Bone marrow-derived HL mitigates bone marrow-derived CETP-mediated decreases in HDL in mice globally deficient in HL and the LDLr

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Abstract **The objective of this study was to determine the combined effects of HL and cholesteryl ester transfer protein (CETP), derived exclusively from bone marrow (BM), on plasma lipids and atherosclerosis in high-fat-fed, atherosclerosis-prone mice. We transferred BM expressing these proteins into male and female double-knockout HL-deficient, LDL receptor-deficient mice ($HL^{-/-}LDLr^{-/-}$). Four BM chimeras were generated, where BM-derived cells expressed *1*) HL but not CETP, *2*) CETP and HL, *3*) CETP but not HL, or *4*) neither CETP nor HL. After high-fat feeding, plasma HDL-cholesterol (HDL-C) was decreased in mice with BM expressing CETP but not HL (17 ± 4 and 19 ± 3 mg/dl, female and male mice, respectively) compared with mice with BM expressing neither CETP nor HL (87 ± 3 and 95 ± 4 mg/dl, female and male mice, respectively, $P < 0.001$ for both sexes). In female mice, the presence of BM-derived HL mitigated this CETP-mediated decrease in HDL-C. BM-derived CETP decreased the cholesterol component of HDL particles and increased plasma cholesterol. BM-derived HL mitigated these effects of CETP. Atherosclerosis was not significantly different between BM chimeras. These results suggest that BM-derived HL mitigates the HDL-lowering, HDL-modulating, and cholesterol-raising effects of BM-derived CETP and warrant further studies to characterize the functional properties of these protein interactions.—** Hime, N. J., A. S. Black, D. J. Bonnet, and L. K. Curtiss. **Bone marrow-derived HL mitigates bone marrow-derived CETP-mediated decreases in HDL in mice globally deficient in HL and the LDLr. *J. Lipid Res.* 2014.** 55: **1864–1875.**

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Bone marrow (BM)-derived macrophages are important cells in the progression of atherosclerosis. In the artery wall, macrophages accumulate lipid (particularly from LDL) and express cytokines, chemokines, and procoagulant factors that augment the atherosclerotic process (1). Macrophages can express proteins that affect the metabolism and composition of lipoproteins. Two of these proteins are cholesteryl ester transfer protein (CETP) (2, 3) and HL (4, 5). CETP expressed from BM-derived cells has been suggested to have proatherosclerotic (2) and antiatherosclerotic properties (3, 6). Likewise, HL expressed from BM-derived cells has been shown to have proatherosclerotic (4, 7) and antiatherosclerotic properties (7, 8).

The effect that systemically expressed CETP and HL individually have on circulating lipoproteins is known. CETP transfers neutral lipids (cholesteryl ester and TG) and phospholipids (PLs) between lipoproteins. The net transfer of cholesteryl ester and TG between lipoprotein particles is a function of both the relative abundance and lipid composition of the various lipoproteins (9, 10). Mass transfer of cholesteryl ester occurs from cholesteryl ester-rich HDLs to VLDLs and LDLs in exchange for TG.

HL hydrolyzes TGs and PLs in lipoproteins. TG-enriched HDLs are the preferred substrate (11, 12). High levels of CETP and HL expression are both associated with low HDL-cholesterol (HDL-C) concentrations (13–18).

Inverse associations between plasma HDL-C concentrations and atherosclerotic risk are well established (19–21). Therefore, the HDL-lowering activities of both CETP and HL are consistent with these proteins promoting atherosclerosis. Species that lack plasma cholesteryl ester transfer activity are usually resistant to atherosclerosis (22). When CETP is genetically introduced into mice that normally lack CETP, they become prone to diet-induced atherosclerosis (23). However, in mice that are atherosclerosis prone through expression of other human genes, the addition of CETP can reduce atherosclerosis (24). Humans that are genetically deficient in CETP have higher HDL-C and do

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Abbreviations: BM, bone marrow; BMT, bone marrow transfer; CETP, cholesteryl ester transfer protein; HDL-C, HDL cholesterol; LDLr, LDL receptor; PL, phospholipid.

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not appear to get premature atherosclerosis (25). Although HL activity can lower HDL, it does not always follow that HL is an atherogenic protein. The absence of HL activity decreases atherosclerosis in apoE-deficient mice (26), and yet it increases atherosclerosis in LDL receptor (LDLr)-deficient mice (27). Also, HL activity is inversely correlated with atherosclerosis in familial hypercholesterolemia (28), and raised HDL associated with HL genetic variants does not protect against atherosclerosis (29). The effect of HL on atherosclerosis is influenced by the underlying lipoprotein phenotype (7).

In individuals with polymorphisms in both the genes encoding for CETP and HL (polymorphisms that reduce protein expression), HDL-C concentration is increased, but so is atherosclerosis or coronary artery disease risk (30, 31). We have previously proposed that expression of CETP and HL by macrophages in the arterial interstitial space may modify HDL to generate lipid-poor apoA-I, a rate-limiting step in reverse cholesterol transport (32). In this way, CETP and HL may together have antiatherogenic properties that are active at sites of atherosclerotic lesion formation. In isolation, both CETP and HL expressed from macrophages are atherogenic in mouse models of atherosclerosis (2, 4). In those studies, macrophage-derived HL did not affect the plasma lipid profile, whereas macrophage-derived CETP increased VLDL/LDL and decreased HDL. We propose that the HDL-lowering effect of macrophage-derived CETP may be more pronounced in the absence of HL, or conversely, macrophage-derived HL may raise HDL in the presence of macrophage-derived CETP. Previously, we elucidated the effect of BM-derived HL in the presence of CETP through bone marrow transfer (BMT) studies in $LDLr^{-/-}$ mice that expressed human CETP (8). Those studies showed that the absence of BM-derived HL decreased HDL and increased diet-induced aortic atherosclerosis. However, as all BM donor and recipient mice in those studies expressed CETP, it was not possible to determine whether any effects were a consequence of the combined actions of both HL and CETP. This current study addresses this concern. The double-knockout, $HL^{-/-}LDLr^{-/-}$, BM recipient mice in the BMTs in this study lack HL (and do not express human CETP). The BM donor mice in the BMTs either lack both HL and CETP, express HL but not CETP, express CETP but not HL, or express both HL and CETP. Therefore, the chimeric mice generated by the BMTs have CETP and/or HL expression confined to BM-derived cells. This study demonstrates conclusively the effect of the combined, BM-derived expression of both HL and CETP on plasma HDL concentrations.

MATERIALS AND METHODS

Animals

All mice in this study were backcrossed onto a C57BL/6J background and bred in-house. LDLr-deficient mice ($LDLr^{-/-}$; strain B6.129S7-$Ldlr^{tm1Her}$/J) and human CETP transgenic mice [CETPtg; strain B6.CBA-Tg(CETP)5203Tall/J] with CETP expression driven by the human promoter were originally purchased from Jackson Laboratories (Bar Harbor, ME). HL-deficient mice ($HL^{-/-}$; strain B6.129P2-$Lipc^{tm1Unc}$/J) were kindly provided by Dr. Santamarina-Fojo (National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD). CETPtg mice deficient in the LDLr (CETPtg$LDLr^{-/-}$) and CETPtg mice deficient in HL (CETPtg$HL^{-/-}$) were generated by crossing CETPtg mice with $LDLr^{-/-}$ and $HL^{-/-}$ mice, respectively, and then crossing the progeny (heterozygous for the human CETP transgene) with $LDLr^{-/-}$ and $HL^{-/-}$ until the functional LDLr and HL genes were eliminated. CETPtg$LDLr^{-/-}$ and CETPtg$HL^{-/-}$ mice (heterozygous for the human CETP transgene) were always crossed with $LDLr^{-/-}$ and $HL^{-/-}$ mice, respectively, keeping mice heterozygous for the human CETP transgene. Double-knockout mice deficient in both HL and LDLr ($HL^{-/-}LDLr^{-/-}$) were generated by crossing $HL^{-/-}$ mice with $LDLr^{-/-}$ mice and then crossing the heterozygous progeny until the functional HL and LDLr genes had been eliminated. Genotyping was performed on DNA from tail clippings using protocols and primers as provided by Jackson Laboratories (http://jaxmice.jax.org/pub-cgi/protocols/protocols.sh?objtype =prot_list). The mice were weaned at 4 weeks of age and fed ad libitum a standard mouse chow (Diet No. 7019, Harlan Teklad, Madison, WI). During study periods, mice were fed a high-fat, atherogenic diet containing 15.8% fat, 1.25% cholesterol (w/w), and no cholate (No. 94059, Harlan Teklad). All mice were housed three to four per cage in autoclaved, filter-top cages with autoclaved water and kept in a 12 h light/dark cycle. Animal care and use for all procedures was conducted in accordance with guidelines approved by the Institutional Animal Care and Use Committee of the Scripps Research Institute. This experimentation conforms to Public Health Service Policy on Humane Care and Use of Laboratory Animals.

BMT

BMTs were performed as previously described (33). Briefly, BM recipient $HL^{-/-}LDLr^{-/-}$ mice (aged 9–13 weeks) received 10 Gy γ-irradiation followed by reconstitution of BM by intravenous injection of BM containing 2×10^6 nucleated cells extracted from the femurs and tibias of 8- to 14-week-old donor mice. The donor mice were either C57BL/6J WT (human CETP absent, HL present), CETPtg (human CETP present, HL present), $HL^{-/-}$ (human CETP absent, HL absent), or CETPtg$HL^{-/-}$ (human CETP present, HL absent). These BMTs generated the following four types of chimeric mice where human CETP and murine HL were only BM derived: WT BM, $HL^{-/-}LDLr^{-/-}$ (CETP absent and HL present); CETPtg BM, $HL^{-/-}LDLr^{-/-}$ (CETP and HL both present); $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ (CETP and HL both absent); and CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ (CETP present and HL absent) (**Table 1**). Successful BM reconstitution was confirmed by RT-PCR of blood cell lysates 5 weeks after BMT (**Fig. 1A–D**). Expression of human CETP was also confirmed by polyacrylamide gel electrophoresis and Western blot using anti-human CETP monoclonal antibody kindly provided by Dr. Ross Milne (University of Ottawa Heart Institute, Ottawa, Canada) (Fig. 1E). Recipient mice were fed a chow diet for 5 weeks after BMT to allow for reconstitution of BM, after which they were fed the high-fat diet for a further 15 weeks. Periodically, mice were fasted overnight and weighed, and venous blood was drawn from the retro-orbital sinus into a heparinized capillary tube for determination of plasma lipids.

Measurement of plasma HL and CETP activity

Plasma HL activity in venous blood drawn from fasted mice 10 min after the intravenous injection of heparin (500 U/kg) was determined using a substrate of [^{3}H]triolein in Intralipid as previously described (8). Plasma CETP activity was determined from the transfer of fluorescent neutral lipid provided in a CETP activity

TABLE 1. Chimeric mice generated through BMT

BM Donor Mouse	BM Recipient Mouse	Human CETP Transgene Expression in Chimera	Murine HL Expression in Chimera
WT	$HL^{-/-}LDLr^{-/-}$	None	BM derived
CETPtg	$HL^{-/-}LDLr^{-/-}$	BM derived	BM derived
$HL^{-/-}$	$HL^{-/-}LDLr^{-/-}$	None	None
$CETPtgHL^{-/-}$	$HL^{-/-}LDLr^{-/-}$	BM derived	None

assay kit (BioVision, Mountain View, CA). Plasma from fasted mice was used, and the assay conducted as previously described (8).

Measurement of plasma cholesterol, TG, HDL-C, HDL-TG, and HDL-PL

Cholesterol and TG concentrations were measured in triplicate in plasma from fasted mice by colorimetric enzymatic assays (Thermo Electron Corp., Melbourne, Australia, and Raichem, San Diego, CA, respectively). PL concentration was also measured by colorimetric enzymatic assay (Wako Diagnostics, Richmond, VA). Plasma HDL lipid concentrations were determined by first precipitating VLDL and LDL with 50,000 molecular weight dextran sulfate and $MgCl_2$ (34, 35). Briefly, plasma (0.02 ml) from fasted mice was added to 0.06 ml of PBS and 0.008 ml of precipitating reagent (dextran sulfate, 10 g/l; $MgCl_2$, 0.5 M). The mixture was vortexed briefly and incubated at room temperature for 10 min before centrifugation at 5,000 rpm for 30 min. An aliquot (0.04 ml) of the supernatant containing HDL was removed for measurement of HDL-C, HDL-TG, and HDL-PL by colorimetric enzymatic assay.

Quantification of the extent of atherosclerosis

Atherosclerosis in the aorta from the proximal ascending aorta to the bifurcation of the iliac artery was assessed as described (33, 36). The dissected aorta was cut longitudinally under a dissecting microscope, pinned flat on black wax, and stained with Sudan IV. Pinned aortas were digitally photographed at a fixed magnification, and total en face aortic areas and atherosclerotic lesion areas calculated using Adobe Photoshop CS2 version 9.0.2 and NIH Scion Image version 1.63 software. Results are reported as the lesion area as a percentage of total en face aortic area.

A second assessment of atherosclerosis was determined from atherosclerotic lesions of the aortic root (aortic sinus). The method has previously been described (37) but was performed with modifications (36). Hearts were fixed (Tissue-Tek OCT Compound, Sakura, Torrance, CA), frozen, and sectioned on a Leica cryostat. For each aortic sinus cusp, sections were collected from the beginning of the sinus (defined as when a valve leaflet became visible on cryostat sectioning) for a distance of 500 μm distally. Sections (10 μm thick) were stained with Oil Red O and counterstained with Gill hematoxylin 1 (Fisher Scientific, Pittsburgh, PA). Stained sections were digitally photographed, and the lesion volume in the 500 μm proximal portion of each cusp was estimated from the lesion area of four sections spaced 140 μm apart. Lesion volume was calculated from an integration of the measured cross-sectional areas using Adobe Photoshop CS2 version 9.0.2 and NIH Scion Image version 1.63 software (36).

Data analysis and statistics

Values are expressed as mean ± SEM. Plasma lipid concentrations obtained while mice were chow fed were acquired from blood collected 5 weeks after BMT and immediately prior to 15 weeks of high-fat feeding. Plasma lipid concentrations obtained while mice were fed a high-fat diet were acquired from the mean of plasma lipid concentrations after 10 and 15 weeks of high-fat feeding (15 and 20 weeks after BMT). All lipid assays were done in triplicate. Statistical comparisons of plasma lipid concentrations were made within diets (chow and high fat) and between chimeric mice that differed by a single BM-derived protein. Thus, the following pairs of chimeric mice were compared: WT BM, $HL^{-/-}LDLr^{-/-}$ and CETPtg BM, $HL^{-/-}LDLr^{-/-}$ (in the presence of BM-derived HL, without and with BM-derived CETP); WT BM, $HL^{-/-}LDLr^{-/-}$ and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ (in the absence of CETP, with and without BM-derived HL); CETPtg BM, $HL^{-/-}LDLr^{-/-}$ and $CETPtgHL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ (in the presence of BM-derived CETP with and without BM-derived HL); and $CETPtgHL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ (in the absence of HL, with and without BM-derived CETP). These four comparisons were examined using a one-way ANOVA with a Bonferroni's multiple comparison test to determine which pairs of chimeric mice had statistically significant different plasma lipid concentrations. This same strategy was used

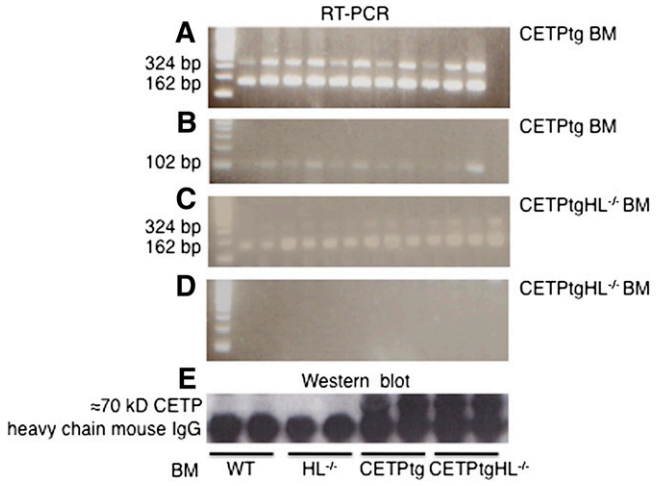

Fig. 1. Detection of the human CETP transgene and murine HL mRNA in blood cell lysates and the human CETP transgene protein in plasma of BM chimeric mice following BMT. Shown in A–D are the 162, 324, and 102 bp products of RT-PCR of the human CETP transgene, murine interleukin-2, and murine HL, respectively, on 3% agarose gels with ethidium bromide detection. The human CETP transgene and murine interleukin-2 were probed for in A and C. Murine HL was probed for in B and D. At the left of each gel is a 100 bp ladder with the bottom band being 100 bp. A and B are results from RT-PCR of blood cell lysates from CETPtg BM, $HL^{-/-}LDLr^{-/-}$ mice. Both the products of RT-PCR of the human CETP transgene (162 bp) and murine HL (102 bp) are evident in A and B, respectively. C and D are results from RT-PCR of blood cell lysates from $CETPtgHL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice. The 162 bp product of RT-PCR of the human CETP transgene is evident in C. The 102 bp product of RT-PCR of murine HL is absent in D. E shows the Western blot of plasma from $HL^{-/-}LDLr^{-/-}$ mice after BMT using anti-human CETP monoclonal antibody to detect the human CETP transgene. The ~70 kDa human CETP is detected in plasma from mice with BM donated from CETPtg and $CETPtgHL^{-/-}$ mice but not when mice have BM from WT and $HL^{-/-}$ mice.

to examine differences in the extent of atherosclerosis. Atherosclerosis data were analyzed by D-Agostino and Pearson omnibus normality test and found to be normally distributed ($P > 0.05$). Correlations between high-fat-fed plasma HDL-C concentrations and atherosclerosis severity were examined using the Pearson's correlation test. All statistical tests were conducted using Prism version 5.0b software.

RESULTS

BMTs were performed in irradiated $HL^{-/-}LDLr^{-/-}$ double-knockout mice using BM from WT, CETPtg, $CETPtgHL^{-/-}$, and $HL^{-/-}$ mice. The chimeric mice that resulted from the BMTs were fed a high-fat diet to promote atherosclerosis in the atherosclerosis-prone, LDLr-deficient mice. The four different chimeras enabled an examination of atherosclerosis and plasma lipid concentrations in the presence of BM-derived HL but not BM-derived CETP (WT BM), in the presence of both BM-derived HL and BM-derived CETP (CETPtg BM), in the presence of BM-derived CETP but not BM-derived HL ($CETPtgHL^{-/-}$ BM), and in the absence of both BM-derived HL and BM-derived CETP ($HL^{-/-}$ BM) (Table 1). Four weeks after BMT, human CETP mRNA was detected by RT-PCR in blood cell lysates of those mice with BM-derived cells expressing human CETP (Fig. 1A, C). Murine HL mRNA was detected in blood cell lysates of those mice with BM-derived cells expressing HL (Fig. 1B) but not in those mice completely deficient in HL (Fig. 1D). The ~70 kDa human CETP was detected in plasma from mice with BM-derived cells expressing human CETP (Fig. 1E).

Four weeks after BMT, postheparin plasma from mice with BM-derived HL had no greater ability to hydrolyze [^{3}H]triolein in Intralipid than plasma from mice with a complete absence of HL (results not shown). Thus, any lipolytic activity in plasma that resulted from BM-derived HL could not be detected by HL assay. This is consistent with the small amount of HL mRNA in plasma suggested by the faint banding by RT-PCR (Fig. 1B) and the lack of HL protein on Western blot (result not shown). It is also possible that some "breakthrough" lipid hydrolysis by lipoprotein lipase is detected in the HL assay, and this masks any small difference in plasma lipolytic activity between mice that are globally deficient in HL but differ in BM expression of HL. Likewise, neutral lipid-transfer activity was not statistically different in plasma from mice with BM-derived CETP and mice deficient in CETP. Surprisingly, the mean neutral lipid-transfer activity was lower in plasma from mice with BM-derived CETP, both in the presence and absence of BM-derived HL, although the differences were not statistically significant (**Tables 2**, **3**). Interestingly, neutral lipid-transfer activity was less in chow-fed mice with both BM-derived CETP and BM-derived HL (CETPtg BM chimeras) compared with plasma from mice with BM-derived CETP but without BM-derived HL ($CETPtgHL^{-/-}$ BM chimeras) ($P < 0.05$ for both female and male mice). In female chow-fed mice, neutral lipid-transfer activity was higher in plasma from mice without BM-derived HL and BM-derived CETP ($HL^{-/-}$ BM chimeras) compared with plasma from mice with BM-derived HL and without BM-derived CETP (WT BM chimeras) ($P < 0.01$). This commercially available CETP activity assay appears to measure neutral lipid-transfer activity that is not applicable to CETP as those chimeras without BM-derived CETP reported significant neutral lipid-transfer activity. We suspect that CETP activity derived solely from BM-derived cells is too low to be measured in venous plasma. High-fat feeding increased plasma neutral lipid-transfer activity between 5.7- and 9.4-fold. This is consistent with our previous observations (8) and 4- to 10-fold increases in transcription of CETP in this mouse model with high-fat feeding (38), although the activity we have measured is not specific to CETP. There were no statistically significant differences in neutral lipid-transfer activity in plasma from high-fat fed mice.

In chow-fed female mice, 4 weeks after BMT, plasma HDL-C and HDL-PL concentrations were both higher in $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice than in WT BM, $HL^{-/-}LDLr^{-/-}$ mice ($P < 0.001$ for both HDL-C and HDL-PL) (**Table 4**). HDL-PL and HDL-TG concentrations were both higher in $CETPtgHL^{-/-}$ BM chimeras than in CETPtg BM chimeras ($P < 0.001$ HDL-PL; $P < 0.01$ HDL-TG).

TABLE 2. Neutral lipid-transfer activity of plasma from WT BM, CETPtg BM, CETPtg $HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice: female mice

Chimera	Chow Diet[a]	High-Fat Diet[b]	Change in Neutral Lipid-Transfer Activity with High-Fat Feeding
	pmol Neutral Lipid Transfer/ml Plasma/h		Fold Increase
WT BM	1,547 ± 98[c]	12,082 ± 571	7.8
CETPtg BM	1,359 ± 122[d]	12,762 ± 648	9.4
$CETPtgHL^{-/-}$ BM	1,761 ± 51[d]	12,656 ± 664	7.2
$HL^{-/-}$ BM	2,119 ± 120[c]	12,130 ± 471	5.7

Statistical comparisons were made within the same diet and between chimeras that differed by a single BM-derived protein.

[a]These values were obtained from blood drawn 5 weeks after BMT and prior to 15 weeks of high-fat feeding.

[b]These values were obtained from blood drawn after 15 weeks of high-fat feeding.

[c]$P < 0.01$, WT BM (expressing HL but not CETP) compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[d]$P < 0.05$, CETPtg BM (expressing both HL and CETP) compared with $CETPtgHL^{-/-}$ BM (expressing CETP but not HL).

TABLE 3. Neutral lipid-transfer activity of plasma from WT BM, CETPtg BM, CETPtg $HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice: male mice

Chimera	Chow Diet[a]	High-Fat Diet[b]	Change in Neutral Lipid-Transfer Activity with High-Fat Feeding
	pmol Neutral Lipid Transfer/ml Plasma/h		Fold Increase
WT BM	2,268 ± 61	17,181 ± 1,210	7.6
CETPtg BM	1,957 ± 84[d]	13,801 ± 980	7.1
CETPtg$HL^{-/-}$ BM	2,272 ± 101[d]	13,310 ± 1,084	5.9
$HL^{-/-}$ BM	2,550 ± 87	15,290 ± 1,046	6.0

Statistical comparisons were made within the same diet and between chimeras that differed by a single BM-derived protein.

[a]These values were obtained from blood drawn 5 weeks after BMT and prior to 15 weeks of high-fat feeding.

[b]These values were obtained from blood drawn after 15 weeks of high-fat feeding.

[c]$P < 0.01$, WT BM (expressing HL but not CETP) compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[d]$P < 0.05$, CETPtg BM (expressing both HL and CETP) compared with CETPtg$HL^{-/-}$ BM (expressing CETP but not HL).

In the total absence of HL, BM-derived CETP resulted in an increase in HDL-TG ($P < 0.01$). In the presence of BM-derived HL, BM-derived CETP did not significantly increase HDL-TG. Plasma total cholesterol concentrations were higher in $HL^{-/-}$ BM chimeras than in WT BM chimeras ($P < 0.001$) and CETPtg$HL^{-/-}$ BM chimeras ($P < 0.001$). Total cholesterol was higher in CETPtg$HL^{-/-}$ BM chimeras than in CETPtg BM chimeras ($P < 0.01$). Plasma total TG concentrations were higher in $HL^{-/-}$ BM chimeras than in WT BM chimeras ($P < 0.01$) and CETPtg$HL^{-/-}$ BM chimeras ($P < 0.05$).

Far fewer plasma lipid changes were observed in the chow-fed male BM chimeric mice (**Table 5**). HDL-C concentrations were marginally higher in $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice than in CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice ($P < 0.05$). HDL-PL concentrations were higher in $HL^{-/-}$ BM chimeras than in WT BM chimeras ($P < 0.001$). Plasma total cholesterol concentrations were higher in WT BM chimeras than in CETPtg BM chimeras ($P < 0.001$).

Plasma lipid concentrations in high-fat fed mice were obtained from the mean of measurements in fasted mice after 10 and 15 weeks of high-fat feeding (15 and 20 weeks after BMT). In high-fat-fed female mice, plasma HDL-C and HDL-PL concentrations were both higher in $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice than in CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice ($P < 0.001$ for both HDL-C and HDL-PL) (Table 4). HDL-C and HDL-PL concentrations were also higher in CETPtg BM chimeras than in CETPtg$HL^{-/-}$ BM chimeras ($P < 0.001$ for both HDL-C and HDL-PL). HDL-TG concentrations were higher in CETPtg BM chimeras than in CETPtg$HL^{-/-}$ BM chimeras ($P < 0.01$) and marginally higher in WT BM chimeras than in $HL^{-/-}$ BM chimeras ($P < 0.05$). BM-derived CETP did not increase plasma HDL-TG concentrations, either in the presence or absence of BM-derived HL. Plasma total cholesterol concentrations were marginally higher in CETPtg$HL^{-/-}$ BM chimeras than in $HL^{-/-}$ BM chimeras ($P < 0.05$).

TABLE 4. Plasma HDL-C, HDL-PL, HDL-TG, total cholesterol, and total TG concentrations in WT BM, CETPtg BM, CETPtg $HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice: female mice

	Chow Diet[a]				High-Fat Diet[b]			
Chimera	WT BM n = 12	CETPtg BM n = 10	CETPtg$HL^{-/-}$ BM n = 10	$HL^{-/-}$ BM n = 11	WT BM n = 12	CETPtg BM n = 10	CETPtg$HL^{-/-}$ BM n = 10	$HL^{-/-}$ BM n = 11
	mg/dl							
HDL-C	122 ± 2[d]	125 ± 3	131 ± 2	140 ± 3[d]	85 ± 2	69 ± 8[g]	17 ± 4[g,i]	87 ± 3[i]
HDL-PL	229 ± 3[d]	240 ± 4[g]	274 ± 5[g,k]	255 ± 4[d,k]	166 ± 5	149 ± 15[g]	54 ± 7[g,i]	165 ± 6[i]
HDL-TG	45 ± 3	49 ± 3[h]	63 ± 3[h,j]	46 ± 2[j]	32 ± 0.7[f]	33 ± 2[h]	27 ± 0.7[h]	27 ± 1[f]
Cholesterol	390 ± 14[d]	356 ± 7[h]	420 ± 12[h,i]	518 ± 17[d,i]	2,016 ± 115	2,305 ± 170	2,553 ± 153[k]	2,006 ± 76[k]
TG	129 ± 10[e]	102 ± 8	137 ± 9[k]	196 ± 25[e,k]	566 ± 32	448 ± 34	508 ± 36	563 ± 51

Statistical comparisons were made within the same diet and between chimeras that differed by a single BM-derived protein.

[a]These values were obtained from a fasting blood sample drawn 5 weeks after BMT and immediately prior to 15 weeks of high-fat feeding.

[b]These values were obtained from the means of two separate fasting blood draws after 10 and 15 weeks of high-fat feeding.

[c]$P < 0.001$; WT BM (expressing HL but not CETP) were compared with CETPtg BM (expressing both HL and CETP).

[d]$P < 0.001$; WT BM (expressing HL but not CETP) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[e]$P < 0.01$; WT BM (expressing HL but not CETP) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[f]$P < 0.05$; WT BM (expressing HL but not CETP) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[g]$P < 0.001$; CETPtg BM (expressing both HL and CETP) were compared with CETPtg $HL^{-/-}$ BM (expressing CETP but not HL).

[h]$P < 0.01$; CETPtg BM (expressing both HL and CETP) were compared with CETPtg $HL^{-/-}$ BM (expressing CETP but not HL).

[i]$P < 0.001$; CETPtg $HL^{-/-}$ BM (expressing CETP but not HL) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[j]$P < 0.01$; CETPtg $HL^{-/-}$ BM (expressing CETP but not HL) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

[k]$P < 0.05$; CETPtg $HL^{-/-}$ BM (expressing CETP but not HL) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

TABLE 5. Plasma HDL-C, HDL-PL, HDL-TG, total cholesterol, and total TG concentrations in WT BM, CETPtg BM, CETPtg $HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice: male mice

	Chow Diet[a]				High-Fat Diet[b]			
Chimera	WT BM n = 9	CETPtg BM n = 8	CETPtg$HL^{-/-}$ BM n = 8	$HL^{-/-}$ BM n = 8	WT BM n = 9	CETPtg BM n = 8	CETPtg$HL^{-/-}$ BM n = 8	$HL^{-/-}$ BM n = 8
					mg/dl			
HDL-C	147 ± 3	142 ± 6	143 ± 2[k]	160 ± 2[k]	117 ± 3[c]	37 ± 11[c]	19 ± 3[i]	95 ± 4[i]
HDL-PL	267 ± 4[d]	272 ± 11	284 ± 4	304 ± 3[d]	199 ± 3[c]	95 ± 21[c]	63 ± 6[i]	178 ± 8[i]
HDL-TG	37 ± 3	37 ± 3	46 ± 4	35 ± 3	32 ± 1	27 ± 2	25 ± 1	29 ± 0.8
Cholesterol	512 ± 13[e]	426 ± 13[e]	456 ± 14	510 ± 16	2,472 ± 195	2,617 ± 129	2,797 ± 110	2,441 ± 128
TG	206 ± 14	160 ± 7	207 ± 20	241 ± 16	992 ± 88[e]	514 ± 50[e]	590 ± 30[j]	947 ± 75[j]

Statistical comparisons were made within the same diet and between chimeras that differed by a single BM-derived protein.
[a]These values were obtained from a fasting blood sample drawn 5 weeks after BMT and immediately prior to 15 weeks of high-fat feeding.
[b]These values were obtained from the means of two separate fasting blood draws after 10 and 15 weeks of high-fat feeding.
[c]$P < 0.001$; WT BM (expressing HL but not CETP) were compared with CETPtg BM (expressing both HL and CETP).
[d]$P < 0.001$; WT BM (expressing HL but not CETP) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).
[e]$P < 0.01$; WT BM (expressing HL but not CETP) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).
[f]$P < 0.05$; WT BM (expressing HL but not CETP) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).
[g]$P < 0.001$; CETPtg BM (expressing both HL and CETP) were compared with CETPtg $HL^{-/-}$ BM (expressing CETP but not HL).
[h]$P < 0.01$; CETPtg BM (expressing both HL and CETP) were compared with CETPtg $HL^{-/-}$ BM (expressing CETP but not HL).
[i]$P < 0.001$; CETPtg $HL^{-/-}$ BM (expressing CETP but not HL) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).
[j]$P < 0.01$; CETPtg $HL^{-/-}$ BM (expressing CETP but not HL) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).
[k]$P < 0.05$; CETPtg $HL^{-/-}$ BM (expressing CETP but not HL) were compared with $HL^{-/-}$ BM (expressing neither HL nor CETP).

In high-fat-fed male mice, as with female mice, HDL-C and HDL-PL concentrations were higher in $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice than in CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice ($P < 0.001$ for both HDL-C and HDL-PL) (Table 5). However, unlike in female mice, the differences in HDL-C and HDL-PL concentrations, although higher in CETPtg BM chimeras than in CETPtg$HL^{-/-}$ BM chimeras, were not statistically significant. Instead, HDL-C and HDL-PL concentrations were higher in WT BM chimeras than in CETPtg BM chimeras ($P < 0.001$ for both HDL-C and HDL-PL). As was the case in female mice, BM-derived CETP did not increase plasma HDL-TG concentrations [although the mass ratios of the constitutes of HDL (see below) show that CETP did enrich HDL particles in TG]. BM-derived CETP did decrease the plasma total TG concentrations both in the absence ($P < 0.01$) and presence ($P < 0.001$) of BM-derived HL. There was a trend of this also occurring in female mice.

The mass ratio of cholesterol/PL/TG in HDL was similar for the different chimeric mice when chow fed (**Tables 6**, **7**). In high-fat-fed mice, in the complete absence of HL, BM-derived CETP resulted in a marked increase in the PL and TG component of HDL. In female CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ and $HL^{-/-}$BM, $HL^{-/-}LDLr^{-/-}$ mice, the mass ratios of cholesterol/PL/TG in HDL were 1.0:3.2:1.6 and 1.0:1.9:0.3, respectively (Table 6). In male CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ and $HL^{-/-}$BM, $HL^{-/-}LDLr^{-/-}$ mice, the mass ratios of cholesterol/PL/TG in HDL were 1.0:3.3:1.3 and 1.0:1.9:0.3, respectively (Table 7). In the presence of BM-derived HL, BM-derived CETP also resulted in an increase in the PL and TG component of HDL, although to a lesser extent than in the total absence of HL. Conversely, these results demonstrate that BM-derived CETP decreases the cholesterol component in HDL in $HL^{-/-}LDLr^{-/-}$ mice and that BM-derived HL mitigates this decrease.

The extent of atherosclerosis after 15 weeks of high-fat feeding, measured both in the en face aorta from the proximal ascending aorta to the bifurcation of the iliac artery and in the aortic sinus, was not statistically significantly different between the different chimeric mice (**Figs. 2**, **3**). However, by both measures of atherosclerosis, and in both sexes, the mean measured extent of atherosclerosis was greatest in CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice. In female mice, the mean en face aortic lesion area in CETPtg$HL^{-/-}$ BM chimeras was 6.9 ± 1.4% versus 5.4 ± 0.8, 4.8 ± 0.5, and 5.0 ± 0.5% for WT BM, CETPtg BM, and $HL^{-/-}$ BM chimeras, respectively (Fig. 2A). In male mice, the mean en face aortic lesion area in CETPtg$HL^{-/-}$ BM chimeras was 7.8 ± 1.3% versus 4.7 ± 0.6, 6.8 ± 1.0, and 5.9 ± 0.4% for WT BM, CETPtg BM, and $HL^{-/-}$ BM chimeras, respectively (Fig. 2B). In female mice, the mean aortic sinus lesion volume in CETPtg$HL^{-/-}$ BM chimeric mice was 451 ± 19 $\mu m^3 \times 10^6$ versus 395 ± 21, 411 ± 37, and 407 ± 23 $\mu m^3 \times 10^6$ for WT BM, CETPtg BM, and $HL^{-/-}$ BM chimeras, respectively (Fig. 3A). In male mice, the mean aortic sinus lesion volume in CETPtg$HL^{-/-}$ BM chimeric mice was 385 ± 25 $\mu m^3 \times 10^6$ versus 311 ± 21, 373 ± 35, and 376 ± 24 $\mu m^3 \times 10^6$ for WT BM, CETPtg BM, and $HL^{-/-}$ BM chimeras, respectively (Fig. 3B).

TABLE 6. The mass ratio of cholesterol/PL/TG in plasma HDL from WT BM, CETPtg BM, CETPtg $HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice: female mice

	Cholesterol/PL/TG	
Chimera	Chow Diet	High-Fat Diet
WT BM	1.0:1.9:0.4	1.0:2.0:0.4
CETPtg BM	1.0:1.9:0.4	1.0:2.2:0.5
CETPtg$HL^{-/-}$ BM	1.0:2.1:0.5	1.0:3.2:1.6
$HL^{-/-}$ BM	1.0:1.8:0.3	1.0:1.9:0.3

These mass ratios were determined from the plasma HDL-lipid concentrations in Tables 4, 5.

TABLE 7. The mass ratio of cholesterol/PL/TG in plasma HDL from WT BM, CETPtg BM, CETPtg $HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ mice: male mice

	Cholesterol/PL/TG	
Chimera	Chow Diet	High-Fat Diet
WT BM	1.0:1.8:0.3	1.0:1.7:0.3
CETPtg BM	1.0:1.9:0.3	1.0:2.6:0.7
CETPtg$HL^{-/-}$ BM	1.0:2.0:0.3	1.0:3.3:1.3
$HL^{-/-}$ BM	1.0:1.9:0.2	1.0:1.9:0.3

These mass ratios were determined from the plasma HDL-lipid concentrations in Tables 4, 5.

BM-derived CETP lowered plasma HDL-C, and the degree of lowering was dependent on BM-derived HL. Because low plasma HDL-C is a strong predictor of atherosclerotic risk and we observed no statistically significant effects on atherosclerosis in this study, we examined the associations between HDL-C and atherosclerosis in each individual chimeric mouse in order to determine whether this association varied for the different chimeras. For atherosclerosis measured in the en face aorta, inverse correlations between the plasma HDL-C concentration and the extent of atherosclerosis were observed in CETPtg BM, $HL^{-/-}LDLr^{-/-}$, and CETPtg$HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$

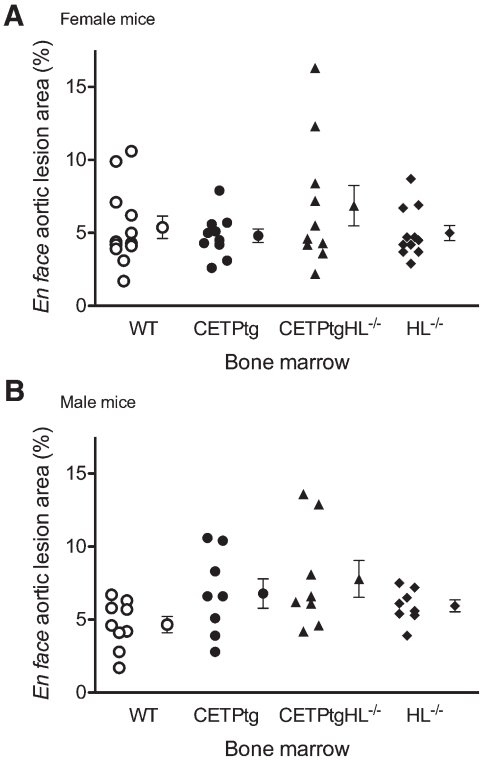

Fig. 2. Atherosclerosis severity in high-fat-fed WT BM, CETPtg BM, CETPtg$HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice as assessed in the en face aorta. After 15 weeks of high-fat feeding, atherosclerosis was assessed in the whole aorta (percent of en face aorta area that contained lesion) in female (A) and male (B) mice. Shown are measurements from individual mice as well as the mean ± SEM. Statistical analysis by one-way ANOVA and Bonferroni's multiple comparison tests showed there to be no statistical difference between the groups.

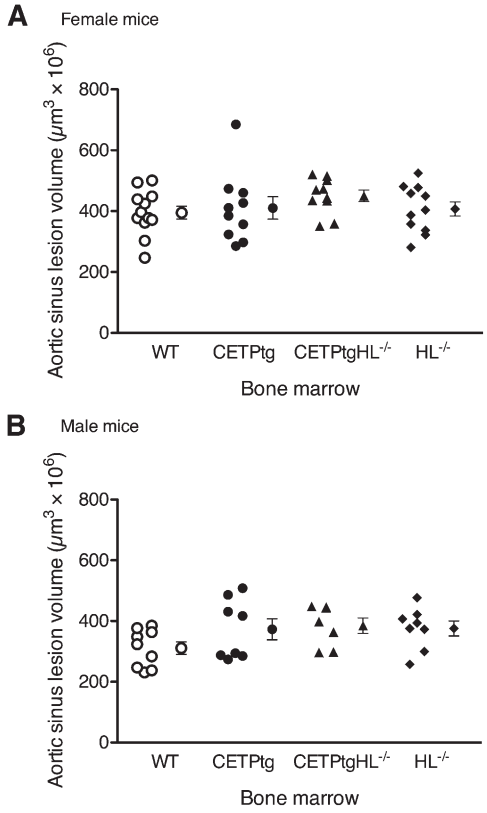

Fig. 3. Atherosclerosis severity in high-fat-fed WT BM, CETPtg BM, CETPtg$HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice as assessed in the aortic sinus. After 15 weeks of high-fat feeding, the volume of atherosclerosis was determined in the first 500 μm segment of the aortic sinus in female (A) and male (B) mice. Shown are measurements from individual mice as well as the mean ± SEM. Statistical analysis by one-way ANOVA and Bonferroni's multiple comparison tests showed there to be no statistical difference between the groups.

mice ($P = 0.03$ and $P = 0.01$, respectively) (**Fig. 4**). There were no correlations between the plasma HDL-C concentration and the extent of atherosclerosis in WT BM and $HL^{-/-}$ BM chimeric mice. For atherosclerosis measured in the aortic sinus, an inverse correlation between the plasma HDL-C concentration and the extent of atherosclerosis was observed in WT BM, $HL^{-/-}LDLr^{-/-}$ mice ($P = 0.0007$) (**Fig. 5**). There were no correlations between the plasma HDL-C concentration and the extent of atherosclerosis in CETPtg BM, CETPtg$HL^{-/-}$ BM, and $HL^{-/-}$ BM chimeric mice.

DISCUSSION

This study demonstrates that plasma HDL is decreased in the presence of BM-derived CETP in high-fat-fed $HL^{-/-}LDLr^{-/-}$ mice and that this effect is mitigated in the presence of BM-derived HL in female $HL^{-/-}LDLr^{-/-}$ mice. BM-derived CETP also decreases the cholesterol in HDL in mice globally deficient in HL and the LDLr, and this effect is mitigated in the presence of BM-derived HL. Also, in high-fat-fed $HL^{-/-}LDLr^{-/-}$ mice, plasma total

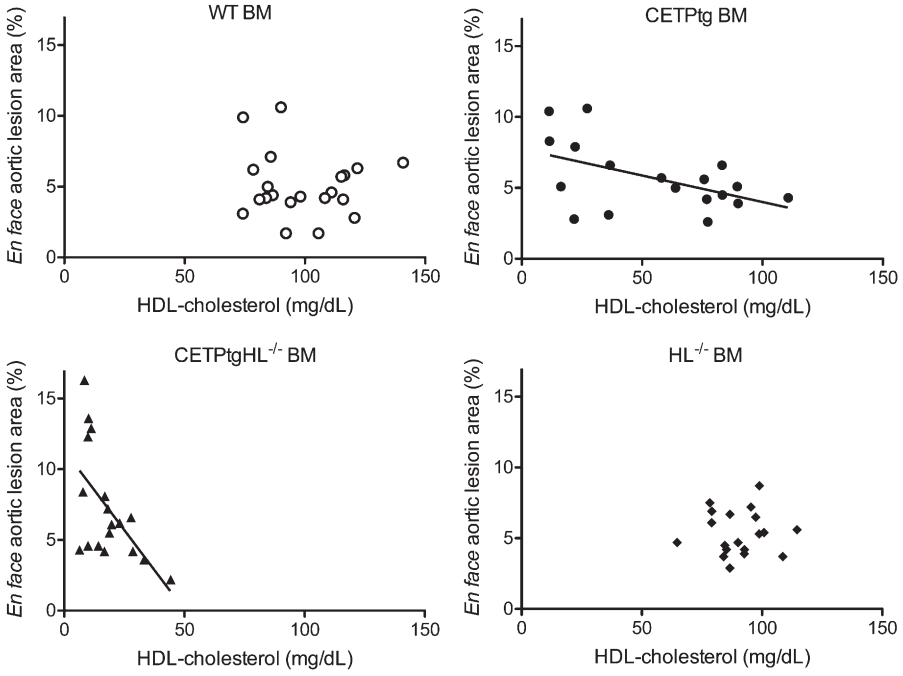

Fig. 4. Associations between aortic atherosclerosis severity (percent of en face aorta area that contained lesion) and plasma HDL-C concentration in WT BM, CETPtg BM, CETPtgHL$^{-/-}$ BM, and HL$^{-/-}$ BM, HL$^{-/-}$LDLr$^{-/-}$ chimeric mice. For each mouse, the extent of aortic atherosclerosis was plotted against the mean plasma HDL-C concentration from blood draws at 10 and 15 weeks of high-fat feeding. The data are from both male and female mice; the numbers of each sex for each chimera are as shown in Tables 4, 5. Regression lines are shown where statistically significant correlations were observed ($P < 0.05$).

cholesterol is increased in the presence of BM-derived CETP, and this increase is mitigated in the presence of BM-derived HL. The extent of atherosclerosis after 15 weeks of feeding a high-fat diet did not differ statistically among the different chimeric mice with combinations of BM-derived CETP and HL. However, by two measures of atherosclerosis, and in both male and female mice, the highest mean extent of atherosclerosis among the four BM chimeric groups was observed in those mice with BM-derived CETP and without BM-derived HL. Together, these results suggest that BM-derived HL mitigates the HDL-lowering, HDL-modulating, cholesterol-raising, and possibly proatherogenic effects of BM-derived CETP. These results show that BM-derived proteins can have significant effects on plasma lipids. Functional studies are required to demonstrate that BM-derived HL and CETP are interacting to produce these effects on plasma lipids.

The results of this study are consistent with our previous results with CETPtgLDLr$^{-/-}$ mice (8). In that study, the only difference between the BMT-generated chimeras was that one group of animals expressed HL systemically and another group lacked expression of HL by BM-derived cells. BM-derived HL increased HDL-C and decreased atherosclerosis in high-fat-fed mice. However, as all animals expressed CETP (from non-BM-derived cells), it was not possible to tell whether the presence of CETP was required to achieve those results. The results of the present study show that in high-fat-fed mice, HDL is greater in mice with BM-derived HL compared with mice without BM-derived HL, but only if BM-derived CETP is also present. In the absence of BM-derived CETP, there is no difference in HDL levels between mice with and without BM-derived HL. Effects of BM-derived HL, in the absence of CETP, were only observed in chow-fed mice. Here, BM-derived HL decreased plasma HDL and, in female HL$^{-/-}$LDLr$^{-/-}$ mice, decreased plasma total cholesterol and TGs. However, with a chow diet, BM-derived CETP did not significantly change HDL in these mice and did not raise plasma total cholesterol. In fact, BM-derived CETP decreased plasma total cholesterol, both in the absence and presence of BM-derived HL. Previous studies in female LDLr$^{-/-}$ mice have shown BM-derived CETP to have no effect on total cholesterol in chow-fed animals (2). This suggests that the actions of BM-derived CETP on cholesterol metabolism are influenced by HL, as we demonstrate in this present study. Consistent with the BMT studies in LDLr$^{-/-}$ mice (2), we also observed that with high-fat feeding BM-derived CETP decreased HDL-C and increased total cholesterol.

We failed to see statistically significant differences in the extent of atherosclerosis due to BM-derived CETP and HL; however, the trend in atherosclerosis is what would be expected from the changes in plasma lipids. The highest mean atherosclerosis was observed in those mice with the lowest HDL-C and highest total cholesterol, CETPtgHL$^{-/-}$ BM, LDLr$^{-/-}$HL$^{-/-}$ that were totally deficient in HL and had BM-derived CETP. In male mice, the lowest mean atherosclerosis was observed in those mice with the highest

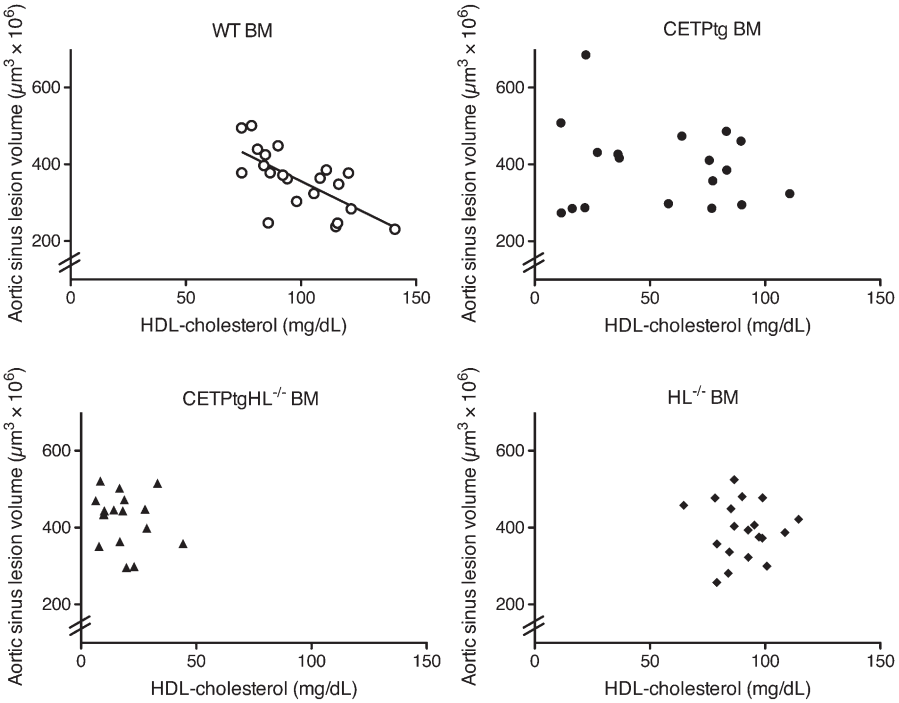

Fig. 5. Associations between atherosclerosis severity in the aortic sinus and plasma HDL-C concentration in WT BM, CETPtg BM, CETPtg$HL^{-/-}$ BM, and $HL^{-/-}$ BM, $HL^{-/-}LDLr^{-/-}$ chimeric mice. For each chimeric mouse, the extent of aortic sinus atherosclerosis was plotted against the mean plasma HDL-C from blood draws at 10 and 15 weeks of high-fat feeding. The data are from both male and female mice; the numbers of each sex for each chimera are as shown in Tables 4, 5. A regression line is shown where a statistically significant correlation was observed ($P < 0.001$).

HDL-C, WT BM, $LDLr^{-/-}HL^{-/-}$ that were totally deficient in CETP and had BM-derived HL. The effects of BM-derived CETP and HL were sex specific, and any effects these proteins exert on atherosclerosis may be HDL dependent. In female mice, BM-derived CETP may increase atherosclerosis, but only when BM-derived HL is absent and where HDL-C is lowest. In male mice, BM-derived CETP may increase atherosclerosis to a similar extent in both the presence and absence of BM-derived CETP, instances where HDL-C concentrations were also decreased to a similar extent. It has been shown previously that BM-derived CETP increases atherosclerosis in female $LDLr^{-/-}$ mice (2). Chow-fed double-knockout mice that are deficient in both HL and the LDLr ($HL^{-/-}LDLr^{-/-}$) have significantly greater atherosclerosis than chow-fed $LDLr^{-/-}$ mice (27). It may be that in our high-fat-fed $HL^{-/-}LDLr^{-/-}$ mice, atherosclerosis is sufficiently advanced such that BM-derived CETP could not significantly enhance the extent of atherosclerosis, even with a drastic decrease in HDL-C.

This study shows that BM-derived CETP has greater effects on HDL and total plasma cholesterol in female mice (and potentially on atherosclerosis) when BM-derived HL is absent. This could either imply that BM-derived HL mitigates the actions of CETP or that BM-derived HL has completely separate and opposite effects on HDL to that of CETP. Given that there was little difference in HDL concentrations between high-fat-fed $HL^{-/-}LDLr^{-/-}$ mice with WT BM and $HL^{-/-}LDLr^{-/-}$ mice with $HL^{-/-}$ BM, it would appear that BM-derived HL has minimal effect on HDL in this animal. Therefore, it is more likely that BM-derived HL mitigates the actions of BM-derived CETP on plasma concentrations of HDL and cholesterol. Functional studies are required to confirm this.

BM-derived CETP did not increase the plasma HDL-TG concentration in high-fat-fed $HL^{-/-}LDLr^{-/-}$ mice. However, the mass ratio of the lipid constituents of HDL show that HDLs were TG-enriched in mice with BM-derived CETP. The enrichment of HDL in TG was markedly greater in the absence of BM-derived HL. We propose that the reason for this is that BM-derived HL hydrolyzes the TG in HDL, thereby limiting the extent to which CETP can increase TG in these particles. Furthermore, it has previously been shown that in $HL^{-/-}LDLr^{-/-}$ mice there is an increase in VLDL and LDL when compared with $LDLr^{-/-}$ mice (27). It may be that in the complete absence of HL there is a larger pool of TG-enriched particles (VLDL and LDL) for CETP-mediated neutral lipid exchange with HDL, hence the enhanced TG enrichment we observed in mice with BM-derived CETP and no HL compared with mice with BM-derived CETP and BM-derived HL.

The situation in which the HDLs were most enriched in TG, $HL^{-/-}LDLr^{-/-}$ mice with BM-derived CETP but no BM-derived HL, was accompanied by the lowest concentration of plasma HDL. Conversely, when both BM-derived CETP and BM-derived HL were present, the enrichment of HDL in TG was less, and the CETP-mediated decrease

in plasma HDL was also less. A model that we proposed previously may offer a mechanism to explain these results (32). In the interstitial space of blood vessels beneath the endothelial layer, CETP expressed by BM-derived macrophages may generate TG-enriched HDL. TG-enriched HDLs are the favored substrate of HL (11). HL expressed by BM-derived macrophages could hydrolyze lipids (particularly TGs) in these particles, thereby reducing the TG enrichment of HDL. As a consequence of HL-mediated hydrolysis of HDL lipids, lipid-poor apoA-I could be generated that will initiate the formation of new HDL particles and thus increase the plasma concentration of HDL (32, 39). Nascent HDL particles in the vascular interstitial space could have the capacity to accept cholesterol, remove it from the body by reverse cholesterol transport, and in turn reduce atherosclerosis (40). The CETP and HL in our BMT-generated chimeras were derived from BM and thus may well be expressed by macrophages. However, we do not know in which cells or tissues these proteins were expressed in our chimeras. Further in vivo studies are also required to fully characterize the size and composition of HDL in mice where CETP and HL are solely derived from BM.

In this study, the female mice that had both BM-derived CETP and HL had higher plasma HDL concentrations, as well as the suggestion of less atherosclerosis, than those mice that had BM-derived CETP in the absence of BM-derived HL. These results support our proposed mechanism of BM-derived CETP and HL interacting to remove cholesterol from the vasculature, thereby reducing atherosclerosis.

In mice (in the absence of CETP), BM-derived HL has been shown to promote atherosclerosis (4). Also, BM-derived CETP decreases HDL-C and increases atherosclerosis in LDLr$^{-/-}$ mice (2). From these results, it would be expected that the combination of both BM-derived CETP and BM-derived HL would favor atherosclerosis; however, our results suggest that the presence of BM-derived CETP and absence of BM-derived HL is the most atherogenic combination. No previous study has examined the effects of BM-derived expression of these two proteins in combination. In humans, the majority of protein expression of both CETP and HL is from the liver, with comparatively little expression from BM (41). While it could be argued that this renders the results presented in this study meaningless in the human context, in order to determine whether the interaction of BM-derived CETP and HL has any effect in vivo, it was important to exclude other sources of these proteins. Mice too express HL primarily from the liver; however, we have previously demonstrated that the mere cessation of HL expression from BM-derived cells results in effects on plasma lipids and atherosclerosis (8). Thus, even if the vast majority of HL expression is from another tissue, it appears that HL expression from BM cells has important effects. This supports the notion that BM-derived CETP and HL may have important effects in humans too.

Mice and humans have some similarities in cholesterol metabolism and the effects of cholesterol on atherosclerosis, but there are differences that make extrapolation of the results presented here to the human condition perilous. Mice do not naturally express CETP and have thus evolved a mechanism for handling cholesterol in CETP's absence. Unlike in humans where HL is primarily bound to glycoproteins, in mice much of the HL is freely circulating in plasma (42). However, HL is expressed from both human and murine BM, and CETP is expressed from human BM (41). The relative expression levels of BM-derived HL and CETP in humans is 1.25:1 (41). Although the absolute expression of each protein in BM is low, the fact that each protein is expressed in relatively equal proportions suggests that there will be sufficient amounts of each protein to act in a coordinated manner. We do not know how this compares with the relative expression levels of these proteins in our BM chimeras. In this study, we saw an increase in neutral lipid-transfer activity with high-fat feeding, as we, and others, have demonstrated previously (8, 38); however, this activity was not specific to CETP. An increase in plasma CETP protein in response to cholesterolemic diets has also been demonstrated in humans (43).

We propose that the results of this study suggest that in an animal that expresses CETP, such as humans, the combined actions of BM-derived CETP and HL at sites of atherosclerosis could have beneficial effects. However, as stated previously, most CETP and HL is expressed in the liver, and these proteins have many effects beyond those that we have proposed. Also, although we know that the CETP and HL in our animals was derived from BM cells, we do not know in which tissues these proteins were expressed. It is not known to what extent any beneficial effects of CETP and HL may limit or oppose atherosclerosis-promoting effects of these proteins. There is, however, limited evidence suggesting that together these two proteins have a protective role. In a case control study of people with increased HDL, it has been suggested that high HDL levels may not be protective against atherosclerosis when CETP is deficient and HL activity low (44). In that study in people with CETP deficiency, HL activity was significantly lower in people with atherosclerosis compared with people without atherosclerosis despite their being no difference in HDL levels between the two groups. Others have shown that increased HDL associated with mutations in both CETP and HL is not inversely associated with atherosclerosis (31, 45). Another study examining the effects of polymorphisms in the CETP and HL genes found that polymorphisms in both genes in the same individual increased the risk of atherosclerosis, and this increase in risk was maintained after adjustment for HDL (30). A multivariate logistic regression analysis of the extent of atherosclerosis in people undergoing clinically indicated coronary angiography found the simultaneous presence of polymorphisms in the CETP and HL genes was an independent predictor of more extensive atherosclerosis (46).

Others have suggested that TG-enrichment of HDL via CETP-mediated lipid exchange and the lipolytic action of HL are driving forces in enhancing clearance of HDL and lowering HDL in insulin resistant, hypertriglyceridemic states (12, 47). With regard to atherosclerotic risk, the rate

of HDL turnover may be more important than the actual amount of HDL. Of course, this disregards other potential antiatherosclerotic effects of HDL such as inhibiting inflammation in artery walls (48) that may be enhanced by increasing the residence time of HDL particles.

We have previously shown that in BM chimeric mice, inverse associations between atherosclerosis severity and plasma HDL-C concentration only occur in those chimeras that have low concentrations of HDL (8). The present study demonstrates similar results. Inverse associations between the extent of en face aortic atherosclerosis and plasma HDL-C concentration were observed in high-fat-fed CETPtg BM and CETPtgHL$^{-/-}$ BM, HL$^{-/-}$LDLr$^{-/-}$ mice. These were the cohorts with the lowest plasma HDL-C concentrations. The steep slope of the regression line for the association in CETPtgHL$^{-/-}$ BM, HL$^{-/-}$LDLr$^{-/-}$ mice (the cohort with the lowest plasma HDL-C concentration) shows that a small increase in plasma HDL-C at low concentrations results in a large decrease in the extent of aortic atherosclerosis. Thus, it appears that even if the turnover of HDL is important in reducing atherosclerosis risk, the actual amount of HDL (particularly at low concentrations) is an important contributor to atherosclerosis.

This study used a complicated scheme to examine the effects of BM-derived CETP and BM-derived HL in a very specific way. Whether the expression of these two proteins from macrophages (or BM-derived cells) results in lipid profiles that afford protection from atherosclerosis in a WT animal is unknown. CETP and HL are present in a variety of tissues, and the actions of these proteins will not be limited to a specific site in the body. In the plasma compartment, CETP-mediated lipid-transfer activity decreases HDL-C as a result of the net mass transfer of cholesteryl ester from HDL to TG-rich lipoproteins and LDL (24). HL also decreases plasma HDL-C (15, 49) via enhanced hepatic uptake (50). Furthermore, mice do not normally express CETP, and therefore, this protein may act differently in mice than in an animal that naturally expresses CETP, such as humans.

What this study demonstrates is that CETP derived solely from BM decreases plasma HDL in mice globally deficient in HL and the LDLr and that BM-derived HL may mitigate this effect. The effects of CETP and HL on lipid metabolism are complex. In combination, these two proteins have different effects than either protein has alone. To label either protein pro- or antiatherogenic is to oversimplify the complex interactions at play.

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