

# Niacin regulates apolipoprotein M expression via liver X receptor- $\alpha$

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**Abstract.** Niacin is currently the most effective drug that increases HDL-C levels. Apolipoprotein M (ApoM) in humans is mainly found in plasma high-density lipoprotein (HDL). Little is known about the role played by niacin in ApoM expression. In this study, the effects of niacin on ApoM expression were assessed as well as the associated mechanism. Human liver cancer cell line HepG2 was treated with niacin alone or with liver X receptor- $\alpha$  (LXR $\alpha$ ) inhibitor at multiple concentrations. The mRNA and protein expression of ApoM were assessed by qRT-PCR and western blotting. Specific LXR $\alpha$  shRNA was transfected into HepG2 cells to further evaluate the regulatory effects of LXR $\alpha$  on ApoM. An *in vivo* model was also established to investigate the LXR $\alpha$  inhibitor on the mouse ApoM levels. The comparisons among groups were evaluated using one-way ANOVA and Student-Newman-Keuls test. It was revealed that in HepG2 cells, niacin dose-dependently increased ApoM gene and protein expression levels. Niacin-induced upregulation of ApoM was attenuated by an LXR $\alpha$  inhibitor or LXR $\alpha$  shRNA, indicating that LXR $\alpha$  mediated this effect. Moreover, niacin treatment resulted in increased LXR $\alpha$  mRNA levels, *in vivo* and *in vitro*; niacin treatment resulted in increased ApoM gene and protein expression levels in mice. In conclusion, niacin upregulates ApoM expression by increasing LXR $\alpha$  expression *in vivo* and *in vitro*.

## Introduction

High-density lipoprotein cholesterol (HDL-C) levels are negatively correlated with coronary heart disease (CHD)

occurrence (1,2). HDL-C exerts anti-atherosclerotic effects via its critical function in reverse cholesterol transport (RCT) (3). Apolipoprotein M (ApoM), a novel apolipoprotein associated with HDL-C, has a critical function in HDL-C metabolism (4,5). ApoM is predominantly found in hepatocytes and renal tubular epithelial cells (6). Reducing hepatic ApoM expression by siRNA decreases HDL-C amounts, increases HDL levels, and suppresses pre- $\beta$  HDL (7). In addition, ApoM-deficient HDL exhibited reduced efficacy compared with normal HDL in promoting cholesterol export from macrophages (7). It is known that ApoM impacts RCT essentially by regulating pre- $\beta$ -HDL synthesis. ApoM is therefore crucial for HDL-C biosynthesis and RCT.

Hepatic ApoM expression is controlled by transcription factors regulating critical steps in liver fat and glucose metabolism. Liver X receptor- $\alpha$  (LXR $\alpha$ ) belongs to nuclear receptors which respond to elevated levels of intracellular cholesterol (8). LXR $\alpha$  is known to enhance transcription of genes that control cholesterol efflux and fatty acid biosynthesis (8). Recent studies have revealed new functions of LXR $\alpha$  as an essential nuclear receptor that regulates ApoM expression (8), suggesting that targeting the LXR $\alpha$ -mediated ApoM expression may be applied for anti-atherosclerosis therapy.

Niacin is widely used clinically as an antihyperlipidemic drug. It increases HDL-C amounts and improves RCT; however, the exact underlying mechanism is largely unknown. The aim of this study was to investigate whether niacin increases ApoM via LXR $\alpha$  in *in vitro* and *in vivo* models. The present research potentially contributes to further investigating the molecular mechanisms of niacin increasing HDL and development of therapeutic strategies against atherosclerosis disease.

## Materials and methods

**Cell lines, mice and reagents.** Eight-week-old male C57BL/6N mice were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences. Human liver cancer cell line HepG2 was obtained from the Cell Center of Xiangya School of Medicine, Central South University.

The following reagents and kits were used in the present study: Quantitative PCR kit (cat. no. A6101; Promega

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Corporation), anti-ApoM antibody (cat. no. ab66379; Abcam), secondary antibody goat anti-mouse horseradish peroxidase (HRP)-conjugated IgG (cat. no. A0216, Beyotime Institute of Biotechnology), niacin (cat. no. 1461003, Sigma-Aldrich; KGaA), DMEM (cat. no. 10569044; Gibco; Thermo Fisher Scientific, Inc.), TRIzol reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.), cell protein extraction kit (cat. no. P0027, Beyotime Institute of Biotechnology); LXR $\alpha$  inhibitor (ECHS; Wako Pure Chemicals Inc.). ApoM and LXR $\alpha$  specific primers were obtained from AuGCT Biotechnology.

**Cell culture and treatment.** DMEM supplemented with 10% fetal bovine serum (FBS) and an antibiotic cocktail (1.0x10<sup>5</sup> U/l penicillin and 1.0x10<sup>5</sup> U/l streptomycin) was employed for liver cancer cell line HepG2 culture in a humid environment containing 5% CO<sub>2</sub> at 37°C. The cells were passaged every other day.

Liver cancer cell line HepG2 cells were treated with niacin at concentrations of 0, 0.25, 0.5, 1.0 and 2.0 mmol/l for 24 h. For LXR $\alpha$  inhibition assays, cells were treated with niacin (0.5 mmol/l) and ECHS (LXR $\alpha$  inhibitor; 100  $\mu$ mol/l) for 24 h. ApoM and LXR $\alpha$  mRNA levels were determined with specific primers.

**Animal experiments.** A total of 80 mice were used in this experiment. Eight-week-old male C57BL/6N mice were housed in single cages under a 12-h light/dark cycle at 24–28°C, with 60–75% relative humidity. The animals were allowed to adapt for one week before treatments. After 12 h of fasting, fasting lipid levels (week 0) were determined, and used as a baseline value for the experimental animals. A normal diet (15 kJ/g feed; proteins, carbohydrates, and fats contributed to 23, 65, and 12% of total energy, respectively) was provided by Xiangya Hospital, Central South University Animal Laboratory. The animals were randomized into control and niacin (1% w/w niacin, and 0.5 g niacin added in every 100-g feed, for 12 weeks) groups. The average intake of each mouse was 4.2–4.5 g/day. At 12 weeks later, fasting blood lipid levels were determined. Liver tissues were harvested at weeks 0, 3, 6, 9 and 12 (n=8/group at each time-point) after the mice were euthanized and death was confirmed when the eyes turned white. Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (2%, 40 mg/kg) and sacrificed by cardiac puncture. The Ethics Committee of the Xiangya Hospital of Central South University reviewed and approved this study.

**Fasting plasma lipids.** At time zero and 12 weeks, 0.2 ml of tail vein blood was obtained under fasting conditions. After 30 min of incubation at room temperature, serum was obtained by centrifugation at 1,000 x g for 15 min. Serum lipid levels were determined by investigators blinded to treatment regimens. Measurements included total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) amounts. Serum TC and TG amounts were assessed by standard enzymatic methods using the kits from Beijing Solarbio Science & Technology Co., Ltd. (cat. nos. BC1985 and BC0625) according to the manufacturer's instructions. LDL-C and HDL-C concentrations were evaluated by the chemical shielding method using kits from Beijing Solarbio Science &

Technology Co., Ltd. (cat. nos. BB-47437-1 and BB-47438-1) according to the manufacturer's instructions.

**Short hairpin RNA (shRNA) transfection.** The pLKO.1-GFP-LXR $\alpha$  shRNA Plasmids were purchased from Santa Cruz Biotechnology, Inc. (cat. no. sc-38829-SH). The transfected shRNA plasmid is a pool of 3 target-specific lentiviral vector plasmids. The three shRNA sequences are: Sequence #1, 5'-CCGGGATCTGGGATGTGCACGAATGCTCGAGCATTCGTGCACATCCCAGATCTTTTTTTG-3'; sequence #2, 5'-CCGGAGTTCTCCAGGGCCATGAATGCTCGAGCATTCATGGCCCTGGAGAACTTTTTTTG-3'; and sequence #3, 5'-CCGGGCAACTCAATGATGCCGAGTTCTCGAGAACTCGGCATCATTGAGTTGCTTTTTT-3'. The control pLKO.1-GFP-shRNA plasmid encodes a scrambled shRNA sequence that will not lead to the specific degradation of any cellular message. shRNA transfection was performed using the Lipofectamine<sup>®</sup> 3000 (cat. no. L3000008; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Briefly, transfection was performed in a six-well tissue culture plate, at a density of 6x10<sup>5</sup> cells with 50–70% confluency in antibiotic-free normal growth medium supplemented with FBS. Plasmid (1  $\mu$ g) was transfected into 4x10<sup>5</sup> cells/well. Subsequently, 72 h later, the cells were collected for downstream assays. The transfection efficiency was determined by RT-qPCR.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA extraction was performed from cells and mice liver tissues using TRIzol according to the manufacturer's instructions. RNA purity and amounts were assessed on a NanoDrop<sup>™</sup> spectrophotometer (Thermo Fisher Scientific, Inc.). First strand cDNA was synthesized with reverse transcriptase (AMV; Beijing Aoke Biotechnology Co., Ltd.) as directed by the manufacturer. The primers used for RT-qPCR were: ApoM forward, 5'-CTGACAACTCTGGGCGTGGA-3' and reverse, 5'-CAGAGCCAGCAGCCATATTGAA-3'; LXR $\alpha$  forward, 5'-AGAACAGATCCGCCTGAAGA-3' and reverse, 5'-AGCCTCTCCACCTGGAGCTGGT-3'. GAPDH was used as a housekeeping gene for normalization with the following primers: Forward, 5'-CCATGTTTCGTCATGGGTGTGAACCA-3' and reverse, 5'-GCCAGTAGAGGCAGGGATGATGTTTC-3'. SYBR green was employed for RT-qPCR at 50°C (2 min) and 95°C (10 min), followed by 40 cycles of amplification at 95°C (15 sec), 61°C (45 sec) and 61°C (10 sec). Data analysis was performed using the 2<sup>- $\Delta\Delta$ C<sub>q</sub></sup> method (9).

**Immunoblotting.** Cell lysates were submitted to centrifugation for 10 min at 4°C and 15,000 x g. Proteins were extracted using RIPA lysis buffer (Shanghai Biyuntian Biotechnology Co., Ltd.) from cultured cells or mouse livers. Protein amounts in the supernatant were assessed by the BCA method. Equal amounts (50  $\mu$ g) of total protein were resolved by 6% SDS-PAGE and subsequently electro-transferred onto PVDF membranes. After blocking with 5% milk for 2 h at room temperature, the membranes were probed with anti-ApoM primary antibody (1:500, molecular weight: 21 kDa), at 4°C overnight, and washed in TBST. Then, HRP-conjugated secondary antibodies (1:2,000) were added for 1 h at room temperature. Immunoreactive bands were detected using

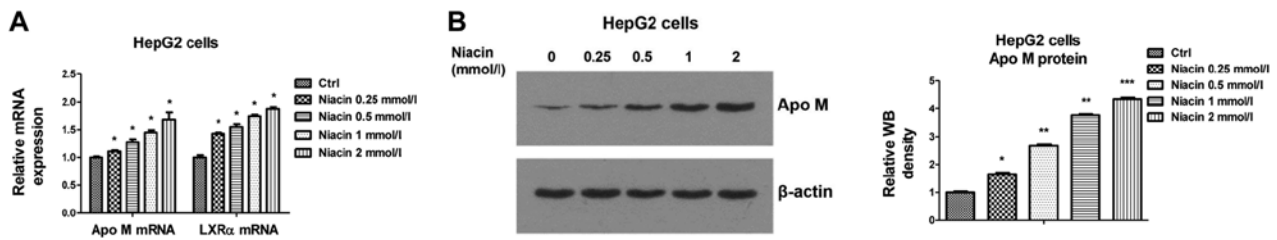


Figure 1. Niacin treatment dose-dependently affects ApoM protein expression. The effects of various niacin concentrations on (A) ApoM and LXRα mRNAs were detected by reverse transcription-quantitative PCR analysis, and (B) ApoM protein levels by western blotting. β-actin was a loading control. \* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$  vs. control group. ApoM, apolipoprotein M; LXRα, liver X receptor-α.

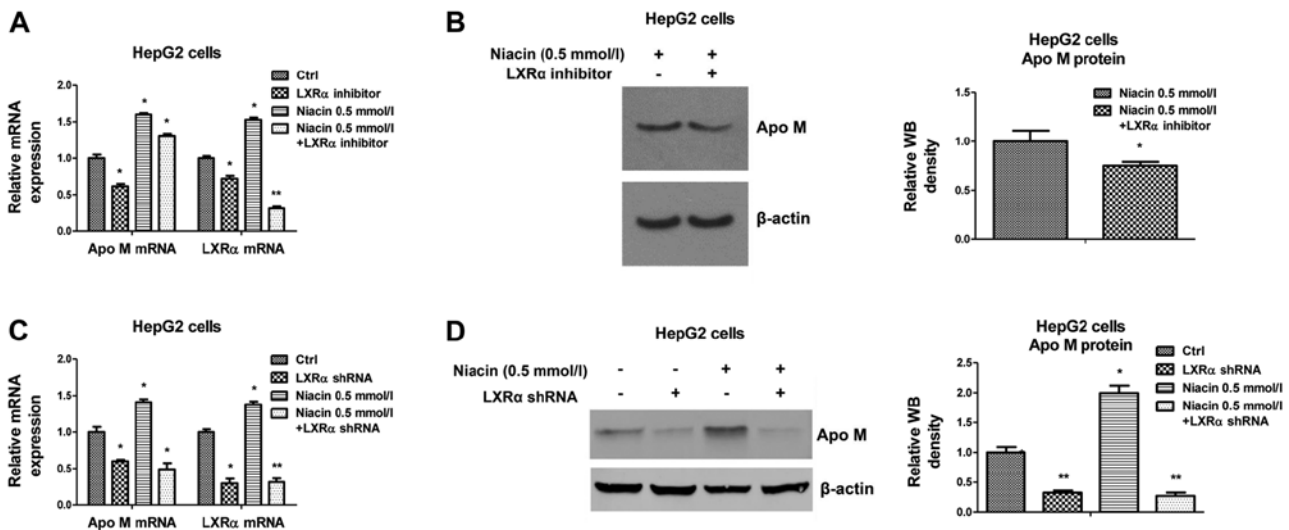


Figure 2. LXRα inhibitor ECHS inhibits niacin-associated upregulation of ApoM protein expression in HepG2 cells. (A) Control, niacin (0.5 mmol/l) alone, LXRα inhibitor alone or in combination with the LXRα inhibitor ECHS (100 μmol/l) was administered to cells for 24 h. ApoM and LXRα mRNAs were detected by RT-qPCR. (B) HepG2 cells were treated with niacin (0.5 mmol/l) alone or in combination with the LXRα inhibitor ECHS (100 μmol/l) for 24 h. ApoM protein amounts were assessed by immunoblotting. (C) HepG2 cells were treated with control plasmid, LXRα shRNA alone, niacin (0.5 mmol/l) alone, or niacin in combination with the LXRα shRNA for 24 h. mRNA expression of ApoM and LXRα was detected by RT-qPCR. (D) Cells that underwent the same treatment as those detected by RT-qPCR were subjected to western blotting analysis. β-actin was a loading control. \* $P<0.05$ ; \*\* $P<0.01$  vs. control group. ApoM, apolipoprotein M; LXRα, liver X receptor-α; RT-qPCR, reverse transcription-quantitative PCR; shRNA, short hairpin RNA.

an ECL western blotting detection kit (Pierce; Thermo Fisher Scientific, Inc.) and assessed with TINA 2.09 image processing software (<http://www.tina-vision.net/index.php>). Semi-quantitative analysis of band intensities was performed against the control group.

**Statistical analysis.** All experimental data are presented as the mean ± standard deviation, and analyzed with the software SPSS 15.0 (SPSS, Inc.). Comparisons among groups were evaluated using one-way ANOVA and Student-Newman-Keuls test. Experiments were performed in triplicate and repeated three times.  $P<0.05$  was considered to indicate a statistically significant difference.

## Results

**Niacin induces ApoM and LXRα expression in HepG2 cells.** Multiple studies have indicated that the liver cancer cell line HepG2, derived from hepatoblastoma (10), is a good model for evaluating cholesterol and lipid metabolism in the liver (11-13). Therefore, HepG2 cells were adopted to assess the effects of niacin on hepatic apolipoprotein M expression.

HepG2 cells were administered various concentrations (0.25-2.0 mmol/l) of niacin for 24 h. Notably, niacin treatment resulted in higher ApoM and LXRα mRNA amounts compared to the control (Fig. 1A). Likewise, ApoM protein expression increased with increasing niacin concentrations (Fig. 1B).

**Effects of the LXRα inhibitor ECHS on niacin-associated ApoM upregulation in HepG2 cells.** To explore the mechanism by which niacin upregulates ApoM, HepG2 cells were administered 0.5 mmol/l niacin in the presence of the LXRα inhibitor ECHS. As revealed in Fig. 2 and Table I, ECHS inhibited niacin-associated ApoM upregulation, both at the mRNA and protein levels (Fig. 2A and B). In addition, the LXRα inhibitor ECHS significantly inhibited the expression of LXRα mRNA (Fig. 2A and Table II). To assess the specificity of LXRα inhibitor, LXRα expression was knocked down by transfection of LXRα shRNA into HepG2 cells. As anticipated, knockdown of LXRα protein expression by shRNA significantly inhibited the basal level of ApoM and the niacin-stimulated ApoM (Fig. 2C and D). Collectively, these results consistently demonstrated LXRα positively regulated ApoM.

Table I. LXR $\alpha$  inhibitor, ECHS, inhibits ApoM mRNA expression induced by niacin.

Group	Niacin (0.5 mmol/l) group	Niacin (0.5 mmol/l) + LXR $\alpha$ inhibitor
ApoM mRNA	1.60 $\pm$ 0.02	1.37 $\pm$ 0.06 <sup>a</sup>

<sup>a</sup>P<0.05 vs. control group. ApoM, apolipoprotein M; LXR $\alpha$ , liver X receptor- $\alpha$ .

Table II. LXR $\alpha$  inhibitor, ECHS, inhibits LXR $\alpha$  mRNA expression.

Group	Control group	Niacin (0.5 mmol/l)	Niacin (0.5 mmol/l) + LXR $\alpha$ inhibitor
LXR $\alpha$ mRNA	1.00 $\pm$ 0.03	1.53 $\pm$ 0.03 <sup>a</sup>	0.31 $\pm$ 0.02 <sup>a,b</sup>

<sup>a</sup>P<0.05 vs. control group; <sup>b</sup>P<0.05 vs. niacin group. LXR $\alpha$ , liver X receptor- $\alpha$ .

Table III. Fasting blood lipid levels in mice after niacin treatment.

Group	TG	TC	LDL	HDL
Control group (n=10)	0.85 $\pm$ 0.02	1.47 $\pm$ 0.04	0.55 $\pm$ 0.03	0.57 $\pm$ 0.03
Niacin group (n=10)	0.73 $\pm$ 0.02 <sup>a</sup>	1.31 $\pm$ 0.03 <sup>a</sup>	0.23 $\pm$ 0.04 <sup>a</sup>	0.85 $\pm$ 0.05 <sup>a</sup>

<sup>a</sup>P<0.05 vs. control group. TG, total triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

*Fasting blood lipid levels are reduced in mice treated with niacin.* In comparison with control values, TG, TC, and LDL-C levels in niacin-treated animals were significantly decreased (P<0.05), whereas HDL-C amounts were markedly increased (P<0.05) (Table III).

During the total 12-week niacin treatment, ApoM and LXR $\alpha$  mRNA levels in the murine liver were assessed by real-time RT-PCR at weeks 0, 3, 6, 9 and 12. ApoM protein expression was determined by immunoblotting at week 12. ApoM mRNA and protein levels were significantly increased in the niacin group compared with the control group (Fig. 3A and B, Table IV). In addition, LXR $\alpha$  mRNA amounts were significantly elevated after treatment with niacin (Fig. 3C, Table V).

## Discussion

Multiple epidemiological findings indicate that HDL-C levels are negatively associated with risk of CHD (13). Elevated HDL-C and RCT induction are considered to be crucial for CHD prevention and treatment. ApoM, a novel lipid transfer protein, is a major component of HDL. Plasma HDL-C levels are increased in mice overexpressing ApoM. Conversely, ApoM gene silencing results in a 25% decrease of plasma HDL-C levels. Lack of ApoM leads to complete loss of pre- $\beta$ -HDL, which significantly reduces the amounts of cholesterol flowing from macrophages to HDL. This affects the RCT function of HDL (7); thus, ApoM plays an important role in RCT and HDL formation.

Table IV. Effect of niacin on the ApoM mRNA expression in mice liver.

Group	Control group (n=10)	Niacin group (n=10)
ApoM mRNA	1.00 $\pm$ 0.03	1.39 $\pm$ 0.04 <sup>a</sup>

<sup>a</sup>P<0.05 vs. control group. ApoM, apolipoprotein M.

Table V. Effect of niacin on the LXR $\alpha$  mRNA expression in mice liver.

Group	Control group (n=10)	Niacin group (n=10)
LXR $\alpha$ mRNA	1.00 $\pm$ 0.02	1.45 $\pm$ 0.03 <sup>a</sup>

<sup>a</sup>P<0.05 vs. control group. LXR $\alpha$ , liver X receptor- $\alpha$ .

Niacin is currently the most effective drug that increases HDL-C amounts (14), resulting in reduced coronary events (15,16). The ARBITER 2 (17) study demonstrated that combination of niacin with statins could reverse atherosclerosis in CHD patients with reduced HDL-C amounts, who mainly benefit from the inductive effect of niacin on HDL-C amounts. Niacin may increase HDL-C primarily by preventing the liver from removing ApoA1, which increases

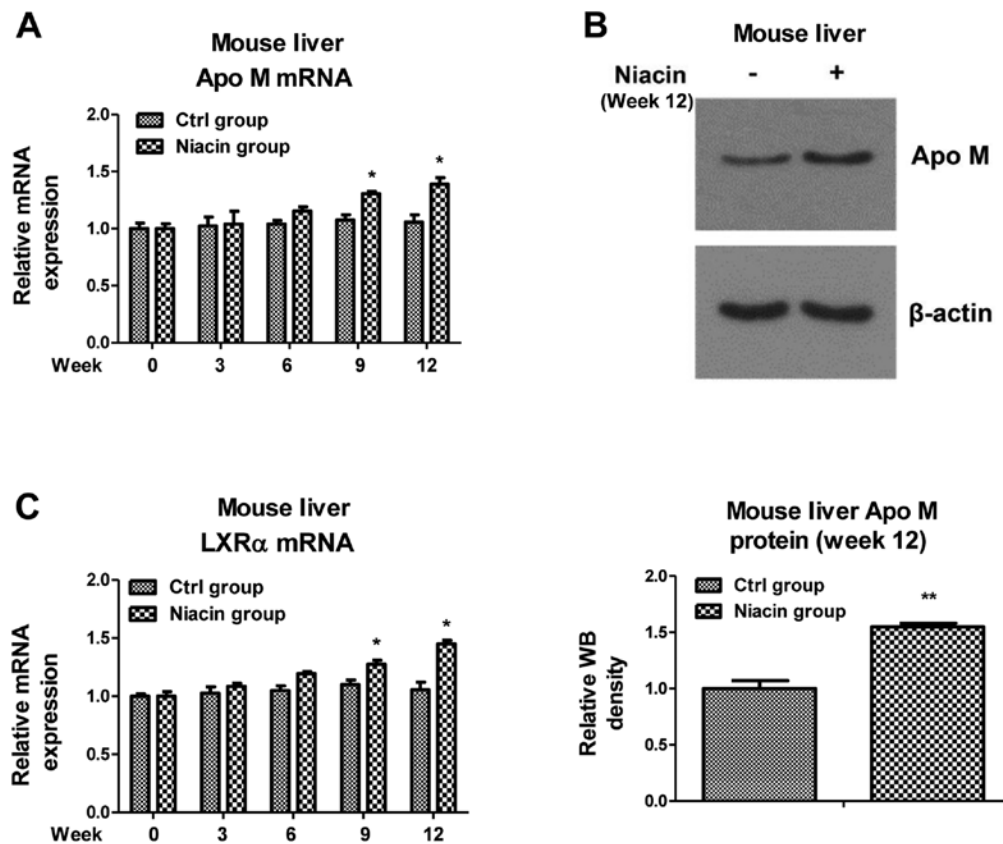


Figure 3. Effects of niacin on ApoM levels in mice. (A) ApoM mRNA was detected in the control group and the Niacin group by RT-qPCR at weeks 0, 3, 6, 9 and 12 of niacin treatments. (B) ApoM protein expression at week 12 was measured in the control group and the niacin group by western blotting. (C) The LXR $\alpha$  mRNA expressions were detected in the control group and the niacin group by RT-qPCR at weeks 0, 3, 6, 9 and 12 of niacin treatments. \* $P < 0.05$ ; \*\* $P < 0.01$  vs. control group. ApoM, apolipoprotein M; LXR $\alpha$ , liver X receptor- $\alpha$ ; RT-qPCR, reverse transcription-quantitative PCR.

ApoA1 concentrations as well as the amounts of large ApoA1 containing HDL particles (18). In addition, niacin preserves the ability to retain ApoA1 and augment RCT. Furthermore, niacin promotes cholesterol efflux from fat cells to ApoA1 by activating PPAR- $\gamma$ -LXR $\alpha$ -ABCA1 signaling (19). No reported studies have investigated whether niacin increases HDL-C and promotes cholesterol efflux by regulating ApoM.

The present study firstly demonstrated that niacin increased ApoM mRNA and protein levels *in vivo* and *in vitro*, indicating that niacin may control HDL-C levels and promote cholesterol efflux via ApoM upregulation. These findings provide novel insights into the mechanism by which niacin augments HDL-C levels, revealing that ApoM is one of the niacin targets in lipid regulation.

LXR $\alpha$  is a ligand-activated transcription factor that regulates lipid metabolism and inflammation (20,21). LXR $\alpha$  induced by a specific ligand forms a heterodimer with retinol receptor, and binds to the promoter of target genes for regulation (22). LXR $\alpha$  is an intracellular cholesterol sensor that regulates genes that control cholesterol absorption, secretion, degradation, and efflux, and has a critical function in the maintenance of cholesterol homeostasis in cells (23). Target genes of LXR $\alpha$  include ATP binding cassette transporter A1 (ABCA1), ABCG5, ABCG8, lipoprotein, cholesterol ester transfer protein (CETP), lipoprotein lipase (LPL), fatty acid synthase (FAS) and element binding protein 1C (SREBP-1C). LXR $\alpha$  is an essential factor in the regulation of lipid and

cholesterol metabolism (24-28). A recent study revealed that ApoM is a target gene of LXR $\alpha$ , which is recruited to the proximal ApoM promoter region -241/+42; in addition, LXR $\alpha$  ligands (oxysterols) overtly induced human ApoM gene expression and promoter activity in HepG2 cells (29). Niacin promotes cholesterol efflux via induction of LXR $\alpha$  expression, which is considered a possible mechanism behind the elevated HDL-C levels (18). The present study demonstrated that niacin increased LXR $\alpha$  expression *in vivo* and *in vitro*. As aforementioned, the LXR $\alpha$  inhibitor ECHS reduced ApoM expression induced by niacin, and knocking down LXR $\alpha$  protein expression by shRNA significantly inhibited the level of niacin-stimulated ApoM, suggesting that LXR $\alpha$  signaling is involved in niacin-associated regulation of ApoM metabolism.

Previous findings indicate that niacin affects PPAR- $\gamma$ , which is a regulator of LXR $\alpha$  (19). Whether niacin regulates ApoM through the PPAR- $\gamma$ -LXR $\alpha$ -ApoM pathway remains unknown. Recently, ApoM was reported to serve as a carrier for the bioactive lipid sphingosine-1-phosphate (S1P) on HDL particles. S1P induces five distinct G-protein-coupled receptors (S1P-receptors 1-5), affecting multiple biological processes, including lymphocyte trafficking, lipid metabolism, angiogenesis, and atherosclerosis (29). The ApoM-S1P axis induces S1P-receptor-1, and is responsible for several HDL-associated functions (30-32). Additionally, evidence indicates that niacin affects S1P levels in plasma, red blood cells and platelets (33).

Whether niacin impacts lipid metabolism and atherosclerosis through the ApoM-S1P axis requires further investigation.

In summary, it was first demonstrated that ApoM is a niacin target in lipid regulation. The mechanism by which niacin upregulates ApoM may involve LXRA regulation. ApoM regulation may constitute a novel mechanism for increasing HDL levels and promoting RCT. The present findings provide novel insights into the anti-atherosclerotic mechanism of niacin.

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## Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

LY, TL, SPZ and SDZ conceived and designed the experiments. LY and TL performed the experiments, collected and analyzed the data. LY and TL wrote the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The Ethics Committee of Xiangya Hospital of Central South University reviewed and approved the present study.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

- Gotto AM Jr and Brinton EA: Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: A working group report and update. *J Am Coll Cardiol* 43: 717-724, 2004.
- Assmann G and Gotto AM Jr: Hdl cholesterol and protective factors in atherosclerosis. *Circulation* 109 (23 Suppl 1): III8-III14, 2004.
- Miller NE: High-density lipoprotein: A major risk factor for coronary atherosclerosis. *Baillieres Clin Endocrinol Metab* 1: 603-622, 1987.
- Xu N and Dahlbäck B: A novel human apolipoprotein (apom). *J Biol Chem* 274: 31286-31290, 1999.
- Luo G, Zhang X, Nilsson-Ehle P and Xu N: Apolipoprotein M. *Lipids Health Dis* 3: 21, 2004.
- Zhang XY, Dong X, Zheng L, Luo GH, Liu YH, Ekström U, Nilsson-Ehle P, Ye Q and Xu N: Specific tissue expression and cellular localization of human apolipoprotein M as determined by in situ hybridization. *Acta Histochem* 105: 67-72, 2003.
- Wolfrum C, Poy MN and Stoffel M: Apolipoprotein M is required for prebeta-HDL formation and cholesterol efflux to HDL and protects against atherosclerosis. *Nat Med* 11: 418-422, 2005.
- Zhang X, Zhu Z, Luo G, Zheng L, Nilsson-Ehle P and Xu N: Liver X receptor agonist downregulates hepatic apoM expression in vivo and in vitro. *Biochem Biophys Res Commun* 371: 114-117, 2008.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- López-Terrada D, Cheung SW, Finegold MJ and Knowles BB: Hep G2 is a hepatoblastoma-derived cell line. *Human Pathology* 40: 1512-1515, 2009.
- Ríos-Marco P, Ríos A, Jiménez-López JM, Carrasco MP and Marco C: Cholesterol homeostasis and autophagic flux in perifosine-treated human hepatoblastoma HepG2 and glioblastoma U-87 MG cell lines. *Biochem Pharmacol* 96: 10-19, 2015.
- Jin FY, Kamanna VS and Kashyap ML: Niacin accelerates intracellular ApoB degradation by inhibiting triacylglycerol synthesis in human hepatoblastoma (HepG2) cells. *Arterioscler Thromb Vasc Biol* 19: 1051-1059, 1999.
- Zhu D, Ganji SH, Kamanna VS and Kashyap ML: Effect of gemfibrozil on apolipoprotein B secretion and diacylglycerol acyltransferase activity in human hepatoblastoma (HepG2) cells. *Atherosclerosis* 164: 221-228, 2002.
- Carlson LA: Nicotinic acid: The broad-spectrum lipid drug. A 50th anniversary review. *J Intern Med* 258: 94-114, 2005.
- McKenney J: New perspectives on the use of niacin in the treatment of lipid disorders. *Arch Intern Med* 164: 697-705, 2004.
- Brown BG, Zhao XQ, Chait A, Fisher LD, Cheung MC, Morse JS, Dowdy AA, Marino EK, Bolson EL, Alaupovic P, *et al*: Simvastatin and niacin, antioxidant vitamins, or the combination for the prevention of coronary disease. *N Engl J Med* 345: 1583-1592, 2001.
- Taylor AJ, Sullenberger LE, Lee HJ, Lee JK and Grace KA: Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol (ARBITER) 2: A double-blind, placebo-controlled study of extended-release niacin on atherosclerosis progression in secondary prevention patients treated with statins. *Circulation* 110: 3512-3517, 2004.
- Kamanna VS and Kashyap ML: Mechanism of action of niacin on lipoprotein metabolism. *Curr Atheroscler Rep* 2: 36-46, 2000.
- Wu ZH and Zhao SP: Niacin promotes cholesterol efflux through stimulation of the PPARgamma-LXRalpha-ABCA1 pathway in 3T3-L1 adipocytes. *Pharmacology* 84: 282-287, 2009.
- Ulven SM, Dalen KT, Gustafsson JA and Nebb HI: LXR is crucial in lipid metabolism. *Prostaglandins Leukot Essent Fatty Acids* 73: 59-63, 2005.
- Valledor AF: The innate immune response under the control of the LXR pathway. *Immunobiology* 210: 127-132, 2005.
- Bensinger SJ and Tontonoz P: Integration of metabolism and inflammation by lipid-activated nuclear receptors. *Nature* 454: 470-477, 2008.
- Steffensen KR and Gustafsson JA: Putative metabolic effects of the liver X receptor (LXR). *Diabetes* 53 (Suppl 1): S36-S42, 2004.
- Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, *et al*: A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. *Mol Cell* 7: 161-171, 2001.
- Schwartz K, Lawn RM and Wade DP: ABC1 gene expression and ApoA-I-mediated cholesterol efflux are regulated by LXR. *Biochem Biophys Res Commun* 274: 794-802, 2000.
- Luo Y and Tall AR: Sterol upregulation of human CETP expression in vitro and in transgenic mice by an LXR element. *J Clin Invest* 105: 513-520, 2000.
- Sato M, Kawata Y, Erami K, Ikeda I and Imaizumi K: LXR agonist increases the lymph HDL transport in rats by promoting reciprocally intestinal ABCA1 and apo A-I mRNA levels. *Lipids* 43: 125-131, 2008.
- Khovidhunkit W, Moser AH, Shigenaga JK, Grunfeld C and Feingold KR: 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: 1728-1736, 2003.
- Arkensteijn BW, Berbee JF, Rensen PC, Nielsen LB and Christoffersen C: The apolipoprotein m-sphingosine-1-phosphate axis: Biological relevance in lipoprotein metabolism, lipid disorders and atherosclerosis. *Int J Mol Sci* 14: 4419-4431, 2013.



30. Ruiz M, Frej C, Holmér A, Guo LJ, Tran S and Dahlbäck B: High-density lipoprotein-associated apolipoprotein M limits endothelial inflammation by delivering sphingosine-1-phosphate to the sphingosine-1-phosphate receptor 1. *Arterioscler Thromb Vasc Biol* 37: 118-129, 2017.
31. Ruiz M, Okada H and Dahlbäck B: HDL-associated ApoM is anti-apoptotic by delivering sphingosine 1-phosphate to S1P1 & S1P3 receptors on vascular endothelium. *Lipids Health Dis* 16: 36, 2017.
32. Frej C, Mendez AJ, Ruiz M, Castillo M, Hughes TA, Dahlbäck B and Goldberg RB: A shift in ApoM/S1P between HDL-particles in women with type 1 diabetes mellitus is associated with impaired anti-inflammatory effects of the ApoM/S1P complex. *Arterioscler Thromb Vasc Biol* 37: 1194-1205, 2017.
33. Błażnio-Zabielska A, Baranowski M, Wójcik B and Górski J: Reduction of ceramide de novo synthesis in solid tissues changes sphingolipid levels in rat plasma, erythrocytes and platelets. *Adv Med Sci* 61: 72-77, 2016.



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