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Regulatory Role for Phosphatidylcholine Transfer Protein/StarD2 in the Metabolic Response to Peroxisome Proliferator Activated Receptor Alpha (PPAR α)

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Summary

Phosphatidylcholine transfer protein (PC-TP, a.k.a. StarD2) is abundantly expressed in liver and is regulated by PPAR α . When fed the synthetic PPAR α ligand fenofibrate, *Pctp*^{-/-} mice exhibited altered lipid and glucose metabolism. Microarray profiling of livers from fenofibrate fed wild type and *Pctp*^{-/-} mice revealed differential expression of a broad array of metabolic genes, as well as their regulatory transcription factors. PC-TP expression in cell culture controlled the activities of both PPAR α and HNF4 α , suggesting that the mechanism by which it modulates hepatic metabolism is at least in part via activation of transcription factors that govern nutrient homeostasis.

Keywords

START domain; fibrate drug; lipid; glucose; microarray; gene transcription

Introduction

Phosphatidylcholine transfer protein (PC-TP, a.k.a. StarD2) is a 25 kDa cytosolic lipid binding protein with exquisite specificity for phosphatidylcholines [1]. It is a member of the steroidogenic acute regulatory protein-related lipid transfer (START) domain superfamily of proteins that mediate intracellular lipid transport and metabolism, as well as cellular signaling and gene transcription [2–4]. PC-TP is robustly expressed in oxidative tissues [5] and studies of *Pctp*^{-/-} mice have revealed key roles in the regulation of lipid and glucose metabolism [6, 7], potentially by regulating the balance of fatty acids and fatty acyl-CoAs within cells [8].

PPAR α is a transcription factor that is enriched in oxidative tissues and regulates nutrient metabolism [9]. PPAR α itself binds phosphatidylcholines [10] and is activated by endogenous polyunsaturated fatty acids [9]. The PC-TP gene promoter contains consensus DNA response

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elements for binding PPAR α [11], and accumulating evidence suggests that PPAR α controls PC-TP expression: Fibrate drugs, which are synthetic ligands that activate PPAR α [9], upregulate hepatic PC-TP mRNA [12] and protein [13,14] in mice.

The aim of the current study was to explore the contributions of PC-TP expression to PPAR α -mediated regulation of lipid and glucose homeostasis. In mice administered a fenofibrate-supplemented diet, the absence of PC-TP expression altered lipid and glucose metabolism. Consistent with diverse roles for PC-TP in mediating the effects of PPAR α , expression profiling in livers of fenofibrate fed *Pctp*^{-/-} compared with wild type mice revealed a broad array of differentially expressed metabolic genes. The absence of PC-TP expression also led to differential expression of transcription factors that control hepatic lipid and glucose metabolism. Acute siRNA-mediated knockdown of PC-TP in culture cells altered the transcriptional activities of both PPAR α and HNF4 α , suggesting a mechanism for the broader impact of this specific lipid binding protein on nutrient metabolism within the liver.

Materials and Methods

Animal and diets

Wild type C57BL/6J mice, as well as *PPAR* α ^{-/-} and wild type 129S3/svImJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). *Pctp*^{-/-} mice [15] and wild type littermate controls on an FVB/NJ genetic background were as previously described [16]. A fenofibrate-supplemented chow diet (0.2% wt/wt) was prepared (Bioserve, Frenchtown, NJ) by pelleting fenofibrate obtained from Sigma (St. Louis, MO) together with standard rodent diet 5001 (Purina, St. Louis, MO). Experiments were conducted using male mice at 8–12 w of age. Preliminary experiments revealed that *Pctp*^{-/-} and wild type mice consumed the same amount of fenofibrate-supplemented chow. Mice were euthanized, blood collected and tissues harvested as described [7]. Protocols for animal use and euthanasia were approved by the institutional committee of Harvard Medical School.

Analytical techniques

Plasma concentrations of triglycerides, cholesterol, nonesterified fatty acids (NEFA) and β -hydroxybutyrate were determined enzymatically [7]. Hepatic triglyceride and cholesterol concentrations were measured following organic extraction [17]. Distributions of cholesterol among lipoprotein particles were determined by FPLC [17]. Plasma insulin concentrations were measured by ELISA [18]. Western and northern blot analyses were as described [11, 19]. Quantitative polymerase chain reaction (qPCR) was performed with RPL32 as an invariant control [7], using primers listed in Supplemental Table 1.

Hepatic triglyceride production rates

Rates of hepatic triglyceride production were measured after 4 h of fasting [7].

Glucose and insulin tolerance tests

After a 4 h fast, baseline glucose concentrations were measured using a OneTouch Ultra glucose monitor (LifeScan, Milpitas, CA). This was immediately followed by glucose or insulin tolerance tests [7].

Microarray analysis of gene expression

Total RNA was extracted from livers of mice fed the fenofibrate-supplemented diet for 7 d (wild type, n = 6; *Pctp*^{-/-}, n = 6) using Trizol (Invitrogen). Following purification (RNeasy Mini kit, Qiagen, Valencia, CA), cDNA was synthesized, cleaned up and transcribed *in vitro* using appropriate Affymetrix kits (Affymetrix, Inc., Santa Clara, CA). cRNA samples (20 μ g)

were hybridized to GeneChip Mouse Genome 430A 2.0 Arrays (one mouse liver per GeneChip) at 45°C in an Affymetrix hybridization oven. Microarrays were prepared and scanned using a Model 450 Fluidics Station and a Model 3000 7G scanner, each controlled by GeneChip Operating Software (Affymetrix).

Microarray data were analyzed by two independent methods. The first utilized DNA-Chip Analyzer (dChip) [20]. Data for individual GeneChips were normalized using the “invariant normalization” function of dChip. Normalized intensity values for individual genes expression were filtered out if a strong signal for the gene was not detected on greater than 30% of the GeneChips. Fold changes (*Pctp*^{-/-} vs. wild type) of at least 1.2 were considered to be significant for *P* < 0.05 by two-tailed Student's *t*-test. Microarray data were also analyzed using Bayesian Analysis of Differential Gene Expression (BADGE) version 1.0 [21]. This method computes the posterior probability that each gene was regulated more than one fold in *Pctp*^{-/-} compared with wild type mice. Differentially regulated genes were classified according to biological functions using GenMAPP [22].

Cell culture and knockdown of endogenous PC-TP

Human embryonic kidney (HEK) 293T cells in culture [19] were plated at densities of 3×10^4 cells/well in BD Falcon 12-well plates (BD Biosciences, San Jose, CA). After 24 h, cells were transfected (Lipofectamine, Invitrogen) with 20 nM siRNA (5'-CCAGUAUGUUAAGAACUC-dTdT-3') to knockdown endogenous PC-TP expression in HEK 293T cells [19] or scrambled Negative siRNA Control #1 (Applied Biosystems/Ambion, Austin, TX). Following 6 h of transfection, fresh media was added for 48 h.

Transcriptional activities of PPAR α and HNF4 α

To measure PPAR α activity, HEK 293T cells were cotransfected with 70 ng of a PPAR α promoter-firefly luciferase reporter plasmid (PPRE₃-tk-luc; gift from Dr. Jorge Plutzky, Harvard Medical School, Boston, MA), 10 ng of the PPAR α expression plasmid pcDNA3.1-hPPAR α (gift from Dr. John Chiang, Northeastern Ohio University College of Medicine, Rootstown, OH) plus 1.4 ng of an expression plasmid encoding renilla *luciferase* (pRL-tk, Promega, Madison, WI) as a control for transfection efficiency. Because in preliminary experiments fenofibrate concentrations ranging up to 100 μ M failed to activate PPAR α , this was instead accomplished using bovine serum albumin (BSA)/docosahexaenoic acid (DHA) (1:5 mol:mol, 0.6 mM DHA) complexes [23]. BSA/DHA or BSA alone was added to the media for 4 h at the end of the 24 h transfection period. HNF4 α activity was similarly measured using 70 ng of HNF4 α promoter-firefly luciferase (HNF4-tk-luc) and 70 ng of the HNF4 α expression plasmid pCMX-HNF4 α (gifts from Dr. Chiang). After 24 h incubation in OPTI-MEM (Invitrogen), cells were harvested using Passive Lysis Buffer (Promega). Luciferase activity was measured using a Dual-Luciferase reporter assay system (Promega).

Statistics

Data are reported as means $\pm$ SEM. Differences between groups were analyzed using a two-tailed unpaired Student's *t*-test or ANOVA.

Results

PPAR α -mediated regulation of PC-TP expression

Figure 1 demonstrates the regulation of PC-TP expression by PPAR α . In keeping with our prior observation that PC-TP is expressed more robustly in livers of C57BL/6J compared with FVB/NJ mice [16], PC-TP mRNA was readily detected in livers of chow fed C57BL/6J mice by northern blot analysis, but was not apparent in FVB/NJ mice at similar X-ray film exposure

times (Figure 1A). There was marked upregulation of mRNA by fenofibrate feeding, with transcript levels reaching similar levels in the two mouse strains. Figure 1B quantifies this finding by qPCR, which revealed a 3-fold upregulation of PC-TP mRNA in C57BL/6J mice and 20-fold upregulation in FVB/NJ mice. Consistent with transcriptional regulation, Figure 1C shows that variations in protein expression reflected changes in mRNA levels. Figure 1D demonstrates that, in the absence of PPAR α , upregulation of PC-TP in response to fenofibrate did not occur. Based upon the more robust fenofibrate-mediated upregulation of PC-TP, FVB/NJ mice were utilized for subsequent studies.

Influence of PC-TP expression on lipid metabolism in fenofibrate fed mice

Figure 2 shows the influence of PC-TP on key parameters of lipid metabolism in mice fed chow or a fenofibrate supplemented diet. Plasma triglycerides were not different in chow fed *Pctp*^{-/-} and wild type mice and were not influenced significantly by fenofibrate feeding (Figure 2A). Plasma cholesterol concentrations were not different on a chow diet (Figure 2B). Whereas fenofibrate feeding did not affect plasma cholesterol concentrations in wild type mice, values for *Pctp*^{-/-} mice were decreased. Not shown is that the lipoprotein profile by FPLC did not differ for fenofibrate fed mice. Figure 2C shows that hepatic triglyceride concentrations were higher in chow fed mice lacking PC-TP. Whereas fenofibrate feeding increased hepatic triglycerides in wild type mice, values for *Pctp*^{-/-} mice were unchanged, so that hepatic triglycerides were lower in *Pctp*^{-/-} mice after fenofibrate treatment. Hepatic concentrations of cholesterol were increased in chow fed *Pctp*^{-/-} mice (Figure 2D). Whereas fenofibrate increased hepatic cholesterol concentrations in wild type mice, there was no change in *Pctp*^{-/-} mice. As a result, hepatic cholesterol concentrations were lower in fenofibrate fed *Pctp*^{-/-} mice. Hepatic phospholipid concentrations were higher in chow fed *Pctp*^{-/-} mice (Figure 2E). Because they increased in wild type but not *Pctp*^{-/-} mice, hepatic phospholipid concentrations were the same in both genotypes following fenofibrate feeding.

As previously reported [7], hepatic triglyceride production rates were higher in chow fed *Pctp*^{-/-} mice (Figure 2F). Fenofibrate increased production rates to similar levels in both genotypes. Plasma NEFA concentrations were lower in chow fed *Pctp*^{-/-} mice (Figure 2G). Whereas values remained the same in wild type mice following fenofibrate feeding, NEFA concentrations increased in the absence of PC-TP expression. As also reported previously [7], plasma concentrations of β -hydroxybutyrate were the same in chow fed *Pctp*^{-/-} and wild type. Whereas there was no change in wild type mice, fenofibrate feeding decreased β -hydroxybutyrate concentrations in *Pctp*^{-/-} mice (Figure 2H).

PC-TP expression regulates glucose metabolism in fenofibrate treated mice

The effects of PC-TP expression on glucose homeostasis in chow and fenofibrate fed mice are illustrated in Figure 3. Fasting plasma glucose concentrations were reduced in *Pctp*^{-/-} mice (Figure 3A) in the absence of changes in plasma insulin (Figure 3B). Indicative of delayed clearance, glucose tolerance tests (Figure 3C) revealed that peak plasma glucose concentrations were achieved at 30 min in wild type mice and at 60 min in *Pctp*^{-/-} mice. The absence of PC-TP expression was associated with an increase in the area under the curve (AUC, inset to Figure 3C). Consistent with these findings, insulin tolerance tests in *Pctp*^{-/-} mice (Figure 3D) revealed decreased glucose clearance at each point in time and an increase in AUC (inset).

Differentially regulated hepatic genes in fenofibrate fed *Pctp*^{-/-} mice

Microarray analysis was utilized to examine the influence of PC-TP expression on hepatic gene expression in response to PPAR α activation. On the GeneChip, 14,000 genes were represented by 22,690 probes. According to dChip analysis, 727 hepatic genes met the pre-specified criteria for altered regulation. In *Pctp*^{-/-} compared with wild type mice, 414 genes were upregulated and 313 genes were downregulated. For analysis by BADGE, an expected false positive rate

of 0.30% selected the genes with more than 99.85% and less than 0.15% chance of being more expressed in wild type mice. Under these conditions, 757 genes were differentially expressed, with 89 upregulated and 668 downregulated genes in *Pctp*^{-/-} mice. Comparison of the two approaches demonstrated that 263 genes were differentially expressed according to both algorithms. Table 1 shows the 53 differentially regulated genes related to lipid and glucose metabolism grouped using GenMapp. Differential expression of selected genes was validated by qPCR and consistently demonstrated higher fold changes than observed by microarray analysis. Testing of selected genes using chow fed mice revealed that these differences did not pre-exist fenofibrate treatment.

For genes related to triglyceride metabolism (Figure 2), there was variable regulation in response to fenofibrate. In livers of *Pctp*^{-/-} mice, some lipogenic genes were differentially upregulated, whereas others were suppressed. Whereas SREBP1c, which controls genes of fatty acid synthesis, was upregulated, anticipated target genes [24] were not identified by the microarray analysis. These included ACC α , which was not represented on the GeneChip, and FAS, which fell below the threshold for upregulation by dChip analysis. Testing by qPCR (Table 1) demonstrated that both of these mRNA transcripts were expressed at higher levels in *Pctp*^{-/-} compared with wild type mice. The relative suppression observed for Lipin-1 and CPT1a in fenofibrate fed mice lacking PC-TP are both consistent with downregulation of PPAR α [9,25]. In contrast to the mixed regulation of genes governing triglyceride metabolism, Table 1 demonstrates more consistent upregulation of mRNA transcripts for proteins that mediate synthesis of cholesterol (e.g. 3-hydroxy-3-methylglutaryl-Coenzyme A reductase and squalene epoxidase) and phospholipids (i.e. CT α and PSS1), as well as lipoprotein turnover (i.e. LDLr and ABCA1). Upregulation of cholesterol metabolic genes is in line with increased mRNA levels of the transcription factor SREBP2 [24]. In keeping with reduced fasting plasma glucose concentrations but impaired glucose and insulin tolerance in *Pctp*^{-/-} mice (Figure 3), the associated pattern of differential regulation genes appeared to be more complex. This involved altered expression of mRNAs encoding several key transcription factors that play critical roles in regulating glucose metabolism (e.g. HNF4 α , PGC-1 α , FoxO1 and HIF-1 α).

Regulation of PPAR α and HNF4 α activity by PC-TP in vitro

Potential regulation of PPAR α and HNF4 α activities by PC-TP expression was examined using HEK 293T cells. PC-TP siRNA treatment led to >90% reductions in PC-TP expression compared to scrambled siRNA treatment and non-transfected cells (inset to Figure 4A). PC-TP knockdown did not affect transcriptional activity of PPAR α that was attributable to endogenous or overexpressed protein (Figure 4A). However, in the presence of the PPAR α ligand DHA [9], the transcriptional activities of endogenous and overexpressed PPAR α were both increased further in the setting of PC-TP knockdown. Although fenofibrate was not effective in HEK 293T cells under these conditions, the more potent fibrate WY-14,643 (1 μ M) similarly increased the activity of overexpressed PPAR α following knockdown of PC-TP (data not shown). Endogenous HNF4 α activity was reduced with PC-TP knockdown (Figure 4B), an effect that was also observed when HNF4 α was overexpressed.

Discussion

The objective of this study was to explore the contribution of PC-TP to the metabolic response of lipids and glucose to activation of PPAR α . The response of PC-TP to fenofibrate feeding both confirms and extends observations in the literature that PPAR α regulates PC-TP [13,14, 26]. The demonstration in two different strains of mice that levels of expression of mRNA and protein are tightly coupled suggests that transcriptional control is the main mechanism that governs PC-TP levels in the liver.

Our observation that hepatic triglyceride concentrations increased by 91% in FVB/NJ wild type mice fed 0.2% fenofibrate for 7 d is consistent with published reports concerning the effects of fibrates drugs. When fed 0.2% fenofibrate for 14 d, C57BL/6 mice exhibited a 58% increase in hepatic triglyceride concentrations [27], whereas a much more modest, non-significant increase was observed for Sv/129 mice gavaged daily for 7 d with 10 mg/kg of WY-14,643 [28]. Unlike the C57BL/6 mice in which fenofibrate reduced plasma triglyceride concentrations by 80% [27], we observed a much more modest 19% decrease, which did not achieve significance. These differences may have been attributable to duration or methods of feeding, type of fibrate drug or mouse strain.

We previously reported that hepatic triglyceride concentrations were lower in chow fed *Pctp*^{-/-} compared with wild type mice [7], whereas in the current study values for *Pctp*^{-/-} mice were higher. This was due to variable hepatic triglyceride concentrations for the wild type mice because there was no difference in values for *Pctp*^{-/-} mice in the two studies. The higher values previously published for livers of wild type mice were attributable to the longer duration of fasting (16 h compared vs 4 h), which would be expected to increase hepatic triglyceride concentrations [28]. Considering the central regulatory role of PPAR α during fasting [9], the absence of changes in *Pctp*^{-/-} mice during fasting or following fenofibrate feeding suggests that PC-TP contributes to the metabolic response that follows PPAR α activation. This notion is further supported by the influence of PC-TP expression on fenofibrate-mediated control of hepatic triglyceride production rates, as well as plasma concentrations of NEFA and β -hydroxybutyrate.

We have provided evidence that NEFA from adipose tissue provided the source for increased hepatic triglyceride secretion rates in chow fed *Pctp*^{-/-} mice [7]. This occurred in the setting of marked reductions in hepatic fatty acid synthesis, which were accompanied by downregulation of SREBP1c and its target genes. By contrast in fenofibrate fed mice, the absence of PC-TP was associated with differential upregulation of SREBP1c, which was accompanied in *Pctp*^{-/-} mice by relative increases in fatty acid synthetic genes, including ACC α and FAS. The relative reduction in lipin-1 [25] appears to have contributed to the reduced hepatic triglyceride concentrations in fenofibrate fed *Pctp*^{-/-} mice compared with fenofibrate fed wild type controls. The marked decrease in plasma β -hydroxybutyrate concentrations in response to fenofibrate feeding of *Pctp*^{-/-} mice is in keeping with the reduction in CPT1a expression, which was most likely attributable to relative downregulation of PPAR α [9], PGC-1 α and PGC-1 β [29]. Our data do not provide insights into the relative upregulation in *Pctp*^{-/-} mice of FGF21, a PPAR α regulated gene that promotes fatty acid oxidation and ketogenesis [30].

In fenofibrate fed *Pctp*^{-/-} mice, the relative increases in the expression of SREBP2 and its target genes, cholesterol biosynthetic enzymes and the LDL receptor [24], appear to represent a compensatory response to the observed reduction in hepatic cholesterol concentrations. This may have occurred secondary to increased expression of ABCA1, which promotes apolipoprotein AI-mediated cholesterol efflux from hepatocytes during HDL formation [31]. It is noteworthy that the decrease in hepatic cholesterol was not accompanied by a decrease in hepatic phospholipids in *Pctp*^{-/-} mice. It is possible that an increase in phospholipid synthesis associated with hepatic upregulation of CT α and PSS1 [32] contributed to increased SREBP2 processing by reducing the relative concentration of cholesterol concentration in the endoplasmic reticulum [33]. The relative reduction in ABCG8 argues against the possibility that the increase in biliary cholesterol excretion decreased hepatic cholesterol contents [34], and the decreased relative expression of SHP and increased LRH-1 mRNA make it unlikely that conversion of cholesterol to bile salts was increased [35].

In contrast to chow fed mice [7], fenofibrate fed *Pctp*^{-/-} mice exhibited reduced fasting plasma glucose concentrations, but decreased glucose clearance and insulin responsiveness. The reduction in fasting plasma glucose concentrations was presumably explained by the reduced relative expression levels of PGC-1 α and FoxO1, leading to decreased PEPCK expression [36,37]. The relative increase in expression of GK may have contributed and was likely due to the higher expression of both HNF4 α and HIF-1 α [38]. Downregulation of IRS2 may have played a role in reducing both glucose clearance and the sensitivity of plasma glucose to insulin [39]. However, additional experiments will be required to dissect the influence of PC-TP on insulin signaling in the context of PPAR α activation.

Because many of the differentially expressed genes were themselves regulated by PPAR α [9], we explored *in vitro* the possibility that PC-TP expression *per se* could influence PPAR α activity. Appreciating that the reductionist system in which PC-TP was acutely silenced in HEK 293T cells using siRNA does not reflect the complex changes that occur in livers of fenofibrate fed *Pctp*^{-/-} mice, the observed changes in ligand-activated PPAR α activity and intrinsic activity of HNF4 α suggest that PC-TP may modulate the activity of these transcription factors. In this connection, we have reported that PC-TP enhances the acyl-CoA thioesterase activity of thioesterase superfamily member 2 (Them2) [8]. This could modulate the intracellular balance of fatty acids and fatty acyl-CoAs, which contributes to the regulation of PPAR α and HNF4 α [26]. Taken together, our findings are in keeping with a primary role for PC-TP in fatty acid metabolism [7], which secondarily regulates transcription factors that govern nutrient homeostasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ABC	ATP-binding cassette
ACC	Acetyl-coenzyme A carboxylase
BADGE	Bayesian Analysis of Differential Gene Expression
CPT	Carnitine palmitoyltransferase
CT	CTP:phosphocholine cytidyltransferase
DHA	docosahexaenoic acid
FAS	fatty acid synthase
FGF	fibroblast growth factor
FOX	forkhead box
GK	glucokinase
IRS	insulin receptor substrate
LDLr	low density lipoprotein receptor
LRH	liver receptor homolog
HIF	hypoxia inducible factor
HNF	hepatocyte nuclear factor
NEFA	non-esterified fatty acid
PEPCK	phosphoenolpyruvate carboxykinase

PGC	peroxisome proliferator activated receptor, gamma, coactivator
PC-TP	phosphatidylcholine transfer protein
PPAR	peroxisome proliferator activated receptor
PSS	phosphatidylserine synthase
SHP	short heterodimer partner
SREBP	sterol regulatory element binding protein
START	steroidogenic acute regulatory protein-related lipid transfer

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Figure 1. Upregulation of PC-TP expression in response to fenofibrate is mediated by PPAR α activation

Mice were fed chow (C) a fenofibrate-supplemented diet (F) for 7 d prior to A) northern blot analysis, B) qPCR (C57BL/6J, n = 6/group; FVB/NJ, n = 8/group) and C) western blot analysis. D) qPCR of PC-TP mRNA harvested from PPAR α ^{-/-} (n = 4/group) and wild type 129S3/svImJ (n = 6/group) mice. **P* < 0.05, wild type vs. Pctp^{-/-} mice.

Figure 2. PC-TP expression regulates the response of lipid metabolism to fenofibrate

Wild type mice (open bars) and *Pctp*^{-/-} mice (solid bars) were fed 7 d with chow (C: wild type, n = 7 – 8; *Pctp*^{-/-}, n = 6 – 8) or fenofibrate supplemented (F: wild type, n = 6 – 8; *Pctp*^{-/-}, n = 6 – 8) diets prior to measuring plasma concentrations of (A) triglycerides and (B) cholesterol; hepatic concentrations of (C) triglycerides, (D) cholesterol and (E) phospholipids; (F) hepatic triglyceride secretion rates (please note that values for chow fed *Pctp*^{-/-} and wild type mice in this panel were replotted from reference [7]); and plasma concentrations of (G) NEFA and (H) β-hydroxybutyrate. **P* < 0.05, wild type vs. *Pctp*^{-/-} mice; †*P* < 0.05, C vs. F.

Figure 3. Influence of PC-TP on the response of glucose metabolism to fenofibrate

(A) Wild type mice (open bars) and *Pctp*^{-/-} mice (solid bars) were fed 7 d with a fenofibrate supplemented diet (wild type, n = 8; *Pctp*^{-/-}, n = 6) prior to measuring plasma concentrations of (A) glucose and (B) insulin. The same mice (wild type, ○; *Pctp*^{-/-}, ●) were then subjected to (C) glucose and (D) insulin tolerance tests. Inserts provide AUC values (wild type, open bars; *Pctp*^{-/-}, closed bars). **P* < 0.05, wild type vs. *Pctp*^{-/-} mice.

Figure 4. Regulation of PPAR α and HNF4 α transcriptional activity by PC-TP *in vitro*

(A) Influence of PC-TP expression on PPAR α transcriptional activity. HEK 293T cells were treated with scrambled siRNA (open bars) or PC-TP siRNA (closed bars). PPAR α transcriptional activity was determined according to firefly *luciferase* activity expressed by PPRE₃-tk-luc in the absence (–) or presence (+) of exogenous PPAR α expression (pcDNA-hPPAR α), as well as BSA or DHA complexed to BSA (DHA/BSA). The inset western blot shows the effects of PC-TP siRNA treatment compared with scrambled siRNA or non-transfected control cells. (B) Influence of PC-TP expression on HNF4 α transcriptional activity. HEK 293T cells were treated with siRNA as described in panel A. HNF4 α transcriptional activity was determined according to firefly *luciferase* activity expressed by HNF4 α -TK-luc in the absence (–) or presence (+) of exogenous HNF4 α expression (pCMX-HNF4 α). Data are representative of three independent experiments, each experiment was performed in triplicate. * $P < 0.05$, PC-TP vs. scrambled siRNA.

Table 1

Genes Involved in Lipid and Glucose Metabolism that are Differentially Expressed in Fenofibrate Fed *Pctp*^{-/-} vs. Wild Type Mice^a

Gene	Abbreviation ^b	Affymetrix ID	dChip ^c	BADGE ^c	qPCR ^d
Triglyceride Metabolism					
Fibroblast growth factor 21 ^e	FGF21	1422916_at	3.00	2.95	11.22
Lipase, endothelial ^e		1421262_at, 1450188_s_at	2.09	2.17	
Phosphatidic acid phosphatase 2A		1422620_s_at	1.56	1.49	
Stearyl-Coenzyme A desaturase 2		1415823_at	1.55		
Aquaporin 9		1421605_a_at	1.47	1.62	
ATP-binding cassette, sub- family D (ALD), member 2		1419748_at	1.45	1.72	
Glycerol-3-phosphate acyltransferase, mitochondrial		1425834_a_at	1.39	1.54	
Acyl-CoA synthetase long- chain family member 1 ^e		1422526_at		1.22	5.80
Fatty acid synthase	FAS	1423828_at	1.07 ^f		4.30
Apolipoprotein M		1419095_a_at, 1419096_at	-1.41	-1.40	
Lipin 2		1452837_at	-1.47		
Camitine palmitoyltransferase 1a, liver	CPT1a	1434866_x_at	-1.53		
Lipin 1		1418288_at, 1426516_a_at	-2.40	-2.08	
Acetyl-coenzyme A carboxylase alpha	ACCα	not present			2.39
Cholesterol and Phospholipid Metabolism					
NAD(P) dependent steroid dehydrogenase-like		1451799_at	2.78		
Acetoacetyl-CoA synthetase		1423797_at, 1456081_a_at	2.63	3.08	

Gene	Abbreviation ^b	Affymetrix ID	dChip ^c	BADGE ^c	qPCR ^d
3-hydroxy-3-methylglutaryl-Coenzyme A reductase ^e		1427229_at	1.78	2.07	2.34
Low density lipoprotein receptor ^e	LDLr	1450383_at, 1421821_at	1.75	1.96	
ATP-binding cassette, subfamily A (ABC1), member 1 ^e	ABCA1	1421839_at, 1450392_at	1.75	2.03	
Phosphatidylserine synthase 1	PSSI	1441866_s_at		1.53	
Mevalonate (diphospho) decarboxylase		1417303_at, 1448663_s_at	1.68		
Squalene epoxidase ^e		1415993_at	1.39		2.33
Sterol-C4-methyl oxidase-like		1423078_a_at	1.38		
Phosphate cytidyltransferase 1, choline, alpha isoform	CTa	1424453_at	1.34	1.44	
Phosphatidylinositol transfer protein, cytoplasmic 1		1452940_x_at, 1455204_at	-1.51		
ATP-binding cassette, subfamily G (WHITE), member 8	ABCG8	1420656_at	-1.69	-1.55	
Glucose Metabolism					
Glucokinase ^e	GK	1419146_a_at, 1425303_at	2.76	2.43	
Ribose 5-phosphate isomerase A		1418337_at	1.57	1.49	
Dihydrodipolamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)		1452005_at		1.43	
Isocitrate dehydrogenase 3 (NAD+) alpha		1432016_a_at		1.40	
Phosphoglucosyltransferase 1		1453283_at		1.37	
Pyruvate dehydrogenase E1 alpha 1		1449137_at	1.34		
6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 1		1427213_at	-1.41		
Solute carrier family 2		1434773_a_at	-1.55		

Gene	Abbreviation ^b	Affymetrix ID	dChip ^c	BADGE ^c	qPCR ^d
(facilitated glucose transporter), member 1					
Insulin receptor substrate 2	IRS2	1443969_at	-1.81		
Phosphoenolpyruvate carboxykinase 1, cytosolic ^e	PEPCK	1423439_at	-1.88	-2.63	
Glutamate oxaloacetate transaminase 1, soluble		1450970_at	-2.67		
Transcriptional Factors					
Nuclear receptor subfamily 0, group B, member 2 ^e	SHP	1449854_at	2.26		
Sterol regulatory element binding factor 1	SREBP1c	1426690_a_at	1.62	1.67	14.06
Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)		1421866_at	1.59	1.49	
Retinoic acid receptor, beta		1454906_at	1.53	1.44	
Hypoxia inducible factor 1, alpha subunit	HIF-1α	1448183_a_at	1.50	1.74	
Hepatic nuclear factor 4, alpha ^e	HNF4α	1421983_s_at	1.49	1.60	6.84
Sterol regulatory element binding factor 2 ^e	SREBP2	1426744_at	1.47		9.60
Nuclear receptor subfamily 2, group F, member 2 (COUP-TFII)		1416158_at	1.34	1.78	
Peroxisome proliferator activated receptor alpha ^e	PPARα	1449051_at	-1.36		
Nuclear receptor subfamily 5, group A, member 2 ^e	LRH-1	1449706_s_at	-1.41		
CCAAAT/enhancer binding protein (C/EBP), delta		1423233_at	-1.56		
Forkhead box O1 ^e	FoxO1	1416982_at, 1416983_s_at	-1.60	-1.51	
Forkhead box O3a		1434831_a_at, 1434832_at	-1.68	-1.61	
Peroxisome proliferative activated receptor,	PCG-1β	1449945_at	-2.35	-1.85	

Gene	Abbreviation ^b	Affymetrix ID	dChip ^c	BADGE ^c	qPCR ^d
gamma, coactivator 1 beta Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha ^e	PCG-1α	1437751_at, 1456395_at, 1460336_at	-3.15	-2.21	

^aData are expressed as ratios of hepatic gene expression values for *Pcyp*^{-/-} mice/wild type mice after feeding a fenofibrate-supplemented chow diet for 7 d as determined by dChip or BADGE algorithms. Selected genes were tested by quantitative real-time PCR (qPCR) for differential expression. Genes were assigned to categories using GenMAPP.

^bCommon gene abbreviation used in the text.

^cWhere more than one Affymetrix ID is listed, values represent averages of fold changes.

^dqPCR was performed (wild type, n = 6 – 7; *Pcyp*^{-/-}, n = 4 – 6) and differences were determined to be significant at *P* < 0.05.

^eqPCR was performed using cDNA prepared from livers of chow fed mice (wild type, n = 4; *Pcyp*^{-/-}, n = 4), which revealed no significant difference for these genes.

^fFalls below the threshold for significance by dChip algorithm.