

## RESEARCH PAPER

Activation of peroxisome proliferator-activated receptor- $\alpha$  in mice induces expression of the hepatic low-density lipoprotein receptorZ Huang<sup>1</sup>, X Zhou<sup>1</sup>, AC Nicholson<sup>1</sup>, AM Gotto Jr<sup>2</sup>, DP Hajjar<sup>1,4</sup> and J Han<sup>1,3,4</sup><sup>1</sup>Department of Pathology, Center of Vascular Biology, Weill Cornell Medical College, New York, NY, USA; <sup>2</sup>Department of Medicine, Weill Cornell Medical College, New York, NY, USA and <sup>3</sup>Department of Biochemistry and Molecular Biology, College of Life Sciences, Nankai University, Tianjin, China

**Background and purpose:** Mutations in the low-density lipoprotein receptor (*LDLR*) gene cause familial hypercholesterolaemia in humans and deletion of the *LDLR* induces lesion development in mice fed a high-fat diet. *LDLR* expression is predominantly regulated by sterol regulatory element-binding protein 2 (*SREBP2*). Fenofibrate, a peroxisome proliferator-activated receptor  $\alpha$  (*PPAR $\alpha$* ) ligand, belongs to a drug class used to treat dyslipidaemic patients. We have investigated the effects of fenofibrate on hepatic *LDLR* expression.

**Experimental approach:** The effects of fenofibrate on hepatic *LDLR* expression (mRNA and protein) and function were evaluated by both *in vitro* (with AML12 cells) and *in vivo* experiments in mice.

**Key results:** Fenofibrate increased *LDLR* expression and LDL binding in a mouse hepatoma cell line, AML12 cells. Fenofibrate restored sterol-inhibited hepatocyte *LDLR* expression. Mechanistic studies demonstrated that induction of *LDLR* expression by fenofibrate was dependent on *PPAR $\alpha$*  and sterol regulatory elements (*SRE*). Specifically, fenofibrate induced *LDLR* expression by increasing maturation of *SREBP2* and phosphorylation of protein kinase B (*Akt*) but had no effect on *SREBP* cleavage-activating protein. *In vivo*, a high-fat diet suppressed *LDLR* expression in mouse liver while elevating total and LDL cholesterol levels in plasma. However, fenofibrate restored *LDLR* expression inhibited by high-fat diets in the liver and reduced LDL cholesterol levels in plasma.

**Conclusions and implications:** Our data suggest that fenofibrate increased hepatic *LDLR* expression in mice by a mechanism involving *Akt* phosphorylation and *LDLR* gene transcription mediated by *SREBP2*.

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**Keywords:** *Akt*; fenofibrate; *LDLR*; *PPAR $\alpha$* ; *SREBP2*

**Abbreviations:** *Akt*, protein kinase B; *LDLR*, low-density lipoprotein receptor; *PPAR $\alpha$* , peroxisome proliferator-activated receptor  $\alpha$ ; *SCAP*, *SREBP* cleavage-activating protein; *SRE*, sterol regulatory element; *SREBP2*, sterol regulatory element-binding protein 2

## Introduction

Expression of the low-density lipoprotein receptor (*LDLR*) is regulated at the transcriptional and/or translational levels. Cholesterol and its derivatives inhibit *LDLR* expression that results in a reduction of cellular cholesterol uptake. In this feedback regulatory pathway, the sterol regulatory element-binding protein 2 (*SREBP2*) has a crucial function (Goldstein *et al.*, 2006). *SREBPs* are transcription factors belonging to the basic helix–loop–helix–leucine zipper (*bHLH-Zip*) family. They

consist of three isoforms, namely *SREBP-1a*, *SREBP-1c* and *SREBP2* (Goldstein *et al.*, 2006). *SREBP1* is responsible for fatty acid synthesis and metabolism, whereas *SREBP2* controls expression of *LDLR* and 3-hydroxy-3-methylglutaryl coenzyme A (*HMG-CoA*) reductase, the rate-limiting enzyme for cholesterol synthesis (Brown and Goldstein, 1997; Shimano, 2001). *SREBP 1* and *2* are synthesized in the rough endoplasmic reticulum (*ER*) as precursor proteins and bind to the *SREBP* cleavage-activating protein (*SCAP*). In sterol-depleted cells, *SCAP* chaperones *SREBP* from the *ER* to the Golgi where the *SREBP* is cleaved by two Golgi residing proteases, site-1 protease (*S1P*) and site-2 protease (*S2P*). The released *bHLH-Zip* domain of *SREBP* or nuclear form of *SREBP* (*nSREBP*) translocates into the nucleus, and then binds to the sterol regulatory element (*SRE*) in the promoter region of target genes, including the

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LDLR, and activates transcription. In contrast, cholesterol binds to the sterol-sensing domain of SCAP in lipid-loaded cells and causes a change in SCAP conformation, allowing SCAP to bind to a pair of ER membrane proteins, Insig1 and Insig2. The formation of the SREBP/SCAP/Insig complex prevents the processing of SREBP and inhibits the expression of SREBP target genes (Nohturfft *et al.*, 1999; Yang *et al.*, 2002; Adams *et al.*, 2003; Radhakrishnan *et al.*, 2004).

Peroxisome proliferator-activated receptors (PPARs) including PPAR $\alpha$ , PPAR $\beta/\delta$  and PPAR $\gamma$  are ligand-activated transcription factors that regulate lipid metabolism, glucose homeostasis, inflammation and the development of adipocyte differentiation (Gilde *et al.*, 2006). Both PPAR $\alpha$  and PPAR $\gamma$  ligands are anti-atherogenic in mouse models of atherosclerosis (Li *et al.*, 2000; Duez *et al.*, 2002). They have been shown to decrease cardiovascular end points in clinical trials (Helsinki Heart Study, Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) and Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive)) (Dormandy *et al.*, 2005; Keech *et al.*, 2005; Backes *et al.*, 2007). Fibric acid derivatives (such as, fenofibrate) are PPAR $\alpha$  ligands used in the treatment of dyslipidaemia in patients with diabetes. They reduce plasma triglyceride levels, increase high-density lipoprotein (HDL) cholesterol levels and shift the distribution of LDL subfractions towards larger, less atherogenic particles (Vu-Dac *et al.*, 1995; Berthou *et al.*, 1996).

Increased hepatic LDLR expression is one of the most effective means to lower plasma cholesterol levels (Bays and Stein, 2003). In some clinical studies, PPAR $\alpha$  agonists have been shown to reduce LDL cholesterol levels, particularly in the presence of hypertriglyceridaemia with unrevealed mechanisms. In patients with hypertriglyceridaemia, LDL cholesterol may increase, but this is due to an increase in particle size but not in particle number (Davidson *et al.*, 2006).

In this study, we hypothesized that fenofibrate, a PPAR $\alpha$  ligand, might reduce plasma LDL cholesterol levels through increased hepatic LDLR expression. We provide studies that show for the first time that fenofibrate increased LDLR expression and uptake of LDL by hepatocytes in a manner dependent on the activation of PPAR $\alpha$ . Our *in vivo* studies further show that administration of fenofibrate in mouse induced liver LDLR expression and reduced plasma LDL cholesterol levels, and that this process occurred through the activation of SREBP2 and phosphorylation of Akt.

## Methods

### Cell culture

The AML12 murine hepatocyte cell line (ATCC CRL-2254) (Wu *et al.*, 1994) was purchased from ATCC (Manassas, VA, USA) and cultured in complete growth medium prepared as follows: 1:1 of Dulbecco's modified Eagle's medium (DMEM) and Ham's F12 medium with 5  $\mu\text{g mL}^{-1}$  insulin, 5  $\mu\text{g mL}^{-1}$  transferrin, 5 ng  $\text{mL}^{-1}$  selenium, 40 ng  $\text{mL}^{-1}$  dexamethasone, 10% foetal bovine serum (FBS) and 50  $\mu\text{g mL}^{-1}$  penicillin/streptomycin. Cells were switched to serum-free medium and received treatments when confluent.

Human kidney 293 cell line was also purchased from ATCC and cultured in complete DMEM medium (10% FBS and 50  $\mu\text{g mL}^{-1}$  penicillin/streptomycin).

### Animals

All animal procedures were in strict compliance with NIH guidelines. C57BL wild-type mice (at the age of 10 weeks and about 25 g) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and maintained in fully accredited facilities (AAALAC) at Weill Medical College of Cornell University. Mice were divided into three groups at the time of the experiments. Group 1 was left on normal chow, whereas the other two groups were initially fed with a high-fat diet for 2 weeks. They were then further divided as follows: a high-fat diet alone or a high-fat diet plus fenofibrate (50 mg per 100 g food). We assumed that the intake of fenofibrate by mice was about 50 mg  $\text{day}^{-1}$  per kg body weight, based on the following calculation: a 25 g mouse eats 2.5 g food a day, thus the intake of fenofibrate is  $2.5 \times 50/100/0.025 = 50$ . After 10 days of treatment with fenofibrate, mice were killed and blood or liver was individually collected. Plasma was prepared by centrifuging blood samples for 5 min at  $400 \times g$  and was kept at  $-20^\circ\text{C}$  for assay of total, LDL and HDL cholesterol levels. The liver was used to extract cellular total RNA.

### Isolation of total RNA and northern blot analysis of LDLR, HMG-CoA reductase and SCAP mRNA expression

Total cellular RNA was extracted from cells or liver and used to determine the levels of mouse *LDLR*, *HMGCR* (HMG-CoA reductase), *SCAP* and *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) mRNA by northern blot analysis as described (Han *et al.*, 2004). Probes were generated by reverse transcription-PCR based on the published sequence. The sequences of primers are *LDLR*-f: GACTGCAAGGACATGACCGA (781-801); *LDLR*-r: CGTTGGTGAAGAGCAGATA (1221-1201); *HMGCR*-f: CGTTGGCTGGAGACGGCA (121-138), *HMGCR*-r: CACTGACATGCAGCCGAA (798-781); *SCAP*-f: TTCTGGCAGTCGATGTGTC (573-592); and *SCAP*-r: GACCA CCAGGTATGGGAAGA (1309-1280).

### Western blot analysis of LDLR, SREBP2, PPAR $\alpha$ , total Akt and phospho-Akt protein expression

After treatment, cells were washed twice with cold phosphate-buffered saline, then scraped and lysed in ice-cold lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 1 mM phenylmethanesulphonyl fluoride, 50 mM sodium fluoride, 1 mM sodium orthovanadate, 50  $\mu\text{g mL}^{-1}$  aprotinin and 50  $\mu\text{g mL}^{-1}$  leupeptin). If the lysis buffer was used to extract cellular proteins for total Akt and phospho-Akt analysis, 1 mM  $\beta$ -glycerol phosphate and 2.5 mM sodium pyrophosphate were added. Cellular lysate was centrifuged at  $400 \times g$  for 15 min at  $4^\circ\text{C}$ , and supernatants were transferred into a new test tube. After measuring the protein content, lysates were loaded and separated on a 12% SDS-polyacrylamide gel electrophoresis and transferred onto nylon-enhanced nitrocellulose membrane. The

membrane was blocked with a solution of TBS/0.1% Tween 20 (TBS-T) containing 5% fat-free dry milk for 1 h, and then incubated with primary antibody overnight at 4 °C followed by washing for 3 × 10 min with TBS-T buffer. The blot was re-blocked with TBS/T containing 5% milk, followed by incubation with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. After washing 3 × 10 min with TBS-T, the membrane was incubated for 1 min in a mixture of equal volumes of western blot chemiluminescence reagents 1 and 2, and then exposed to film before development.

#### Isolation of LDL and analysis of LDL binding to hepatocytes

LDL (1.019–1.063 g mL<sup>-1</sup>) was isolated from normal human plasma by sequential ultracentrifugation. To conduct the binding of LDL to hepatocytes, LDL was fluorescein conjugated with a reactive succinimidyl-ester of carboxyl-fluorescein by using a labelling kit purchased from Princeton Separations (Adelphia, NJ, USA). Hepatocytes were cultured in a four-well slide chamber. After treatment, cells were washed twice with phosphate-buffered saline and then incubated with 30 µg mL<sup>-1</sup> of labelled LDL in serum-free medium for 2 h at 37 °C. After washing twice with phosphate-buffered saline and covering with Vectashield mounting medium (Vector Laboratories Inc., Burlingame, CA, USA), cells were observed with a fluorescent microscope and photographed.

#### Construction and determination of LDLR promoter activity

A mouse LDLR promoter including the SRE (pLDLR, from -331 to +49, SRE in LDLR (ATCACCCAT) locates from -213 to -204) was generated by PCR with primers, pLDLR-f, 5'-CGGCCAAGCTTGCTGTGGGAGGAATTTGAGGA-3' and pLDLR-r, 5'-GGCCACCATGGAAGGAGCAGGGCGATGAC-3', and genomic DNA isolated from mouse liver. The primers contained restriction sites for *Hind*III (5' primer) and *Nco*I (3' primer), respectively. After the sequence was confirmed, the PCR product was digested by enzymes and then sub-cloned into pGL4.10 luciferase reporter vector (Promega, Madison, WI, USA) to generate the LDLR promoter (pLDLR-Luc) and amplified. The LDLR promoter with a mutation in SRE (pLDLR-SREmu-Luc) was generated by using a Quik-Change II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA), DNA of pLDLR-Luc, and the following primers: pLDLR SREmu-f, 5'-gaagattttgaaaATCACGGCATgcagactctccccg-3' (CC in SRE motif (in caps) was replaced by GG (underlined)); pLDLR SREmu-r, 5'-ggggaggagtctgcaATGCCGTGATtttcaaaaatcttc-3'.

About 90% confluent 293 cells in 24-well plates were transfected with 1 µg per well of pLDLR-Luc or pLDLR-SREmu-Luc plus *Renilla* luciferase reporter (RLuc, for internal control) by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After transfection for 8 h, the cells were treated overnight. After washing with phosphate-buffered saline, the cells were lysed and the activity of pLDLR-Luc or pLDLR-SREmu-Luc and RLuc in cellular lysate was determined by using a Dual-Luciferase Reporter Assay System from Promega.

#### Data analysis

All experiments were repeated three times except as indicated, and representative results are presented. Data generated from the cholesterol assays and the activity assays of LDLR promoters were expressed as mean ± s.e.mean and analysed by paired *t*-test.

#### Materials

Fenofibrate, GW7647, GW9662, LY294002 and LY303511 were purchased from CalBiochemistry (San Diego, CA, USA) and prepared in dimethyl sulphoxide. Control and PPARα siRNAs, siRNA transfection medium and reagents, and polyclonal anti-PPARα antibody were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). High-fat diet (21% fat and 0.2% cholesterol) was purchased from Harlan Teklad (Madison, WI, USA). Rabbit anti-mouse LDLR antibody was purchased from R&D Systems (Minneapolis, MN, USA). Rabbit anti-mouse total Akt and phospho-Akt (Ser473) were obtained from Cell Signaling Technology (Beverly, MA, USA). Rabbit anti-aP2 serum was generated by immunization with murine aP2 peptide of DGKSTTIKRRKRDGDKLV (Sun *et al.*, 2003). Rabbit-anti mouse-SREBP2-specific polyclonal antibody was a gift of Dr Joseph Goldstein from The University of Southwestern Medical Center. Total, LDL and HDL cholesterol assay kits were purchased from Wako Chemicals (Richmond, VA, USA). All other reagents were obtained from Sigma-Aldrich Chemicals (St Louis, MO, USA) except as indicated.

## Results

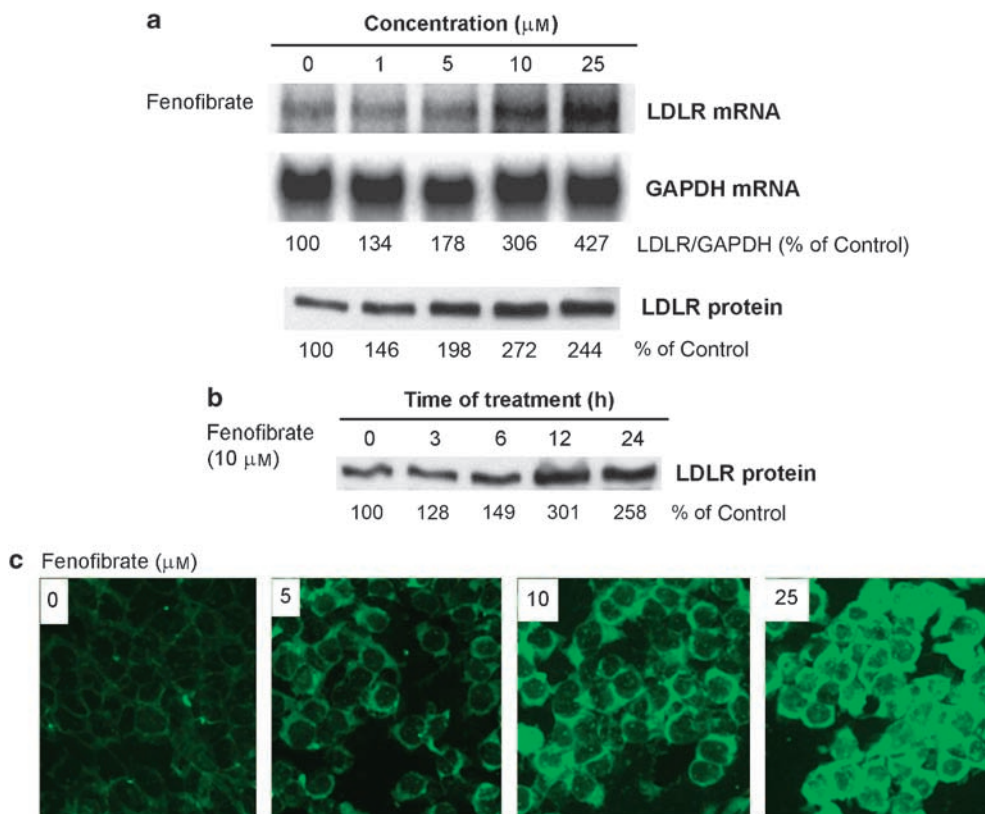
#### Fenofibrate induces LDLR expression in hepatocytes in a PPARα-dependent manner

To study the effects of fenofibrate on hepatic LDLR expression, a mouse hepatocyte cell line, AML12 cells, was treated overnight with different concentrations of fenofibrate in serum-free medium. Changes in LDLR mRNA were assessed by northern blot analysis (Figure 1a). Fenofibrate induced hepatocyte LDLR mRNA expression in a concentration-dependent manner. Maximal induction was observed with 25 µM of fenofibrate.

To determine whether the induction of LDLR mRNA by fenofibrate was associated with the induction of LDLR protein, total cellular protein was extracted from cells after treatment with fenofibrate and used to determine LDLR protein expression by western blot analysis. Consistent with the changes in LDLR mRNA expression, LDLR protein was increased by fenofibrate in a concentration-dependent manner, with maximal induction of LDLR protein expression at a concentration of 10 µM (Figure 1a).

The temporal effects of fenofibrate on LDLR expression were assessed by time course studies. Cells were treated with fenofibrate (10 µM) for up to 24 h and changes in LDLR protein were analysed by western blot. Induction of LDLR protein expression by fenofibrate was first observed at 6 h, and maximal expression was observed at 12 h (Figure 1b).

To correlate the induction of LDLR expression in response to fenofibrate with the LDLR function, LDL binding to



**Figure 1** Fenofibrate induces hepatic LDLR expression and function. (a) Fenofibrate induces LDLR mRNA and protein expression: confluent mouse hepatocytes (AML12 cells) in serum-free medium were treated with fenofibrate at the indicated concentrations overnight. Total cellular RNA or proteins were extracted and used to determine the levels of hepatic LDLR mRNA or protein by northern or western blot analysis as described in the Methods section. (b) Fenofibrate induces LDLR protein expression and time course: hepatocytes in serum-free medium were treated with fenofibrate (10  $\mu\text{M}$ ) for indicated times. Cellular proteins were extracted and used to determine the levels of LDLR protein by western blot analysis. (c) Fenofibrate induces the binding of LDL to hepatocytes: hepatocytes were treated overnight with fenofibrate at the indicated concentrations. LDL binding assays were performed as described in the Methods section. LDLR, low-density lipoprotein receptor.

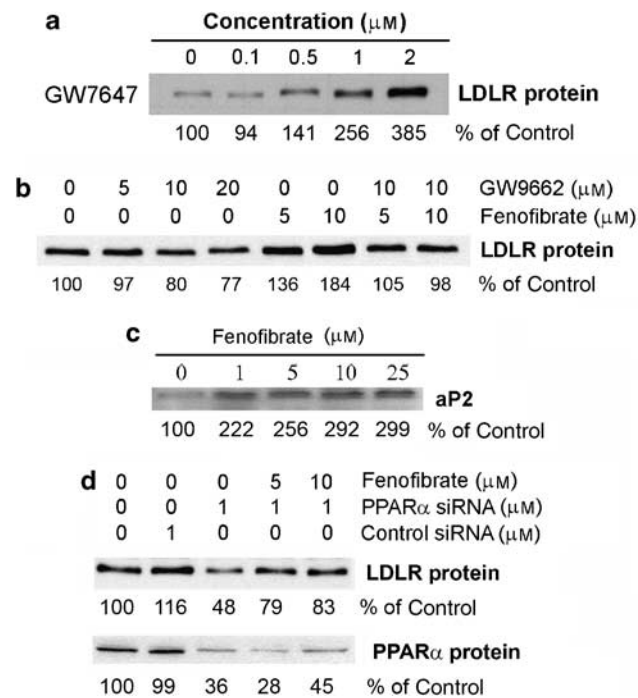
AML12 hepatocytes was assessed. Uptake of LDL by hepatocytes was significantly enhanced by fenofibrate in a concentration-dependent manner (Figure 1c).

To study if the induction of hepatic LDLR expression by fenofibrate was dependent on the activation of PPAR $\alpha$ , we initially treated hepatocytes with another PPAR $\alpha$  agonist, GW7647 (Muoio *et al.*, 2002), and determined its effects on LDLR expression. Similar to fenofibrate, GW7647 induced LDLR expression in a dose-dependent manner (Figure 2a). In contrast, a PPAR $\alpha$  antagonist, GW9662 alone (Brown *et al.*, 2001; Leesnitzer *et al.*, 2002), moderately inhibited hepatic LDLR expression. It also blocked fenofibrate-induced hepatic LDLR expression (Figure 2b). Induction of adipose fatty acid-binding protein (A-FABP or aP2) in the liver is also dependent on PPAR $\alpha$  activity (Motojima, 2000). Thus, increased aP2 expression in hepatocytes in response to fenofibrate will indicate the activation of PPAR $\alpha$ . Indeed, we observed a strong induction of aP2 expression in hepatocytes by fenofibrate (Figure 2c). To further confirm if the induction of hepatic LDLR expression by fenofibrate is PPAR $\alpha$ -dependent, we used siRNA to reduce PPAR $\alpha$  expression. Results in Figure 2d indicate that PPAR $\alpha$  siRNA significantly reduced PPAR $\alpha$  levels, whereas a control siRNA had no effect on PPAR $\alpha$ . PPAR $\alpha$  siRNA alone decreased the expression of hepatocyte LDLR. More importantly, as PPAR $\alpha$

siRNA did not completely inhibit PPAR $\alpha$  expression, as compared with PPAR $\alpha$  siRNA alone, the co-treatment of fenofibrate and PPAR $\alpha$  siRNA induced LDLR expression (Figure 2d).

To examine the physiological relevance of fenofibrate-induced hepatic LDLR expression, wild-type mice were fed with normal chow or a high-fat diet or a high-fat diet containing fenofibrate. The effects of fenofibrate on LDLR mRNA expression in the liver, and the levels of total, LDL and HDL cholesterol in plasma were determined. Compared with normal chow, a high-fat diet increased total and LDL cholesterol levels (163 and 139% of control group, respectively, Table 1). However, fenofibrate abolished the elevation of circulating LDL cholesterol. The increased total cholesterol in mice given fenofibrate was partially due to increased HDL cholesterol (Table 1). To study if the reduction of LDL cholesterol in response to fenofibrate was due to the increased LDLR-mediated LDL clearance, the expression of liver LDLR mRNA in mice was assessed by northern blot analysis. As expected, a high-fat diet inhibited LDLR mRNA expression in mouse liver (43% of group on normal chow, Figure 3). In contrast, the addition of fenofibrate in the high-fat diet abolished the decline of LDLR mRNA levels (96% of group on normal chow, Figure 3).

*Fenofibrate restores LDLR expression inhibited by lipid loading*  
LDLR expression is feedback inhibited by cellular sterol levels. Thus, cholesterol or lipid 'loading' will inhibit LDLR



**Figure 2** Induction of hepatocyte LDLR expression by fenofibrate is PPAR $\alpha$ -dependent. (a) Induction of hepatocyte LDLR expression by GW7647, another PPAR $\alpha$  ligand: confluent hepatocytes were treated with another PPAR $\alpha$  agonist, GW7647 at the indicated concentrations overnight. The expression of LDLR protein was determined by western blot analysis. (b) PPAR $\alpha$  antagonist, GW9662, blocks fenofibrate-induced LDLR expression: confluent hepatocytes were treated with GW9662 or fenofibrate alone or co-treated with GW9662 and fenofibrate at the indicated concentrations overnight. Changes in LDLR protein was assessed by western blot analysis. (c) Induction of hepatocyte aP2 expression by fenofibrate: confluent hepatocytes were treated with fenofibrate at the indicated concentrations overnight. Expression of aP2 was determined by western blot analysis. (d) PPAR $\alpha$  siRNA inhibits LDLR expression: confluent hepatic cells in six-well plates were transfected with control siRNA or PPAR $\alpha$  siRNA for 6 h followed by treatment with fenofibrate at the indicated concentrations overnight. Expression of LDLR and PPAR $\alpha$  proteins were determined by western blot analysis. LDLR, low-density lipoprotein receptor; PPAR $\alpha$ , peroxisome proliferator-activated receptor  $\alpha$ .

**Table 1** Effect of fenofibrate on mouse plasma cholesterol levels ( $\text{mg L}^{-1}$ )

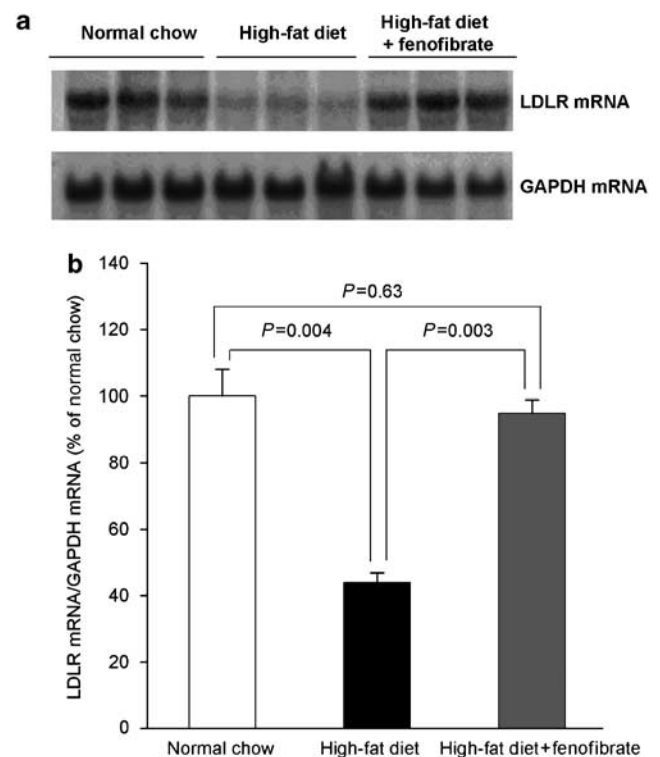
Treatment	Total C	LDL C	HDL C
Control (normal chow)	782 $\pm$ 90	196 $\pm$ 17	465 $\pm$ 50
High-fat diet	1277 $\pm$ 38*	271 $\pm$ 24*	749 $\pm$ 46*
High-fat diet + fenofibrate	1508 $\pm$ 48* <sup>#</sup>	211 $\pm$ 13 <sup>#</sup>	997 $\pm$ 45* <sup>#</sup>

Abbreviations: C, cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

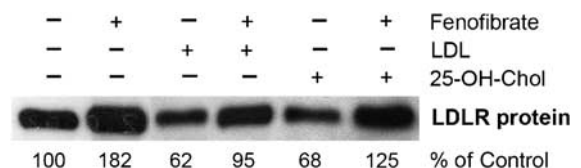
Mice at the age of 10 weeks were divided into three groups. Control group was fed with normal chow. Other two groups were fed a high-fat diet for 2 weeks followed by drug treatment as described in the Methods section. At the end of experiments, plasma and liver were collected from each mouse. Total, LDL and HDL cholesterol levels were determined.

\*Significantly different from control group at  $P < 0.05$ ; <sup>#</sup>significantly different from group of high-fat diet alone at  $P < 0.05$ , by Student's *t*-test ( $n = 5$ ).

expression. This mechanism not only prevents cells from uncontrolled accumulation of lipids but also reduces the clearance of excess LDL cholesterol from the circulation by the liver during hyperlipidaemia. To determine whether fenofibrate would restore LDLR expression suppressed by lipids, cells were treated with LDL or 25-hydroxycholesterol or lipid plus fenofibrate. LDL and 25-hydroxycholesterol inhibited LDLR protein expression in AML12 hepatocytes (Figure 4). This inhibitory effect was abolished in the presence of fenofibrate, suggesting that fenofibrate restores the LDLR expression inhibited by sterols (Figure 4).



**Figure 3** Fenofibrate induces LDLR expression in mouse liver. (a) Mice were treated as described in Table 1. Total RNA was extracted from the mouse liver, and the expression of LDLR mRNA was determined by northern blot analysis. The same blot was re-hybridized with  $^{32}\text{P}$ -labeled GAPDH probe for internal control. (b) Summary of northern blot results indicating the significant difference ( $P < 0.05$ ,  $n = 3$ ) between groups of normal chow and high-fat diet, or groups of high-fat diet and high-fat diet + fenofibrate, but not between groups of normal chow and high-fat diet + fenofibrate. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; LDLR, low-density lipoprotein receptor.

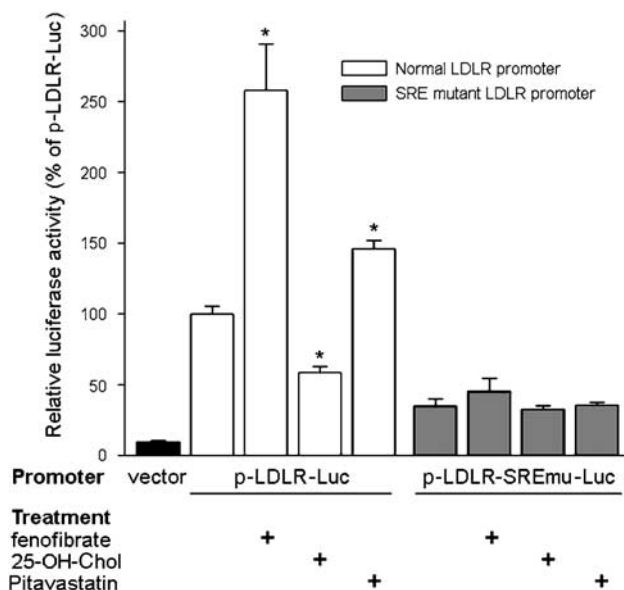


**Figure 4** Fenofibrate restores sterol-inhibited LDLR expression. Hepatocytes were treated with fenofibrate ( $10 \mu\text{M}$ ) in the presence or absence of LDL ( $100 \mu\text{g mL}^{-1}$ ) or 25-hydroxycholesterol (25-OH-Chol,  $10 \mu\text{M}$ ) overnight. Expression of LDLR protein was determined by western blot analysis. LDLR, low-density lipoprotein receptor.

*Induction of LDLR expression by fenofibrate occurs through the activation of SREBP2 and Akt phosphorylation*

LDLR expression is primarily regulated by the activity of SREBP2, which is dependent on cellular sterol levels. Fenofibrate restored sterol-suppressed LDLR expression, suggesting that fenofibrate may modulate the SREBP2 pathway to activate hepatic LDLR expression. Initially, we determined whether the induction of LDLR expression by fenofibrate was SRE-dependent. The kidney cell line, 293 cells, was transfected with LDLR promoter-luciferase constructs, including normal SRE or mutant SRE, followed by treatment with fenofibrate or 25-hydroxycholesterol or pitavastatin, a HMG-CoA reductase inhibitor. The activity of the promoter containing the normal SRE sequence was increased by pitavastatin (47%) and decreased by 25-hydroxycholesterol (42%), as expected. Fenofibrate treatment significantly increased the activity of the LDLR promoter containing normal SRE (>2-fold) (Figure 5). Mutation in the SRE reduced promoter activity and disabled the regulation of promoter activity by pitavastatin and 25-hydroxycholesterol. Fenofibrate did not influence the activity of the SRE mutant LDLR promoter, also suggesting an essential role of SRE in the regulation of LDLR transcription in response to fenofibrate.

SRE-dependent induction of LDLR promoter activity by fenofibrate indicated that fenofibrate may activate SREBP2. Thus, expression and maturation of SREBP2 in response to

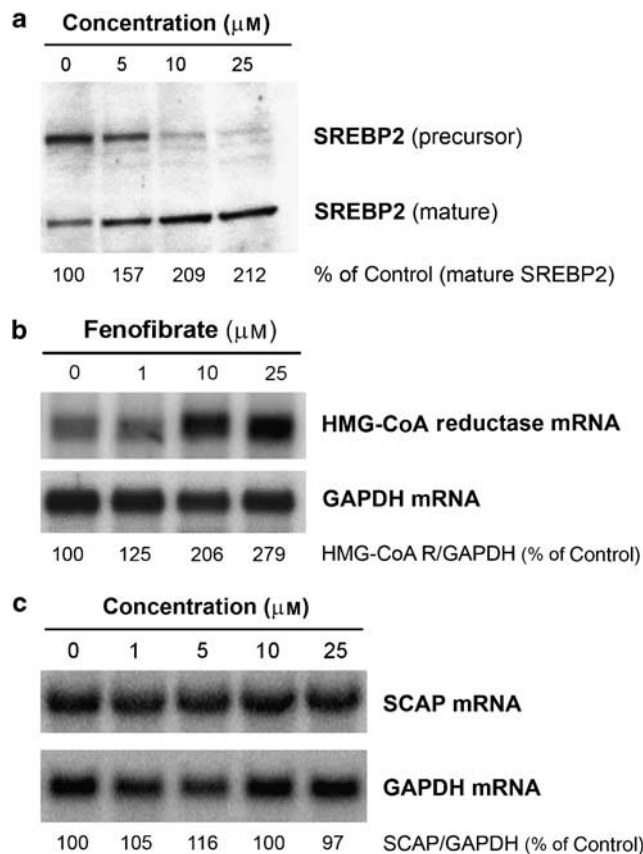


**Figure 5** Induction of hepatic LDLR expression by fenofibrate is sterol regulatory elements (SRE)-dependent. Confluent 293 kidney cells were transfected with plasmid DNA of normal LDLR promoter (pLDLR-luc) or SRE mutant LDLR promoter (pLDLR-SREmu-Luc) plus *Renilla* luciferase reporter (RLuc) followed by treatment with fenofibrate (10  $\mu$ M) or 25-hydroxycholesterol (25-OH-Chol, 10  $\mu$ M) or pitavastatin (10  $\mu$ M) overnight in serum-free medium. Promoter activity was analysed as described in the Methods section. The relative luciferase activity of the LDLR promoter was calculated based on the activity of co-transfected RLuc. The activity of normal LDLR promoter without treatment was defined as 100%. \*Significantly different from normal LDLR promoter without treatment at  $P < 0.05$  by Student's *t*-test ( $n = 4$ ). LDLR, low-density lipoprotein receptor.

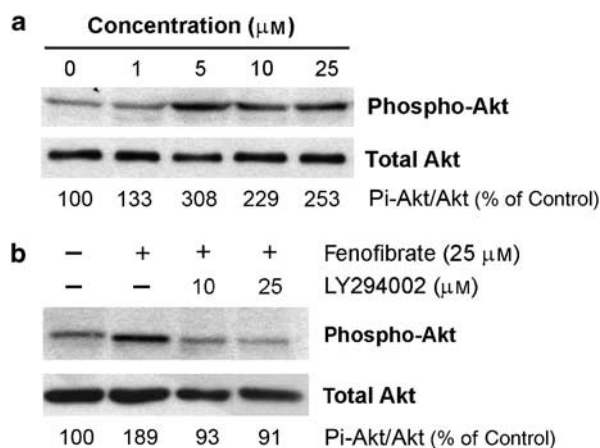
fenofibrate was determined by western blot analysis. Results shown in Figure 6a demonstrate that fenofibrate did not induce SREBP2 expression. However, fenofibrate increased the amount of mature SREBP2 and decreased SREBP2 precursor, suggesting that fenofibrate enhanced the proteolysis of SREBP2. To further confirm the activation of SREBP2 by fenofibrate, we evaluated the expression of HMG-CoA reductase mRNA, another target gene regulated by SREBP2 activity. HMG-CoA reductase mRNA was also increased in response to fenofibrate (Figure 6b).

Levels of SCAP or translocation of the SCAP-SREBP2 complex from the ER to the Golgi affect the maturation of SREBP2. To test if fenofibrate was able to induce SCAP expression, its effect on SCAP mRNA expression was assessed by northern blot analysis. Results shown in Figure 6c indicate that SCAP expression was unaffected by fenofibrate treatment.

The activity of the protein kinase Akt influences the SREBP2 processing and expression of the LDLR (Du *et al.*, 2006). To study if the activation of SREBP2 by fenofibrate



**Figure 6** Fenofibrate induces maturation of sterol regulatory element-binding protein 2 (SREBP2) and expression of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, but has no effect on the expression of SREBP cleavage-activating protein (SCAP). (a) Hepatocytes were treated with fenofibrate at the indicated concentrations overnight. Total cellular proteins were extracted and used to determine the levels of precursor of SREBP2 (P) and mature SREBP2 (N) by western blot analysis. Hepatocytes in serum-free medium were treated with fenofibrate at the indicated concentrations overnight. Total cellular RNA was collected and used to determine the expression of HMG-CoA reductase mRNA (b) and SCAP mRNA (c), respectively, by northern blot analysis.



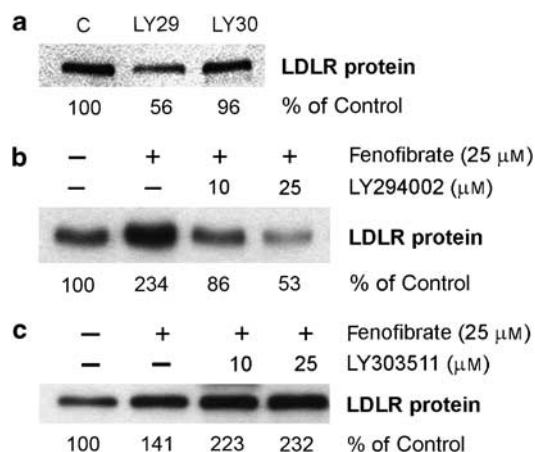
**Figure 7** Fenofibrate increases Akt phosphorylation. Hepatocytes were treated with fenofibrate at the indicated concentrations overnight (a), or treated with fenofibrate (25 μM) in the presence or absence of LY294002 at the indicated concentrations overnight (b). Cellular protein was extracted and used to determine total Akt and phospho-Akt expressions by western blot analysis as described in the Methods section.

was initially related to Akt phosphorylation, total Akt and phosphorylated Akt in response to fenofibrate treatment was assessed by western blot analysis. Figure 7a demonstrates that fenofibrate increased phosphorylated Akt but had no effect on total Akt expression. Furthermore, LY294002, a phosphoinositide 3-kinase (PI3K, the primary activator of Akt phosphorylation) inhibitor (Song *et al.*, 2005), blocked the induction of Akt phosphorylation by fenofibrate (Figure 7b).

To correlate changes in Akt activity with expression of the LDLR in response to fenofibrate, we treated hepatocytes with a PI3K inhibitor, LY294002, or a negative control for the PI3K inhibitor, LY303511. Results in Figure 8a demonstrate that the PI3K inhibitor reduced LDLR expression. The negative control for the PI3K inhibitor had little effect on LDLR expression. We then evaluated whether the PI3K inhibitor was able to block fenofibrate-induced LDLR expression. Consistent with changes in phospho-Akt, the induction of LDLR expression by fenofibrate was blocked by a PI3K inhibitor, LY294002 (Figures 8b), whereas the negative control for PI3K inhibitor (LY303511) slightly enhanced fenofibrate-induced LDLR expression (Figure 8c).

## Discussion

Clearance of excess plasma LDL cholesterol occurs through binding of LDL to the hepatic LDLR. Thus, increased LDLR expression in the liver can reduce plasma LDL cholesterol. Statins, the most commonly used therapeutic agents to lower plasma LDL cholesterol, act to increase liver LDLR expression by inhibiting cholesterol synthesis in hepatocytes. Herein, we demonstrate for the first time that fenofibrate induces the expression of LDLR mRNA and protein in a mouse hepatocyte cell line (AML12 cells) and that the induction of LDLR expression enhances LDL uptake. The induction of hepatic LDLR expression is not a



**Figure 8** Induction of hepatic low-density lipoprotein receptor (LDLR) expression by fenofibrate is blocked by phosphoinositide 3-kinase (PI3K) inhibition. (a) Confluent hepatocytes were treated with the PI3K inhibitor (LY294002, LY29, 10 μM) or the negative control for the PI3K inhibitor (LY303511, LY30, 10 μM) overnight. (b) Cells were treated with fenofibrate (25 μM) in the absence or presence of PI3K inhibitor (LY294002) at the indicated concentrations overnight. (c) Cells were treated with fenofibrate (25 μM) in the absence or presence of the negative control for the PI3K inhibitor (LY303511) at the indicated concentrations overnight. Changes in LDLR protein were assessed by western blot analysis.

fenofibrate-specific effect but rather a PPARα ligand-dependent effect (that is, dependent on the activation of PPARα). Administration of fenofibrate induced expression of the LDLR in mouse liver reducing the plasma levels of LDL cholesterol. The increase in hepatic LDLR expression by fenofibrate is sustainable and dependent on the SRE and the activity of SREBP2, the primary transcriptional regulator of LDLR expression. Moreover, our data show that increased phosphorylation of Akt results in enhanced SREBP2 processing.

Induction of hepatic LDLR expression by fenofibrate was SRE-dependent (Figure 5). Suppression of hepatic LDLR expression by lipid treatment *in vitro* or by feeding mice a high-fat diet is also dependent on the action of lipids on SRE in the LDLR promoter (Brown and Goldstein, 1997). Therefore, fenofibrate was able to restore sterol-inhibited hepatic LDLR expression both *in vitro* and *in vivo* (Figures 3 and 4). The SRE-dependent induction of hepatic LDLR expression by fenofibrate suggests that the activity of SREBP2 is required. Indeed, we observed that fenofibrate reduced the precursor of SREBP2 but increased mature SREBP2, indicating that the proteolysis of SREBP2 is enhanced. In addition, we observed that fenofibrate increased the expression of HMG-CoA reductase, another target gene of SREBP2. SCAP is the molecule chaperoning SREBP2 from the ER to the Golgi for SREBP2 processing. Interestingly, we observed that the expression of SCAP was not altered in fenofibrate-treated AML12 hepatocytes. Thus, the enhancement of ER-to-Golgi transport of SCAP-SREBP2 most likely occurs by a different mechanism.

Protein kinase B (Akt) is a critical regulator of cell growth, proliferation and survival, and is itself activated by PI3K (Song *et al.*, 2005). Akt activity is also involved in

SREBP2-mediated gene transcription. Inhibition of PI3K by a specific inhibitor, LY294002, reduced SREBP2 processing from its precursor to a mature form, thereby inhibiting the expression of SREBP2 target genes, including the *LDLR* (Du *et al.*, 2006). Induction of SREBP2 maturation by sterol depletion (for example, statin treatment) or by growth factors (for example, epidermal growth factor, vascular endothelial growth factor and insulin/insulin-like growth factor-1) in different cell types can involve the activation of Akt phosphorylation. This process can also be blocked by PI3K-specific inhibitors (Borradaile *et al.*, 2003; Zhou *et al.*, 2004; Porstmann *et al.*, 2005). Inhibition of Akt phosphorylation does not affect the binding of SCAP–Insig1, but disrupts the transport of SCAP–SREBP2 from the ER to the Golgi (Du *et al.*, 2006). We interpret our studies to suggest that Akt activity can play a critical role in fenofibrate-induced hepatic LDLR expression, as fenofibrate induced phosphorylation of Akt (Figure 7). Fenofibrate-induced Akt activity, as well as hepatic LDLR expression, was blocked by a PI3K inhibitor (Figures 7 and 8). Taken together, our data demonstrate that fenofibrate can induce hepatic LDLR expression by the activation of Akt and consequently the maturation of SREBP2.

The effects of fenofibrate on serum total and LDL cholesterol levels have been well examined in patients. Although it did not greatly lower serum total and LDL cholesterol levels (~10%) in patients with isolated hypercholesterolaemia (LDL cholesterol > 1300 mg L<sup>-1</sup> but triglyceride levels are normal; Wierzbicki, 2006), fenofibrate did significantly reduce serum total and LDL cholesterol levels in patients with combined hypercholesterolaemia (LDL cholesterol > 1300 mg L<sup>-1</sup>, triglycerides > 2000 mg L<sup>-1</sup> and < 10 000 mg L<sup>-1</sup>), either as a monotherapy or a combined therapy with a statin (Frost *et al.*, 2001; Malik *et al.*, 2001; Athyros *et al.*, 2002; Melenovsky *et al.*, 2002; Sebestjen *et al.*, 2002; Vega *et al.*, 2003; Wang *et al.*, 2003; Derosa *et al.*, 2004; Winkler *et al.*, 2004; Saklamaz *et al.*, 2005; Grundy *et al.*, 2005; Iovine *et al.*, 2006; Muhlestein *et al.*, 2006; Arca *et al.*, 2007; Chatley *et al.*, 2007). In these studies, fenofibrate alone at a dose about 200 mg day<sup>-1</sup> decreased total cholesterol by 10–20% in combined hypercholesterolaemic patients after a few months (1–6) treatment. A similar decrease was observed in serum LDL cholesterol. In a similar period of treatment, statin therapy alone reduced total and LDL cholesterol by a greater degree than fenofibrate (that is, 20–30% by cerivastatin and simvastatin, and 30–40% by atorvastatin). Statins have little effect on HDL and only a moderate effect on triglycerides. In contrast, fenofibrate can increase HDL cholesterol and apolipoprotein AI/II levels, and significantly decrease triglycerides. LDL consists of a heterogeneous group of particles. Among these particles, the small or dense subfractions of LDL are believed the most atherogenic. Statins decrease all subfractions of LDL to a similar degree. In contrast, fenofibrate can decrease the small or dense LDL more than the large or light LDL. Thus, statins and fenofibrate have different impacts on the composition of LDL in serum (Frost *et al.*, 2001; Vega *et al.*, 2003; Winkler *et al.*, 2004; Arca *et al.*, 2007). For example, atorvastatin decreased the dense LDL (dLDL) apoB in total LDL apoB by 5%, whereas fenofibrate reduced it by 36% (Winkler *et al.*,

2004). Simvastatin alone had little effect on LDL large/small ratio (1.12 vs 0.94 by placebo). In combination with fenofibrate, simvastatin increased this ratio to 2.37 (Vega *et al.*, 2003). More importantly, a combination therapy of fenofibrate and a statin improved lipid profiles in patients with combined hypercholesterolaemia in a synergistic manner and lowered the dose of statin, potentially reducing side effects (Athyros *et al.*, 2002; Vega *et al.*, 2003; Derosa *et al.*, 2004; Grundy *et al.*, 2005; Muhlestein *et al.*, 2006). Fenofibrate also demonstrated several beneficial effects on non-lipid biochemical risk factors, such as C-reactive protein, Lp(a) and oxidation of LDL (Athyros *et al.*, 2002; Melenovsky *et al.*, 2002; Wang *et al.*, 2003). Combined hypercholesterolaemia is very often observed in type II diabetic patients. These studies suggest the application of fenofibrate alone or in combination with a statin in the treatment of lipid disorders in these patients.

Effects of fibrates on cholesterol homeostasis were also investigated in different animal models. Administration of fenofibrate to rats reduced plasma cholesterol levels associated with increased LDLR expression in the liver (Staels *et al.*, 1992). In contrast, fenofibrate induced expression of cholesterol synthesis genes in mice (Le Jossic-Corcus *et al.*, 2004). Administration of fenofibrate to mice increased the expression of HMG-CoA reductase and farnesyl pyrophosphate synthases, although the effect of fenofibrate on plasma cholesterol levels and expression of LDLR in the liver was not examined in this report. Induction of HMG-CoA reductase and farnesyl pyrophosphate synthase expression by fibrates was PPAR $\alpha$ -dependent. Interestingly, fenofibrate induced expression of SREBP1 but not SREBP2 at mRNA levels in mouse liver (Le Jossic-Corcus *et al.*, 2004). Similarly, we observed that fenofibrate enhanced the proteolysis, but not the expression of SREBP2 protein (Figure 6a). Effect of fibrates on the expression of SREBP target genes including LDLR may depend on the ligand and the duration of treatment. WY14643 is a more selective PPAR $\alpha$  ligand but had less inductive effect than fenofibrate on HMG-CoA reductase in mouse liver (Le Jossic-Corcus *et al.*, 2004). Fenofibrate induced LDLR expression in rat liver only after 3 days of administration, whereas WY14643 had no effect on LDLR expression in mouse liver after the same duration of treatment (Staels *et al.*, 1992; Anderson *et al.*, 2004). Gemfibrozil induced LDLR expression, whereas clofibrate had no effect on LDLR expression in rat liver. In contrast to the results in clinical studies, administration of fibrates to rats reduced apoA-I levels in plasma and suppressed expression of apoA genes in the liver (Staels *et al.*, 1992). In a recent report, fenofibrate decreased plasma LDL cholesterol levels in human apolipoprotein (apo) E2 knockin mice (Hennuyer *et al.*, 2005). Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a natural inhibitor of LDLR expression. Recently, fenofibrate has been reported to suppress the expression of PCSK9 in human hepatocytes. This may be another potential mechanism by which fenofibrate induces hepatic LDLR expression (Kourimate *et al.*, 2008).

In summary, our studies demonstrate for the first time that fenofibrate increased hepatic LDLR expression in a PPAR $\alpha$ -dependent manner and reduced plasma LDL cholesterol



levels in mice. Activation of SREBP2 and Akt phosphorylation was required for fenofibrate-induced hepatic LDLR expression. Whether a similar PPAR $\alpha$ -dependent mechanism operates in humans remains to be determined.

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## Conflict of interest

The authors state no conflict of interest.

## References

- Adams CM, Goldstein JL, Brown MS (2003). Cholesterol-induced conformational change in SCAP enhanced by Insig proteins and mimicked by cationic amphiphiles. *Proc Natl Acad Sci USA* **100**: 10647–10652.
- Anderson SP, Dunn C, Laughter A, Yoon L, Swanson C, Stulnig TM *et al.* (2004). Overlapping transcriptional programs regulated by the nuclear receptors peroxisome proliferator-activated receptor  $\alpha$ , retinoid X receptor, and liver X receptor in mouse liver. *Mol Pharmacol* **66**: 1440–1452.
- Arca M, Montali A, Pigna G, Antonini R, Antonini TM, Luigi P *et al.* (2007). Comparison of atorvastatin versus fenofibrate in reaching lipid targets and influencing biomarkers of endothelial damage in patients with familial combined hyperlipidemia. *Metabolism* **56**: 1534–1541.
- Athyros VG, Papageorgiou AA, Athyrou VV, Demitriadis DS, Kontopoulos AG (2002). Atorvastatin and micronized fenofibrate alone and in combination in type 2 diabetes with combined hyperlipidemia. *Diabetes Care* **25**: 1198–1202.
- Backes JM, Gibson CA, Ruisinger JF, Moriarty PM (2007). Fibrates: what have we learned in the past 40 years? *Pharmacotherapy* **27**: 412–424.
- Bays H, Stein EA (2003). Pharmacotherapy for dyslipidaemia—current therapies and future agents. *Expert Opin Pharmacother* **4**: 1901–1938.
- Berthou L, Duverger N, Emmanuel F, Langouet S, Auwerx J, Guillouzo A *et al.* (1996). Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J Clin Invest* **97**: 2408–2416.
- Borradaille NM, De Dreu LE, Huff MW (2003). Inhibition of net HepG2 cell apolipoprotein B secretion by the citrus flavonoid naringenin involves activation of phosphatidylinositol 3-kinase, independent of insulin receptor substrate-1 phosphorylation. *Diabetes* **52**: 2554–2561.
- Brown MS, Goldstein JL (1997). The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**: 331–340.
- Brown PJ, Stuart LW, Hurley KP, Lewis MC, Winegar DA, Wilson JG *et al.* (2001). Identification of a subtype selective human PPAR $\alpha$  agonist through parallel-array synthesis. *Bioorg Med Chem Lett* **11**: 1225–1227.
- Chatley P, Badyal DK, Calton R, Khosla PP (2007). Combination therapy of low-dose atorvastatin and fenofibrate in mixed hyperlipidemia. *Methods Find Exp Clin Pharmacol* **29**: 217–221.
- Davidson MH, Bays HE, Stein E, Maki KC, Shalwitz RA, Doyle R (2006). Effects of fenofibrate on atherogenic dyslipidemia in hypertriglyceridemic subjects. *Clin Cardiol* **29**: 268–273.
- Derosa G, Cicero AE, Bertone G, Piccinni MN, Ciccarelli L, Roggeri DE (2004). Comparison of fluvastatin + fenofibrate combination therapy and fluvastatin monotherapy in the treatment of combined hyperlipidemia, type 2 diabetes mellitus, and coronary heart disease: a 12-month, randomized, double-blind, controlled trial. *Clin Ther* **26**: 1599–1607.
- Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK *et al.* (2005). Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* **366**: 1279–1289.
- Du X, Kristiana I, Wong J, Brown AJ (2006). Involvement of Akt in ER-to-Golgi transport of SCAP/SREBP: a link between a key cell proliferative pathway and membrane synthesis. *Mol Biol Cell* **17**: 2735–2745.
- Duez H, Chao YS, Herandez M, Torpier G, Poulain P, Mundt S *et al.* (2002). Reduction of atherosclerosis by the peroxisome proliferator-activated receptor  $\alpha$  agonist fenofibrate in mice. *J Biol Chem* **277**: 48051–48057.
- Frost RJ, Otto C, Geiss HC, Schwandt P, Parhofer KG (2001). Effects of atorvastatin versus fenofibrate on lipoprotein profiles, low-density lipoprotein subfraction distribution, and hemorheologic parameters in type 2 diabetes mellitus with mixed hyperlipoproteinemia. *Am J Cardiol* **87**: 44–48.
- Gilde AJ, Fruchart JC, Staels B (2006). Peroxisome proliferator-activated receptors at the crossroads of obesity, diabetes, and cardiovascular disease. *J Am Coll Cardiol* **48**: A24–A32.
- Goldstein JL, Bose-Boyd RA, Brown MS (2006). Protein sensors for membrane sterols. *Cell* **124**: 35–46.
- Grundy SM, Vega GL, Yuan Z, Battisti WP, Brady WE, Palmisano J (2005). Effectiveness and tolerability of simvastatin plus fenofibrate for combined hyperlipidemia (the SAFARI trial). *Am J Cardiol* **95**: 462–468.
- Han J, Parsons M, Zhou X, Nicholson AC, Gotto Jr AM, Hajjar DP (2004). Functional interplay between the macrophage scavenger receptor class B type I and pitavastatin (NK-104). *Circulation* **110**: 3472–3479.
- Hennuyer N, Tailleux A, Toppier G, Mezdoor H, Fruchart JC, Staels B *et al.* (2005). PPAR $\alpha$ , but not PPAR $\gamma$ , activators decrease macrophage-laden atherosclerotic lesions in a nondiabetic mouse model of mixed dyslipidemia. *Arterioscler Thromb Vasc Biol* **25**: 1897–1902.
- Iovine C, Lilli S, Gentile A, Patti L, Di ML, Cipriano P *et al.* (2006). Atorvastatin or fenofibrate on post-prandial lipaemia in type 2 diabetic patients with hyperlipidaemia. *Eur J Clin Invest* **36**: 560–565.
- Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR *et al.* (2005). Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* **366**: 1849–1861.
- Kourimate S, Le May C, Langhi C, Jarnoux AL, Ouguerram K, Zaïr Y *et al.* (2008). Dual mechanisms for the fibrate-mediated repression of proprotein convertase subtilisin/Kexin type 9. *J Biol Chem* **283**: 9666–9673.
- Le Jossic-Corcus C, Duclos S, Ramirez LC, Zaghini I, Chevillard G, Marti P *et al.* (2004). Effects of peroxisome proliferator-activated receptor  $\alpha$  activation on pathways contributing to cholesterol homeostasis in rat hepatocytes. *Biochim Biophys Acta* **1683**: 49–58.
- Leesnitzer LM, Parks DJ, Bledsoe RK, Cobb JE, Collins JL, Consler TG *et al.* (2002). Functional consequences of cysteine modification in the ligand binding sites of peroxisome proliferator activated receptors by GW9662. *Biochemistry* **41**: 6640–6650.
- Li AC, Brown KK, Silvestre MJ, Willson TM, Palinski W, Glass CK (2000). Peroxisome proliferator-activated receptor  $\gamma$  ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J Clin Invest* **106**: 523–531.
- Malik J, Melenovsky V, Wichterle D, Haas T, Simek J, Ceska R *et al.* (2001). Both fenofibrate and atorvastatin improve vascular reactivity in combined hyperlipidaemia (fenofibrate versus atorvastatin trial—FAT). *Cardiovasc Res* **52**: 290–298.
- Melenovsky V, Malik J, Wichterle D, Simek J, Pisarikova A, Skrha J *et al.* (2002). Comparison of the effects of atorvastatin or fenofibrate on nonlipid biochemical risk factors and the LDL particle size in subjects with combined hyperlipidemia. *Am Heart J* **144**: E6.
- Motojima K (2000). Differential effects of PPAR $\alpha$  activators on induction of ectopic expression of tissue-specific fatty acid

- binding protein genes in the mouse liver. *Int J Biochem Cell Biol* **32**: 1085–1092.
- Muhlestein JB, May HT, Jensen JR, Horne BD, Lanman RB, Lavasani F *et al.* (2006). The reduction of inflammatory biomarkers by statin, fibrate, and combination therapy among diabetic patients with mixed dyslipidemia: the DIACOR (Diabetes and Combined Lipid Therapy Regimen) study. *J Am Coll Cardiol* **48**: 396–401.
- Muoio DM, Maclean PS, Lang DB, Li S, Houmard JA, Way JM *et al.* (2002). Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR)  $\alpha$  knock-out mice. Evidence for compensatory regulation by PPAR $\delta$ . *J Biol Chem* **277**: 26089–26097.
- Nohturfft A, Bose-Boyd RA, Scheek S, Goldstein JL, Brown MS (1999). Sterols regulate cycling of SREBP cleavage-activating protein (SCAP) between endoplasmic reticulum and Golgi. *Proc Natl Acad Sci USA* **96**: 11235–11240.
- Porstmann T, Griffith B, Chung YL, Delpuech O, Griffith JR, Downward J *et al.* (2005). PKB/Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. *Oncogene* **24**: 6465–6481.
- Radhakrishnan A, Sun LP, Kwon HJ, Brown MS, Goldstein JL (2004). Direct binding of cholesterol to the purified membrane region of SCAP: mechanism for a sterol-sensing domain. *Mol Cell* **15**: 259–268.
- Saklamaz A, Comlekci A, Temiz A, Caliskan S, Ceylan C, Alacacioglu A *et al.* (2005). The beneficial effects of lipid-lowering drugs beyond lipid-lowering effects: a comparative study with pravastatin, atorvastatin, and fenofibrate in patients with type IIa and type IIb hyperlipidemia. *Metabolism* **54**: 677–681.
- Sebestjen M, Zegura B, Keber I (2002). Both cerivastatin and fenofibrate improve arterial vasoreactivity in patients with combined hyperlipidaemia. *J Intern Med* **251**: 77–85.
- Shimano H (2001). Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res* **40**: 439–452.
- Song G, Ouyang G, Bao S (2005). The activation of Akt/PKB signaling pathway and cell survival. *J Cell Mol Med* **9**: 59–71.
- Staels B, Van TA, Andreu T, Auwerx J (1992). Fibrates influence the expression of genes involved in lipoprotein metabolism in a tissue-selective manner in the rat. *Arterioscler Thromb* **12**: 286–294.
- Sun L, Nicholson AC, Hajjar DP, Gotto Jr AM, Han J (2003). Adipogenic differentiating agents regulate expression of fatty acid binding protein and CD36 in the J744 macrophage cell line. *J Lipid Res* **44**: 1877–1886.
- Vega GL, Ma PT, Cater NB, Filipchuk N, Meguro S, Garcia-Garcia AB *et al.* (2003). Effects of adding fenofibrate (200 mg/day) to simvastatin (10 mg/day) in patients with combined hyperlipidemia and metabolic syndrome. *Am J Cardiol* **91**: 956–960.
- Vu-Dac N, Schoonjans K, Kosykh V, Dallongeville J, Fruchart JC, Staels B *et al.* (1995). Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. *J Clin Invest* **96**: 741–750.
- Wang TD, Chen WJ, Lin JW, Cheng CC, Chen MF, Lee YT (2003). Efficacy of fenofibrate and simvastatin on endothelial function and inflammatory markers in patients with combined hyperlipidemia: relations with baseline lipid profiles. *Atherosclerosis* **170**: 315–323.
- Wierzbicki AS (2006). FIELDS of dreams, fields of tears: a perspective on the fibrate trials. *Int J Clin Pract* **60**: 442–449.
- Winkler K, Weltzien P, Friedrich I, Schmitz H, Nickell HH, Hauck P *et al.* (2004). Qualitative effect of fenofibrate and quantitative effect of atorvastatin on LDL profile in combined hyperlipidemia with dense LDL. *Exp Clin Endocrinol Diabetes* **112**: 241–247.
- Wu JC, Merlino G, Fausto N (1994). Establishment and characterization of differentiated, nontransformed hepatocyte cell lines derived from mice transgenic for transforming growth factor alpha. *Proc Natl Acad Sci USA* **91**: 674–678.
- Yang T, Espenshade PJ, Wright ME, Yabe D, Gong Y, Aebersold R *et al.* (2002). Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* **110**: 489–500.
- Zhou RH, Yao M, Lee TS, Zhu Y, Martins-Green M, Shyy JY (2004). Vascular endothelial growth factor activation of sterol regulatory element binding protein: a potential role in angiogenesis. *Circ Res* **95**: 471–478.



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