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Liver X receptor agonist downregulates hepatic apoM expression in vivo and in vitro

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ABSTRACT

It has been demonstrated that apolipoprotein M (apoM), a recently discovered HDL apolipoprotein, has antiatherosclerotic properties, which may be mediated by the enhancement of reversed cholesterol transportation and/or hepatic cholesterol catabolism. The detailed mechanisms are unknown yet. Liver X receptor (LXR) belongs to the nuclear receptor superfamily and is a ligand-activated transcription factor involved in the regulation of lipid metabolism and inflammation. Activation of LXR in the cell cultures results in an enhancement of cholesterol efflux to apoAI. In the present study, we investigated effects of the LXR agonist, T0901317 on hepatic apoM expression in vivo and in vitro. Serum apoM levels in mice given T0901317 at 10 mg or 100 mg/kg for 7 days were reduced by 12–17% ($P < 0.05$). In HepG2 cell cultures, apoM mRNA levels were significantly lower in presence of 25 μM T0901317 (37.1%) than in control cells ($P < 0.001$). A similar reduction was found by the addition of 9-*cis* retinoic acid (RA). Twenty-five micromolar T0901317 together with 100 nM RA decreased apoM mRNA expression by 65% ($P < 0.001$). Thus, the LXR agonist T0901317 significantly downregulates apoM mRNA expression in vivo and in vitro, which indicates that apoM is another novel target gene regulated by the LXR. The combination of RA and T0901317 showed additive effects, which suggests that apoM expression can be modulated by LXR/RXR pathway.

Apolipoprotein (apo) M, a novel high-density lipoprotein (HDL) apolipoprotein, was initially isolated from postprandial triglyceride-rich lipoproteins (TGRLP) and cloned by Xu and Dahlback [1]. ApoM is predominantly confined to HDL in plasma, and in minor proportions confined to TGRLP and low-density lipoprotein (LDL) [1,2]. Hepatocyte nuclear factor-1α (HNF-1α) is an activator of apoM gene promoter [3]. Deficiency of HNF-1α mouse shows lack of apoM expression [3]. ApoM mRNA levels could be regulated by platelet activating factor (PAF), leptin, transforming growth factor-beta (TGF-β), epidermal growth factor (EGF), hepatic growth factor (HGF) and hyperglycemia in vivo and/or in vitro [4–8]. In transgenic mouse models, apoM has a strong protective effect against atherosclerosis [9]. However, the physiological and patho-physiological roles of apoM in man remain to be clarified. Evidences indicate that apoM may be involved in the inflammatory activities in vivo and the potential immuno- and inflamm-reactive property of apoM may contribute to the anti-inflammatory function of HDL, as generally acknowledged as a significant antiatherogenesis mechanism [10,11].

Liver X receptor (LXR) was initially identified as orphan member of the nuclear receptor superfamily that forms obligate heterodimers with retinoid X receptor (RXR) [12]. LXR–RXR can be activated by the endogenous oxysterols and by synthetic agonists such as T0901317 [13]. Two subtypes of LXR have been identified: LXRα (LXRA, i.e. NR1H3: nuclear receptor subfamily 1, group H, member 3, RLD-1) and LXRβ (LXRB, i.e. NR1H2, OR-1). LXRα contains a DNA-binding domain and a ligand-binding domain; the amino acid sequences of these domains are 77% identical to those of LXRβ. LXRα occurs in liver, spleen, kidney, adipose, and small intestine, whereas LXRβ is ubiquitously expressed. Activated LXR must form LXR/RXR heterodimers with RXR to regulate lipid metabolism. LXRα/β are believed to regulate cholesterol and lipid homeostasis, and LXR is also a regulator of glucose metabolism. It has been suggested that LXR agonists possess anti-inflammatory properties [14]. LXR has been established to regulate intracellular cholesterol levels by transactivating the expression of cholesterol 7α-hydroxylase (CYP7A), cholesterol ester transfer protein (CETP), and ATP-binding cassette transporter A1 (ABCA1), which modulates cholesterol efflux and mediates reverse cholesterol transport from peripheral tissues. The LXR agonist, T0901317, could elevate HDL cholesterol and phospholipid in C57BL-6J mice and generates enlarged HDL particles that are enriched in cholesterol, apoAI,

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apoE, and phospholipid [15,16]. Previous studies have shown that LXR upregulated apoAI, apoAIV, apoB, apoCI, apoCIV, apoD, and apoE, while apoAV was downregulated [17–19]. In this study, we examined if apoM expression is also regulated by the LXR–RXR pathway.

Materials and methods

Cells, animals, and reagents. The human hepatoblastoma cell line, HepG2, was from American Type Culture Collection (ATCC, Manassas, VA). Male C57BL-6J mice were purchased from National Resource Center for Mutant Mice (Nanjing University, China). T0901317 was purchased from Cayman Chemical Company (Ann Arbor, MI). 9-*Cis* RA was from Sigma–Aldrich (St. Louis, USA). ABI PRISM 7700 sequence detection system, real-time RT-PCR reagents and control probe GAPDH were purchased from the Applied Biosystems Inc. (Foster City, CA, USA). Rabbit anti-mouse apoM, primary antibody, was from Abnova Corporation, Taiwan. Alkaline phosphatase (AP) conjugated Affinipure goat anti-rabbit IgG, secondary antibody, was from Jackson Immunoresearch Laboratories, Inc., USA. BCIP/NBT Color Development Substrate was from Sino-American Biotechnology Company (Luoyang, China). Quantity One Software v4.6.2 was from Bio-Rad. Cell culture flasks (25 cm^2) and 6-well cell culture clusters were from Costar, USA.

Procedure of animal experiments. Twenty-four male C57BL-6J mice weighing 20–22 g, 6-weeks old, were used in the study. Mice were acclimatized one week prior to the experiment and were housed in standard cages at 22 °C and 40–60% relative humidity with a 12-h light/dark cycle, maintained on standard chow and water ad libitum. The mice were randomly assigned to and housed in four different cages containing sawdust bedding (six mice per cage and group). After 12-h fasting, blood samples (about 0.5 ml per mouse) were collected from the orbital venous plexus under anesthesia. The blood samples were placed under 37 °C for 60 min to clot and then centrifuged for 10 min at 2000 rpm, 0.2 ml serum was collected from each mouse and stored at −80 °C until further experiments. The mice were quarantined for one week after the operations. Saline (0.9%), DMSO alone, low-dose (10 mg/kg/day) or high-dose (100 mg/kg/day) T0901317 was then administrated by the gavage tube once daily for one week. T0901317 was first dissolved in DMSO (50 mg/ml) and then diluted by 0.9% saline prior to administration. The total liquor volume for one mouse per day is 0.3 ml. The amounts of DMSO were same in the DMSO control group and low- or high-dose T0901317 group. After fasting for 12 h on day 8, the blood samples were obtained from the right orbital plexus under anesthesia and the mice were sacrificed. Samples were centrifuged and serum stored at −80 °C.

Cell cultures. HepG2 cells were grown in RPMI 1640 with 10% fetal calf serum (FCS) in the presence of benzylpenicillin (100 U/ml) and streptomycin (100 μg/ml) under standard culture conditions (5% CO_2, 37 °C). Cells were seeded in 25-cm^2 cell culture flasks or in 6-well cell culture clusters, and grown to 50–70% confluence. Prior to experiments, cells were washed twice with phosphate buffered saline (PBS) and once with serum-free RPMI 1640 without antibiotics. Then the experimental medium, containing RPMI 1640 with 0.5% human serum albumin (HSA) and different concentrations of T0901317 (1 μM and 25 μM), RA (1 nM and 100 nM) and T0901317 together with RA were added.

Isolation of total RNA and real-time RT-PCR. Total RNA of HepG2 cells was isolated by the guanidinium thiocyanate method [20]. Primer Express software (Applied Biosystems) was used to design human apoB and apoM primers and probes based on the TaqMan assay. In order to avoid amplifying the DNA template, the apoM specific primers that are spanned an 81-bp intron was designed to amplify a 66-bp product. The primers were 5′-tgccccggaaatggatcta and 5′-cagggcggccttcagtt, and the probe was 5′-FAM-cacctgactgaagggagcacagatctca-TAMRA. Relative standard curves for apoM and GAPDH were performed to compensate for the efficiency of PCR. A serial dilution of human apoM cDNA was used to generate a standard curve by plotting the cycle threshold versus the log of input cDNA. The apoB, apoM, and GAPDH standards were linear with the input of cDNA. Quantification of apoB mRNA levels or apoM mRNA levels is relative to GAPDH mRNA levels, and was performed on an ABI PRISM 7700 Sequence Detector. The real-time RT-PCR was performed in two steps in a 25 μl reaction mixture containing 1 μl TaqMan Universal PCR Master Mix, 22.5 pmol of both forward and reverse primers, 5 pmol probe and 50 ng of the total RNA templates. Thermal cycling conditions included the following steps: 25 °C 10 min, 48 °C 30 min, and 95 °C 5 min to do reverse transcription, and then the reaction mixture was preheated for 2 min at 50 °C and for 10 min at 95 °C to activate Taq polymerase. After that, a 40-cycle two-step PCR was performed consisting of 15s at 95 °C and 1 min at 60 °C. All experiments were performed at least three times in triplicate.

Determination of serum apoM concentration. Serum apoM levels were semi-quantitatively examined by a dot-blot analysis with a specific rabbit anti-mouse apoM antibody. Two-microliter serum samples were applied to the Hybond-C membrane in triplicate. All samples were applied to the same membrane. The membrane was quenched in Tris–HCl buffer in presence of 4% Tween and 3% BSA for 3 h, and sequentially incubated with primary antibody (1:1000 dilution in Tris–HCl buffer) overnight at 4 °C. After washing by Tris–HCl buffer three times the membrane was then incubated with AP conjugated secondary antibody for 2 h at room temperature. The development of AP activity was performed with a commercial visualization system according to the manufacturer's instructions. The relative amount of apoM were analyzed with Quantity One Software, and presented as volume (intensity/mm^2).

Statistical analyses. Statistical analysis was performed with Graphpad Prism 5.0 software (GraphPad Software Inc.). Results are expressed as means ± SE. Multiple comparisons were performed with one-way ANOVA/Turkey, and comparisons between after and before T0901317 treatment of each group were statistically evaluated by the paired *t*-test. Significance was established at a P value less than 0.05.

Results

Administration of T0901317 decreases serum apoM levels in C57BL-6J mice

As shown in Fig. 1, mice given T0901317 at 100 mg/kg/day for 7 days had a significant lower serum apoM levels than mice receiving the lower dose T0901317 or controls. As T0901317 must be dissolved in the solvent DMSO we used DMSO as control too. It is indicated that administration of DMSO could also decrease serum apoM levels (Fig. 1), however there is no statistic significance between saline group and DMSO group.

Effects of T0901317 and RA on apoB and apoM mRNA expression in HepG2 cells

We also investigated T0901319 and RA on apoM and apoB expression in HepG2 cells. The higher dose T0901317 significantly downregulated apoM mRNA levels in HepG2 cells, whereas it did not influence apoB mRNA levels (Fig. 2A). There were no obvious effects on mRNA levels of apoM by 1 μM T0901317. At 25 μM T0901317, apoM mRNA levels were decreased by 37.1% compared to the control ($P < 0.001$). RA could also significantly inhibit apoM expression in HpeG2 cells in a dose- and time-dependent manner (Figs. 2B and 3). At high concentration of RA, apoB mRNA levels were moderately decreased (Fig. 2B). As shown in Fig. 4 , the combination of T0901317 at 25 μM and RA at 100 nM resulted in a further decrease of apoM mRNA levels, indicating that the two agents have additive effects (Fig. 4).

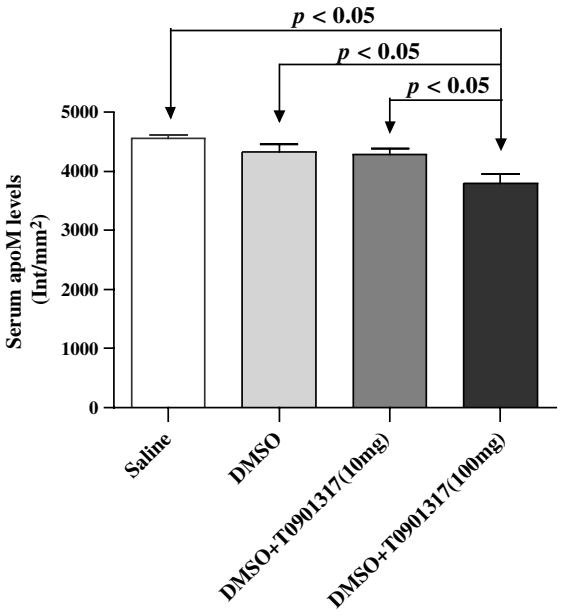

Fig. 1. Serum apoM levels in C57BL mice treated by T0901317. ApoM concentrations were determined by dot-blotting analyses as described in Materials and methods. Data are expressed as the intensity/mm^2 that was analyzed by the software of Quantity One. Data are means ± SE (n = 6 for each sample group).

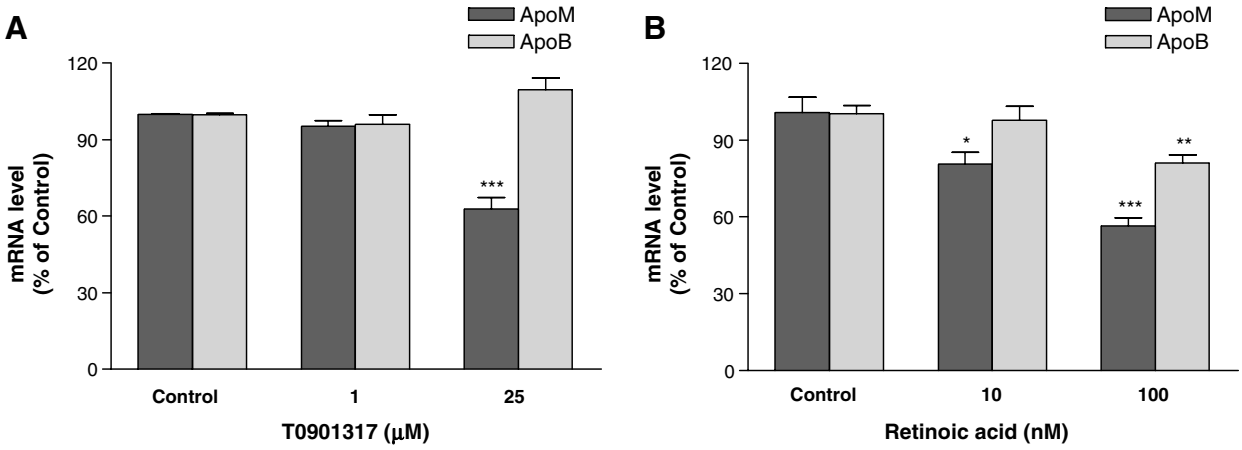

Fig. 2. Effects of T0901317 and retinoic acid (RA) on mRNA levels of apoB and apoM in HepG2 cells. HepG2 cells were grown in the culture medium with different concentrations of T0901317 (A) or RA (B) for 24 h. ApoB and apoM mRNA levels were determined by real-time RT-PCR. Each experimental group contains six replicates and real-time RT-PCR was run in triplicates. The control group is represented as 100%. Data are means ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group.

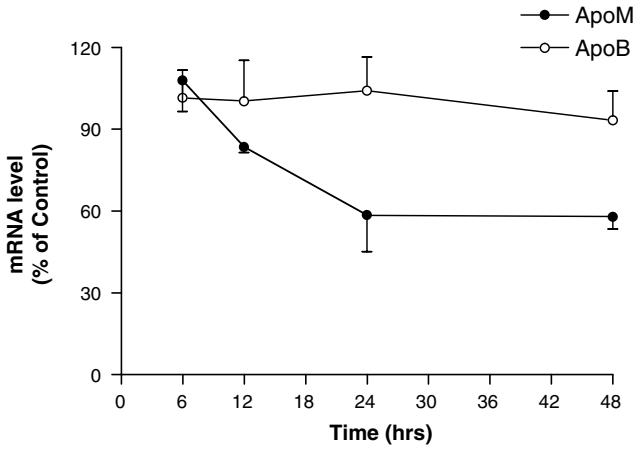

Fig. 3. Time curves of RA on apoM and apoB expression. HepG2 cells were grown in culture medium with 100 nM RA. ApoM and apoB mRNA levels were determined by real-time RT-PCR. Each experimental group contains three replicates and real-time RT-PCR was run triplicates. The control group is represented as 100%. Data are means ± SE.

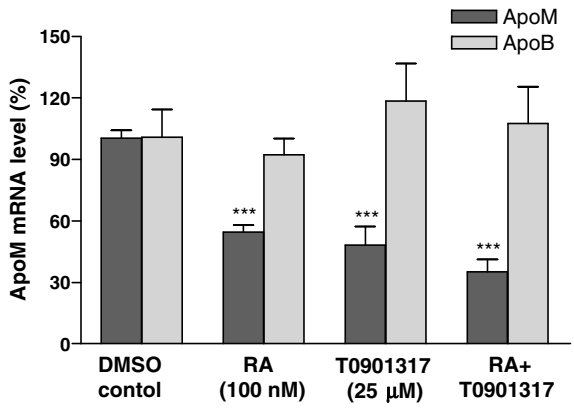

Fig. 4. Additive effect of RA on T0901317 mediated apoM inhibition. HepG2 cells were cultured with RA (100 nM) or T0901317 (25 μM) alone, or cultured with RA and T0901317 together for 24 h. DMSO was used as in the control group. ApoM or apoB mRNA levels were determined by real-time RT-PCR. Each experimental group contains six replicates and real-time RT-PCR was run in triplicates. Data are means ± SE. The control group is represented as 100%. $^{***}P < 0.001$ vs. control group.

Discussion

ApoM is a recently discovered human apolipoprotein predominantly present in HDL, and the physiological and patho-physiological roles of apoM in man remain to be clarified [1]. LXR plays an important role in the transcriptional regulation of lipid homeostasis and inflammation [12,21]. LXR is an intracellular sterol sensor that regulates expression of genes controlling cholesterol absorption, excretion, catabolism, and cellular efflux and has been found to play a central role in maintaining cellular cholesterol homeostasis [22]. LXR forms heterodimers with RXR, which bind to characteristic DNA sequences termed LXR-response elements (LXRE) located in the 5′-flanking region of target genes. Target genes of LXR include ABCA1, ABCG5, ABCG8, apoE, CETP, lipoprotein lipase (LPL), fatty acid synthase (FAS), and sterol-response element-binding protein 1c (SREBP-1c), suggesting that LXR is a key regulator in lipid and cholesterol metabolism [23–27]. T0901317 is a potent and selective agonist for both LXRα and LXRβ and elevates HDL cholesterol in C57BL-6J mice [28]. It has been reported that the apoAV gene is regulated by LXR, RXR, peroxisome proliferator-activated receptor-α (PPARα) and farnesoid X receptor (FXR) ligands [29]. Previous studies have shown that LXR regulates several apolipoproteins including apoA and apoE. Thus it is interesting to study whether and how LXR regulates apoM.

In the present study, we demonstrated that the LXR agonist T0901317 decreased apoM in vivo and in vitro and in combination of RA had an additive effect. Our data demonstrated that high-dose T0901317 significantly downregulated apoM levels in C57BL-6J mice and in HepG2 cells, while there were no obvious effects with low-dose T0901317. Our studies also indicate that RA reduced apoM expression in HepG2 cells in a dose- and time-dependent manner. These data suggest that apoM might be a novel LXR target gene.

Treatment of HepG2 cell lines with the LXR ligand T0901317, which elevates SREBP-1c mRNA and protein levels, results in downregulation of apoAV mRNA. There are two putative E-box elements that were able to bind specifically SREBP-1c on the human apoAV promoter sequence [30]. On activation, LXR builds heterodimers with another nuclear receptor, RXR, and bind to specific DR4-type LXR-response elements (LXREs) in the promoter region of their target genes, thus regulating their expressions. If apoM is a direct target gene of LXR, there is LXRE on apoM gene too. Also, it is probable that LXR regulates apoM expression indirectly through LXR target genes such as ABCA1, ABCG1, ABCG5, ABCG8,

LPL, CETP, phospholipid transfer protein (PLTP), and so on. Of course, direct and indirect pathways may coexist. For example, there are both direct and indirect mechanisms for the regulation of fatty acid synthase gene by LXR.

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References

[1] N. Xu, B. Dahlback, A novel human apolipoprotein (apoM), J. Biol. Chem. 274 (1999) 31286–31290.
[2] G. Luo, X. Zhang, P. Nilsson-Ehle, N. Xu, Apolipoprotein M, Lipids Health Dis. 3 (2004) 21.
[3] S. Richter, D.Q. Shih, E.R. Pearson, C. Wolfrum, S.S. Fajans, A.T. Hattersley, M. Stoffel, Regulation of apolipoprotein M gene expression by MODY3 gene hepatocyte nuclear factor-1alpha: haploinsufficiency is associated with reduced serum apolipoprotein M levels , Diabetes 52 (2003) 2989–2995.
[4] N. Xu, X.Y. Zhang, X. Dong, U. Ekstrom, Q. Ye, P. Nilsson-Ehle, Effects of platelet-activating factor, tumor necrosis factor, and interleukin-1alpha on the expression of apolipoprotein M in HepG2 cells , Biochem. Biophys. Res. Commun. 292 (2002) 944–950.
[5] G. Luo, M. Hurtig, X. Zhang, P. Nilsson-Ehle, N. Xu, Leptin inhibits apolipoprotein M transcription and secretion in human hepatoma cell line, HepG2 cells, Biochim. Biophys. Acta 1734 (2005) 198–202.
[6] N. Xu, M. Hurtig, X.Y. Zhang, Q. Ye, P. Nilsson-Ehle, Transforming growth factor-beta down-regulates apolipoprotein M in HepG2 cells, Biochim. Biophys. Acta 1683 (2004) 33–37.
[7] N. Xu, P. Nilsson-Ehle, M. Hurtig, B. Ahren, Both leptin and leptin-receptor are essential for apolipoprotein M expression in vivo, Biochem. Biophys. Res. Commun. 321 (2004) 916–921.
[8] N. Xu, B. Ahren, J. Jiang, P. Nilsson-Ehle, Down-regulation of apolipoprotein M expression is mediated by phosphatidylinositol 3-kinase in HepG2 cells, Biochim. Biophys. Acta 1761 (2006) 256–260.
[9] C. Wolfrum, M.N. Poy, M. Stoffel, Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis, Nat. Med. 11 (2005) 418–422.
[10] C. Christoffersen, M. Jauhiainen, M. Moser, B. Porse, C. Ehnholm, M. Boesl, B. Dahlback, L.B. Nielsen, Effect of apolipoprotein M on high density lipoprotein metabolism and atherosclerosis in low density lipoprotein receptor knock-out mice, J. Biol. Chem. 283 (2008) 1839–1847.
[11] N. Venteclef, A. Haroniti, J.J. Tousaint, I. Talianidis, P. Delerive, Regulation of anti-atherogenic apolipoprotein M gene expression by the orphan nuclear receptor LRH-1, J. Biol. Chem. 283 (2008) 3694–3701.
[12] S.M. Ulven, K.T. Dalen, J.A. Gustafsson, H.I. Nebb, LXR is crucial in lipid metabolism, Prostaglandins Leukot. Essent. Fatty Acids 73 (2005) 59–63.
[13] K.A. Houck, K.M. Borchert, C.D. Hepler, J.S. Thomas, K.S. Bramlett, L.F. Michael, T.P. Burris, T0901317 is a dual LXR/FXR agonist, Mol. Genet. Metab. 83 (2004) 184–187.
[14] N. Zelcer, P. Tontonoz, Liver X receptors as integrators of metabolic and inflammatory signaling, J. Clin. Investig. 116 (2006) 607–614.
[15] S. Fernandez-Veledo, I. Nieto-Vazquez, C.M. Rondinone, M. Lorenzo, Liver X receptor agonists ameliorate TNFalpha-induced insulin resistance in murine brown adipocytes by downregulating protein tyrosine phosphatase-1B gene expression, Diabetologia 49 (2006) 3038–3048.
[16] M. Nilsson, T.M. Stulnig, C.Y. Lin, A.L. Yeo, P. Nowotny, E.T. Liu, K.R. Steffensen, Liver X receptors regulate adrenal steroidogenesis and hypothalamic–pituitary–adrenal feedback, Mol. Endocrinol. 21 (2007) 126–137.
[17] Y. Liang, X.C. Jiang, R. Liu, G. Liang, T.P. Beyer, H. Gao, T.P. Ryan, S. Dan Li, P.I. Eacho, G. Cao, Liver X receptors (LXRs) regulate apolipoprotein AIV-implications of the antiatherosclerotic effect of LXR agonists, Mol. Endocrinol. 18 (2004) 2000–2010.
[18] S. Hummasti, B.A. Laffitte, M.A. Watson, C. Galardi, L.C. Chao, L. Ramamurthy, J.T. Moore, P. Tontonoz, Liver X receptors are regulators of adipocyte gene expression but not differentiation: identification of apoD as a direct target, J. Lipid Res. 45 (2004) 616–625.
[19] P.A. Mak, B.A. Laffitte, C. Desrumaux, S.B. Joseph, L.K. Curtiss, D.J. Mangelsdorf, P. Tontonoz, P.A. Edwards, Regulated expression of the apolipoprotein E/C-I/C-IV/C-II gene cluster in murine and human macrophages. A critical role for nuclear liver X receptors alpha and beta, J. Biol. Chem. 277 (2002) 31900–31908.
[20] P. Chomczynski, N. Sacchi, Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction, Anal. Biochem. 162 (1987) 156–159.
[21] A.F. Valledor, The innate immune response under the control of the LXR pathway, Immunobiology 210 (2005) 127–132.
[22] K.R. Steffensen, J.A. Gustafsson, Putative metabolic effects of the liver X receptor (LXR), Diabetes 53 (Suppl. 1) (2004) S36–S42.
[23] A. Chawla, W.A. Boisvert, C.H. Lee, B.A. Laffitte, Y. Barak, S.B. Joseph, D. Liao, L. Nagy, P.A. Edwards, L.K. Curtiss, R.M. Evans, P. Tontonoz, A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis, Mol. Cell 7 (2001) 161–171.
[24] K. Schwartz, R.M. Lawn, D.P. Wade, ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR, Biochem. Biophys. Res. Commun. 274 (2000) 794–802.
[25] 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.
[26] M. Sato, Y. Kawata, K. Erami, I. Ikeda, K. Imaizumi, LXR agonist increases the lymph HDL transport in rats by promoting reciprocally intestinal ABCA1 and apo A-I mRNA levels , Lipids 43 (2008) 125–131.
[27] W. Khovidhunkit, A.H. Moser, J.K. Shigenaga, C. Grunfeld, K.R. Feingold, Endotoxin down-regulates ABCG5 and ABCG8 in mouse liver and ABCA1 and ABCG1 in J774 murine macrophages: differential role of LXR, J. Lipid Res. 44 (2003) 1728–1736.
[28] T. Plosch, T. Kok, V.W. Bloks, M.J. Smit, R. Havinga, G. Chimini, A.K. Groen, F. Kuipers, Increased hepatobiliary and fecal cholesterol excretion upon activation of the liver X receptor is independent of ABCA1, J. Biol. Chem. 277 (2002) 33870–33877.
[29] H. Jakel, M. Nowak, A. Helleboid-Chapman, J. Fruchart-Najib, J.C. Fruchart, Is apolipoprotein A5 a novel regulator of triglyceride-rich lipoproteins?, Ann Med. 38 (2006) 2–10.
[30] H. Jakel, M. Nowak, E. Moitrot, H. Dehondt, D.W. Hum, L.A. Pennacchio, J. Fruchart-Najib, J.C. Fruchart, The liver X receptor ligand T0901317 down-regulates APOA5 gene expression through activation of SREBP-1c, J. Biol. Chem. 279 (2004) 45462–45469.