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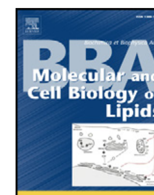

Regulatory role for phosphatidylcholine transfer protein/StarD2 in the metabolic response to peroxisome proliferator activated receptor alpha (PPARα)

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ABSTRACT

Phosphatidylcholine transfer protein (PC-TP, a.k.a. StarD2) is abundantly expressed in liver and is regulated by PPARα. When fed the synthetic PPARα 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 PPARα and HNF4α, suggesting that the mechanism by which it modulates hepatic metabolism is at least in part via activation of transcription factors that govern nutrient homeostasis.

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1. 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].

Abbreviations: ABC, ATP-binding cassette; ACC, Acetyl-Coenzyme A carboxylase; BADGE, Bayesian Analysis of Differential Gene Expression; CPT, Carnitine palmitoyltransferase; CT, CTP:phosphocholine cytidylyltransferase; 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; RPL32, Ribosomal protein L32; SHP, short heterodimer partner; SREBP, sterol regulatory element binding protein; START, steroidogenic acute regulatory protein-related lipid transfer

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PPARα is a transcription factor that is enriched in oxidative tissues and regulates nutrient metabolism [9]. PPARα itself binds phosphatidylcholines [10] and is activated by endogenous polyunsaturated fatty acids [9]. The PC-TP gene promoter contains consensus DNA response elements for binding PPARα [11], and accumulating evidence suggests that PPARα controls PC-TP expression: Fibrate drugs, which are synthetic ligands that activate PPARα [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 PPARα-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 PPARα, 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 PPARα and HNF4α, suggesting a mechanism for the broader impact of this specific lipid binding protein on nutrient metabolism within the liver.

2. Materials and methods

2.1. Animal and diets

Wild type C57BL/6J mice, as well as *PPARα*$^{-/-}$ and wild type 129S3/svlmJ 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.

2.2. Analytical techniques

Plasma concentrations of triglycerides, cholesterol, non-esterified 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.

2.3. Hepatic triglyceride production rates

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

2.4. 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].

2.5. 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 µg) 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 gene 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].

2.6. 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′-CCAGUAUGUUAAAGAACUC-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.

2.7. Transcriptional activities of PPARα and HNF4α

To measure PPARα activity, HEK 293T cells were cotransfected with 70 ng of a PPARα promoter-firefly luciferase reporter plasmid ($PPRE_3$-tk-luc; gift from Dr. Jorge Plutzky, Harvard Medical School, Boston, MA), 10 ng of the PPARα expression plasmid pcDNA3.1-hPPARα (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 µM failed to activate PPARα, 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. HNF4α activity was similarly measured using 70 ng of HNF4α promoter-firefly luciferase (HNF4-tk-luc) and 70 ng of the HNF4α expression plasmid pCMX-HNF4α (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).

2.8. Statistics

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

3. Results

3.1. PPARα-mediated regulation of PC-TP expression

Fig. 1 demonstrates the regulation of PC-TP expression by PPARα. 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 (Fig. 1A). There was marked upregulation of mRNA by fenofibrate feeding, with transcript levels reaching similar levels in the two mouse strains. Fig. 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, Fig. 1C shows that variations in protein expression reflected changes in mRNA levels. Fig. 1D demonstrates that, in the absence of PPARα, 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.

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

Fig. 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

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