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Journal of Nutritional Biochemistry 25 (2014) 669–674

Journal of Nutritional Biochemistry

Fibrates and fish oil, but not corn oil, up-regulate the expression of the cholesteryl ester transfer protein (CETP) gene☆

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Received 20 August 2013; received in revised form 7 February 2014; accepted 12 February 2014

Abstract

Cholesteryl ester transfer protein (CETP) is a plasma protein that reduces high density lipoprotein (HDL)-cholesterol (chol) levels and may increase atherosclerosis risk. *n*-3 and *n*-6 polyunsaturated fatty acids (PUFAs) are natural ligands, and fibrates are synthetic ligands for peroxisome proliferator activated receptor-alpha (PPARα), a transcription factor that modulates lipid metabolism. In this study, we investigated the effects of PUFA oils and fibrates on CETP expression. Hypertriglyceridemic CETP transgenic mice were treated with gemfibrozil, fenofibrate, bezafibrate or vehicle (control), and normolipidemic CETP transgenic mice were treated with fenofibrate or with fish oil (FO; *n*-3 PUFA rich), corn oil (CO, *n*-6 PUFA rich) or saline. Compared with the control treatment, only fenofibrate significantly diminished triglyceridemia (50%), whereas all fibrates decreased the HDL-chol level. Elevation of the CETP liver mRNA levels and plasma activity was observed in the fenofibrate (53%) and gemfibrozil (75%) groups. Compared with saline, FO reduced the plasma levels of nonesterified fatty acid (26%), total chol (15%) and HDL-chol (20%). Neither of the oil treatments affected the plasma triglyceride levels. Compared with saline, FO increased the plasma adiponectin level and reduced plasma leptin levels, whereas CO increased the leptin levels. FO, but not CO, significantly increased the plasma CETP mass (90%) and activity (23%) as well as increased the liver level of CETP mRNA (28%). In conclusion, fibrates and FO, but not CO, up-regulated CETP expression at both the mRNA and protein levels. We propose that these effects are mediated by the activation of PPARα, which acts on a putative PPAR response element in the CETP gene.

Keywords: CETP expression; Fish oil; Omega-3 polyunsaturated fatty acids; Fibrates

1. Introduction

The cholesteryl ester transfer protein (CETP) is a 74-kDa glycoprotein that transfers neutral lipids. During this process, cholesteryl esters (CE) from high density lipoprotein (HDL) are transferred to apolipoprotein B-containing lipoproteins (LP) in exchange for triglycerides (TGs) [1]. Because CETP reduces the plasma levels of HDL-cholesterol (CHOL) and increases the plasma levels of low density lipoprotein (LDL)-CHOL, CETP activity may increase cardiovascular disease (CVD) risk. However, depending on the metabolic context, CETP may have protective effects against the development of atherosclerosis, as verified by experimental hypertriglyceridemia [2], castration [3,4] and in other settings [5,6]. The pharmacological inhibition of CETP as an antiatherogenic therapy has been pursued in the last decade. The first clinical trial using the inhibitor torcetrapib was interrupted because of an unexpected increase in mortality in the treated group [7–9]. Another clinical trial with dalcetrapib showed no benefits [10]. Currently, two new CETP inhibitors, anacetrapib and evacetrapib, are in Phase III trials. Both were effective in increasing the HDL concentration and decreasing the LDL concentration; however, the benefits regarding CVD still need to be confirmed [11].

The biochemical pathway and physiological function of CETP have been well characterized. CETP gene expression can be influenced by inflammatory and hormonal stimuli and diet composition [12]. The detailed positive regulation of the CETP gene by CHOL, either endogenous or from the diet, has been elucidated by Tall's group [13–15]. Lou and Tall [16] demonstrated that CETP expression is up-regulated by the liver X receptor alpha in response to a high-CHOL diet. More recently, Gautier et al. [17] demonstrated that CETP expression is also up-regulated by bile acids through farnesoid X receptor activation. However, the effects of specific dietary fatty acids on CETP expression remain less well understood. Compared with a diet rich in saturated

Abbreviations: apoB, apolipoprotein B; BEZA, bezafibrate; CVD, cardiovascular disease; CHOL, cholesterol; CETP, cholesteryl ester transfer protein; CE, cholesteryl esters; CO, corn oil; FXR, farnesoid X receptor; FENO, fenofibrate; FO, fish oil; GEM, gemfibrozil; LP, lipoproteins; LPL, lipoprotein lipase; LXRα, liver X receptor alpha; *n*-3, omega-3; *n*-6, omega-6; PPAR, peroxisome proliferator-activated receptors; PUFA, polyunsaturated fatty acids; PPRE, PPAR response element; S, saline; TG, triglycerides.

☆ Funding: This study was supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP # 06/59786-0, 2011/50400-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq # 304532/2010-0).

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http://dx.doi.org/10.1016/j.jnutbio.2014.02.008
0955-2863/

fatty acids, a monounsaturated fatty acid-rich diet stimulates CETP protein and mRNA expression in treated transgenic mice [18].

Some of the drugs commonly used to reduce hypertriglyceridemia or hypercholesterolemia might exert their effects through CETP modulation. Fibrates have been used in clinical practice to reduce TG levels for more than 5 decades [19]. These drugs are known to reduce plasma TG levels by 30 to 50% and to raise HDL-C levels by 2 to 20%, whereas their effects on low density lipoprotein-cholesterol (LDL-C) are more variable [20]. Fibrates and glitazones are synthetic ligands for peroxisome proliferator-activated receptors (PPAR) α and γ, respectively [21], which are transcription factors that modulate many genes related to lipid metabolism [22,23]. More than 300 PPAR target genes have been identified [24].

Fatty acids, especially long-chain polyunsaturated fatty acids (PUFA) and their metabolites, such as prostaglandins and leukotrienes, are natural PPARα and γ ligands [24]. Omega-3 (*n*-3) fatty acids can be obtained from fatty fish, especially menhaden, tuna, salmon and anchovy [25], whereas omega-6 (*n*-6) fatty acids are highly concentrated in nuts, seeds and vegetable oils, such as corn and soy oil [26]. The intake of PUFA, particularly eicosapentanoic acid (EPA, 20:5) and docosahexanoic acid (DHA, 22:6), has been shown to be related to health benefits and cardiovascular protection [27] and is specifically used to treat hypertriglyceridemia [28].

Because a potential PPAR response element (PPRE) sequence has been identified in the CETP gene regulatory region [18], we hypothesized that CETP might be regulated by PPAR agonists. Moreover, fibrates' effects on CETP are still controversial, and there are reports indicating that fibrates decrease [29–33], have no effect [34–38] or increase [39–41] CETP levels. There are few data on the effects of *n*-3 PUFA on CETP. It was previously demonstrated that fish oil (FO) treatment down-regulates CETP expression in hamsters [42] and reduces plasma CETP activity in hypercholesterolemic subjects [43], whereas EPA and DHA treatment had no effect on plasma CETP activity in normolipidemic subjects [44].

Considering the relevance of understanding the regulation of CETP gene expression and the previous controversial results, the aim of this study was to investigate whether synthetic (fibrates) and natural [fish and corn oil (CO)] PPAR ligands could modulate CETP expression and its effects on the plasma lipid profile. For this purpose, we use the human CETP transgenic mouse model, which is particularly useful to study gene expression, since the transgene encompass the natural promoter and regulatory flanking regions.

2. Methods and materials

2.1. Animals and treatments

All experimental protocols were approved by the university's Committee for Ethics in Animal Experimentation (CEUA/UNICAMP, protocol # 1107–1) and are in accordance with the "Principles of Laboratory Animal Care" (NIH publication no. 85–23, revised 1985). Transgenic mice expressing a human natural promoter-driven CETP gene, line 5203 [13], were crossbred with human apolipoprotein CIII transgenic mice, line 3707 [45], to obtain CETP (normolipidemic) and CIII/CETP (hypertriglyceridemic) mice. Founder mice for these colonies were kindly provided by Dr. Alan R. Tall (Columbia University, New York, NY, USA) in 1996. Because the transgene inserted into the mouse genome includes large amounts of natural flanking sequences at 5′ (3.2 kb) and 3′ (2 Kb) regions of the gene, the human CETP transgenic mice are especially useful to study human CETP gene regulation. The mice were housed in a conventional facility, in a temperature-controlled room at 22±1°C on a 12-h light/dark (6 am/6 pm) cycle and had free access to food (rodent chow diet; Nuvital CR1, Colombo, Brazil) and tap water. The chow diet composition was (% by weight) 23% protein, 5.0% total fat, 33% carbohydrates and 21% fiber, with a total of 269 kcal/100 g. CETP-expressing mice were screened by assaying plasma CETP activity. The apo CIII transgenic mice had plasma TG levels above 300 mg/dl, whereas the nontransgenic TG levels were below 100 mg/dl. During the entire period of treatment, mice were housed in three to four per cage in wood-based bedding, and they were treated daily at 11 to 12 am (light period).

In the first study, groups of 3-month-old CIII/CETP hypertriglyceridemic mice were randomly separated in three experimental groups and one control group. Mice were treated orally for 14 days with three different fibrates: micronized fenofibrate (FENO, 100 mg/kg body wt, Allergan, S. Paulo, Brazil), gemfibrozil (GEM, 200 mg/kg body wt, Roche, S. Paulo, Brazil) and bezafibrate (BEZA, 100 mg/kg body wt, Parke-Davis, S. Paulo, Brazil), whereas the control mice received the vehicle (2% arabic gum, Sigma-Aldrich, St. Louis, MO, USA). In addition, a normolipidemic CETP transgenic mice group was treated with FENO as above. In the second study, groups of 3-month-old CETP mice (normolipidemic) were randomly separated in two experimental groups and one control group. Mice were treated orally for 14 days with 10 ml/kg of omega-3-rich oil (menhaden fish, FO), omega-6-rich oil (corn, CO) or saline (S). FO contained ~15% EPA and ~20% DHA, and the CO contained ~56% linoleic acid (see oils and diet full fatty acids composition in supplemental data, Table S1). Body weight and food intake were recorded twice a week (11 to 12 am). After 12 h of fasting (8 pm/8 am), blood samples were obtained from the retroorbital plexus of anesthetized mice (intraperitoneal injection of ketamine and xilasine, 50 and 10 mg/kg). After mice death by exsanguination, livers were removed, weighed and quickly frozen in liquid nitrogen. Liver samples were stored at −70°C until further use.

2.2. Plasma biochemical analyses

The mice were submitted to 12 h of fasting before blood collection. The levels of total CHOL, TGs (Chod-Pap; Roche Diagnostic GmbH, Mannheim, Germany) and nonesterified fatty acids (NEFA, Wako Chemicals, Neuss, Germany) were determined using enzymatic colorimetric assays according to the manufacturer's instructions. Blood glucose (GLUC) concentrations were measured using a GLUC analyzer (Accu-Chek Advantage, Roche Diagnostic, Switzerland). Adiponectin and leptin plasma concentrations were determined using a mouse Enzyme-Linked Immunosorbent Assay (ELISA) (Linco Research, MO, USA). Plasma CETP concentrations were determined using mouse ELISA (ALPCO - American Laboratory Products Company, Salem, USA).

2.3. Fast-protein liquid chromatography

Plasma pools (200 μl) from treated and control mice were fractionated by fast-protein liquid chromatography (FPLC) using an HR10/30 Superose 6 column (Amersham-Pharmacia Biotech, Uppsala, Sweden) equilibrated with Tris-buffered S (pH 7.2), as previously described [46]. The total CHOL level was determined enzymatically in each FPLC fraction.

2.4. Intravascular lipases activities

Total lipase (TL) activity was determined as previously described [41]. Briefly, overnight-fasted mice plasmas, obtained before (basal) and 10 min after heparin iv injection (100 U/kg body weight), were incubated with a ^{3}H-triolein/arabic gum substrate (9,10 3H (N)-triolein, New England Nuclear, Boston, MA, USA) in 0.2 M Tris–HCl buffer, pH 8.5, 37°C, during 1 h. In parallel tubes, the lipoprotein lipase (LPL) was inhibited with 2-M NaCl, and hepatic lipase (HL) activity was determined. The hydrolyzed labeled free fatty acids were extracted with methanol/chloroform/heptane (1.4:1.25:1), 0.14 M K_2CO_3/H_3BO_3, pH 10.5, dried under N_2, and their radioactivity were determined in a liquid scintillation solution in a LS6000 Beckman Beta Counter. The LPL activity was calculated as the difference between the total and the HL activities.

2.5. CETP activity (exogenous assay)

A mixture of human very low density lipoprotein (VLDL) and LDL (100-μg protein) was incubated with 10,000 dpm of human HDL_3 labeled with cholesteryl [1-^{14}C]oleate (Amersham Life Sciences, Buckinghamshire, England) and 5 μl of mouse plasma as a source of CETP in a final volume of 100 μl. Blanks were prepared with Tris-saline-ethylenediamine tetraacetic acid (S-EDTA) buffer (10-mM Tris, 140-mM NaCl, 1-mM EDTA, pH 7.4), and the negative controls were plasma from nontransgenic mice. The mixtures were incubated for 4 h at 40°C. After incubation, 400 μl of Tris-saline-EDTA (TSE) was added, and the apo B-containing LPs were precipitated with 50 μl of a 1.6% dextran sulfate-1 M $MgCl_2$ solution (1:1 v/v). The tubes were maintained at room temperature for 10 min and then centrifuged (10,000 rpm) at 4°C. Radioactivity was measured in the supernatant using an ACS-Aqueous Counting Scintillant (Amersham Biosciences, NJ, USA) in an LS6000 Beckman Beta Counter. The percentage of cholesteryl [1-^{14}C]oleate transferred from HDL to VLDL+LDL was calculated as [(dpm in blank tube - dpm in sample tube)/dpm in blank tube] × 100.

2.6. RNA extraction and RT-PCR

Total liver RNA was extracted from 50 to 100 mg of tissue using the TRIzol reagent (Invitrogen, Grand Island, NY, USA). The integrity of the RNA was assessed using Tris-borate 1.2% agarose gels stained with ethidium bromide. The amount and purity of the RNA were determined by optical density readings at 260 and 280 nm (Gene Quant, Amersham-Pharmacia Biotech). Genomic DNA contamination was excluded by running a polymerase chain reaction (PCR) on the RNA samples, and contaminated samples were reextracted until totally decontaminated. cDNA was prepared in duplicate and was obtained from 2 μg of total RNA by reverse transcription using an Applied Biosystems kit (High-Capacity cDNA reverse transcription kit) according to the manufacturer's instructions. CETP mRNA expression was determined by real-time reverse transcription polymerase chain reaction (RT-PCR) (Step One Real-time PCR System, Applied Biosystems, Foster City,

Table 1
Fasting plasma lipid levels and glycemia in CIII/CETP mice treated with different fibrates or placebo (control)

	Control	BEZA	FENO	GEM
GLUC (mg/dl)	71±8	72±9	83±5	103±56*
TG (mg/dl)	576±64	441±67	282±37*	566±25
CHOL (mg/dl)	111±8	108±11	85±2	102±6

Mean±SE (n=6–9). ANOVA, Tukey–Kramer post-hoc test.
* $P \leq .05$ *vs.* control.

CA, USA) using a SybrGreen PCR Master Mix and the following primers: CETP, 5′-CAGATCAGCCACTTGTCCAT-3′ and 5′-CAGCTGTGTGTTGATCTGGA-3′; and β-actin, 5`-GGACTCATCGTACTCCTGCTT-3` and 5`-GAGATTACTGCTCTGGCTCCT-3`. Gene expression was quantified using the ΔΔCT method by measuring the threshold cycle normalized to β-actin and then expressed relative to the control groups.

2.7. Statistical analyses

The results are presented as the means±S.E.M., and the number of mice (n) is indicated. Since two-way analysis of variance (ANOVA) showed no interaction between sex and response to treatments, male and female data were pooled. The pooled data were analyzed using one-way ANOVA with Tukey's post-hoc test. Two groups' comparisons were done by Student t test. The differences between groups were considered significant at $P<.05$.

3. Results

Hypertriglyceridemic CETP mice were used to study the effects of synthetic PPAR ligands (fibrates) on CETP expression. Compared with the control treatment, treatment with FENO, but not BEZA or GEM, reduced the body weight by 13% (data not shown). The liver and perigonadal adipose tissue weights were not affected by any of the fibrate treatments (supplemental data: Table S2). The GLUC and lipid plasma levels are presented in Table 1. Compared with the control, only the FENO treatment had the expected effect of diminishing triglyceridemia (50%). No effects of the fibrate treatments were observed on the CHOL levels. GEM treatment had the adverse effect of enhancing glycemia (45%), as also previously reported with ciprofibrate treatment [41].

To investigate whether PPAR ligands modulate CETP expression, we determined the hepatic CETP mRNA and plasma CETP levels, the latter of which was estimated by an activity assay using exogenous substrates (Fig. 1). Two of the fibrates, FENO and GEM, significantly increased both plasma CETP activity (Fig. 1A) and the liver mRNA level (Fig. 1B).

The CHOL distributions in the plasma LP fractions of the CIII/CETP transgenic mice treated with the different fibrates or vehicle (control) are shown in Fig. 2. VLDL-CHOL was reduced by the FENO treatment, in agreement with its effect of reducing TG levels. After 2 weeks of treatment, all fibrates diminished the HDL-CHOL levels, in agreement with the effect of elevating the plasma CETP levels.

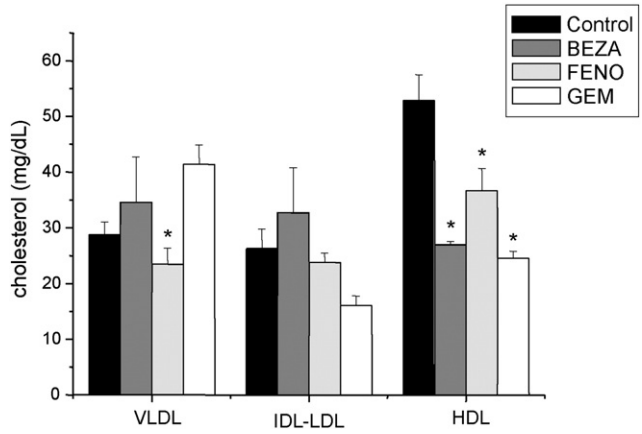

Fig. 2. LP-CHOL profile in CIII/CETP transgenic mice treated with different fibrates or a placebo (control). Mean±SE (n=3–4). ANOVA, Tukey–Kramer post-hoc test. * $P \leq .05$ *vs.* control.

Since FENO was the most effective fibrate in reducing TG and increasing CETP expression, we also tested FENO effects on CETP transgenic mouse without hypertriglyceridemic background (Table 2). Endorsing the results found in CIII/CETP mice, CETP-only mice also presented reduction in plasma TG and increased CETP expression and plasma activity. In addition, FENO treatment positively regulated the PPARα target gene, the LPL plasma levels.

Because synthetic PPARα ligands increased CETP expression, we tested whether natural PPAR ligands, n-3 and n-6 PUFA-rich oils, had the same effects. For this purpose, CETP transgenic mice on a normolipidemic background were treated with fish oil (FO), corn oil (CO) or saline (S) for 2 weeks. The CO and FO treatments did not affect food intake or body, liver, perigonadal or subcutaneous adipose tissue weights after this 2-week period of treatment (Supplemental data: table S3).

The fasting plasma levels of lipids and GLUC are shown in Table 3. Glycemia and triglyceridemia were similar in all groups. NEFA levels were reduced by FO (26%). CO appeared to have the same effect, but the changes did not reach statistical significance. Compared with S, FO reduced the plasma CHOL levels (15%), whereas compared with CO, FO reduction was 20%. Other researchers have also demonstrated that FO reduces CHOL levels [47–49].

Because adipokine expression can be affected by PPAR activity, we determined the leptin and adiponectin plasma levels (Fig. 3). FO reduced leptin (13% *vs.* CO) and increased the adiponectin levels (27% *vs.* S and 31% *vs.* CO). The changes in the adipokine levels were likely caused by the differential activation of PPARα [50] and γ [51,52].

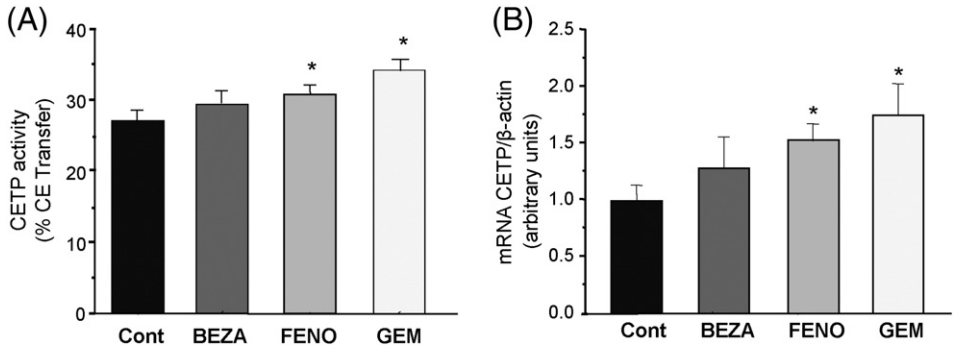

Fig. 1. Plasma CETP activity (A) and liver CETP mRNA level (B) in CIII/CETP transgenic mice treated with different fibrates or a placebo (control). Mean±SE (n=6–9). ANOVA, Tukey–Kramer post-hoc test. * $P \leq .05$ *vs.* control.

Table 2
Plasma triglycerides (TG), post heparin lipases and CETP activities and liver CETP expression of CETP mice treated with fenofibrate (FENO) or placebo (control)

	Control	FENO
TG (mg/dl)	73±7	50±2*
LPL (nmol/ml/h)	228±19	430±56*
HL (nmol/ml/h)	887±83	970±67
TL (nmol/ml/h)	1115±84	1400±94*
CETP activity (%CE transfer)	29.0±3.3	39.5±2.0*
CETP mRNA (fold change)	1.0±0.37	2.5±0.51*

Mean±SE (n=4–8). Student t test.
* $P \leq .05$ *vs.* control.

Table 3
Fasting plasma concentrations of lipids and glucose (GLUC) in CETP mice treated with FO, CO or S

	S	CO	FO
GLUC (mg/dl)	81±5	83±5	75±4
TG (mg/dl)	58±5	51±4	51±4
NEFA (nmol/dl)	1.32±0.12	1.00±0.09	0.97±0.08*
CHOL (mg/dl)	86±4	92±3	73±4*#

Mean±SE (n=23–25). ANOVA, Tukey–Kramer post-hoc test: * $P \leq .05$ *vs.* S; # $P \leq .05$ *vs.* CO.

We next evaluated the effects of the oil treatments on CETP expression and the CETP plasma levels. FO significantly increased the CETP plasma concentration (90% *vs.* S and 83% *vs.* CO) (Fig. 4A) and activity (23% *vs.* S and 19% *vs.* CO) (Fig. 4B). The same tendency was observed for the abundance of CETP liver mRNA (28% *vs.* S and 69% *vs.* CO) (Fig. 4C). It is important to emphasize that, compared with S, CO had no effect at all on the CETP plasma levels or mRNA abundance.

In order to confirm that FO treatment was indeed a PPARα agonist, we analyzed the liver mRNA expression of LPL, an established PPARα target gene (Fig. 5). In fact, FO increased LPL expression by 65% *vs.* S and 73% *vs.* CO.

The LP-CHOL profiles of the CETP mice treated with S, CO or FO are shown in Fig. 6. FO reduced HDL-CHOL by 20% when compared with S and CO, although these effects were statistically significant in females only (supplemental data: Fig. S1).

4. Discussion

In this study, we hypothesized that the CETP gene is up-regulated by PPAR ligands based on two main findings: (1) a potential PPRE consensus sequence is present in the CETP human gene [18] and (2) ciprofibrate treatment stimulated CETP gene expression in both normo- and hypertriglyceridemic backgrounds [41]. In fact, the present data show that FENO, GEM and *n*-3 PUFA-rich FO increase CETP expression, likely through PPAR activation.

In this study, we used two assays to quantify the plasma CETP levels. The exogenous CETP activity assay is independent of the endogenous LPs as substrates and, thus, is indicative of CETP mass [37]. The second assay, the ELISA, is more sensitive and directly quantifies the CETP mass (Fig. 4A *vs.* B). Most studies in humans that have demonstrated a reduction by or no effect of fibrates on the plasma CETP activity have used CE transfer assays, which are dependent on the endogenous LP substrates [32–34,36,38,53,54]. Thus, their data reflect the decreased availability of TG-rich LPs as CE acceptors, which is a major effect of fibrates. One study that used the CETP exogenous assay demonstrated that GEM increased CETP in the plasma of diabetic patients [39]. Other studies have directly determined the CETP mass after fibrate treatments [36,37,54] and demonstrated no alterations in the CETP levels in hyperlipidemic subjects. In animal models, controversial results have been reported. ApoE*3Leiden.CETP (hyperlipidemic) mice had their CETP plasma (endogenous) activity and liver mRNA levels diminished by FENO [29] and their CETP mass reduced by tesaglitazar, a ligand of both PPARα and PPARγ [30]. In contrast, the present data and previous studies using human CETP transgenic mice have demonstrated that FENO [40] and ciprofibrate [41] increase CETP liver mRNA and plasma (exogenous) activity or mass. In addition, Beyer et al. [40] used three different synthetic PPARα ligands (FENO, Wy14643 and LY970) and also studied natural CETP-expressing hamsters and HepG2 cells. Therefore, four distinct PPARα agonists tested on two animal models and in cell culture have demonstrated the up-regulation of CETP gene expression.

Only a few studies have investigated CETP modulation by natural PPAR ligands, such as those present in PUFA-rich oils, and these studies have shown no effect [44] or a reduction [43] in the plasma CETP (exogenous) activity in humans treated with FO. Recently, Ishida et al. [55] demonstrated that DHA, but not EPA, increased CETP mRNA expression and plasma activity in hamsters fed a high-fat diet, whereas Chadli et al. [56] showed no effects of FO on the plasma levels of CETP when using the same animal model. Our data fit best with the results of Ishida et al. [55].

In the present study, the increases in the plasma CETP concentration after FO treatment were greater than the increases in hepatic mRNA abundance, which might reflect increased CETP secretion by extrahepatic tissues, such as the spleen, small intestine, kidneys and adipose tissues [13].

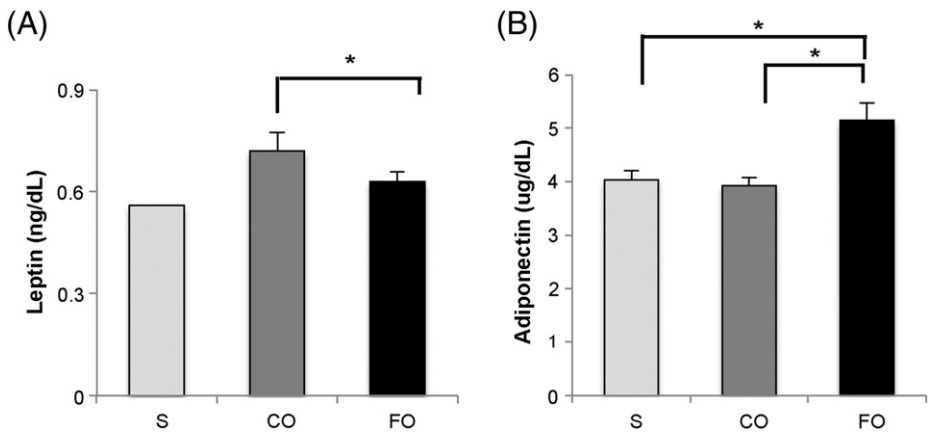

Fig. 3. Plasma leptin (A) and adiponectin (B) levels in CETP transgenic mice treated with S, CO or FO. Mean±SE (9–11 per group). ANOVA, Tukey–Kramer post-hoc test. * $P \leq .05$.

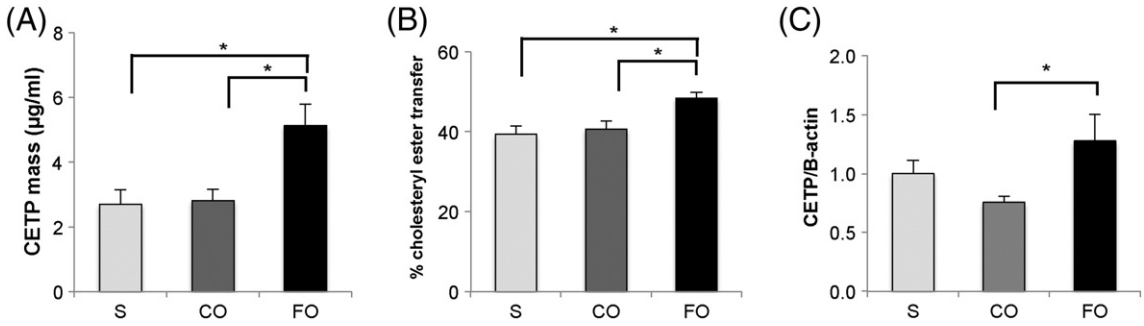

Fig. 4. CETP plasma levels (A), activity (B) and liver mRNA level (C) in CETP transgenic mice treated with saline (S), corn (CO) or fish (FO) oil. Mean±SE (12–14 per group). ANOVA, Tukey–Kramer post-hoc test: * $P \leq .05$.

Modest elevations in HDL-CHOL levels in human plasma have been previously demonstrated after treatments with FENO [57] and EPA and DHA [28]. This effect is attributed to a PPRE in the human apo A1 gene promoter region [58]. In fact, the results from the Framingham Offspring Study have demonstrated that the HDL response to PUFA intake is dependent on a polymorphism in the apoA1 promoter region [59]. Different from the human gene, the mouse apo A1 gene has a nonfunctional PPRE [60]. Therefore, in our study, the diminished HDL-CHOL level after all fibrate and FO treatments can be explained by the increase in the CETP expression associated with the lack of an effect on the mouse apo A1 gene.

Although both CO and FO are sources of PPAR ligands, it is known that *n*-6 and *n*-3 fatty acids exhibit fairly different biological effects, which may be partially explained because *n*-6 and *n*-3 PUFA have distinctive affinities for specific PPAR subtypes. For example, EPA has a higher affinity for PPARα than other PUFA do [61]. Our data reinforce the differences between the effects of *n*-6 and *n*-3 fatty acids and demonstrate that only *n*-3 PUFA (FO) is potent enough to increase CETP expression.

Other PPARα targets, such as LPL and plasma adiponectin levels were up-regulated by *n*-3 PUFA and FENO treatment, supporting the hypothesis that CETP modulation by *n*-3 PUFA and fibrates occurs through PPARα activation pathway.

In conclusion, we have demonstrated that fibrates and FO, but not CO, up-regulate human CETP gene expression at both the mRNA and protein levels. Because these are known PPARα ligands, we postulate that these effects are mediated by PPARα activation, which acts on a putative PPRE in the CETP gene. The final outcome of these treatments regarding the LP profile will depend on the responsiveness of other determinant genes and the species' genetic backgrounds. These findings are relevant to the understanding of the CETP gene regulation, a putative therapeutic target for CVD.

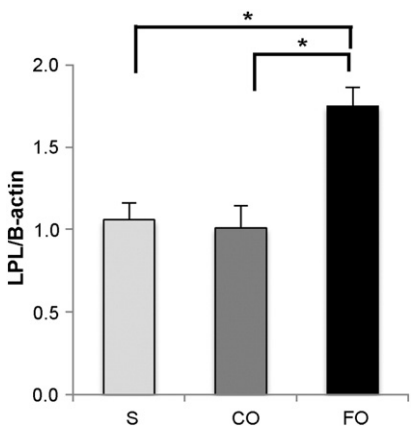

Fig. 5. Liver LPL mRNA level in CETP transgenic mice treated with saline (S), corn (CO) or fish (FO) oil. Mean±SE (12–14 per group). ANOVA, Tukey–Kramer post-hoc test: * $P \leq .05$.

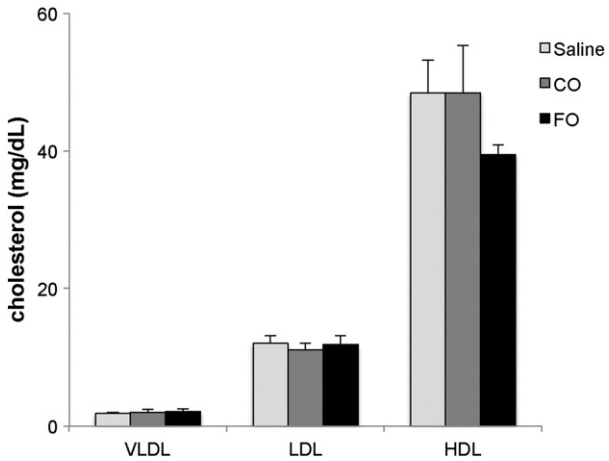

Fig. 6. LP-CHOL profile in CETP transgenic mice treated with saline (S), corn (CO) or fish (FO) oil. Mean±SE (six pools per group). ANOVA, Tukey–Kramer post-hoc test: nonsignificant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2014.02.008.

Acknowledgments

We are especially grateful for the technical assistance of Letícia Paiva Silveira.

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