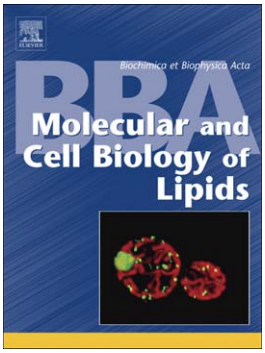

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Differential Regulation of the Human versus the Mouse Apolipoprotein AV Gene by PPARalpha

Implications for the study of pharmaceutical modifiers of hypertriglyceridemia in mice

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ABSTRACT

Mice have been used widely to define the mechanism of action of fibric acid derivatives. The fibrates are pharmacological agonists of the peroxisome proliferator-activated receptor α (PPARα), whose activation in human subjects promotes potent reduction in plasma levels of triglycerides (TG) with concomitant increase in those of HDL-cholesterol. The impact of PPARα agonists on gene expression in humans and rodents is however distinct; such distinctions include differential regulation of key genes of lipid metabolism. We evaluated the question as to whether the human and murine genes encoding apolipoprotein apoAV, a regulator of plasma concentrations of TG-rich lipoproteins, might be differentially regulated in response to fibrates. Fenofibrate, a classic PPARα agonist, repressed expression of mouse *Apoa5 in vivo* in a mouse model transgenic for the human *APOA5 gene;* by contrast, expression of the human ortholog was up-regulated. Our findings are consistent with the presence of a functional PPAR-binding element in the promoter of the human *APOA5* gene; this element is however degenerate and non-functional in the corresponding mouse *Apoa5* sequence, as demonstrated by reporter assays and gel shift analyses. These data further highlights the distinct mechanisms which are implicated in the metabolism of TG-rich lipoproteins in mice as compared to man. They equally emphasize the importance of the choice of a mouse model for investigation of the impact of pharmaceutical modifiers on hypertriglyceridemia.

Introduction

Hypertriglyceridemia is an independent and predictive risk factor for atherosclerosis and a key feature of the metabolic syndrome [1-3].

The gene encoding apolipoprotein (apo) *APOA5* is recognized as a potential determinant of plasma levels of triglyceride (TG) and TG-rich lipoproteins in mice[4]. Indeed, overexpression of *APOA5* in genetically-modified mouse models consistently leads to reduction in circulating TG concentrations, whereas apoAV deficiency is associated with hypertriglyceridemia [5, 6]. *In vitro* and *in vivo* experiments in mice support a role for apoAV in the potentiation of lipoprotein lipase (LPL) activity [7-9]. LPL is a central player in determining plasma TG levels, as LPL catalyses the hydrolysis of the hydrophobic core of TG-rich lipoproteins (TRL), including chylomicrons and VLDL. In addition, apoAV has been proposed to enhance VLDL remnant removal by facilitating particle binding to the LDL receptor and interaction of these particles with the LDL receptor-related protein (LRP) and mosaic type-1 receptor (SorLA) [10, 11]

Fibrates are lipid-modifying agents which are widely employed in the treatment of hypertriglyceridemia. Thus, pharmacological activation of the nuclear receptor peroxisome proliferator-activated receptor-α (PPARα, NR1C1) by fibrates lowers plasma TG levels not only by increasing conversion of fatty acids to acyl-CoA derivatives and fatty acid oxidation (β-oxidation), but in addition, by stimulating intravascular LPL-mediated lipolysis of TRL [12-14]. Following heterodimerization of activated PPARα with the retinoid X receptor (RXR), the nuclear receptor complex binds to peroxisome proliferator response elements (PPREs). As a direct consequence, expression of target genes implicated in fatty acid metabolism (L-FABP, mitochondrial 3-hydroxy-3-methylglutaryl-Coenzyme A synthase) and β-oxidation

(acyl-CoA oxidase, ACOX) [15-18] is regulated. In addition, fibrates activate the expression of the *LPL* gene while concomitantly repressing that of the LPL inhibitor, *APOC3* [19, 20].

Recent studies have shown that primate *APOA5* expression is regulated by PPARα. In human hepatic cells, activation of PPARα by several agonists induces elevation in *APOA5* mRNA levels. Indeed, analysis of the human *APOA5* promoter region revealed the presence of a functional PPRE, consisting of a direct repeat (DR1) motif 5'-AGGTTAAAGGTCA-3' located at –271 nt from the transcription start site [21, 22]. Consistent with this finding, 14 days of treatment with 0.3 mg/kg/day of the PPARα agonist LY570977 L-lysine in the cynomolgus monkey resulted in a 2-fold increase in plasma apoAV concentration and a 50% decrease in TG levels [23]. These findings demonstrated that pharmacological activation of PPARα can lead to increase in plasma apoAV levels, thereby shedding new light on the molecular mechanisms whereby PPARα agonists lower plasma TG levels in primates. It can therefore be proposed that apoAV contributes to the hypotriglyceridemic effect of PPARα agonists by enhancing LPL activity upon PPARα activation.

Peroxisome proliferators were originally characterized in rodents as agents that cause peroxisome proliferation and hepatocarcinoma when chronically administered [24]. On the other hand, PPAR agonists are not associated with an elevated risk of liver cancer or peroxisome proliferation in humans, indicating a species difference in the effects of fibrates in hepatic tissue [25]. Differential regulation of genes involved in lipid metabolism by pharmacological activation of PPARα has equally been reported between species. Thus, whereas the major HDL apolipoprotein gene *APOAI* is induced by PPARα in humans, the mouse orthologous gene is down regulated [26]. Recently, Dorfmeister et al. have shown that a fish oil

diet rich in polyunsaturated fatty acids or rosiglitazone, both of which are PPARγ activators, increased *Apoa5* mRNA levels in livers of obese and insulin-resistant Zucker rats, but tended to diminish both liver and plasma apoAV. However, they also observed a lack of increase in mRNA levels in primary rat hepatocytes treated with PPAR-α or -γ specific agonists, thereby suggesting that rat *Apoa5* may be insensitive to stimulation by PPAR agonists, and that the effects of rosiglitazone in Zucker rats are not directly mediated by PPARγ [27].

In the present study, we evaluated the question as to whether the human and murine *APOA5* genes could be differentially regulated by fibrates. We confirmed that the human *APOA5* promoter activity is up-regulated following PPARα and fenofibrate treatment in a hepatic cell line. In contrast, the proximal promoter region of the mouse *Apoa5* gene was not responsive. *In vivo* analysis of mice transgenic for the human *APOA5* gene (h*APOA5*-mice) confirmed these results. Indeed, short term oral gavage of h*APOA5*-mice with fenofibrate resulted in a dose-dependent increase of human *APOA5* mRNA levels in the liver, whereas those of mouse *Apoa5* were concomitantly slightly down-regulated. These data establish that hepatic expression of the mouse and human *APOA5* genes is regulated in an opposing manner by fibrates *in vivo*, and further highlight a critical difference in the regulation of TRL metabolism between mice and humans.

Materials and methods

Animal protocols— All animal procedures were performed with approval from the Direction Départementale des Services Vétérinaires, Paris, France, under strict compliance with European Community Regulations. The animals were housed in a conventional animal facility on a 6 am to 6 pm dark/light cycle. They were weaned at 21 days and fed a normal mouse chow diet *ad libitum* (RM1; Dietex France). Human *APOA5* transgenic mice in an FVB genetic background were generously provided by Dr E.M. Rubin (Genome Sciences Department, Lawrence Berkeley National Lab, CA). The *APOA5* transgene was maintained in a hemizygous state by breeding transgenic animals with wild-type FVB mice. Genotyping of the mice were performed as previously described [5]

2-3 month old h*APOA5* male mice and wild-type control male littermates were given an oral gavage of 0.2 ml fenofibrate (Sigma) for five days, either once daily at a dose of 100 mg/kg or twice a day at a dose of 125 mg/kg. h*APOA5* transgenic and control non-treated groups received 0.2 ml of vehicle only (0.5% hydroxypropyl methylcellulose, 1% Tween 80). Four hours after the last dose, blood was collected under isoflurane anaesthesia. The animals were then sacrificed by cervical dislocation and livers were collected, rinsed in ice-cold PBS, and snap-frozen in liquid nitrogen. RNAs were prepared from frozen tissue specimens using TRIzol reagent (Invitrogen).

Plasma and lipid analyses - Blood samples were collected in EDTA-coated Microvette tubes (Sarstedt) by retro-orbital bleeding using heparinized micro-hematocrit capillary tubes. Plasma samples were stored frozen at -80°C. Total cholesterol (Roche Diagnostics) and triglyceride (Biomérieux) concentrations were measured by enzymatic colorimetric assays. Plasma lipoproteins were fractionated by

gel filtration on two Superose 6 (Amersham Biosciences) columns connected in series using a BioLogic DuoFlow Chromatography System (BioRad) [28].

Real Time PCR Quantification of mRNAs— cDNA preparation and quantitative PCR analysis were performed as previously described [29]. The sequences of forward and reverse primers are shown in Table I. The specificity of the primers was verified by showing that the real time reverse transcriptase (RT)-PCR reaction product generated a single band after agarose gel electrophoresis. In addition, each couple of primers was tested in successive dilutions of cDNA to analyze and validate its efficiency. The levels of expression of the target genes were normalized to mouse ribosomal protein S3 (*Rps3*) expression to compensate for variations in input RNA amounts (*Rps3* levels were unaffected by fenofibrate treatment).

Plasmids— p–617/+18 hAvLUC containing the 5' flanking region of the human *APOA5* gene (-617 to +18) cloned in front of the promoter-less luciferase gene has been previously described [21]. To generate the luciferase reporter plasmids p-1831/+17 and p-617/+17 mAvLUC, C57BL/6 mouse genomic DNA was amplified by PCR using the primer pairs mAPOAV-1831f 5'-AGT CGG TAC CGG CGT GGC TCA CTG TTT TTA-3' and mAPOAV+17r 5'-AGT CAG ATC TCA CCT GCT CGG TTC TGG G-3', and mAPOAV-617f 5'-AGT CGG TAC CTG TGA GGG AAG ACT CTT GAG G-3' and mAPOAV+17r, respectively. The PCR products were digested at their 5' and 3' ends by KpnI and BglII restriction enzymes, respectively (restriction sites underlined in the primer sequences), and subsequently cloned in KpnI/BglII-digested pGL3 basic vector (Promega). The sequences were verified in the final constructs by DNA sequencing. Expression plasmid for PPARα has been previously described [21].

Cell Transfection and Reporter Assays— Human hepatoblastoma Hep3B cell lines were cultured in Dulbecco's modified Eagle medium (Invitrogen) supplemented with 10% (v/v) foetal calf serum. On day 0, cells were seeded on 24-well plates at a density of 10^5 cells/well. On day 1, cells were transfected with FuGENE 6 transfection reagent (Roche Applied Science) according to the manufacturer's instructions. Typically, each well of a 24 well plate received 250 ng of human or mouse *APOA5* promoter constructs, 15 ng of a β-galactosidase expression plasmid and, either 85 ng of the pSG5-PPARα expression plasmid or a corresponding amount of empty pSG5 vector. After incubation for 5h, the medium was replaced by fresh complete medium in the presence of either 5 μM fenofibrate (Sigma) or vehicle (DMSO). On day 4, cell extracts were prepared in lysis buffer (Promega) and β-galactosidase and luciferase activities were determined as described previously [30]. Luciferase values were normalized to β-galactosidase activities. Transfection data represent the mean (+/- standard deviation) of four independent experiments each performed in triplicate.

***In Vitro* Transcription/Translation and Electro Mobility Shift Assays (EMSAs)—** Human PPARα and RXRα proteins were synthesized *in vitro* from the expression plasmid using TNT® Quick Coupled transcription/translation system (Promega) according to the instructions of the manufacturer. In order to obtain an unprogrammed lysate as a negative control for EMSA, a reaction was performed with the empty vector pSG5. Double-stranded oligonucleotides were radiolabelled by fill-in with the Klenow fragment of DNA polymerase I and used as probes. Samples were electrophoresed at 4 ºC on a 4.5% polyacrylamide gel in 0.5X TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA, pH 8.0). Gels were dried and analyzed using PhosphorImager STORM 860 and ImageQuant software (Amersham Biosciences).

Statistical analysis.

The statistical significance of the differences between groups was evaluated using the unpaired two-tailed Student t-test. $P<0.05$ was considered significant.

Results

Presence of a functional PPRE in the proximal flanking region of the human *APOA5* 5' gene, but not in the mouse *Apoa5* gene –

Previously, we identified two PuGGTCA hexamers binding sites separated by a single nucleotide (DR1) between nt –271 and –259 in the promoter sequence of the human *APOA5* gene. Luciferase assay and gel shift assays allowed us to demonstrate that this *APOA5* DR1 is a genuine PPRE [21]. Interestingly, comparison of the corresponding human, baboon, chimpanzee, mouse and rat 5' flanking sequences surrounding the DR1/PPRE element revealed that the human *APOA5* DR1 element is highly conserved among primates, but not in the rat or mouse gene promoters (Fig1A). Indeed, alignment of the sequences reveals four nucleotide differences in the latter species as compared to the human *APOA5* DR1 (hDR1) sequence. Electrophoretic mobility shift assay analysis revealed that these nucleotide changes in the mouse *Apoa5* sequence abolished binding of the PPAR-RXR heterodimer (Fig1B, lane 8), whereas a specific protein–DNA complex was clearly detected when a probe for the human *APOA5*-DR1 sequence was incubated in the presence of the recombinant PPARα and RXR proteins (Fig1B, lane 7). These data are in agreement with recent studies demonstrating that both HNF-4 [31] or RORα [32], nuclear factors which bind to response elements consisting of the core recognition sequence AGGTCA, bound to the human *APOA5*-DR1sequence, but not to the corresponding mouse sequence.

Mouse *APOA5* promoter is not regulated by PPARα -

To search for other potential PPREs in mouse *Apoa5* promoter, we cloned 1.8 kb (–1831/+18mAvLUC) and 0.6 kb (–617/+18mAvLUC) of the 5' flanking region upstream of the transcription start site of the murine *Apoa5* gene sequence into the

firefly luciferase pGL3-basic vector. In Hep3B cells, the luciferase activities of these constructs remain unchanged when co-transfected with PPARα expression vector alone or in combination with the PPARα agonist fenofibrate (Fig 2). Conversely, in the same experiment, a human promoter construct previously demonstrated to respond to PPARα activation [21] displayed a statistically significant increase in activity of approximately 3.5- and more than 4.5-fold when co-transfected with a PPARα expression vector in absence or presence of fenofibrate, respectively. Of note, in contrast to what we previously described with the PPARα agonist GW9003 using similar experimental conditions [21], the enhanced effect of fenofibrate on the induction of human *APOA5* promoter activity by the over-expression of PPARα was only modest and did not reach statistical significance. This discrepancy may be due to the difference of affinity between the two agonists for PPARα and/or the amount of effective drug available in the cell. Taken together, these data demonstrate that the proximal 5' region in the mouse gene is not responsive to PPARα *in vitro*.

Fibrate treatment upregulates human *APOA5* gene expression, but downregulates that of mouse *Apoa5* gene *in vivo* -

To determine whether the mouse *Apoa5* gene could respond to PPARα agonists through distant regulatory regions, we treated mice for a short period with fenofibrate at two different dosages, and evaluated mouse *Apoa5* mRNA levels in the liver. These studies were performed concomitantly in wild-type (WT) mice and in mice transgenic for a 26-kbp fragment of human chromosome XI solely containing the entire *APOA5* gene, including its flanking regulatory regions [5]. Under the conditions tested, human *APOA5* mRNA levels measured by Q-PCR were increased modestly, and dose dependently (1.3- and 1.6-fold), when h*APOA5*-mice received an oral daily gavage of

fenofibrate (100 or 250 mg) (Fig 3A). Comparatively, mouse *Apoa5* gene expression was decreased similarly (20%) at the two fenofibrate doses in both WT and h*APOA5*-mice (Fig 3B). As a control for the efficacy for short term fenofibrate treatment, we quantified the level of expression of genes known to be either repressed or activated by PPARα. Thus, we observed that fenofibrate treatment decreased the steady state mRNA levels of both m*Apoa1* (Fig3C) and m*Apoc3* (Fig 3D). In contrast, m*Lpl* (Fig 3F) and m*Acox* (Fig.3E) mRNA levels were strongly upregulated in fenofibrate-fed mice. For all these genes, the effects were comparable in both WT and h*APOA5*-mice.

Taken together, these results demonstrate an inverse response of the mouse and human *APOA5* genes to PPARα agonist treatment, in which transcription of the human gene is activated *in vivo*, whereas hepatic mRNA levels of the murine ortholog are downregulated in response to fenofibrate treatment.

Impact of fenofibrate treatment on plasma lipid and lipoprotein levels in WT and h*APOA5* mice-

The impact of a 5-day fibrate treatment in both WT and h*APOA5*-mice on plasma lipid and lipoprotein levels was evaluated at the 250 mg/kg dosage. Lipid concentrations were determined at the time of sacrifice, which was performed 4-h after the final gavage of non-fasted mice. Plasma TG levels were lower in h*APOA5*-mice as compared to WT mice (Fig. 4A), as previously reported [5]. In addition, a slight and similar reduction in plasma TG levels was observed in both drug treated-groups, but this reduction did not reach statistical significance. Surprisingly, plasma total cholesterol (TC) (Fig. 4B) levels were found to be statistically lower in h*APOA5*-mice than in WT untreated-mice. Plasma TC was equally found to be lower in

fenofibrate-fed h*APOA5*-mice as compared to untreated-h*APOA5*-mice; such a reduction was not observed in the WT group. However, fenofibrate treatment markedly and similarly modified the distribution of lipoprotein cholesterol in both WT and h*APOA5*-mice. Indeed, cholesterol content was reduced in fractions corresponding to HDL particles of normal size, with appearance of larger HDL particles (Fig. 4C). Such specific change in HDL particle distribution is, in all likelihood, a direct consequence of down-regulation of hepatic scavenger receptor SR-BI expression at the protein level by fibrates which has been previously reported in mice [33]. Indeed, reduced expression of SR-BI consistently results in the appearance of larger HDL, rich in cholesterol, but equally in elevation of plasma levels of HDL-C (and plasma TC) in mice [28]. In the present study, the concomitant marked reduction of mouse *APOA1* gene expression following fenofibrate-250 treatment in the WT group (Fig. 3C) most likely masked the HDL-C raising effect of knockdown expression of SR-BI; thus resulting in no net change in plasma TC levels (Fig. 4B) but in the presence of large HDL particles and a decrease in the level of regularly sized HDLs (Fig. 4C).

Discussion

It is well established that PPARα activation has distinct effects in human and mice, especially on peroxisome proliferation [25]. In addition, with respect to lipid metabolism, it is established that PPARα agonists differentially regulate genes between humans and rodents including, for example, the *APOA1 or PLTP genes*. Here, we demonstrate that mouse *Apoa5* is repressed by fenofibrate treatment in a mouse model transgenic for the human *APOA5 gene*, whereas the human ortholog is up-regulated. These findings are consistent with the presence of a functional PPRE in the human *APOA5* promoter, which is degenerate in the corresponding mouse *Apoa5* sequence. These observations substantiate the differential impact of PPARα activators on lipid metabolism in human and rodents.

Fibrates are potent pharmacological agents for reduction of plasma levels of triglycerides and TG-rich lipoproteins in humans, as they notably induce elevation in LPL activity through PPAR-mediated activation of *LPL* gene expression, but equally repress expression of the *APOC3 gene*, an inhibitor of LPL activity. Although we observed a marked increase in hepatic *Lpl* expression and a concomitant decrease in *Apoc3* expression associated with fenofibrate treatment in WT mice, nonetheless there was only a trend towards fall in TG levels in these animals as compared to untreated-controls. A similar observation was made in *hAPOA5* mice, as we did not observe an additional reduction in plasma TG levels in h*APOA5*-mice in response to drug treatment, despite evidence both of activation of human *APOA5* gene expression and similar PPAR-mediated changes in expression of *Lpl* and *Apoc3* genes as seen in the WT group. The experimental conditions (non-fasting chow-fed mice, emulsifier molecules used for oral gavage) may have masked a net lowering effect of fenofibrate

on plasma TG levels and in particular in hAPOA5-mice. In the latter group, one could have expected diminished TG concentrations following h*APOA5* activation. Such activation was nonetheless only moderate and the concomitant reduction in levels of mouse *Apoa5* in response to drug treatment may have introduced a confounding effect. Moreover, it should be mentioned that up-regulation of h*APOA5* was not confirmed in plasma, which represents a limitation to the present study. Nevertheless, these results may equally raise the question of the role of ApoAV in the overall TG lowering action of fibrates. Notably, if ApoAV and TG plasma levels were found inversely correlated in cynomolgus monkeys receiving the potent and selective PPARα agonist LY570977 L-lysine, the marked fall in plasma TG preceded the elevation of plasma levels of ApoAV in this study [23]. Finally, apparent contradictions are evident in the literature with respect to the relationship between plasma TG and apoAV levels. Indeed, whereas transgenic mice overexpressing *hAPOA5* displayed significant reduction in plasma TG concentrations, and whereas apoA-V deficient mice exhibited a net increase in plasma TG levels, it has been recently demonstrated that apoAV concentrations are positively correlated with TG levels in normolipidemic h*APOA5* mice, as reported in humans [34].

Whereas consistent associations between common polymorphisms of the *APOA5* gene and plasma TG levels have been reported, the demonstration of such genetic associations with plasma cholesterol levels is less clear. Nonetheless, a number of studies have highlighted associations of genetic variants of *APOA5* with circulating HDL-C levels in populations of different ethnic origin [5, 6, 35-39], thereby suggesting that apoAV may contribute to regulation of HDL-C levels. Indeed, apoAV is mainly present in HDL during the fasting period, which could suggest a potential role of apoAV in HDL-cholesterol metabolism. In the present study, significantly

lower plasma TC and HDL-C levels were observed in chow-fed h*APOA5*-mice as compared to wild-type controls. Such differences were not reported in the original publication describing the generation of h*APOA5*-mice, although a trend existed [5]. However, it is of note that adenovirus-mediated overexpression of apoAV in mice resulted in a profound decrease in TG concentrations but equally plasma cholesterol levels[6]. Moreover, cross-breeding of mice overexpressing human *APOA5* with either human *APOC3* transgenic mice or ApoE2 knock-in mice produced a decrease in plasma cholesterol concentrations [7, 40]. Further studies focusing on the role of apoAV in cholesterol and HDL metabolism are clearly needed to understand whether there exists a potential direct action of apoAV or indirect consequences of apoAV activities on HDL-C levels and HDL particle distribution. Interestingly, fenofibrate treatment resulted in significant diminution in plasma cholesterol levels in h*APOA5*-mice, an effect not observed in the WT group. Whether increase in hapoAV levels following fenofibrate treatment may have contributed to the diminished cholesterol levels observed in transgenic mice remains speculative and requires additional studies.

Hepatic mRNA levels of the mouse *Apoa1* and *Apoc3* genes were markedly reduced in response to oral gavage with fenofibrate. This effect, which was dose-dependent (Fig. 3CD), may result from PPARα-mediated induction of the nuclear receptor Rev-erbα [20, 41]. Indeed, the presence of a response element for this repressor in the promoters of rodent *Apoa1* [42] and *Apoc3* genes support this hypothesis. The sequence motifs for Rev-erbα and the nuclear hormone receptor RORα are closely related [43] which explains that the Rev-erbα response element present in the *apoa1* gene is also a RORα element [42, 44]. Human *APOA5* is a target gene for ROR

through binding to the half-core AGGTCA DR1/PPRE site[45], whereas mouse *apoa5* is not, due to a nucleotide difference in the half-core site which prevents RORα binding to the mouse promoter [32]. Consistently, mouse *Apoa5* gene expression is not affected in the staggerer mutant mouse which carries a deletion in the gene encoding for RORα [32]. Thus, the modest decrease of m*Apoa5* mRNA levels that we observed in the livers of fenofibrate-fed mice is unlikely to result from PPAR-induced Rev-erbα repression mechanism, as most likely occured for the *Apoa1* and *Apoc3* genes. This hypothesis is consistent with the fact that the activity of the mouse *Apoa5* promoter was not repressed by PPARα and fenofibrate treatment in transient transfection assays (Fig. 2).

In conclusion, this study provides additional evidence that extrapolation from mouse to man of experimental findings involving the pharmacological action of fibric acid or their derivatives on lipid metabolism must be conducted with considerable caution. Nonetheless, comparison of h*APOA5*-mice with non-transgenic controls in response to treatment with PPAR agonists may prove useful in deciphering the physiological significance of apoAV in the regulation of TG metabolism in fasting or non-fasting conditions, and equally in normo- or hyperlipidemic contexts. In the latter context, cross-breeding of h*APOA5*-mice with the ApoE2 knock-in mouse model recently allowed demonstration of an atheroprotective effect of hapoAV overexpression on a background of mixed dyslipidemia; this effect was further enhanced upon h*APOA5* gene activation by fenofibrate[40]. Finally, such studies may highlight a potential mechanism by which apoAV could impact HDL-C levels. In this respect, crossbreeding of h*APOA5*-mice with the recently-described hyperlipidemic E3L.CETP mouse model [46], in which the dual ability of fibrates to potently reduce

plasma TG and concomitantly increase HDL-C was clearly demonstrated, may constitute an interesting approach.

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Disclosures

The authors have no financial conflicts of interest.

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FIGURE LEGENDS

FIGURE 1. The human *APOA5*, but not mouse *Apoa5*, 5' flanking region contains a functional PPRE **(*A)***, Sequence comparison of the human DR1/PPRE element present in the 5' flanking region of the human *APOA5* gene to the corresponding baboon, chimpanzee, mouse and rat sequences. The *gray boxes* denote the nucleotide differences between the primate and rodent sequences. The hexameric sites are in *boldface type* and their orientations are indicated by *arrows*. (***B)*** EMSAs were performed using labelled double-stranded oligonucleotides corresponding to the human *APOA5* gene sequence spanning nt –275 to –247 (hDR1) or the corresponding sequence in mouse *Apoa5* gene (mDR1). Oligonucleotides were incubated with either *in vitro* transcribed/translated PPARα, or RXRα, or PPARα and RXRα, or with unprogrammed reticulocyte lysates (pSG5). The PPARα/RXRα-hDR1 complex is indicated by an *arrow*.

FIGURE 2. **Transactivation of the human but not the mouse *APOA5* gene promoter by PPARα and fenofibrate.** Hep3B cells were transfected with plasmids containing a luciferase reporter gene driven by either the –617/+18 5'-flanking region of the human *APOA5* gene or the –617/+18 or -1831/+18 5'-flanking regions of the mouse *Apoa5* gene. The *APOA5* promoter constructs were co-transfected with the PPARα expression vector (or corresponding control vector pSG5) in the presence of either 5 μM fenofibrate or vehicle (DMSO). Results are expressed as -fold induction over control. **, $p < 0.001$

FIGURE 3. Gene activation in livers of wild-type and h*APOA5*-mice treated with fenofibrate. Wild-type (WT) and h*APOA5* transgenic mice were treated with vehicle (*control*), 100 mg/kg/day (fenofibrate-100 group), or 250 mg/kg/day (fenofibrate-250 group) of fenofibrate for 5 days. *A*, Total RNA was extracted for analysis by real time RT-PCR as described under "Experimental Procedures". human *APOA5 (A)*, mouse *Apoa5* (B), mouse *Apoa1* (C), mouse *Apoc3* (D), mouse *Acox* (E) and mouse *Lpl* (F)

mRNA levels, normalized to mouse *Rps3* content, are expressed relative to untreated WT animals set as 100% (except for the h*APOA5* gene whose 100% reference corresponds to untreated-h*APOA5*-mice) (mean±S.D.). Only data for control animals of the fenofibrate-250 group study are shown for more clarity. Significant differences compared with the corresponding untreated controls are as follows: *, $p < 0.05$; **, $p < 0.001$ N.D., not detected

Figure 4. Plasma lipid parameters in fenofibrate-treated mice: Levels (mg/dl) of triglycerides (A) and total cholesterol (B) and lipoprotein cholesterol distribution (C) were determined in plasma from non-fasted wild-type (WT) or h*APOA5* mice treated with vehicle (*control*) or 250 mg/kg/day of fenofibrate for 5 days. Approximate elution volumes for particles in the size ranges of VLDL, LDL, and HDL are indicated in (C). Significant differences are as follows: *, $p < 0.05$; **, $p < 0.001$; NS: Not significantly different from respective controls

TABLE 1

Gene Accession number	Full name	Sense primer	Antisense primer	Amplicon
hAPOAV NM_052968.3	Human apolipoprotein A-V	5'-AGCTGGTGGGCTGGAATTT-3'	5'-GGCCACCTGCTCCATCAG-3'	77 bp
mAPOAV NM_080434.3	Mouse apolipoprotein A-V	5'-CCTTACGCAGAACGCTTGGT-3'	5'-TCCTTCGCCTTACGTGTGAGT-3'	146 bp
mLPL NM_008509.2	Mouse Lipoprotein Lipase	5'-GAGCCAAGAGAAGCAGCAAG-3'	5'-CCATCCTCAGTCCCAGAAAA-3'	103 bp
mAPOAI NM_009692.3	Mouse apolipoprotein A-I	5'-AGCGGCAGAGACTATGTGTC-3'	5'-ACGGTTGAACCCAGAGTGTC-3'	98 bp
mAPOC3 NM_023114.3	Mouse apolipoprotein C-III	5'-GTACAGGGCTACATGGAACAA-3'	5'-TATCGGACTCCTGCACGCTACT-3'	70 bp
mAcox1 NM_015729.2	Mouse acyl-Coenzyme A oxidase 1	5'-GGGAGTGCTACGGGTTACATG-3'	5'-CCGATATCCCCAACAGTGATG-3'	91 bp
mGUSb NM_010368.1	Mouse glucuronidase, beta	5'-CTCATCTGGAATTTCGCCGA-3'	5'-GGCGAGTGAAGATCCCCTTC-3'	82 bp

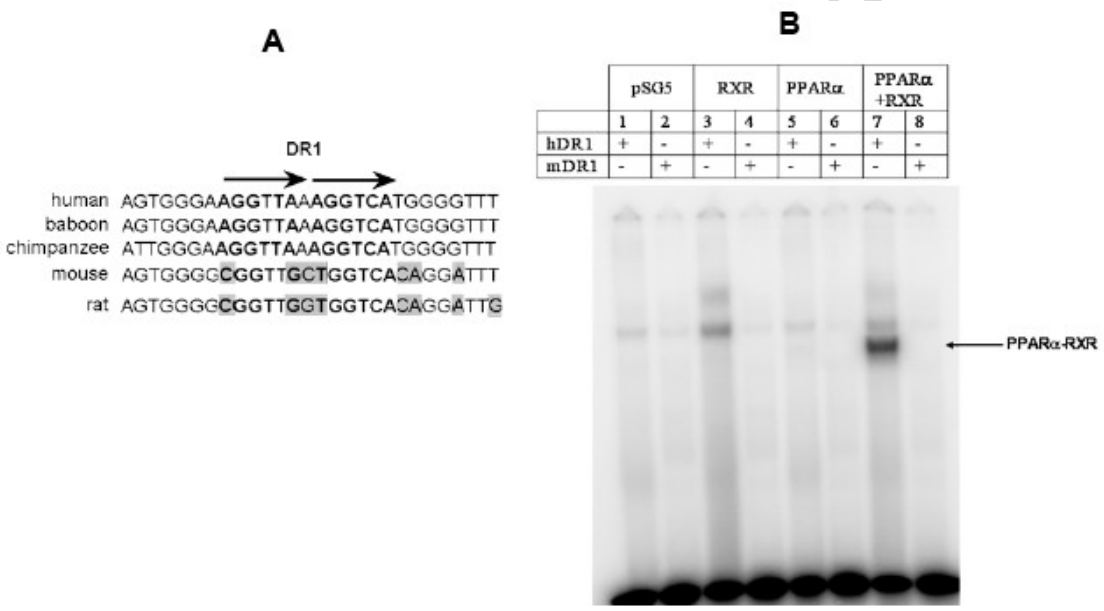


Fig.1

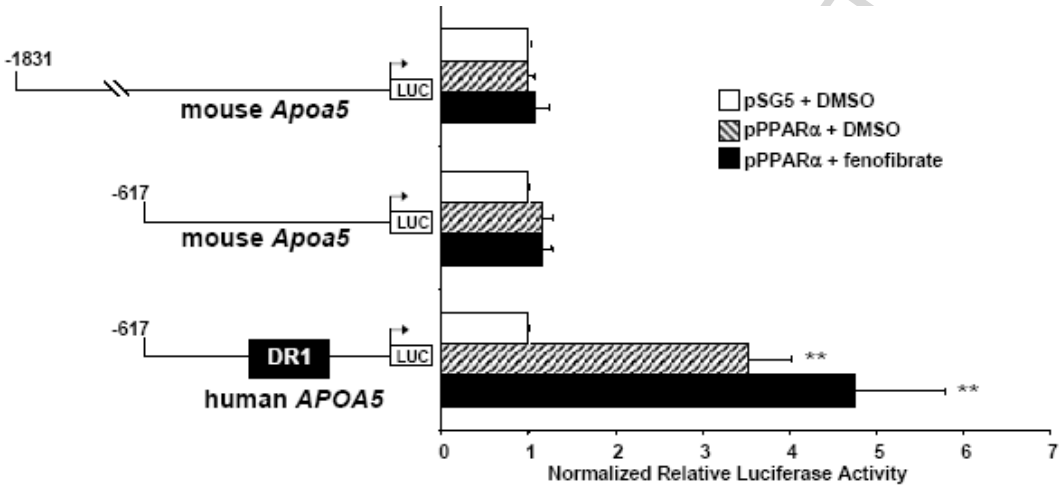


Fig.2

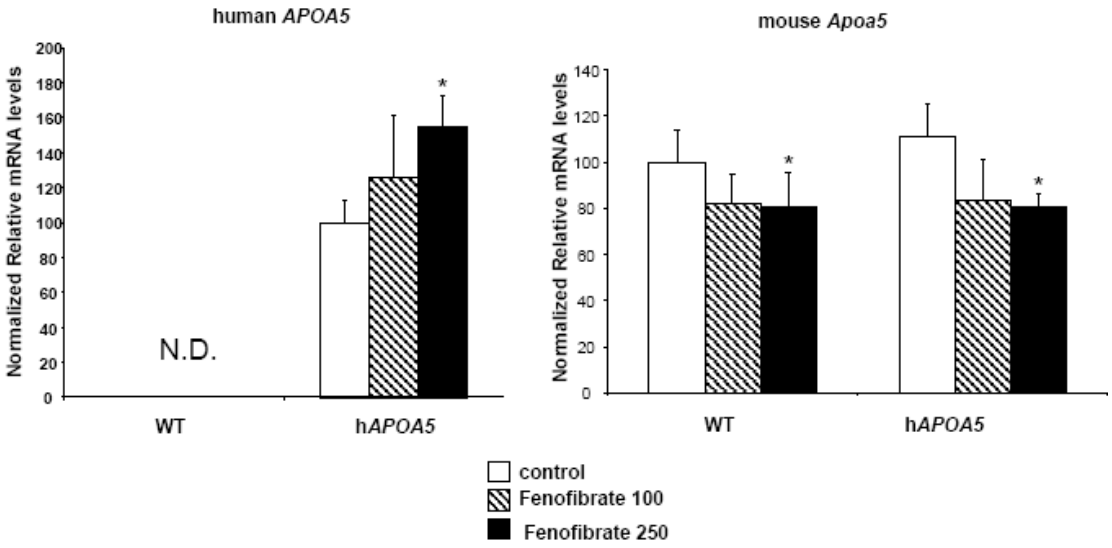
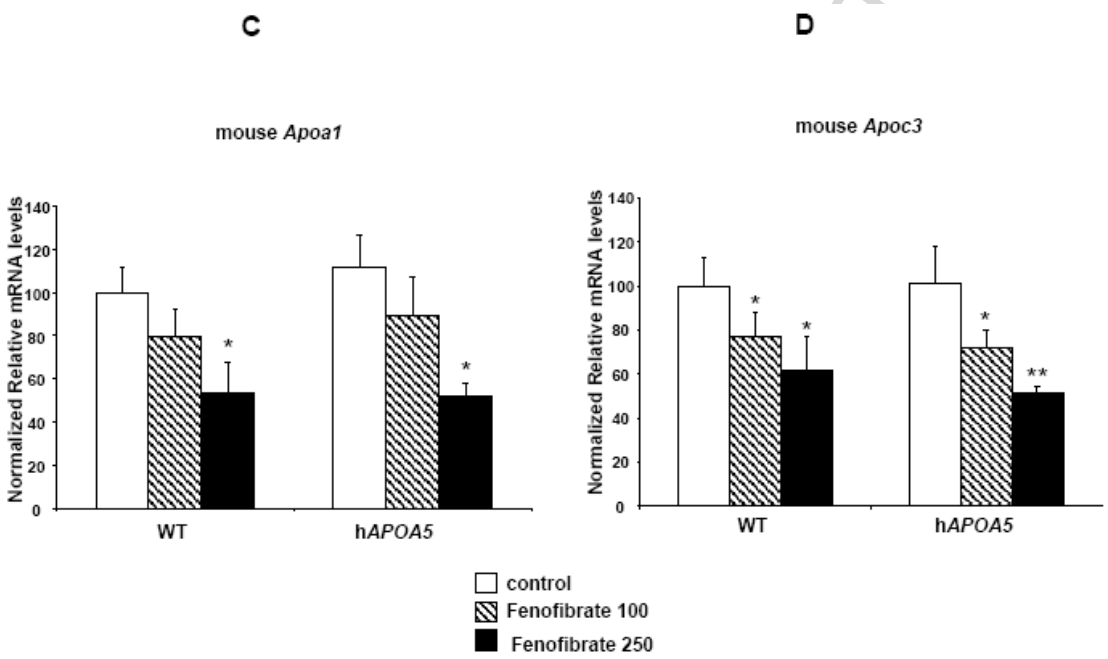


Fig.3AB



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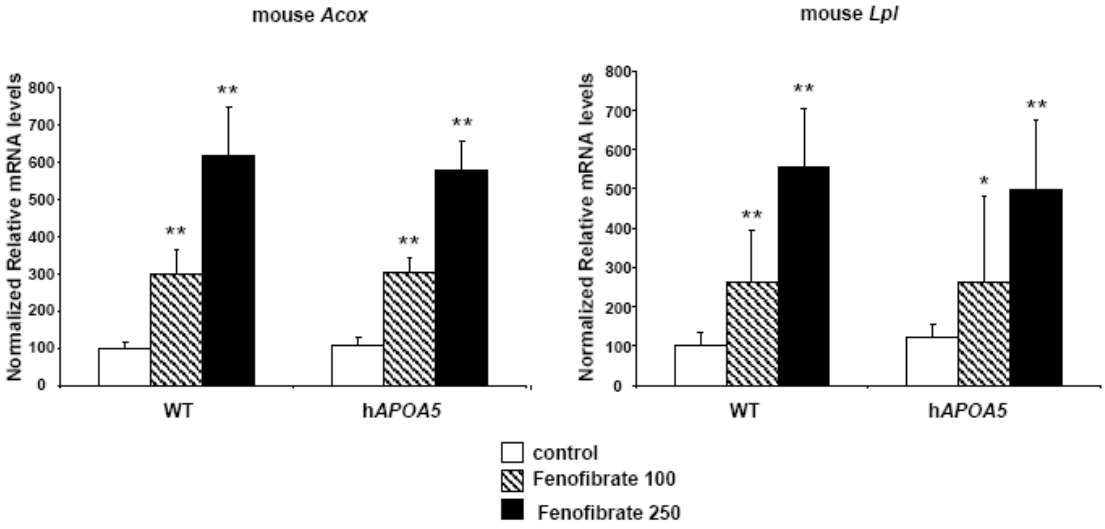

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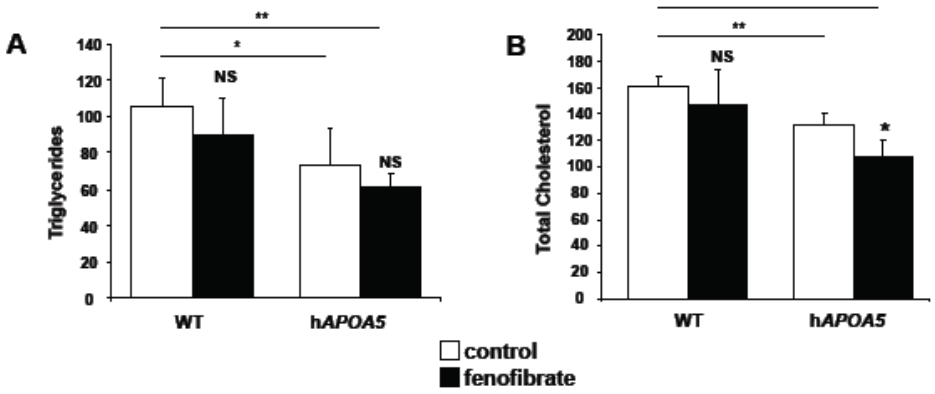

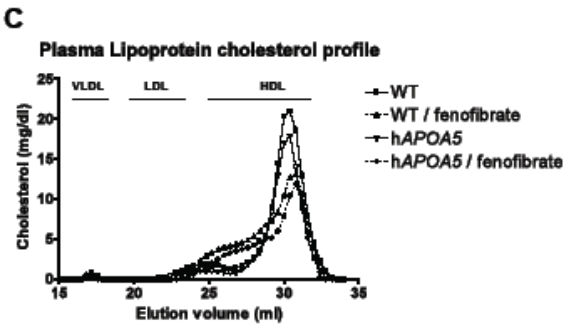

Fig.4