Homozygous disruption of *Pctp* modulates atherosclerosis in apolipoprotein E-deficient mice

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Abstract **Phosphatidylcholine transfer protein (PC-TP) is a cytosolic phospholipid binding protein and a member of the steroidogenic acute regulatory-related transfer domain superfamily. Its tissue distribution includes liver and macrophages. PC-TP regulates hepatic lipid metabolism, and its absence in cholesterol-loaded macrophages is associated with reduced ATP binding cassette transporter A1-mediated lipid efflux and increased susceptibility to apoptosis induced by unesterified cholesterol. To explore a role for PC-TP in atherosclerosis, we prepared PC-TP-deficient/apolipoprotein E-deficient (*Pctp*$^{-/-}$/*Apoe*$^{-/-}$) mice and littermate *Apoe*$^{-/-}$ controls. At 16 weeks, atherosclerosis was increased in chow-fed male, but not female, *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice. This effect was associated with increases in plasma lipid concentrations. By contrast, no differences in atherosclerosis were observed between male or female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$ controls fed a Western-type diet for 16 weeks. At 24 weeks, atherosclerosis in chow-fed male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice tended to be reduced in proportion to plasma cholesterol. The attenuation of atherosclerosis in female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice fed chow or the Western-type diet for 24 weeks was not attributable to changes in plasma cholesterol or triglyceride concentrations. These findings suggest that PC-TP modulates the development of atherosclerosis, in part by regulating plasma lipid concentrations.—**Wang, W-J., J. M. Baez, R. Maurer, H. M. Dansky, and D. E. Cohen. **Homozygous disruption of *Pctp* modulates atherosclerosis in apolipoprotein E-deficient mice. *J. Lipid Res.* 2006.** 47: **2400–2407.**

Supplementary key words phosphatidylcholine transfer protein • steroidogenic acute regulatory-related transfer domain • cholesterol • triglycerides • aorta • macrophage

Phosphatidylcholine transfer protein (PC-TP) is a 25 kDa cytosolic protein that binds phosphatidylcholines exclusively (1, 2). PC-TP (also known as StarD2) is a member of the steroidogenic acute regulatory-related lipid transfer (START) domain protein superfamily (3). It is expressed in a variety of tissues and is enriched in liver (1, 4, 5) and macrophages (6). Although PC-TP promotes the exchange of phosphatidylcholine between membranes in vitro (1), its biological function remains elusive.

Using stably transfected Chinese hamster ovary cells, we demonstrated that overexpression of PC-TP promotes apolipoprotein A-I-mediated efflux of phospholipids and cholesterol (7). When mouse peritoneal macrophages cultured from wild-type and PC-TP-deficient (*Pctp*$^{-/-}$) mice were loaded with esterified cholesterol using oxidized LDL (6), the absence of PC-TP expression was associated with decreased apolipoprotein A-I-mediated lipid efflux attributable to lower expression levels of Abca1. Moreover, lack of PC-TP expression increased susceptibility to unesterified cholesterol-induced apoptosis of macrophages in vitro. Consistent with a role in reverse cholesterol transport, in vivo studies using *Pctp*$^{-/-}$ mice have demonstrated that PC-TP expression regulates the size and hepatic uptake of HDL particles (8) as well as the response of biliary lipid secretion to dietary cholesterol (5) and that the absence of PC-TP expression leads to compensatory alterations in hepatic cholesterol metabolism (9).

These apparent roles in cholesterol efflux from macrophages and in the biliary elimination of plasma cholesterol suggest that PC-TP expression may influence the development of atherosclerosis. To test this hypothesis, we prepared mice with homozygous disruption of both *Pctp* and apolipoprotein E (*Apoe*) genes. *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ and littermate *Apoe*$^{-/-}$ mice were challenged with either chow or a Western-type diet for 16 and 24 weeks. Compared with *Apoe*$^{-/-}$ controls at 16 weeks, atherosclerosis in chow-fed male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice was increased. These differences did not persist when the comparison was adjusted for plasma lipid concentrations and were not observed in Western-type diet-fed mice. At 24 weeks, the absence of

Manuscript received 23 June 2006 and in revised form 7 August 2006.

Published, JLR Papers in Press, August 28, 2006.
DOI 10.1194/jlr.M600277-JLR200

Abbreviations: apoE, apolipoprotein E; PC-TP, phosphatidylcholine transfer protein; START, steroidogenic acute regulatory-related transfer.

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PC-TP expression was associated with attenuated atherosclerosis in chow-fed male and female mice as well as in female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice fed the Western-type diet. In male mice, this could be attributed to changes in plasma lipids. However, in female mice, adjustment for plasma lipids did not entirely eliminate the influence of PC-TP expression on atherosclerotic lesion area, suggesting that PC-TP expression within the arterial wall predisposes to atherosclerosis after extended periods of hyperlipidemia.

MATERIALS AND METHODS

Mice

Pctp$^{-/-}$ mice backcrossed for eight generations to a C57BL/6J genetic background (5) were used to create *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice. Mice were crossed with *Apoe*$^{-/-}$ C57BL/6J mice purchased from the Jackson Laboratory (Bar Harbor, ME). The heterozygous F1 generation was intercrossed to create *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ double-null mice as well as *Apoe*$^{-/-}$ littermate controls. Genotyping for *Pctp* was as described by van Helvoort et al. (10), with *Apoe* genotyping performed according to the Jackson Laboratory protocol (http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh).

Diets and experimental design

Mice were weaned either onto chow (4% fat, 0.02% cholesterol; catalog No. D110804; Research Diets, New Brunswick, NJ) or a Western-type diet (21% fat, 0.2% cholesterol; catalog No. TD 88137; Harlan Teklad, Madison, WI). After feeding for 16 or 24 weeks, mice were anesthetized with intraperitoneal injections of ketamine (87 mg/kg body weight; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (13 mg/kg body weight; Lloyd Laboratories, Shenandoah, IA). At 9 AM, blood was collected by cardiac puncture. The circulatory system was then perfused via the left ventricle with 10 ml of PBS immediately after severing the superior vena cava. The liver was removed and snap-frozen in liquid nitrogen. The aorta was dissected from the heart to the iliac bifurcation and fixed in 3 ml of 10% phosphate-buffered formalin. The heart was transected, and the top half was placed in OCT solution (Tissue-Tek®, Torrance, CA) for 2 min. The aortic root was then placed in a 15 × 15 × 15 mm base mold (Fisher Scientific, Fairlawn, NJ) containing OCT and fixed on dry ice. Samples were stored at −80°C before sectioning. Blood samples were anticoagulated by the addition of EDTA. Plasma was separated by centrifugation. All experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee.

Analytical techniques

Quantification of atherosclerosis. For en face analysis of aortic atherosclerosis, aortas were prepared and stained for quantification of atheromatous lesions (11). Briefly, adventitial tissue surrounding aortas was carefully removed. Samples were then rinsed in 70% ethanol before lipid staining for 5 min with 0.5% Sudan IV, 35% ethanol, and 50% acetone. Aortas were destained for 1 min in 80% ethanol, cut open longitudinally with scissors, and then pinned open on a bed of hard wax. Samples were photographed using a microscope (Omano OMVT) fitted with a digital camera (Olympus C-5000). Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD; http://rsb.info.nih.gov/ij) to quantify the percentage of total aortic area occupied by atheromatous lesions.

Aortic sinus atherosclerosis was quantified in cross-sections of mouse hearts in the region of the aortic root according to the accumulation of neutral lipids and macrophages. Aortic roots were sectioned at −20°C using a Minotome PLUS™ cryostat (Triangle Biomedical Sciences, Inc., Durham, NC). Frozen serial sections were prepared, with the aortic sinus at the level of the three valves as the starting point. Sections were collected onto positively charged slides at three sections per slide (12). Serial sections of 6 μm thickness were fixed in 10% phosphate-buffered formalin for 10 min. Sections were then rinsed with running water for 15 min before staining for lipid accumulation with 0.5% Oil Red O in propylene glycol (Sigma, St. Louis, MO) (13). Sections were then counterstained with Gill's hematoxylin solution (Sigma) and then mounted using glycerol-gelatin (Sigma). Each section was photographed using a Nikon DXM1200F digital camera linked to a Nikon Optiphot-2 microscope (Nikon Instruments, Inc., Melville, NY). Images were analyzed using ImagePro (MedicaCybernetics, Silver Spring, MD) to quantify atherosclerotic lesions. Aortic sinus lesion areas were determined by averaging values obtained from five to nine sections per mouse.

Macrophage accumulation in atherosclerotic lesions was quantified by immunohistochemistry using an avidin-biotin-peroxidase method (14). Briefly, a monoclonal anti-mouse Mac 3 antibody (PharMingen, San Diego, CA) was used at a 1:900 dilution to immunostain macrophages in mouse heart sections. Sections were then exposed using a biotinylated rabbit anti-rat IgG (H+L) mouse absorbed antibody (Vector Laboratories, Burlingame, CA) diluted 1:200 in PBS with 5% rabbit serum. Sections were incubated for 30 min with avidin-biotin complex at a 1:100 dilution in PBS prepared according to the manufacturer's specifications (Vectastain ABC kit instructions; Vector Laboratories). Immunostaining was performed using 3-amino-9-ethylcarbazole (Dako, Carpinteria, CA) followed by counterstaining with Gill's hematoxylin solution. Lesional macrophage contents were determined using ImagePro as the area percentage of color in each section.

Sections were stained for apoptotic cells using the ApopTag®-Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon International, Temecula, CA) according to the manufacturer's specifications. Apoptotic cells were counted using a Leica DMLB 100T microscope at 400× magnification.

Analyses of plasma lipids. Plasma total cholesterol and triglyceride concentrations were determined enzymatically using reagents from Roche (Indianapolis, IN) and Sigma, respectively. Lipoproteins were separated by fast-protein liquid chromatography (15) after equal volumes of plasma were pooled. Cholesterol concentrations in individual fractions were determined enzymatically (15).

Western blot analysis. Protein expression in liver homogenates was determined by Western blot analysis using antibodies to PC-TP (16) and apoE (Biodesign International, Saco, ME). Blots were stripped and reprobed with β-actin antibody (Sigma) to control for differences in protein loading. Detection was by enhanced chemiluminescence.

Statistical analyses

Data are reported as means ± SEM. Differences between experimental groups were analyzed using a two-tailed unpaired Student's *t*-test or Mann-Whitney *U*-test. The primary analysis in these studies was to examine the influence of genotype on atherosclerosis. This involved eight comparisons based on diet, sex, and time. Therefore, a Bonferroni adjustment was made to the *P* value to account for multiple testing: $P < 0.0063$ was considered significant for these comparisons. Linear regression

analysis was used to assess the influence of PC-TP expression on atherosclerosis after adjusting for the contributions of plasma cholesterol and triglyceride concentrations. Because of the relatively small number of mice (n = 7–17/group), plasma cholesterol and triglyceride concentrations were added separately in the regression model to estimate the confounding effect.

RESULTS

To examine the influence of PC-TP expression on the development of atherosclerosis, we created *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ littermate control C57BL/6J mice. The absence of PC-TP in the double-null mice and the lack of apoE expression in both genotypes were confirmed by Western blot analysis (data not shown). Both genotypes of mice reproduced normally and did not exhibit genotype-specific differences in weight during the course of these experiments.

Figure 1 demonstrates the influence of PC-TP expression on aortic atherosclerosis in *Apoe*$^{-/-}$ mice. After 16 weeks, there was a trend ($P < 0.05$) toward increased atherosclerotic lesion area in chow-fed male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice (Fig. 1A), which was not observed for chow-fed female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice. PC-TP expression did not influence atherosclerosis in mice of either gender when fed the Western-type diet for 16 weeks. At 24 weeks, lesion area tended to decrease by 21% and 23% in aortas of chow-fed *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ male and female mice, respectively, compared with their littermate *Apoe*$^{-/-}$ controls (Fig. 1B). The absence of PC-TP expression reduced aortic atherosclerosis by 22% in Western-type diet-fed female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice compared with gender-matched *Apoe*$^{-/-}$ controls (Fig. 1B–D).

To determine whether changes in aortic atherosclerosis might be attributable to alterations in plasma lipids, we measured cholesterol and triglyceride concentrations (**Fig. 2**) as well as the distribution of cholesterol among plasma lipoproteins (**Fig. 3**). Plasma total cholesterol concentrations were increased and plasma triglyceride concentrations tended to increase in male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ versus *Apoe*$^{-/-}$ mice fed chow for 16 weeks (Fig. 2A, C). The Western-type diet increased cholesterol and triglyceride concentrations in both male and female mice. Except for a trend toward increased cholesterol and triglyceride concentrations in male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice, there were no significant variations attributable to PC-TP expression. At 24 weeks, there were no differences in cholesterol or triglyceride concentrations attributable to PC-TP expression for chow-fed male or female mice, other than a trend toward reduced plasma cholesterol concentrations in male

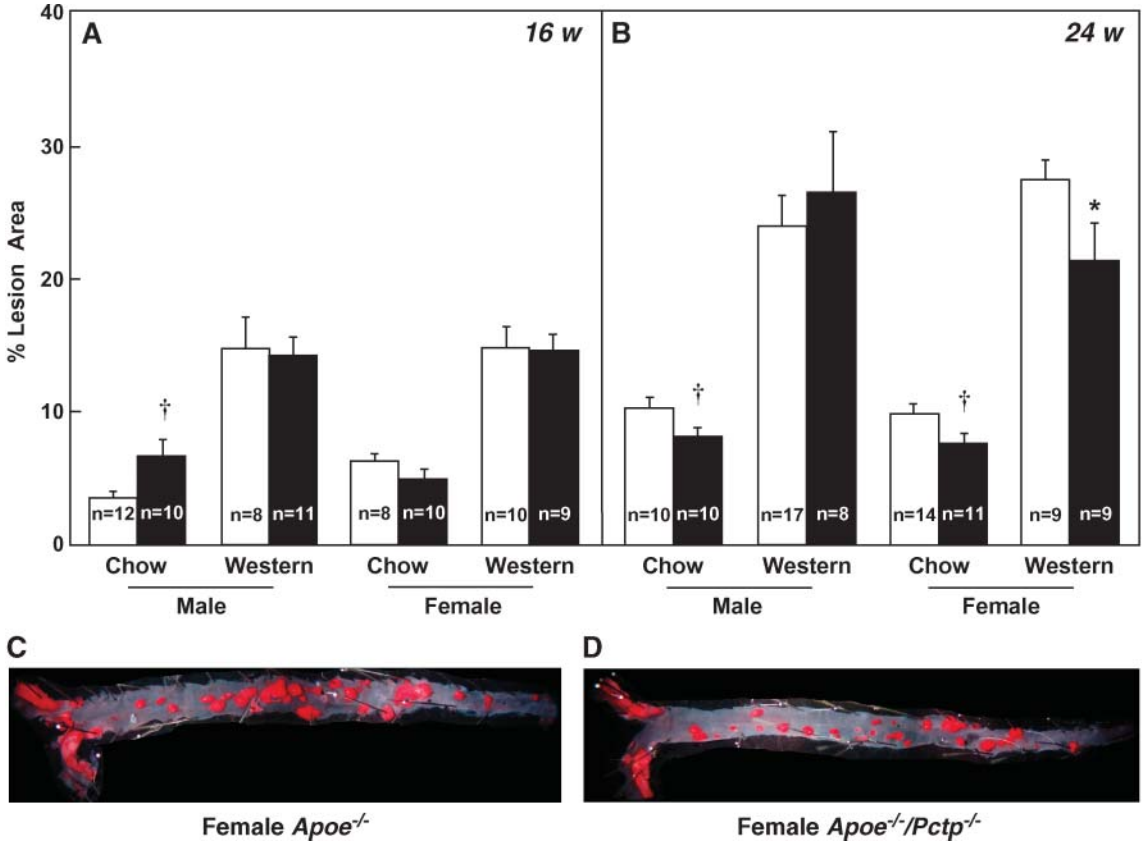

Fig. 1. Influence of phosphatidylcholine transfer protein (PC-TP) expression on aortic atherosclerosis in apolipoprotein E-deficient (*Apoe*$^{-/-}$) mice. A, B: Atherosclerotic lesions were quantified in aortas of male and female *Apoe*$^{-/-}$ (open bars) and *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ (closed bars) mice after feeding chow or a Western-type diet for 16 (A) or 24 (B) weeks. The number of mice per group is indicated within each bar. C, D: Representative en face images of Sudan IV-stained aortas in female *Apoe*$^{-/-}$ (C) and *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ (D) mice after 24 weeks of Western-type diet feeding. Error bars represent SEM. * $P < 0.0063$, † $P < 0.05$, *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ versus *Apoe*$^{-/-}$ mice.

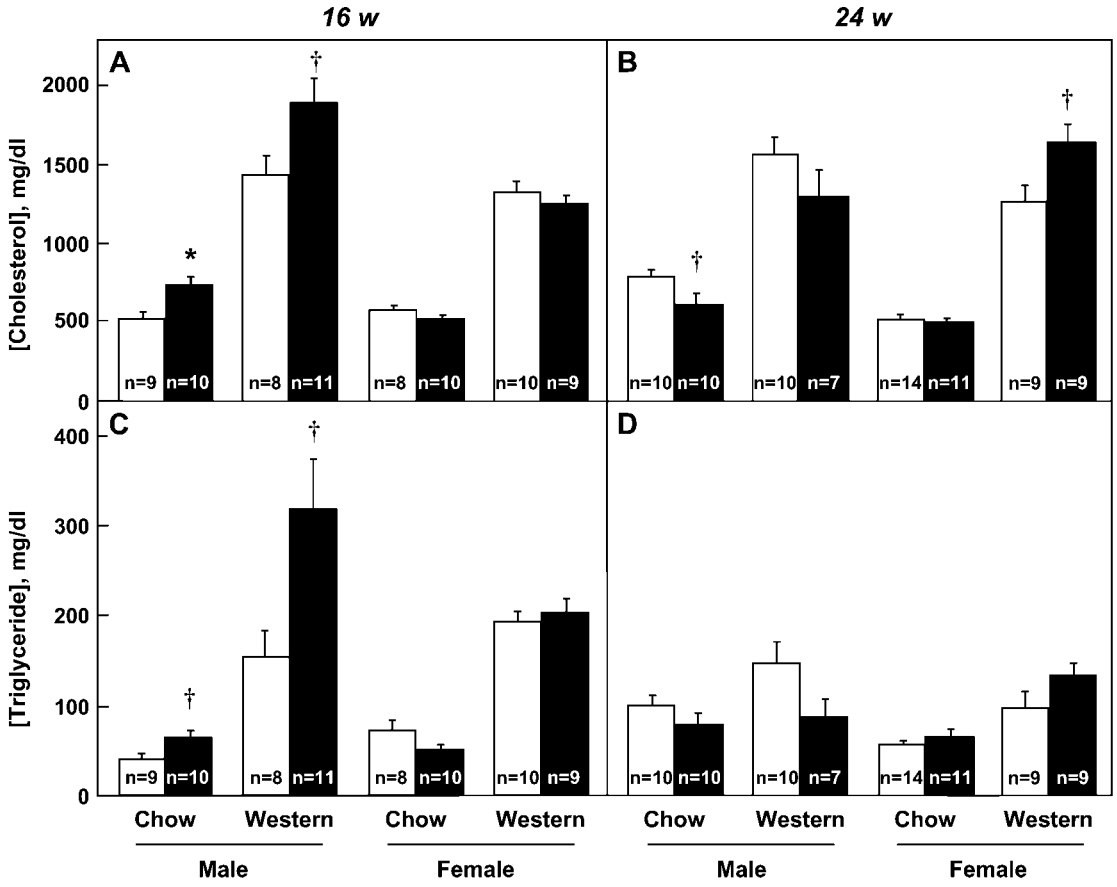

Fig. 2. Influence of PC-TP expression on plasma lipid concentrations in *Apoe*$^{-/-}$ mice. Plasma concentrations of cholesterol (A, B) and triglycerides (C, D) in male and female *Apoe*$^{-/-}$ (open bars) and *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ (closed bars) mice after feeding chow or a Western-type diet for 16 weeks (A, C) or 24 weeks (B, D). The number of mice per group is indicated within each bar. Error bars represent SEM. * $P < 0.0063$, † $P < 0.05$, *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ versus *Apoe*$^{-/-}$ mice.

Pctp$^{-/-}$/*Apoe*$^{-/-}$ mice (Fig. 2B, D). In female but not male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice fed the Western-type diet for 24 weeks, cholesterol concentrations tended to increase by 21% compared with littermate *Apoe*$^{-/-}$ control mice. As illustrated by Fig. 3, the Western-type diet markedly increased the proportion of VLDL cholesterol in plasma. However, there were no clear genotype-dependent differences in the pattern of distribution of plasma cholesterol among lipoproteins in female mice at 16 weeks (Fig. 3A) or 24 weeks (Fig. 3B). The same was true for male mice (data not shown).

To confirm the observation that aortic atherosclerosis in female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice was attenuated after 24 weeks of the Western-type diet (Fig. 1B–D) despite increased plasma cholesterol (Fig. 2B), we quantified aortic sinus atherosclerosis based on Oil Red O staining of cardiac sections from the same mice (**Fig. 4**). Consistent with results of the en face analysis, average lesion area was reduced by 13% for female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice fed a Western-type diet for 24 weeks compared with littermate control *Apoe*$^{-/-}$ mice.

To estimate the extent to which the influence of PC-TP expression on lesion size could be attributed to its influence on plasma lipid concentrations, we performed a regression analysis. **Table 1** presents the influence of PC-TP expression on lesion area quantified by the en face method and the effect of adjustments for plasma cholesterol and triglyceride concentrations. The influence of PC-TP expression before adjustment reflects the results presented in Fig. 1A. Increases in *P* values after adjusting for cholesterol and triglycerides observed in chow-fed male mice at 16 and 24 weeks suggest confounding effects of plasma lipids. For chow-fed and Western-type diet-fed female mice at 24 weeks, adjustment for plasma lipids largely did not change *P* values substantially, suggesting that the influence of genotype remained after correcting for plasma cholesterol or triglyceride concentrations.

Because PC-TP is expressed in macrophages (6), we examined the macrophage content of aortic sinus lesions (**Fig. 5A**). Although there was a modest reduction of macrophage content in Western-type diet-fed female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice at 24 weeks (Fig. 5B), this did not achieve statistical significance. Similarly, there was no difference in the number of apoptotic cells per section (Fig. 5C).

DISCUSSION

Although the cellular function of PC-TP is not known, its expression influences hepatobiliary lipid metabolism as well

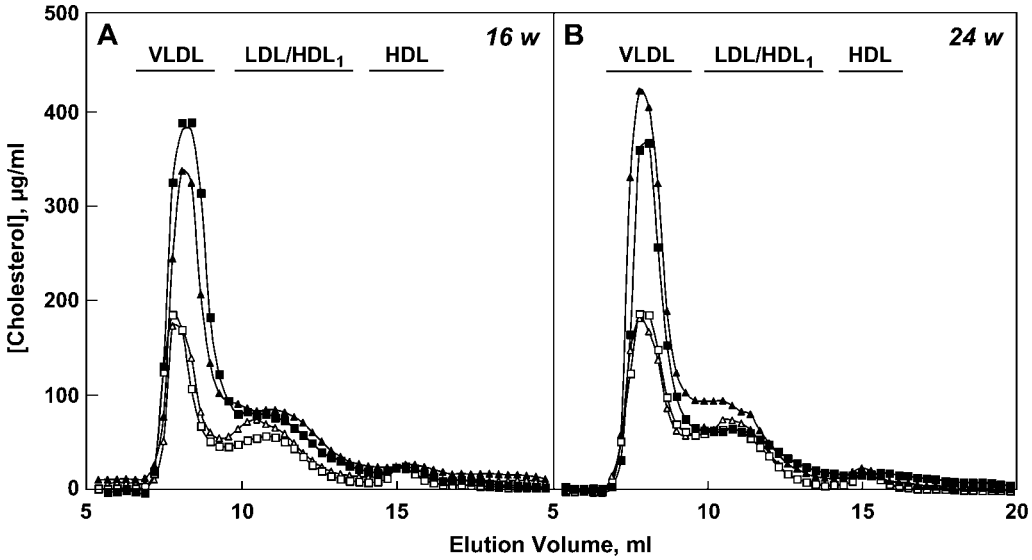

Fig. 3. Distribution of lipoprotein cholesterol in female *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ and *Apoe*$^{-/-}$ mice. Equal volumes of pooled plasma were fractionated by fast-protein liquid chromatography for female *Apoe*$^{-/-}$ mice (triangles) and *Apoe*$^{-/-}$/*Pctp*$^{-/-}$ mice (squares) after feeding chow (open symbols) or a Western-type diet (closed symbols) for 16 (A) and 24 (B) weeks ($n \geq 8$ mice/pooled sample). Distributions of cholesterol among VLDL, LDL/HDL$_1$, and HDL (15) are indicated in each panel.

as the response of macrophages to cholesterol loading. These observations prompted us to examine a role for PC-TP in the progression of atherosclerosis in *Apoe*$^{-/-}$ mice. The main findings were that aortic atherosclerosis at 16 weeks tended to increase in chow-fed male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice compared with littermate *Apoe*$^{-/-}$ mice. By contrast, at 24 weeks, atherosclerosis was attenuated in chow-fed male and female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice as well as in female double-null mice fed the Western-type diet. Whereas the magnitudes of the observed differences were relatively modest and the pathophysiological importance of PC-TP overall in modulating atherosclerosis is not certain, we will discuss these data in a consistent biological context.

The increase of atherosclerosis in the absence of PC-TP expression at 16 weeks was associated with differences in plasma triglyceride concentrations and cholesterol concentrations in chow-fed male *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice. Moreover, the likelihood that the observed difference in atherosclerosis was attributable to the increases in plasma cholesterol and triglyceride concentrations is supported by the regression analysis. In this connection, we have observed abnormalities in both cholesterol (5, 8, 9) and triglyceride metabolism in *Pctp*$^{-/-}$ mice (8, 17–19).

At 24 weeks, atherosclerosis was attenuated in the absence of PC-TP expression in chow-fed male mice. Regression analysis (Table 1) suggested that this effect could be attributed to differences in plasma lipid concentrations. By contrast, in chow-fed and Western-type diet-fed female mice, *P* values were not altered after adjustment for plasma cholesterol and triglyceride concentrations. Moreover, in Western-type diet-fed female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice, aortic atherosclerosis was reduced despite trends toward increased plasma cholesterol concentrations. It is important to note that because of the relatively small sample sizes, the use of regression analysis to adjust for plasma concentrations of cholesterol and triglycerides was exploratory in nature, and we did not use rigorous statistical methods to adjust for multiple testing.

Because aortic atherosclerosis and aortic sinus lesion area determined by cross-sectional analysis are correlated in *Apoe*$^{-/-}$ mice (20, 21), we sought to validate this finding, which was obtained using the en face approach. Consistent with a proatherogenic effect of PC-TP expression, aortic sinus lesional area was decreased in Western-type diet-fed female *Pctp*$^{-/-}$/*Apoe*$^{-/-}$ mice compared with *Apoe*$^{-/-}$ mice at 24 weeks to the extent predicted based on a previously published linear correlation between en face and aortic sinus measurements (20).

In the absence of differences in plasma lipid concentrations that might explain the reduction of atherosclerosis in the absence of PC-TP expression at 24 weeks, a plausible mechanism may be altered macrophage function. In a study of mouse peritoneal macrophages from *Pctp*$^{-/-}$ and wild-type mice, we observed that the absence of PC-TP expression increased the susceptibility of macrophages to apoptotic, but not necrotic, cell death in response to loading with unesterified cholesterol (6). The influence of macrophage apoptosis on atherosclerosis is dependent on a balance of apoptosis and phagocytosis (22, 23). In general, phagocytosis of apoptotic macrophages in early atherosclerotic lesions is robust. Therefore, increased rates of apoptosis tend to diminish atherosclerotic lesion size. In advanced lesions, the development of necrotic cores is attributable in part to ongoing apoptosis in the setting of decreased phagocytosis. Considering that a lack of PC-TP expression sensitizes macrophages to unesterified cholesterol-induced apoptosis, it is attractive to speculate that this mechanism

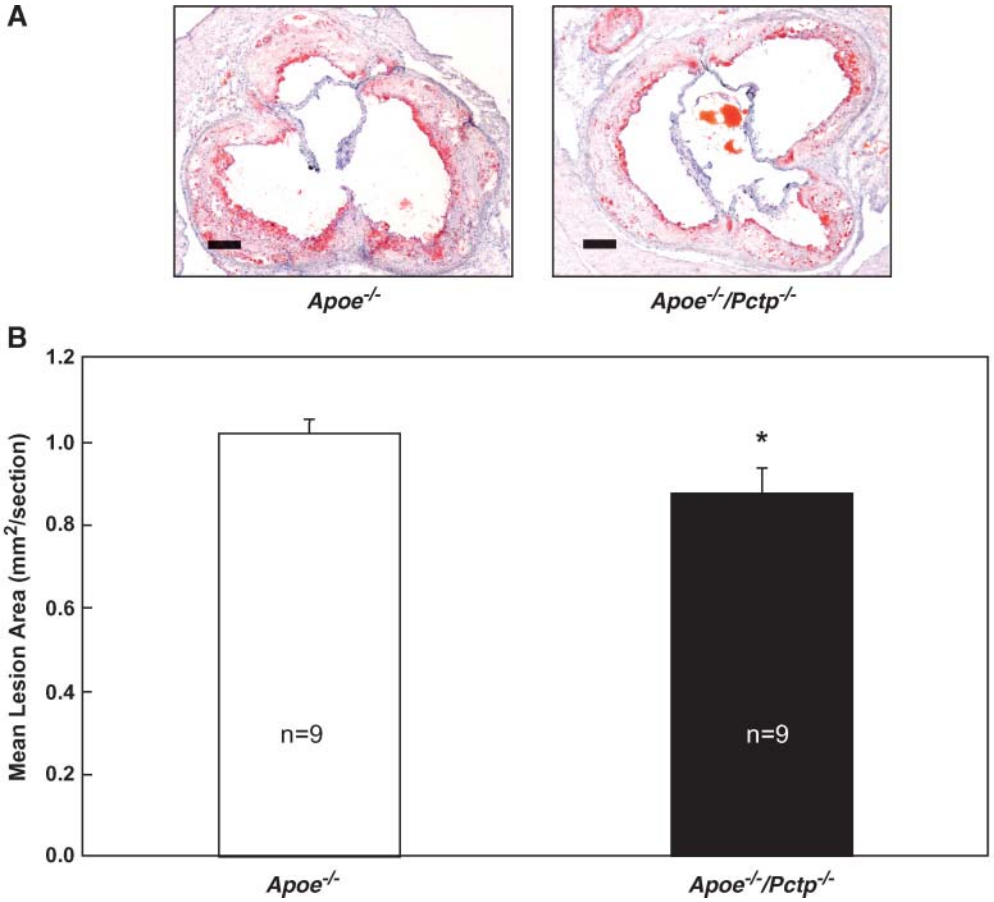

Fig. 4. Aortic sinus lesion sizes in Western-type diet-fed female mice. A: Representative Oil Red O-stained sections of female $Apoe^{-/-}$ (left panel) and $Apoe^{-/-}/Pctp^{-/-}$ (right panel) mice after 24 weeks of Western-type diet feeding. Bars = 200 μm. B: Areas occupied by atherosclerotic lesions. The number of mice per group is indicated within each bar. Error bars represent SEM. * $P < 0.05$, $Apoe^{-/-}/Pctp^{-/-}$ versus $Apoe^{-/-}$ mice.

accounts for the reduction of atherosclerosis that was observed in $Pctp^{-/-}/Apoe^{-/-}$ compared with $Apoe^{-/-}$ female mice at 24 weeks. In support of this possibility, we observed a trend toward decreased lesional macrophage contents. Moreover, very few apoptotic cells were observed in female $Pctp^{-/-}/Apoe^{-/-}$ or $Apoe^{-/-}$ mice fed the Western-type diet for 24 weeks, suggesting that phagocytosis was robust at this time and could have compensated for an increase in apoptotic rate to yield similar numbers of apoptotic cells at steady state. However, this study did not permit a definite answer to this question, and additional studies of advanced plaque morphology and cellular contents at later time points will be required to determined whether increased macrophage apoptosis in vivo contributes mechanistically to attenuated atherosclerosis in the absence of PC-TP expression.

TABLE 1. Regression analysis for the influence of PC-TP expression on atherosclerosis

Weeks on Diet	Sex	Diet	PC-TP Expression[a]	Cholesterol[b]	Triglycerides[c]
16	Male	Chow	0.0378	0.1634	0.0777
		Western-type	0.5919	0.6999	0.5682
	Female	Chow	0.0832	0.0853	0.2244
		Western-type	0.9025	0.9133	0.9356
24	Male	Chow	0.0312	0.1320	0.0639
		Western-type	0.7932	0.5741	0.4716
	Female	Chow	0.0457	0.0366	0.0327
		Western-type	0.0062	0.0860	0.0263

PC-TP, phosphatidylcholine transfer protein.

[a] *P* value for the difference of lesion size in PC-TP-deficient/apolipoprotein E-deficient ($Pctp^{-/-}/Apoe^{-/-}$) mice compared with $Apoe^{-/-}$ mice as quantified by the en face method.

[b] *P* value for the difference of lesion size in $Pctp^{-/-}/Apoe^{-/-}$ mice compared with $Apoe^{-/-}$ mice after adjusting for the influence of plasma cholesterol concentration on lesion size.

[c] *P* value for the difference of lesion size in $Pctp^{-/-}/Apoe^{-/-}$ mice compared with $Apoe^{-/-}$ mice after adjusting for the influence of plasma triglyceride concentration on lesion size.

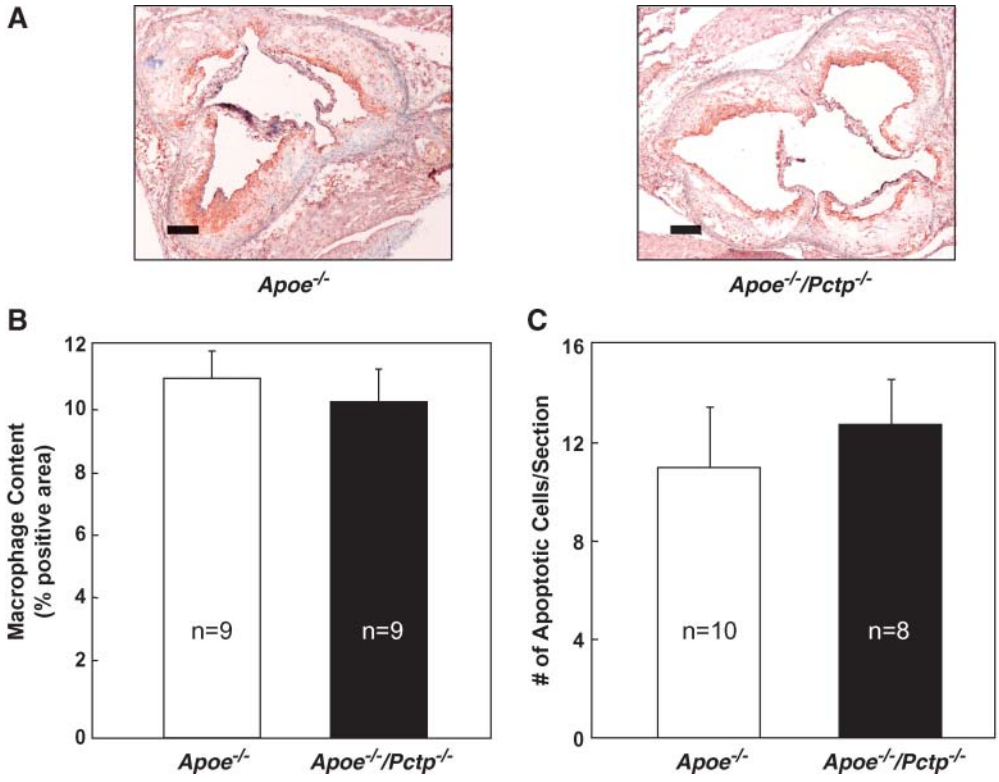

Fig. 5. Macrophage contents of aortic sinus lesions in female *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$*/Pctp*$^{-/-}$ mice fed Western-type diets for 24 weeks. A: Representative sections from female *Apoe*$^{-/-}$ (left panel) and *Apoe*$^{-/-}$*/Pctp*$^{-/-}$ (right panel) mice that were immunostained using monoclonal anti-mouse Mac 3 antibody after 24 weeks of Western-type diet feeding. Bars = 200 μm. B: Areas occupied by macrophages. C: Number of apoptotic cells per section. The number of mice per group is indicated within each bar. Error bars represent SEM.

Emerging data suggest that other START domain proteins, in addition to PC-TP, may play key roles in atherosclerosis. StarD5 is a cholesterol and oxysterol binding protein (24) that is also enriched in macrophages (25). Although its function is not known, StarD5 has been localized to the cytosol and Golgi apparatus (25). In response to cholesterol loading of macrophages that results in endoplasmic reticulum stress, StarD5 is upregulated (26). This suggests that the protein functions to restore normal endoplasmic reticulum function or trigger macrophage apoptosis (26). By contrast, liver-enriched StarD4 is a sterol-regulatory binding element protein-2 target gene that is believed to play a distinct role from StarD5 in cholesterol homeostasis (26, 27). Based on these putative functions, both StarD4 and StarD5 would be expected to influence the development of atherosclerosis.

In summary, these experiments have demonstrated that PC-TP expression appears to reduce lesion size early during the course of atherogenesis in male mice but is later associated with increased atherosclerosis in female *Apoe*$^{-/-}$ mice. In keeping with an emerging role for PC-TP in hepatic lipid metabolism (5, 8, 9, 17–19), early lesion sizes appeared to correlate with variations in plasma lipid concentrations. By contrast, differences in female mice at 24 weeks were not attributable to differences in plasma lipid concentrations and may have reflected local events within the vasculature, such as accelerated apoptosis of macrophages that lack PC-TP expression. This possibility could be specifically addressed in future experiments by the creation of a macrophage-specific *Pctp* knockout mouse or by using a bone marrow transplant approach to replace macrophages in *Apoe*$^{-/-}$ recipient mice with macrophages harvested from the bone marrows of *Pctp*$^{-/-}$*/Apoe*$^{-/-}$ donor mice. These and other studies concerning the mechanisms by which PC-TP, as well as StarD4 and StarD5, influences the progression of atherosclerosis should help elucidate the biological functions of START domain proteins.

This work was supported by National Institutes of Health Grants DK-56626 and DK-48873, by an Established Investigator Award from the American Heart Association, and by an International HDL Research Awards Program grant to D.E.C. J.M.B. was supported by a Minority Access to Research Careers Predoctoral Fellowship (GM-64043) from the National Institutes of Health. The authors thank Drs. Peter Libby and Galina Sukhova for helpful discussions and access to essential equipment required for the histologic analysis of aortic sinus atherosclerosis and Eugenia Shvartz for technical assistance.

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