mRNA by $1,25(\text{OH})_2\text{D}_3$ (10 nM) treatment (Fig. 4E). These data provide direct evidence that $1,25(\text{OH})_2\text{D}_3$ upregulates leptin expression.

Finally, we assessed the requirement of VDR to mediate the stimulatory effect of $1,25(\text{OH})_2\text{D}_3$ on leptin. As shown in Fig. 5, whereas incubation of WT adipose tissue culture with $1,25(\text{OH})_2\text{D}_3$ upregulated leptin expression, $1,25(\text{OH})_2\text{D}_3$ had no effects on leptin in adipose tissue obtained from VDR(–/–) mice (Fig. 5A). As a control, $1,25(\text{OH})_2\text{D}_3$ had no effects on resistin in either WT or VDR(–/–) adipose tissue (Fig. 5A). On the other hand, $1,25(\text{OH})_2\text{D}_3$ -induced upregulation of leptin was more robust in the adipose tissue obtained from aP2-hVDR Tg mice compared with the adipose tissue from WT mice (Fig. 5B), suggesting that this stimulatory action was determined by VDR status in adipocytes.

Discussion

In this study, we provided experimental evidence that the vitamin D hormone directly upregulates leptin expression

Figure 3

Stimulatory effects of low-calcemic vitamin D analog on leptin expression in mice. WT mice were treated with vehicle or RO-27-5646 at a daily dose of 1.5 µg/kg for 1 week. (A and B) Northern blot analysis (A) and PhosphorImaging quantitation (B) of leptin mRNA in the epididymal fat of vehicle- or RO-27-5646-treated mice. (C) Real-time RT-PCR quantitation of leptin mRNA transcript in these mice. (D) Serum leptin levels determined by ELISA. ** $P < 0.01$; *** $P < 0.001$ vs vehicle; $n = 5-7$.

and secretion in the white adipose tissue in mice. This regulation may contribute to the effect of the vitamin D endocrine system on energy metabolism in the body. We and other groups have previously reported a potential

role of adipocyte VDR signaling in energy metabolism based on observations from VDR(−/−) mice (Narvaez *et al.* 2009, Wong *et al.* 2009). Using an adipocyte-specific hVDR Tg model, we recently demonstrated that adipocyte VDR suppresses energy expenditure and promotes obesity (Wong *et al.* 2011). Here, we showed that adipose leptin expression and serum leptin levels were positively correlated with adipose VDR status in VDR(−/−) and aP2-hVDR Tg mice (Figs 1 and 2); however, as the leptin status is mainly determined by adiposity, these animal data cannot prove whether leptin is regulated by the vitamin D hormone. By treating mice with a vitamin D analog, we provided evidence that vitamin D may directly induce adipose leptin expression (Fig. 3), as this short-term analog treatment did not significantly alter the status of mouse adiposity. More direct evidence for vitamin D upregulation of leptin came from experiments with adipose tissue cultures, in which we demonstrated that incubation of the adipose tissue with 1,25(OH)₂D₃ resulted in induction of leptin mRNA expression and protein secretion and that this regulation was VDR dependent (Figs 4 and 5). Our results are different from an early report by Menendez *et al.* (2001) showing that treatment of human adipose tissue cultures with vitamin D inhibited leptin secretion. In the study of Menendez *et al.*, human adipose tissues were treated with 1,25(OH)₂D₃ for up to 96 h, and a marked suppression of leptin secretion was only observed at 72–92 h, suggesting that the inhibitory effects of 1,25(OH)₂D₃ may be indirect. Our data reported here were from mice, and we treated mouse adipose tissues for only 24–48 h *ex vivo*. The change of leptin within this short time frame suggests that the stimulatory effect of 1,25(OH)₂D₃ on leptin is direct. In fact, we showed that this effect is VDR dependent. The reason for the discrepancy between these two studies is unclear but could be due to species differences or different experimental conditions. Further investigations are needed to confirm or refute these human data regarding the effect of vitamin D on leptin production.

A growing number of epidemiological studies have shown an inverse correlation between adiposity or BMI and serum 1,25(OH)₂D₃ and 25-hydroxyvitamin D levels in humans (Wortsman *et al.* 2000, Arunabh *et al.* 2003, Parikh *et al.* 2004, Snijder *et al.* 2005), but whether low vitamin D levels play any roles in the development of obesity is unknown. Given the importance of leptin in energy homeostasis, there is a great interest to understand its potential relationship with vitamin D. Recently, a reverse correlation between serum 25-hydroxyvitamin D and leptin has been reported in obese human subjects

Figure 4

Vitamin D stimulation of leptin production in adipose tissue cultures. (A) RT-PCR detection of VDR mRNA expression in adipose tissue. Total RNAs isolated from epididymal white fat (WF) and kidney (K) of WT mice were subject to RT-PCR amplification. Arrow indicates the 392 bp VDR cDNA fragment. C, No RT control. (B) ELISA quantitation of leptin secretion into the cultured media at the dose of 10 nM define 1.25VD. * $P < 0.05$ vs ethanol. (C and D) Dose-dependent stimulation of leptin in epididymal

fat culture by 1,25-dihydroxyvitamin D₃. Epididymal fat was cultured in the presence of ethanol (E) or different doses (0.1–100 nM) of 1.25VD for 24 h. Leptin and resistin mRNA levels were analyzed by northern blotting (C) and quantified by PhosphorImaging (D). * $P < 0.05$ vs ethanol. (E) Real-time RT-PCR quantitation of leptin mRNA transcript in the epididymal fat culture treated with 10 nM 1.25VD. ** $P < 0.01$ vs E.

(Maetani *et al.* 2009, Grethen *et al.* 2012). This relationship is consistent with the low vitamin D status observed in obesity by the other studies mentioned earlier and the high serum leptin level known in obesity. As vitamin D is highly fat soluble, the low-vitamin D status seen in the obese population is believed to be a result of excess vitamin D trapped in the fat tissue (Wortsman *et al.* 2000). In this case, the trapped vitamin D could increase the local concentration of vitamin D in the fat, resulting in enhancement of its actions on adipocytes including on leptin expression. Therefore, it is plausible that behind the observed inverse relationship between serum vitamin D and leptin status is the fact that high intra-adipose vitamin D concentration stimulates leptin secretion in obesity. This explanation is consistent with our results reported here. More studies are required to further examine this speculation.

The implication of vitamin D upregulation of leptin is unclear. While the body's leptin status is mainly determined by adiposity, vitamin D may participate in fine-tuning leptin levels in the circulation. Interestingly, leptin has been shown to affect 1,25(OH)₂D₃ biosynthesis by downregulating 25-hydroxyvitamin D 1 α -hydroxylase (Matsunuma & Horiuchi 2007), and this regulation seems to be mediated by fibroblast growth factor 23 (Tsuji *et al.* 2010). In this regard, upregulating leptin might be one of

the multiple feedback loops by which the vitamin D hormone self-regulates its concentration in the body. Indeed, the complex interplay between vitamin D and leptin and its underlying biological implications warrant further investigations.

Figure 5

VDR-dependent stimulation of leptin expression by vitamin D. (A) Epididymal fat obtained from WT and VDR(-/-) mice was incubated with ethanol (E) or 10 nM 1,25-dihydroxyvitamin D₃ (VD) for 24 and 48 h. Leptin and resistin mRNA levels were analyzed by northern blotting. (B) Epididymal fat obtained from WT and hVDR Tg mice was incubated with ethanol (E), 10 or 100 nM 1,25-dihydroxyvitamin D (1.25VD) for 24 h. Leptin mRNA transcript levels were quantified by real-time RT-PCR. ** $P < 0.01$ vs WT.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported in part by grants from National Institutes of Health (R01HL085793) and from National Natural Science Foundation of China (Grant No. 30971401 and 81170065).

Acknowledgements

The authors thank Dr Milan Uskokovic (BioXell, Inc.) for providing RO-27-5654.

References

- Arunabh S, Pollack S, Yeh J & Aloia JF 2003 Body fat content and 25-hydroxyvitamin D levels in healthy women. *Journal of Clinical Endocrinology and Metabolism* **88** 157–161. (doi:10.1210/jc.2002-020978)
- Blumberg JM, Tzameli I, Astapova I, Lam FS, Flier JS & Hollenberg AN 2006 Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. *Journal of Biological Chemistry* **281** 11205–11213. (doi:10.1074/jbc.M510343200)
- Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer HF, Lieben L, Mathieu C & Demay M 2008 Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocrine Reviews* **29** 726–776. (doi:10.1210/er.2008-0004)
- Donato J Jr, Cravo RM, Frazao R & Elias CF 2011 Hypothalamic sites of leptin action linking metabolism and reproduction. *Neuroendocrinology* **93** 9–18. (doi:10.1159/000322472)
- Fruhbeck G, Gomez-Ambrosi J, Muruzabal FJ & Burrell MA 2001 The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. *American Journal of Physiology. Endocrinology and Metabolism* **280** E827–E847.
- Grethen E, Hill KM, Jones R, Cacucci BM, Gupta CE, Acton A, Considine RV & Peacock M 2012 Serum leptin, parathyroid hormone, 1,25-dihydroxyvitamin D, fibroblast growth factor 23, bone alkaline phosphatase, and sclerostin relationships in obesity. *Journal of Clinical Endocrinology and Metabolism* **97** 1655–1662. (doi:10.1210/jc.2011-2280)
- Haussler MR, Whitfield GK, Haussler CA, Hsieh J-C, Thompson PD, Selznick SH, Dominguez CE & Jurutka PW 1998 The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *Journal of Bone and Mineral Research* **13** 325–349. (doi:10.1359/jbmr.1998.13.3.325)
- Kong J & Li YC 2006 Molecular mechanism of 1,25-dihydroxyvitamin D₃ inhibition of adipogenesis in 3T3-L1 cells. *American Journal of Physiology. Endocrinology and Metabolism* **290** E916–E924. (doi:10.1152/ajpendo.00410.2005)
- Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R & Demay MB 1997 Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. *PNAS* **94** 9831–9835. (doi:10.1073/pnas.94.18.9831)
- Li YC, Amling M, Pirro AE, Priemel M, Meuse J, Baron R, Delling G & Demay MB 1998 Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice. *Endocrinology* **139** 4391–4396. (doi:10.1210/en.139.10.4391)
- Maetani M, Maskarinec G, Franke AA & Cooney RV 2009 Association of leptin, 25-hydroxyvitamin D, and parathyroid hormone in women. *Nutrition and Cancer* **61** 225–231. (doi:10.1080/01635580802455149)
- Mantzoros CS 1999 The role of leptin in human obesity and disease: a review of current evidence. *Annals of Internal Medicine* **130** 671–680.
- Matsunuma A & Horiuchi N 2007 Leptin attenuates gene expression for renal 25-hydroxyvitamin D₃-1 α -hydroxylase in mice via the long form of the leptin receptor. *Archives of Biochemistry and Biophysics* **463** 118–127. (doi:10.1016/j.abb.2007.02.031)
- Menendez C, Lage M, Peino R, Baldelli R, Concheiro P, Dieguez C & Casanueva FF 2001 Retinoic acid and vitamin D(3) powerfully inhibit *in vitro* leptin secretion by human adipose tissue. *Journal of Endocrinology* **170** 425–431. (doi:10.1677/joe.0.1700425)
- Naggal S, Na S & Rathnachalam R 2005 Noncalcemic actions of vitamin D receptor ligands. *Endocrine Reviews* **26** 662–687. (doi:10.1210/er.2004-0002)
- Narvaez CJ, Matthews D, Broun E, Chan M & Welsh J 2009 Lean phenotype and resistance to diet-induced obesity in vitamin D receptor knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue. *Endocrinology* **150** 651–661. (doi:10.1210/en.2008-1118)
- Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J & Yanovski JA 2004 The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *Journal of Clinical Endocrinology and Metabolism* **89** 1196–1199. (doi:10.1210/jc.2003-031398)
- Procaccini C, Jirillo E & Matarese G 2012 Leptin as an immunomodulator. *Molecular Aspects of Medicine* **33** 35–45. (doi:10.1016/j.mam.2011.10.012)
- Qiao G, Kong J, Uskokovic M & Li YC 2005 Analogs of 1 α ,25-dihydroxyvitamin D₃ as novel inhibitors of renin biosynthesis. *Journal of Steroid Biochemistry and Molecular Biology* **96** 59–66. (doi:10.1016/j.jsbmb.2005.02.008)
- Schwartz MW, Woods SC, Porte D Jr, Seeley RJ & Baskin DG 2000 Central nervous system control of food intake. *Nature* **404** 661–671.
- Snijder MB, van Dam RM, Visser M, Deeg DJ, Dekker JM, Bouter LM, Seidell JC & Lips P 2005 Adiposity in relation to vitamin D status and parathyroid hormone levels: a population-based study in older men and women. *Journal of Clinical Endocrinology and Metabolism* **90** 4119–4123. (doi:10.1210/jc.2005-0216)
- Tilg H & Moschen AR 2006 Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nature Reviews. Immunology* **6** 772–783. (doi:10.1038/nri1937)
- Tsuji K, Maeda T, Kawane T, Matsunuma A & Horiuchi N 2010 Leptin stimulates fibroblast growth factor 23 expression in bone and suppresses renal 1 α ,25-dihydroxyvitamin D₃ synthesis in leptin-deficient mice. *Journal of Bone and Mineral Research* **25** 1711–1723. (doi:10.1002/jbmr.65)
- Wong KE, Szeto FL, Zhang W, Ye H, Kong J, Zhang Z, Sun XJ & Li YC 2009 Involvement of the vitamin D receptor in energy metabolism: regulation of uncoupling proteins. *American Journal of Physiology. Endocrinology and Metabolism* **296** E820–E828. (doi:10.1152/ajpendo.90763.2008)
- Wong KE, Kong J, Zhang W, Szeto FL, Ye H, Deb DK, Brady MJ & Li YC 2011 Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice. *Journal of Biological Chemistry* **286** 33804–33810. (doi:10.1074/jbc.M111.257568)
- Wortsman J, Matsuoka LY, Chen TC, Lu Z & Holick MF 2000 Decreased bioavailability of vitamin D in obesity. *American Journal of Clinical Nutrition* **72** 690–693.
- Zhang Y, Proenca R, Maffei M, Barone M, Leopold L & Friedman JM 1994 Positional cloning of the mouse obese gene and its human homologue. *Nature* **372** 425–432. (doi:10.1038/372425a0)
- Zhang Z, Zhang Y, Ning G, Deb DK, Kong J & Li YC 2008 Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: blockade of compensatory renin increase. *PNAS* **105** 15896–15901. (doi:10.1073/pnas.0803751105)

Received in final form 25 October 2012

Accepted 16 November 2012

Accepted Preprint published online 16 November 2012