Journal of Steroid Biochemistry & Molecular Biology 113 (2009) 222–226

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Serum cholesterol and expression of ApoAI, LXRβ and SREBP2 in vitamin D receptor knock-out mice☆

Jing-Huan Wang[a,b,c,d,*], Tiina Keisala[a,d], Tiina Solakivi[e], Anna Minasyan[a,d], Allan V. Kalueff[a], Pentti Tuohimaa[a,b,c,d]

[a] Department of Anatomy, Medical School, 33014 University of Tampere, Tampere, Finland
[b] Drug Discovery Graduate School, University of Turku, 20520 Turku, Finland
[c] Department of Clinical Chemistry, Tampere University Hospital, University of Tampere, 33014 Tampere, Finland
[d] Tampere Graduate School in Biomedicine and Biotechnology, University of Tampere, Tampere, Finland
[e] Department of Medical Biochemistry, Medical School, University of Tampere, Finland

ARTICLE INFO

Article history:
Received 15 October 2008
Received in revised form 18 December 2008
Accepted 12 January 2009

Keywords:
Vitamin D receptor
Cholesterol
Mice
High-density lipoprotein-bound cholesterol
Gene expression

ABSTRACT

Vitamin D insufficiency has been reported to be associated with increased blood cholesterol concentrations. Here we used two strains of VDR knock-out (VDR-KO) mice to study whether a lack of vitamin D action has any effect on cholesterol metabolism. In 129S1 mice, both in male and female VDR-KO mice serum total cholesterol levels were significantly higher than those in wild type (WT) mice (20.7% (P = 0.05) and 22.2% (P = 0.03), respectively). In addition, the serum high-density lipoprotein-bound cholesterol (HDL-C) level was 22% (P = 0.03), respectively higher in male VDR-KO mice than in WT mice. The mRNA expression levels of five cholesterol metabolism related genes in livers of 129S1 mice were studied using quantitative real-time PCR (QRT-PCR): ATP-binding cassette transporter A1 (ABCA1), regulatory element binding protein (SREBP2), apolipoprotein A-I (ApoAI), low-density lipoprotein receptor (LDLR) and liver X receptor beta (LXRβ). In the mutant male mice, the mRNA level of ApoAI and LXRβ were 49.2% (P = 0.005) and 38.8% (P = 0.034) higher than in the WT mice. These changes were not observed in mutant female mice, but the female mutant mice showed 52.5% (P = 0.006) decrease of SREBP2 mRNA expression compared to WT mice. Because the mutant mice were fed with a special rescue diet, we wanted to test whether the increased cholesterol levels in mutant mice were due to the diet. Both the WT and mutant NMRI mice were given the same diet for 3 weeks before the blood sampling. No difference in cholesterol or in HDL-C between WT and mutant mice was found. The results suggest that the food, gender and genetic background have an effect on the cholesterol metabolism. Although VDR seems to regulate some of the genes involved in cholesterol metabolism, its role in the regulation of serum cholesterol seems to be minimal.

1. Introduction

Vitamin D is produced in the skin through a photolytic reaction of 7-dehydrocholesterol induced by ultraviolet B radiation at 290–315 nm. The vitamin D formed in the skin or absorbed from diet is hydroxylated in the liver to 25-hydroxyvitamin D and further hydroxylated in the kidney to 1,25-dihydroxyvitamin D (calcitriol) [1]. Calcitriol is the most active ligand for vitamin D receptor (VDR) and after binding to VDR performs its biological functions such as control of calcium homeostasis, cell proliferation and differentiation [2,3]. Lack of sunlight and vitamin D deficiency has been suggested to be associated with an increased blood cholesterol concentration [4]. In postmenopausal women with hormone replacement therapy, vitamin D seems to affect serum lipid levels [5]. Therefore we were interested to study the effect of VDR knock-out in mice on their serum cholesterol level.

SREBP2 is a transcription factor which down-regulates ABCA1 [6] but it increases LDLR expression [7] and is involved in cholesterol synthesis [8]. Many of the LXR target genes are also involved in cholesterol and fatty acid metabolism pathways [9–11]. Thus, LXRs form heterodimer with RXR and act as cholesterol sensors as well as regulators of genes for cholesterol efflux and lipid transport to maintain cholesterol homeostasis [12–15]. Major cholesterol-related targets of LXRs include the ATP-binding cassette transporter family members such as ABCA1 [16,17], which is mutated in Tangier disease. A characteristic feature of these patients is extremely low or absent HDL-C and reduced total cholesterol [18–20]. The main role

☆ This paper corresponds to poster presented at the 18th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology (Seefeld, Austria, 18–21 September 2008).

* Corresponding author at: Department of Anatomy, Medical School, University of Tampere, Medisiinarinkatu 3, FI-33520, Tampere, Finland.
Tel.: +358 3 2156726; fax: +358 3 2156170.
E-mail address: Jinghuan.Wang@uta.fi (J.-H. Wang).

0960-0760/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2009.01.003

of ABCA1 is the mediation of the efflux of excess cholesterol from macrophages and fibroblasts in the sub-endothelial space to acceptors such as HDL and ApoAI [21], which in the circulation interacts first with serum phospholipids and forms nascent discoidal HDL (ndHDL), which triggers cholesterol efflux [21]. The externalized cholesterol is incorporated into ndHDL and further modified. The final products, cholesteryl esters, are delivered back to the liver via LDLR, converted to bile salts, and eliminated through the gastrointestinal tract [21]. LDLR knock-out mice showed altered lipid profile [22]. Therefore, in the present study, we tested the mRNA expression of ABCA1, ApoAI, LDLR, LXRβ and SREBP2 from both wild type and mutant mice.

2. Materials and methods

2.1. Mouse breeding, housing and feeding

VDR-KO mice 129S1 were produced from the line initially generated in the University of Tokyo [23], which have been studied in our laboratory [24–26]. NMRI mice were purchased from Harlan, Nederland. They originate from Swiss mice in the US brought from Lausanne, Switzerland, in 1926 by Clara Lynch. In 1937 the mice came from Lynch to Poiley and were inbred by Poiley known as NIH/PI. At F51 they went to US Naval Medical Research Institute and thus known as NMRI. In 1955, the mice went to Bundes-Forschungsanstalt für Viruskrankheiten and in 1958, to Central Institute for Laboratory Breeding, Hannover. In 1981, they came from Central Institute for Laboratory Breeding, Hannover to Winkelmann (now Harlan Winkelmann). In 1998, they came from Harlan Winkelmann to Harlan Nederland (www.harlaneurope.com), known as HsdWin:NMRI mice, which are abbreviated as "NMRI mice" in the text. Studies using this strain of mice have been reported [27,28]. All the mice were housed in the University of Tampere Laboratory Animal facility with a 12:12-light:dark cycle. The management and experimental procedures in this study were approved by the Ethical Committee of the University of Tampere and performed according to EU legislation. Age-matched wild type and VDR-KO mice were genotyped and used in this study. Mice were 6.5–16 months old. The numbers of 129S1 mice used in the study were, male KO 8 and male WT 9; female KO 6 and female WT 5. The numbers of NMRI mice were, male KO 2 and WT 10, female KO 7 and WT 11. The VDR-KO mice were fed with a special diet containing 2% Ca, 1.25% P and 20% lactose (Lactamin AB, Sweden), to normalize their mineral metabolism. In the studies with NMRI mice, WT mice were switched from normal foods (0.9% Ca, 0.7% P and 20% lactose) to special foods 3 weeks before sample collection.

2.2. Serum sample preparation and tissue sample collection

Mice were sacrificed by carbon dioxide and blood was immediately taken by heart puncture. Blood was allowed to clot, followed by centrifugation at 3000 rpm for 10 min. The serum was stored at −70 °C for further analysis. Small pieces of liver and kidney tissues were taken and dropped into RNAlater (Ambion), and stored at −20 °C for later RNA isolation.

2.3. Measurement of total cholesterol and HDL-C

Total cholesterol and HDL-C concentrations were measured using a photometric CHOD-PAP (Ecoline® S+ Cholesterol, DiaSys Diagnostic Systems GmbH, Germany). HDL-C was determined from the clear supernatant after precipitation of serum apoB-containing lipoproteins with 10% polyethylene glycol (final concentration).

2.4. RNA extraction and real-time quantitative PCR

Liver tissue slices was dropped into ice-cooled trizol (Invitrogen, Carlshad, USA) and homogenized on ice. Total cellular RNA was isolated with TRIzoL reagent following the instructions from the manufacturer. The RNA concentration was calculated from absorbance at 260 nm in a GeneQuant II (Pharmacia Biotech, USA) and A280/A260 was measured to verify the purity of the RNA. The ratio of all the RNA samples fell in 1.9–2.5. Randomly selected RNA samples were subjected to denaturing gel electrophoresis. The ratio of the intensity of the 28S band and that of the 18S band was 1.5–2.0. cDNA was synthesised by using High Capacity Archive Kit (Applied Biosystems, USA). Real-time PCR were performed in ABI PRISM 7000 Detection System (Applied Biosystems, USA) using SYBR Green PCR Master Mix (Applied Biosystems). The programs for the amplification were as following: activation of polymerase at 95 °C for 10 min, followed by 45 cycles of denaturation at 95 °C for 15 s and annealing/extension at 60 °C for 1 min. The analysis of dissociation curves was always performed after 45 cycles. Primers were designed by using Primer Express v2.0 software (Perkin-Elmer Applied Biosystems, Foster City, CA). BLASTn searches were performed to ensure that the primers were gene specific. The primers are mABCA1 (NM_013454) forward 5′-CAACCCCTGCTTCCGTTATC-3′, reverse 5′-GACCTTGTGCATGTCCTTAATGC-3′; mApoAI (NM_009692) forward 5′-CTCCTCCTTGGGCCAACA-3′, reverse 5′-TGACTAACGGTTGAACCCAGAGT-3′; mLDLR (NM_010700) forward 5′-TGTGAAAATGACTCAGACGAACAA-3′, reverse 5′-GGAGATGCACTTGCCATCCT-3′; mLXRβ (Nr1h2) (NM_009473) forward 5′-GATCCTCCTCCAGGCTCTGAA-3′, reverse 5′-TGCGCTCAGGCTCATCCT-3′; mSREBP2 (NM_033218) forward 5′-GTGCGCTCTCGTTTTACTGAAGT-3′, reverse 5′-GTATAGAAGACGGCCTTCACCAA-3′. Mouse *gene β-actin (*NM_007393*) was used as endogenous control. Primers for mβ-actin were as follows: forward:* 5′-GCTTCTTTGCAGCTCCTTCGT-3′ *reverse:* 5′-CCAGCGCAGCGATATCG-3′.

2.5. Statistical analysis

Data of cholesterol and HDL-C level as well as real-time PCR are expressed as the mean value ± standard error. Significance was assessed using the Mann–Whitney *U* test. $^{*}p \leq 0.05$ was considered as significant, $^{**}p < 0.001$ as highly significant and $p > 0.05$ as not significant (NS).

3. Results

3.1. Total cholesterol and HDL-C serum concentration

The mean total cholesterol concentration was significantly higher in 5 female mutant mice in comparison with that of 5 female wild type mice (Table 1). In female mice, there was no statistically significant difference in the serum HDL-C. In male mice, both total cholesterol and HDL-C concentrations were significantly lower in WT mice than in KO mice (Table 1).

3.2. ABCA1, ApoAI, LDLR, LXRβ and SREBP2 mRNA expression in the liver

QRT-PCR data show a significant difference in ApoAI, LXRβ and SREBP2 expressions between WT and mutants (Fig. 1A and B). The relative mRNA levels were 1.0 ± 0.2 and 0.7 ± 0.1 ($P = 0.005$) for ApoAI, 1.1 ± 0.3 and 0.8 ± 0.2 ($P = 0.034$) for LXRβ in mutant and wild type male mice, respectively; 0.9 ± 0.1 vs 1.4 ± 0.3 ($P = 0.006$) for SREBP2 in mutant and wild type female mice, respectively. The female wild type and mutant mice do not show difference in the

Table 1
Serum cholesterol and HDL-C level in wild type and VDR knock-out 129S1 mice.

	WT	VDR-KO	*P* value
Female			
Total cholesterol	2.14 ± 0.09	2.62 ± 0.15	0.028
HDL-C	1.23 ± 0.06	1.35 ± 0.04	0.209
Nr of observations	5	5	
Male			
Total cholesterol	2.54 ± 0.07	3.1 ± 0.27	0.054
HDL-C	1.69 ± 0.05	2.04 ± 0.16	0.034
Nr of observations	9	8	

Serum cholesterol and HDL-C levels of wild type and VDR knock-out mice were determined. Data were analyzed by Mann–Whitney *U* test and presented as mean value ± standard error. The unit used in the table is mmol/L (SI unit). To convert values from SI units to conventional units, divide by the conversion factor. For cholesterol and HDL-C, the convert factor is 0.0259.

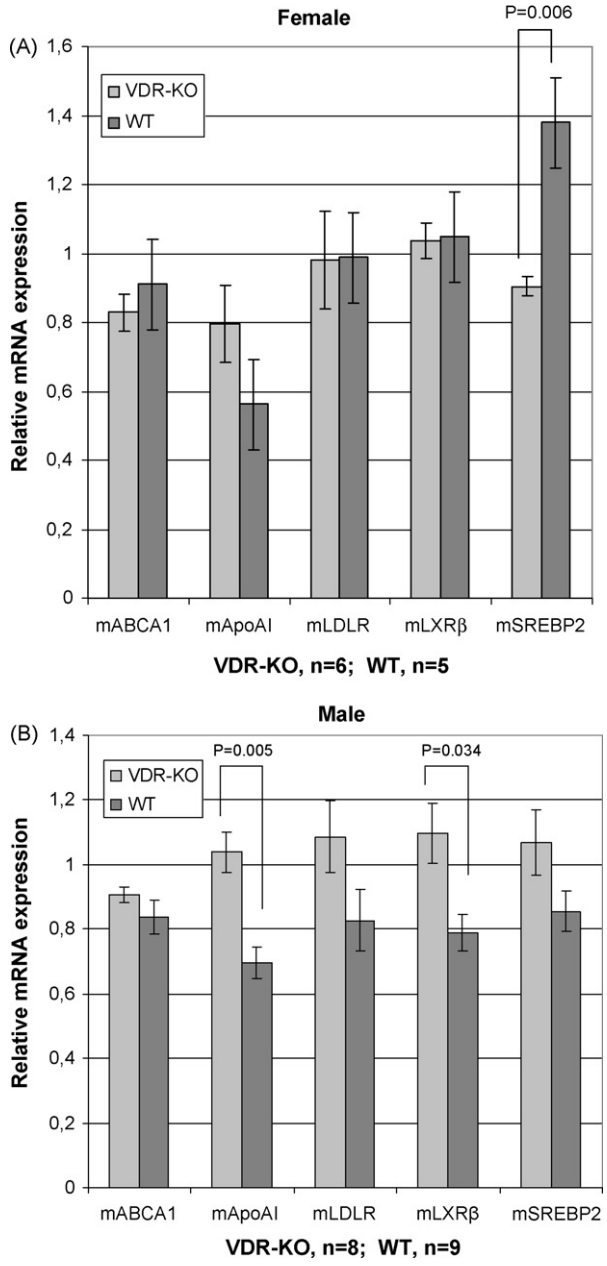

Fig. 1. ABCA1, ApoAI, LDLR, LXRβ and SREBP2 expression in the liver of wild type and VDR knock-out mice. (A) Female and (B) male. Data represented mean value ± standard error from quantitative real-time PCR results and statistically analyzed by Mann–Whitney *U* test. Mouse β-actin was used as housekeeping gene for the calculation of relative corresponding gene expression.

Table 2
Serum cholesterol and HDL-C level in wild type and VDR knock-out NMRI mice.

	Female	Male	*P* value
WT			
Total cholesterol	3.71 ± 0.08	5.15 ± 0.09	0.00108
HDL-C	2.76 ± 0.06	4.14 ± 0.07	0.00011
Nr of observations	11	10	
VDR-KO			
Total cholesterol	3.85 ± 0.07	5.19 ± 0.03	0.0421
HDL-C	2.77 ± 0.05	4.1 ± 0.07	0.01371
Nr of observations	7	2	

3 weeks before sample collection, both wild type and VDR-KO NMRI mice were fed with special foods containing high calcium. Serum cholesterol and HDL-C levels of both types of mice were determined. Data were analyzed by Mann–Whitney *U* test and presented as mean value ± standard error. The unit used in the table is mmol/L (SI unit). To convert values from SI units to conventional units, divide by the conversion factor. For cholesterol and HDL-C, the convert factor is 0.0259.

expression of ApoAI nor LXRβ. There is no significant difference of the expression of ABCA1 and LDLR in mutant and wild type mice.

3.3. The effect of food

We used NMRI strain of VDR-KO mice. Both the WT and mutant mice were given the same foods containing 2% Ca, 1.25% P and 20% lactose for 3 weeks before blood sampling. No difference in cholesterol or in HDL-C between WT and mutant mice was found, but a clear sex difference was observed (Table 2).

4. Discussion

This is the first direct evidence that a nonfunctional VDR can increase serum total cholesterol concentration in both female and male mice. Also HDL-C was increased but only in the male VDR-KO mice. In male animals, the higher total cholesterol level in KO mice was due to increase in both HDL-C and apoB-containing lipoprotein cholesterol. It seems that the mutation of VDR in male and female mice have different effects on lipids, which is consist with relative gene expression changes. In male lacking of functional VDR, ApoAI and LXRβ expression levels were increased, but not in females. In VDR-KO females, expression of SREBP2 was decreased. It has been reported that calcitriol inhibits ApoAI mRNA and protein in the human hepatoma cell line HepG2 VDR- and VDRE-dependently [29]. In our study, male mutant mice had a higher liver ApoAI mRNA expression. Thus, it is possible that VDR knock-out results in a higher ApoAI level, which in turn, increased cholesterol efflux and hence HDL-C.

In mammals there are two forms of LXRs, LXRα/NR1H3 and LXRβ/NR1H2. The expression of LXRα is restricted to kidney, intestine, spleen, and adrenals, with the highest expression levels in the liver [30,31] whereas the LXRβ is ubiquitously expressed [32,33]. Inhibition of LXRα signaling by vitamin D receptor has been reported [34]. Because of the high expression of LXRα in the liver, its role in lipid metabolism has been extensively studied. A search in PUBMED with "LXRβ + lipid" and "LXRα + lipid" revealed 125 and 792 articles, respectively. Thus, in the present study we investigated the LXRβ expression. Interestingly, male mutant mice have higher levels of LXRβ mRNA expression. As one of the target genes for LXRβ, it has been shown that an over-expression of ABCA1 in liver is associated with increased HDL-C levels in transgenic mice and ABCA1 knock-out leads to HDL-C deficiency [35]. Our quantitative real-time PCR results show that the liver ABCA1 mRNA expression levels are unchanged. This suggests factors other than ABCA1 are involved in the serum lipid changes of the mutant mice. Given the role of LXRβ for activating not only ABCA1, but also ABCG1 [36], ABCG5 [37] and ABCG8 [38] it is possible that the

LXRβ exerts its effects on HDL-C levels by activating other cholesterol exporters. In addition, vitamin D inhibits LXRα signaling [34], which thus in VDR-KO mice might be enhanced and consequently contributes to increase ABCG1, ABCG5 and ABCG8 expression as well.

SREBPs are synthesized as precursors complexed with SCAP in the membranes of the endoplasmic reticulum (ER) [39]. When cells are depleted of sterols, SCAP escorts SREBPs from ER to Golgi where the SREBPs are cleaved [40,41] to release the bHLH-Zip domain which travels to the nucleus where it activates genes whose products play roles in the lipid synthesis and uptake [42], including cholesterol [43]. Our results showed that VDR mutant female mice have lower levels of SREBP2 but higher level of cholesterol. Cholesterol has been shown directly bind to sterol-sensing domain of SCAP *in vitro* [44]. The binding of cholesterol to SCAP elicits a conformational change in SCAP causing SCAP to bind to Insigs, endoplasmic reticulum retention proteins that abrogate movement of the SCAP–SREBP complexes to the Golgi apparatus for SREBP processing and activation [44–47]. It has been reported murine Insig-2 promoter harbors a positive vitamin D response element [48]. SREBP2 is a transcription factor which inhibits ABCA1 [6] but stimulate LDLR [7] expression. In the present study, mutant female mice showed lower SREBP2 expression but no changes in ABCA1 nor LDLR were observed.

It seems that the special rescue diet of the mutant mice has a strong effect on the serum lipid levels. In our experiments using NMRI mice, both wild type and mutant mice were fed with special food containing 2% Ca, 1.25% P and 20% lactose 3 weeks before the sampling. No significant difference in cholesterol and/or HDL-C level between wild type and mutant mice was found. Because there were only 2 NMRI male VRD-KO mice available for the studies, it remains unclear whether this is because of the effects of food or the strain of the mice. On the other hand, this might imply that the effect of VDR knock-out on cholesterol metabolism can be rescued by diet. Investigations of all the four VDR-KO mice models to date demonstrated that the lack of functional VDR develops hypocalcemia, rickets, osteomalacia, hyperparathyroidism and alopecia [23,49–51]. Normalization of ionized calcium levels by the special diet normalized these phenotypes, except for alopecia [52,53].

A sex difference of cholesterol and HDL-C was found in both 129S1 and NMRI strains in wild type and mutant mice. Cholesterol changes in postmenopausal women [54] and in rodents after ovariectomy [55,56] are due to a lack of estrogen. Kamei et al. [57] identified changes of gene expression in lipid metabolism including a decrease of SREBP1 in ovariectomized mice. Similarly, here we found that SREBP2 expression was lower in VDR-KO female mice. Previously it was reported that VDR-KO female mice developed uterine hypoplasia in the post-weaning stage due to a lack of estrogen synthesis in the mutant ovaries [23]. Our present study show that the VDR background has different effects in female and male mice, where nonfunctional VDR can increase serum total cholesterol concentration in both female and male mice but HDL-C was increased only in the male VDR-KO mice. This difference might be due to impaired estrogen production in KO female mice.

In conclusion, our study suggests that lack of the functional VDR may lead to an increased serum cholesterol and HDL-C. In addition, this can be partially explained by changes in the expression of cholesterol metabolism related genes such as ApoAI, LXR and SREBP2. Vitamin D deficiency may therefore contribute to cardiovascular diseases such as atherosclerosis [58]. However, gender and diet also have a clear effect on serum lipid concentrations, independent from the VDR knock-out. As such, the role of VDR in the direct regulation of serum cholesterol seems to be minimal.

Acknowledgements

We are grateful to Ms Marianne Kuuslahti for genotyping of the mice, Mrs Anna-Maija Koivisto for help in statistical analysis and Mr Peter Hart for help with language editing. This work was supported by grants from Tampere University Hospital, Drug Discovery Graduate School (University of Turku), the Academy of Finland and the Finnish Cancer Foundation.

References

[1] M.F. Holick, M. Uskokovic, J.W. Henley, J. MacLaughlin, S.A. Holick, J.T. Potts Jr., The photoproduction of 1 alpha,25-dihydroxyvitamin D3 in skin: an approach to the therapy of vitamin-D-resistant syndromes, N. Engl. J. Med. 303 (1980) 349–354.
[2] M. Guzey, J. Luo, R.H. Getzenberg, Vitamin D3 modulated gene expression patterns in human primary normal and cancer prostate cells, J. Cell. Biochem. 93 (2004) 271–285.
[3] X. Dai, K. Yamasaki, L. Yang, K. Sayama, Y. Shirakata, S. Tokumara, Y. Yahata, M. Tohyama, K. Hashimoto, Keratinocyte G2/M growth arrest by 1,25-dihydroxyvitamin D3 is caused by Cdc2 phosphorylation through Wee1 and Myt1 regulation, J. Invest. Dermatol. 122 (2004) 1356–1364.
[4] D.S. Grimes, E. Hindle, T. Dyer, Sunlight, cholesterol and coronary heart disease, QJM 89 (1996) 579–589.
[5] A.-M. Heikkinen, M.T. Tuppurainen, L. Niskanen, M. Komulainen, I. Penttila, S. Saarikoski, Long-term vitamin D3 supplementation may have adverse effects on serum lipids during postmenopausal hormone replacement therapy, Eur. J. Endocrinol. 137 (1997) 495–502.
[6] L. Zeng, H. Liao, Y. Liu, T.S. Lee, M. Zhu, X. Wang, M.B. Stemerman, Y. Zhu, J.Y. Shyy, Sterol-responsive element-binding protein (SREBP) 2 down-regulates ATP-binding cassette transporter A1 in vascular endothelial cells: a novel role of SREBP in regulating cholesterol metabolism, J. Biol. Chem. 279 (2004) 48801–48807.
[7] R. Streicher, J. Kotzka, D. Muller-Wieland, G. Siemeister, M. Munck, H. Avci, W. Krone, SREBP-1 mediates activation of the low density lipoprotein receptor promoter by insulin and insulin-like growth factor-I, J. Biol. Chem. 271 (1996) 7128–7133.
[8] J.D. Horton, I. Shimomura, Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis, Curr. Opin. Lipidol. 10 (1999) 143–150.
[9] J.M. Lehmann, S.A. Kliewer, L.B. Moore, T.A. Smith-Oliver, B.B. Oliver, J.L. Su, S.S. Sundseth, D.A. Winegar, D.E. Blanchard, T.A. Spencer, T.M. Willson, Activation of the nuclear receptor LXR by oxysterols defines a new hormone response pathway, J. Biol. Chem. 272 (1997) 3137–3140.
[10] Y. Luo, A.R. Tall, Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element, J. Clin. Invest. 105 (2000) 513–520.
[11] J.R. Schultz, H. Tu, A. Luk, J.J. Repa, J.C. Medina, L. Li, S. Schwendner, S. Wang, M. Thoolen, D.J. Mangelsdorf, K.D. Lustig, B. Shan, Role of LXRs in control of lipogenesis, Genes Dev. 14 (2000) 2831–2838.
[12] J.J. Repa, D.J. Mangelsdorf, The role of orphan nuclear receptors in the regulation of cholesterol homeostasis, Annu. Rev. Cell. Dev. Biol. 16 (2000) 459–481.
[13] R.K. Tangirala, E.D. Bischoff, S.B. Joseph, B.L. Wagner, R. Walczak, B.A. Laffitte, C.L. Daige, D. Thomas, R.A. Heyman, D.J. Mangelsdorf, X. Wang, A.J. Lusis, P. Tontonoz, I.G. Schulman, Identification of macrophage liver X receptors as inhibitors of atherosclerosis, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 11896–11901.
[14] P. Tontonoz, D.J. Mangelsdorf, Liver X receptor signaling pathways in cardiovascular disease, Mol. Endocrinol. 17 (2003) 985–993.
[15] S.U. Naik, X. Wang, J.S. Da Silva, M. Jaye, C.H. Macphee, M.P. Reilly, J.T. Billheimer, G.H. Rothblat, D.J. Rader, Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo, Circulation 113 (2006) 90–97.
[16] J.J. Repa, S.D. Turley, J.A. Lobaccaro, J. Medina, L. Li, K. Lustig, B. Shan, R.A. Heyman, J.M. Dietschy, D.J. Mangelsdorf, Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers, Science 289 (2000) 1524–1529.
[17] P. Costet, Y. Luo, N. Wang, A.R. Tall, Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor, J. Biol. Chem. 275 (2000) 28240–28245.
[18] S. Rust, M. Rosier, H. Funke, J. Real, Z. Amoura, J.C. Piette, J.F. Deleuze, H.B. Brewer, N. Duverger, P. Denefle, G. Assmann, Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1, Nat. Genet. 22 (1999) 352–355.
[19] A. Brooks-Wilson, M. Marcil, S.M. Clee, L.H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J.A. Collins, H.O. Molhuizen, O. Loubser, B.F. Ouelette, K. Fichter, K.J. Ashbourne-Excoffon, C.W. Sensen, S. Scherer, S. Mott, M. Denis, D. Martindale, J. Frohlich, K. Morgan, B. Koop, S. Pimstone, J.J. Kastelein, J. Genest Jr., M.R. Hayden, Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency, Nat. Genet. 22 (1999) 336–345.
[20] C. Serfaty-Lacrosniere, F. Civeira, A. Lanzberg, P. Isaia, J. Berg, E.D. Janus, M.P. Smith Jr., P.H. Pritchard, J. Frohlich, R.S. Lees, et al., Homozygous Tangier disease and cardiovascular disease, Atherosclerosis 107 (1994) 85–98.
[21] R. Ohashi, H. Mu, X. Wang, Q. Yao, C. Chen, Reverse cholesterol transport and cholesterol efflux in atherosclerosis, QJM 98 (2005) 845–856.

[22] M. Matasconi, P. Parini, B. Angelin, M. Rudling, Pituitary control of cholesterol metabolism in normal and LDL receptor knock-out mice: effects of hypophysectomy and growth hormone treatment, Biochim. Biophys. Acta 1736 (2005) 221–227.
[23] T. Yoshizawa, Y. Handa, Y. Uematsu, S. Takeda, K. Sekine, Y. Yoshihara, T. Kawakami, K. Arioka, H. Sato, Y. Uchiyama, S. Masushige, A. Fukamizu, T. Matsumoto, S. Kato, Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning, Nat. Genet. 16 (1997) 391–396.
[24] A.V. Kalueff, A. Minasyan, T. Keisala, Z.H. Shah, P. Tuohimaa, Hair barbering in mice: implications for neurobehavioural research, Behav. Processes 71 (2006) 8–15.
[25] T. Keisala, A. Minasyan, U. Jarvelin, J. Wang, T. Hamalainen, A.V. Kalueff, P. Tuohimaa, Aberrant nest building and prolactin secretion in vitamin D receptor mutant mice, J. Steroid Biochem. Mol. Biol. 104 (2007) 269–273.
[26] A. Minasyan, T. Keisala, Y.R. Lou, A.V. Kalueff, P. Tuohimaa, Neophobia, sensory and cognitive functions, and hedonic responses in vitamin D receptor mutant mice, J. Steroid Biochem. Mol. Biol. 104 (2007) 274–280.
[27] Y. Lamberty, A.J. Gower, Age-related changes in spontaneous behavior and learning in NMRI mice from middle to old age, Physiol. Behav. 51 (1992) 81–88.
[28] Y. Lamberty, A.J. Gower, J. Gobert, I. Hanin, E. Wulfert, Behavioural, biochemical and histological effects of AF64A following injection into the third ventricle of the mouse, Behav. Brain Res. 51 (1992) 165–177.
[29] K. Wehmeier, A. Beers, M.J. Haas, N.C. Wong, A. Steinmeyer, U. Zugel, A.D. Mooradian, Inhibition of apolipoprotein AI gene expression by 1,25-dihydroxyvitamin D3, Biochim. Biophys. Acta 1737 (2005) 16–26.
[30] R. Apfel, D. Benbrook, E. Lernhardt, M.A. Ortiz, G. Salbert, M. Pfahl, A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily, Mol. Cell. Biol. 14 (1994) 7025–7035.
[31] P.J. Willy, K. Umesono, E.S. Ong, R.M. Evans, R.A. Heyman, D.J. Mangelsdorf, LXR, a nuclear receptor that defines a distinct retinoid response pathway, Genes Dev. 9 (1995) 1033–1045.
[32] D.M. Shinar, N. Endo, S.J. Rutledge, R. Vogel, G.A. Rodan, A. Schmidt, NER, a new member of the gene family encoding the human steroid hormone nuclear receptor, Gene 147 (1994) 273–276.
[33] C. Song, J.M. Kokontis, R.A. Hiipakka, S. Liao, Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 10809–10813.
[34] W. Jiang, T. Miyamoto, T. Kakizawa, S.I. Nishio, A. Oiwa, T. Takeda, S. Suzuki, K. Hashizume, Inhibition of LXRalpha signaling by vitamin D receptor: possible role of VDR in bile acid synthesis, Biochem. Biophys. Res. Commun. 351 (2006) 176–184.
[35] R.R. Singaraja, M. Van Eck, N. Bissada, F. Zimetti, H.L. Collins, R.B. Hildebrand, A. Hayden, L.R. Brunham, M.H. Kang, J.C. Fruchart, T.J. Van Berkel, J.S. Parks, B. Staels, G.H. Rothblat, C. Fievet, M.R. Hayden, Both hepatic and extrahepatic ABCA1 have discrete and essential functions in the maintenance of plasma high-density lipoprotein cholesterol levels in vivo, Circulation 114 (2006) 1301–1309.
[36] M.A. Kennedy, A. Venkateswaran, P.T. Tarr, I. Xenarios, J. Kudoh, N. Shimizu, P.A. Edwards, Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein, J. Biol. Chem. 276 (2001) 39438–39447.
[37] K.E. Berge, H. Tian, G.A. Graf, L. Yu, N.V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, H.H. Hobbs, Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters, Science 290 (2000) 1771–1775.
[38] J.J. Repa, K.E. Berge, C. Pomajzl, J.A. Richardson, H. Hobbs, D.J. Mangelsdorf, Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors alpha and beta, J. Biol. Chem. 277 (2002) 18793–18800.
[39] M.S. Brown, J.L. Goldstein, A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 11041–11048.
[40] J. Sakai, E.A. Duncan, R.B. Rawson, X. Hua, M.S. Brown, J.L. Goldstein, Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment, Cell 85 (1996) 1037–1046.
[41] X. Hua, J. Sakai, M.S. Brown, J.L. Goldstein, Regulated cleavage of sterol regulatory element binding proteins requires sequences on both sides of the endoplasmic reticulum membrane, J. Biol. Chem. 271 (1996) 10379–10384.
[42] J. Yang, R. Sato, J.L. Goldstein, M.S. Brown, Sterol-resistant transcription in CHO cells caused by gene rearrangement that truncates SREBP-2, Genes Dev. 8 (1994) 1910–1919.
[43] J.D. Horton, N.A. Shah, J.A. Warrington, N.N. Anderson, S.W. Park, M.S. Brown, J.L. Goldstein, Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 12027–12032.
[44] A. Radhakrishnan, L.P. Sun, H.J. Kwon, M.S. Brown, J.L. Goldstein, Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain, Mol. Cell 15 (2004) 259–268.
[45] C.M. Adams, J.L. Goldstein, M.S. Brown, Cholesterol-induced conformational change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 10647–10652.
[46] A.J. Brown, L. Sun, J.D. Feramisco, M.S. Brown, J.L. Goldstein, Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism, Mol. Cell 10 (2002) 237–245.
[47] J.D. Feramisco, A. Radhakrishnan, Y. Ikeda, J. Reitz, M.S. Brown, J.L. Goldstein, Intramembrane aspartic acid in SCAP protein governs cholesterol-induced conformational change, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 3242–3247.
[48] S. Lee, D.K. Lee, E. Choi, J.W. Lee, Identification of a functional vitamin D response element in the murine Insig-2 promoter and its potential role in the differentiation of 3T3-L1 preadipocytes, Mol. Endocrinol. 19 (2005) 399–408.
[49] Y.C. Li, A.E. Pirro, M. Amling, G. Delling, R. Baron, R. Bronson, M.B. Demay, Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia, Proc. Natl. Acad. Sci. U.S.A. 94 (1997) 9831–9835.
[50] S.J. Van Cromphaut, M. Dewerchin, J.G. Hoenderop, I. Stockmans, E. Van Herck, S. Kato, R.J. Bindels, D. Collen, P. Carmeliet, R. Bouillon, G. Carmeliet, Duodenal calcium absorption in vitamin D receptor-knockout mice: functional and molecular aspects, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 13324–13329.
[51] R.G. Erben, D.W. Soegiarto, K. Weber, U. Zeitz, M. Lieberherr, R. Gniadecki, G. Moller, J. Adamski, R. Balling, Deletion of deoxyribonucleic acid binding domain of the vitamin D receptor abrogates genomic and nongenomic functions of vitamin D, Mol. Endocrinol. 16 (2002) 1524–1537.
[52] Y.C. Li, M. Amling, A.E. Pirro, M. Priemel, J. Meuse, R. Baron, G. Delling, M.B. Demay, Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice, Endocrinology 139 (1998) 4391–4396.
[53] M. Amling, M. Priemel, T. Holzmann, K. Chapin, J.M. Rueger, R. Baron, M.B. Demay, Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses, Endocrinology 140 (1999) 4982–4987.
[54] S. Okamura, Y. Sawada, T. Satoh, H. Sakamoto, Y. Saito, H. Sumino, T. Takizawa, T. Kogure, C. Chaichantipyuth, Y. Higuchi, T. Ishikawa, T. Sakamaki, Pueraria mirifica phytoestrogens improve dyslipidemia in postmenopausal women probably by activating estrogen receptor subtypes, Tohoku J. Exp. Med. 216 (2008) 341–351.
[55] B.H. Lee, H.H. Lee, J.H. Kim, B.R. Cho, Y.S. Choi, Effects of a soluble fraction of soybean on lipid profiles in ovariectomized rats fed a cholesterolemic diet, J. Med. Food 10 (2007) 521–525.
[56] H.Y. Oh, S. Lim, J.M. Lee, D.Y. Kim, E.S. Ann, S. Yoon, A combination of soy isoflavone supplementation and exercise improves lipid profiles and protects antioxidant defense-systems against exercise-induced oxidative stress in ovariectomized rats, Biofactors 29 (2007) 175–185.
[57] Y. Kamei, M. Suzuki, H. Miyazaki, N. Tsuboyama-Kasaoka, J. Wu, Y. Ishimi, O. Ezaki, Ovariectomy in mice decreases lipid metabolism-related gene expression in adipose tissue and skeletal muscle with increased body fat, J. Nutr. Sci. Vitaminol. (Tokyo) 51 (2005) 110–117.
[58] A. Zittermann, Vitamin D in preventive medicine: are we ignoring the evidence? Br. J. Nutr. 89 (2003) 552–572.