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Plasma carboxyl ester lipase activity modulates apolipoprotein B–containing lipoprotein metabolism in a transgenic mouse model

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Abstract

Pancreatic carboxyl ester lipase (CEL) is in the plasma of many mammals, including humans and rats, but not mice. In vitro, CEL hydrolyzes cholesterol esters of apolipoprotein B–containing lipoproteins (apo B-Lp). To study the effect of CEL on metabolism of apo B-Lp and atherosclerosis in vivo, apo E-knockout (EKO) mice, which have high plasma levels of apo B-Lp and are prone to atherosclerosis, were made to secrete CEL into plasma by introducing a transgene containing a liver-specific promoter and rat CEL complementary DNA. Plasma CEL activity in EKO-CEL mice was comparable with that found in rats. Evidence of modification of apo B-Lp by plasma CEL in vivo was an increase in the free cholesterol to cholesterol ester ratio of apo B-Lp from mice on chow or a Western-type diet. In addition, plasma total cholesterol levels were elevated in EKO-CEL mice, with the elevation found exclusively in the apo B-Lp fraction. Associated with the increase in steady-state apo B-Lp levels was an increase in the plasma half-life of very low-density lipoproteins (VLDL) in EKO-CEL mice, measured by the clearance rate of injected VLDL. Interestingly, despite the increase of apo B-Lp, the atherosclerotic lesion did not differ between EKO and EKO-CEL mice on a Western-type diet. In summary, our results demonstrate that plasma CEL modulates apo B-Lp metabolism in vivo, resulting in reduced VLDL clearance and elevated plasma cholesterol levels.

1. Introduction

Carboxyl ester lipase (CEL; EC 3.1.1.13), also called *bile salt–stimulated lipase* or *cholesteryl ester hydrolase*, is synthesized and secreted by the pancreas. It is a multiple-function lipolytic enzyme [1]. In vitro, it hydrolyzes cholesteryl ester (CE), retinyl ester (RE), triglycerides (TG), lysophospholipids, and ceramides, with the activity against the first 3 substrates having the unusual dependence in vitro on the presence of millimoles-per-liter concentrations of trihydroxy bile salts. Besides the pancreas, CEL is also produced by the mammary gland [2] and the liver [3-5]. In addition, human macrophages and endothelial cells have been shown to synthesize and secrete CEL [6,7].

The major role of CEL was thought to be the liberation from dietary lipids of cholesterol (ie, nonesterified or free cholesterol [FC]) and retinol from their esters by enzyme secreted by the pancreas in preparation for absorption in the intestine. Earlier studies also suggested that CEL might play a role in FC absorption [8-10] and in neonatal development and fat (TG) digestion [11]. Recent studies with CEL gene-targeted mice have agreed that only CE absorption was impaired in the absence of CEL [9,12]. Although the absorption of FC, TG, or RE was normal in one line of CEL-null mice [12], a 40% decrease of RE absorption was observed in a different line [13]. Notably, the lack of CEL does not appear to affect neonatal mouse growth [12].

These results indicate the existence of additional digestive enzyme(s) that can function in roles previously attributed to

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CEL. Interestingly, whereas CEL deficiency does not affect the total amount of cholesterol absorbed in a single meal, CEL in the intestinal lumen has been shown to influence the type of lipoprotein produced by enterocytes [14]. Carboxyl ester lipase promotes the production of large chylomicrons in the intestine, which may act as a protective response to fat feeding [14].

Because it is also expressed outside of the pancreas, the functions of CEL in lipid and lipoprotein metabolism most likely extend beyond the digestive processing of lipids. Carboxyl ester lipase activity is found in the plasma of many mammals, including humans [15] and rats [16], but not mice. It is also found in the arterial vessel wall [17]. In vitro, CEL modifies normal and oxidized apolipoprotein B–containing lipoproteins (apo B-Lp) [17]. Expression of CEL in macrophages has been shown to increase CE accumulation and promote atherosclerosis in transgenic mice [18]. In addition, a recent study shows that mutations in the gene encoding CEL are associated with early-onset diabetes [19].

In the present study, we sought to investigate the effects of plasma CEL on apo B-Lp metabolism and atherosclerosis in vivo. Toward this goal, we took apo E-knockout (EKO) mice, which have high plasma levels of apo B-Lp [20] and are prone to atherosclerosis, and established EKO-CEL mice by introducing a CEL transgene containing a liver-specific promoter, thereby boosting the naturally low level of mouse plasma activity to the level that approximates that of a rat. With this model, we now show that plasma CEL modulates apo B-Lp metabolism in vivo, which results in reduced very low-density lipoprotein (VLDL) clearance and elevated plasma cholesterol levels.

2. Materials and methods

2.1. Generation and genotyping of EKO-CEL mice

The CEL transgene was constructed in a Bluescript plasmid (Stratagene, La Jolla, CA) that had been modified previously to contain β-globin exons 2 and 3 and the intervening intron for greater expression of a transgene [21]. The 324–base pair (bp) hepatic-specific promoter for human apo A-I was inserted into the plasmid multiple cloning site. The complete rat CEL complementary DNA (cDNA) (~2 kilobases) was inserted into the *Eco*RI site in the exon 3 of β-globin (Fig. 1). Transgenic mice were created by microinjecting linearized plasmid construct into male pronuclei of fertilized mouse eggs as described previously [22]. Tail-tip DNA from mice was screened for rat CEL by polymerase chain reaction with primer CE1US, 5′CTCAGTCTCTTGGGTGGTGACTCTG 3′, and primer CE9LS, 5′ GCTCAGTGCCATACCACTCTGAC 3′. Samples from transgenic mice (CEL mice) produced a 600-bp polymerase chain reaction product. No amplification could be detected in DNA obtained from nontransgenic mice. The CEL mice were bred with EKO mice to produce EKO-CEL progeny. The EKO littermates were used as controls in all experiments. Mice were fed Purina (St. Louis, MO) mouse chow or a Western-type diet [23] and had access to water ad libitum.

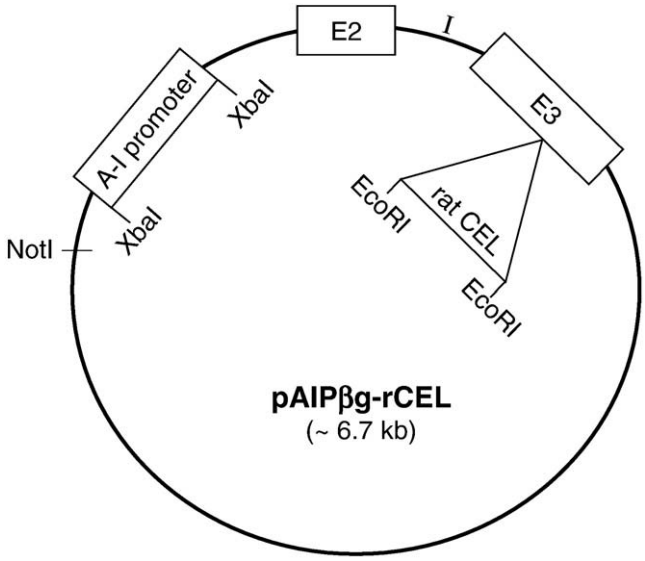

Fig. 1. Construction of rat CEL transgene. A Bluescript plasmid was previously modified to contain β-globin exons 2 and 3 and the intervening intron. The 324-bp hepatic-specific promoter for apo A-I (AI promoter) was inserted into the plasmid multiple cloning site, and the complete rat CEL cDNA (~2 kilobases) was inserted in the *Eco*RI site of exon 3 of β-globin. The plasmid was linearized by restriction digestion with *Not*I and purified for microinjection. E2 indicates exon 2; E3, exon 3; I, intron.

2.2. Determination of plasma CEL activity

Plasma CEL activity was determined radiometrically by using cholesteryl [1-^{14}C] oleate as the substrate, with 50 mmol/L cholate in the reaction [24]. Some plasma samples were delipidated as described previously [25] before CEL activity was determined. The CEL activity was expressed in units of nanomoles of fatty acid (FA) released per hour per milliliter plasma.

2.3. Analysis of plasma lipids and lipoproteins

Blood samples were obtained from mice in the fasted state through retroorbital sinus. Total plasma cholesterol (TC) and TG were determined enzymatically using commercial kits (126012 and 236691, respectively; Boehringer Mannheim, Indianapolis, IN). High-density lipoprotein cholesterol (HDL-C) was isolated from plasma after treatment with the HDL reagent (Sigma, St Louis, MO) and quantified by the same kit as for TC. Non–high-density lipoprotein cholesterol (VLDL cholesterol [VLDL-C] and low-density lipoprotein cholesterol [LDL-C]) was calculated as the difference between TC and HDL-C.

Plasma concentrations of phosphatidyl choline (PC) and lysophosphatidyl choline (lysoPC) were determined by methods modified from Switzer and Ener [26] and Shamir et al [17]. Briefly, plasma lipids were extracted with 21 vol of chloroform-methanol (2:1, vol/vol) and washed with 4 vol of distilled water as described by Folch et al [27]. The

phospholipids were separated by thin-layer chromatography (TLC) on silica gel G (Analtech, Newark, DE) with 2 solvent systems used in the same dimension: chloroform-methanol-water (65:35:6) and chloroform-acetone-methanol-acetic acid-water (6:8:2:2:1). Palmitoyl PC and lysoPC (Sigma) were applied to TLC plates as standards. After visualization with iodine vapor, PC and lysoPC bands were scraped from the plates and assayed for inorganic phosphorus content according to the method of Rouser et al [28]. The ratio of lysoPC to PC was calculated for each sample.

Plasma lipoprotein TC profiles were analyzed by fast protein liquid chromatography (FPLC) using 2 Superose 6 columns (Pharmacia, Piscataway, NJ) in series [29]. Plasma samples (200 μL) pooled from mice in the same experimental group were analyzed. Lipoproteins were eluted at a constant flow rate of 0.3 mL/min with 0.15 mol/L NaCl containing 1 mmol/L EDTA. Total cholesterol in the eluted fractions was determined enzymatically as described above.

2.4. VLDL turnover study

The β-VLDL fraction was isolated from the plasma of EKO mice by ultracentrifugation [23]. Cholesterol ester transport protein was obtained from human lipoprotein–deficient plasma [25]. Radiolabeled VLDL was then prepared in vitro by incorporating ^{3}H-cholesteryl oleoyl ether into β-VLDL through cholesterol ester transport protein [30]. Briefly, an aliquot of β-VLDL was added to an aliquot of human lipoprotein–deficient plasma, which contains 40 times more total protein than the β-VLDL aliquot. Afterward, 1/100 vol of 2% aprotinin solution was added, followed by gradually mixing in of 50 μCi ^{3}H-cholesteryl oleoyl ether solution in ethanol (50 μL). The mixture was capped under N_2 and incubated overnight at 37°C. Radiolabeled β-VLDL was isolated from the mixture by Sephadex G50 columns (Pharmacia). Each mouse received radiolabeled VLDL (1.6×10^6 disintegrations per minute) through the femoral vein. Clearance of radiolabeled β-VLDL was determined as described previously [31]. The half-life ($t_{1/2}$) of the label in the circulation was determined by checking the plasma radioactivities at different time points after injection and performing regression analysis of the data.

2.5. Quantitative atherosclerosis measurements

Mice were anesthetized, and blood was collected via a left ventricular puncture into syringes containing EDTA. The circulatory system was perfused with 0.9% NaCl by cardiac intraventricular canalization. The heart and ascending aorta including the aortic arch were removed, and the heart containing the aortic root was fixed in phosphate-buffered formalin and processed for the quantitative atherosclerosis assay as described previously [32].

2.6. Statistical analysis

Comparison of different groups was performed by 2-tailed Student t test (for normally distributed data), Mann-Whitney rank sum test (for nonnormally distributed data), and repeated-measures analysis of variance (for VLDL turnover study). The SigmaStat software (SPSS Science, Chicago, IL) was used for statistical analyses. $P < .05$ was considered statistically significant.

3. Results

3.1. Plasma CEL activities in EKO and EKO-CEL mice

The EKO-CEL mice were generated as described in experimental procedures. Plasma CEL activities of EKO and EKO-CEL mice were measured radiometrically by using cholesteryl [1-^{14}C] oleate as the substrate. The EKO mice (n = 11; 6 male and 5 female) had very low to undetectable plasma CEL activity (0.5 ± 0.1 nmol of FA per hour per milliliter plasma), whereas EKO-CEL mice (n = 14; 7 male and 7 female) had an activity of 50.1 ± 11.8 nmol of FA per hour per milliliter plasma, which is comparable with that found in rats (30∼90 nmol of FA per hour per milliliter plasma) [16] and about 4-fold higher than that in human plasma (∼12 nmol of FA per hour per milliliter plasma) [15,33]. These relative CEL activities among different species were also confirmed by assaying CEL activities in plasma samples from wild-type mice, EKO mice, EKO-CEL mice, rats, and humans in parallel in our laboratory. To confirm that endogenous lipids were not interfering with the enzyme activity, delipidation of mouse plasma did not increase its measurable CEL activity (data not shown).

3.2. Effect of circulating CEL activity on steady-state levels of plasma lipoprotein lipids

Plasma samples were collected from 2-month–old EKO mice (n=10; 4 female and 6 male) and EKO-CEL mice (n = 10; 5 female and 5 male) on chow and then after 2 weeks of Western diet. Plasma TC, HDL-C, and TG concentrations were determined enzymatically. Non–high-density lipoprotein cholesterol was calculated as the difference between TC and HDL-C. Because our initial analysis did not show significant differences in plasma lipid levels between male and female mice within the same genotype, we pooled the data from male and female mice in this study. Our results showed that EKO-CEL mice had significantly higher plasma TC than EKO mice on either chow or Western diet (Fig. 2). The difference was found exclusively in the non-HDL (apo B-Lp) fraction. No significant differences between the 2 groups were found in the plasma TG concentrations.

Pooled plasma samples from EKO or EKO-CEL mice, respectively, were subjected to lipoprotein cholesterol profile analysis by FPLC (Fig. 3). Consistent with the plasma cholesterol analysis above, VLDL-C from EKO-CEL mice was higher than that from EKO mice either on chow or Western diet. Intermediate-density lipoprotein cholesterol and LDL-C were not significantly different between EKO and EKO-CEL mice. No difference between the 2 groups

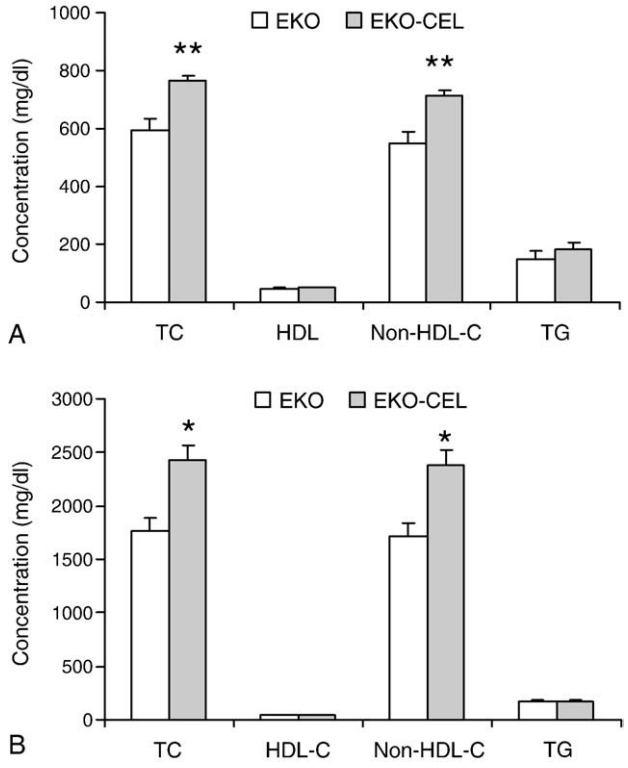

Fig. 2. Plasma lipid concentrations. Plasma samples were collected from 2-month–old EKO mice (n = 10) and EKO-CEL mice (n = 10) on chow (A) or Western diet (B). Plasma TC, HDL-C, and TG concentrations were determined enzymatically by using commercial kits. Non–high-density lipoprotein cholesterol was calculated as the difference between TC and HDL-C. The EKO-CEL mice had significantly higher plasma TC than the EKO mice on either chow or Western diet. The difference was found exclusively in the non–HDL-C fraction. No significant difference between 2 groups was found in the plasma HDL and TG concentrations. * $P < .05$; ** $P < .01$.

was observed in the HDL fractions. The VLDL and LDL fractions of the plasma were collected from the FPLC elution. To determine whether plasma CEL was active as a CE hydrolase using substrate carried by these lipoproteins, FC and CE were determined; and the ratio of FC to CE was calculated (Fig. 4). In both chow- and Western diet–fed mice, FC to CE ratios were significantly higher in the VLDL and LDL fractions from EKO-CEL mice compared with those from EKO mice (Fig. 4). These data indicated that CEL in the plasma of EKO-CEL mice was active.

Carboxyl ester lipase is also a lysophospholipase under some conditions. Thus, pooled plasma samples were also subjected to phospholipid analysis. The lysoPC and PC concentrations in the plasma were determined by lipid extraction, separation on TLC, and colorimetric measurement of inorganic phosphorus. The lysoPC to PC ratios were calculated. No significant difference was found between 2 groups (EKO vs EKO-CEL: 0.65 ± 0.03 vs 0.64 ± 0.01).

3.3. Clearance of VLDL in vivo

Because of the significant increase in non–HDL-C levels in EKO-CEL mice, we were interested in determining kinetically whether this was due to increased apo B-Lp production or decreased clearance. Thus, a bolus of radiolabeled VLDL was administered to chow-fed EKO and EKO-CEL mice by intravenous injection. Plasma radioactivities were determined at different time points after injection. The disappearance of radiolabeled VLDL was significantly diminished in EKO-CEL mice (n = 10) compared with that in EKO mice (n = 9) (Fig. 5). A regression analysis of the percentage recovery of labeled VLDL after injection showed that the plasma $t_{1/2}$ of VLDL was increased by about 22% in EKO-CEL vs EKO mice (152.3 ± 9.4 vs 124.5 ± 14.5 minutes). This degree of clearance delay was roughly comparable with the percentage of increase in the steady-state levels of non–HDL-C (Fig. 2A).

3.4. Effect of circulating CEL on aortic atherosclerosis

The EKO and EKO-CEL mice were fed the Western diet for 10 weeks after weaning and then subjected to aortic root

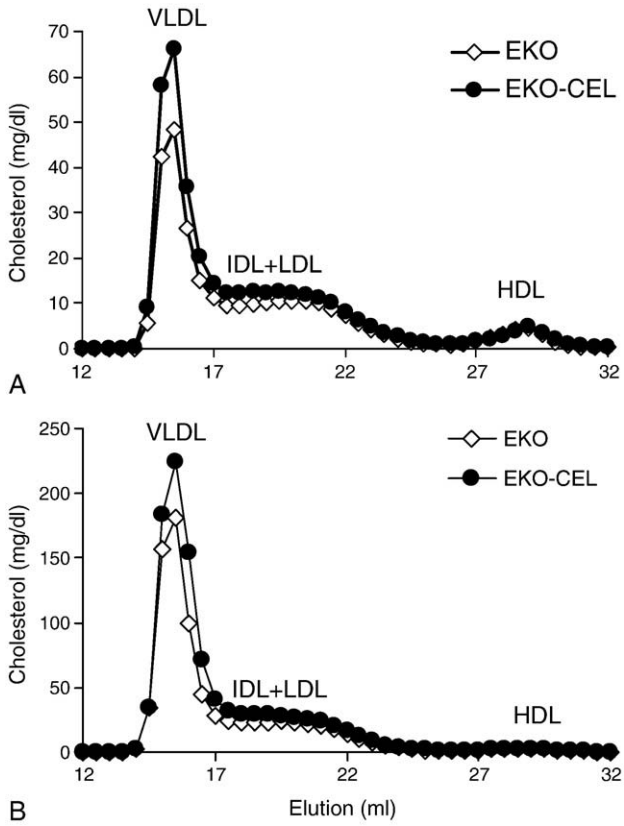

Fig. 3. Plasma lipoprotein cholesterol profiles. Plasma samples were pooled from EKO (n = 10) and EKO-CEL mice (n = 10), respectively, on chow (A) or Western diet (B). Fast protein liquid chromatography was used to separate plasma lipoproteins, and the TC profiles were determined. The VLDL-C from EKO-CEL mice was significantly higher than that from EKO mice on either chow or Western diet. Intermediate-density lipoprotein cholesterol and LDL-C also tended to be higher in EKO-CEL mice. No difference between 2 groups was observed in HDL fraction.

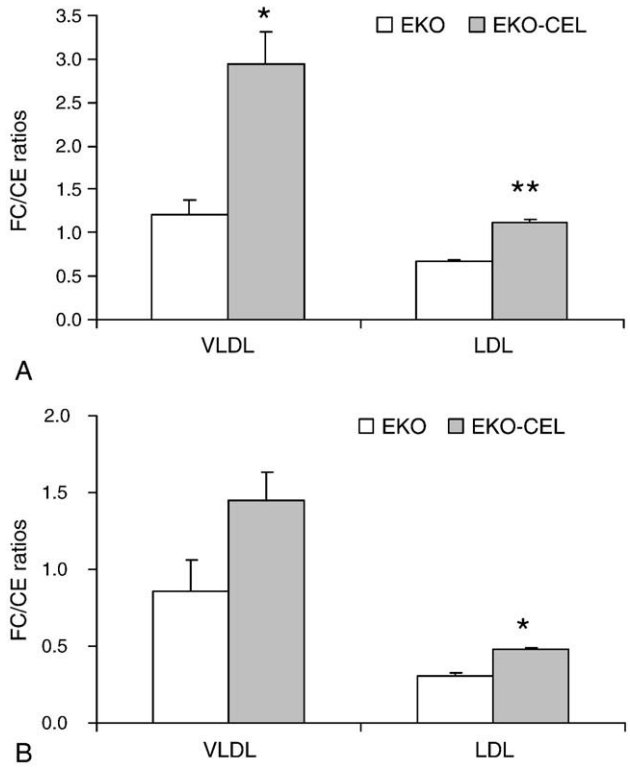

Fig. 4. The FC/CE ratios in apo B-Lp. The VLDL and LDL fractions of the plasma were collected from the FPLC elution. The FC and TC concentrations were determined enzymatically. Concentrations of CE were obtained by subtracting FC from TC. On chow diet (A), the FC/CE ratios were significantly higher in both VLDL and LDL from EKO-CEL mice compared with those from EKO mice. On Western diet (B), the FC/CE ratio in LDL was significantly higher in EKO-CEL mice; and in VLDL, it tended to be higher ($P = .09$) compared with that in EKO mice. * $P < .05$; ** $P < .01$.

lesion analysis. No significant difference in lesion size was observed between EKO (n = 10; 5 male and 5 female) and EKO-CEL (n = 10; 5 male and 5 female) mice (Fig. 6), with

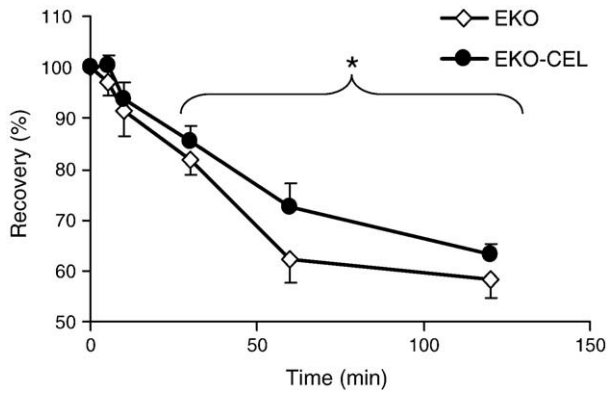

Fig. 5. Clearance of plasma VLDL on chow diet. The turnover rate of VLDL was measured by intravenous injection of a bolus radiolabeled VLDL. Percentage of recovery of radioactivity at different time points after injection was determined. The rate of disappearance of radiolabeled VLDL in EKO-CEL (n = 10) mice was significantly slower than that in EKO (n = 9) mice 30 minutes after injection ($P = .045$ by repeated-measures analysis of variance). * $P < .05$.

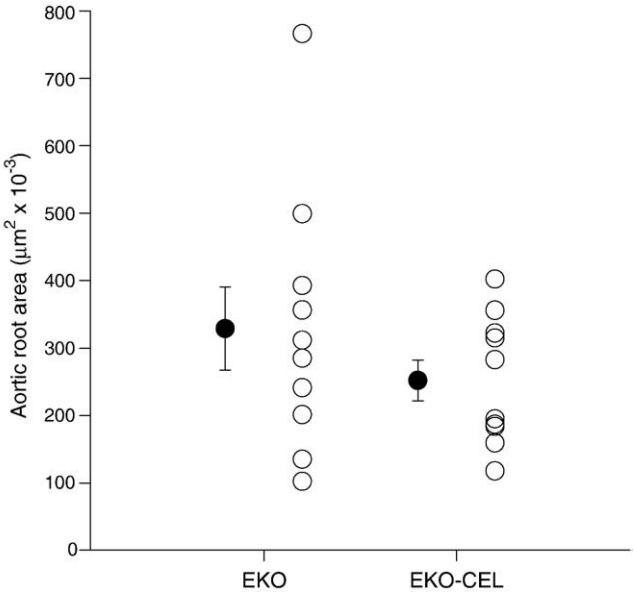

Fig. 6. Atherosclerotic lesion area in the aortic root of mice on Western diet for 10 weeks. No significant difference was observed between EKO (n = 10) and EKO-CEL (n = 10) mice.

both types of mice showing relatively large lesion areas (>200 000 μm^2).

4. Discussion

Carboxyl ester lipase circulates in the plasma of many mammals, including humans [13]. In vitro experiments have shown that CEL modifies normal and oxidized LDL and that human aortic homogenate contains CEL activity [17], suggesting that this enzyme may influence apo B-Lp metabolism and atherosclerosis in vivo. In our mouse model, plasma CEL activity was increased by introduction of a transgene, containing rat CEL cDNA, which resulted in expression levels in plasma comparable with those in the rat and about 4-fold higher than those in human. With the availability of EKO mice, which accumulate apo B-Lp in its plasma and are susceptible to atherosclerosis [23], we were able to investigate the effect of CEL on apo B-Lp metabolism and atherosclerosis in vivo by the establishment of EKO-CEL mice.

Our results show that CEL modifies apo B-Lp composition in vivo, as indicated by significant increases of FC/CE ratios in the VLDL and LDL of EKO-CEL mice either on chow or Western diet (Fig. 4). No difference was found, however, in the FC/CE ratios of HDL among different genotypes (data not shown). These results are consistent with data obtained from previous in vitro experiments. Shamir et al [17] showed that LDL-CE was a better substrate than HDL-CE for CEL in vitro and that CEL interacted with LDL better than with HDL. In agreement with this, Caillol et al [34] reported later that CEL bound to LDL in part by specific interaction with apo B, whereas no interaction was found

with apo A-I, the principal protein of HDL. Thus, the present results in vivo are consistent with the lipoprotein interactions in vitro.

In vitro, CEL also hydrolyzes TG and lysoPC [17]. In vivo, plasma CEL appears to hydrolyze CE preferentially because no differences in the present studies were observed in the TG levels and lysoPC/PC ratios between EKO-CEL and EKO mice. Thus, although CEL is thought to function in the intestinal lumen as a lysophospholipase [13], it appears that plasma conditions do not support a significant level of this particular activity or that the substrate is not in a form that is catalytically available.

The EKO-CEL mice on both types of diet had significantly higher plasma TC concentrations than the EKO mice, with the differences found exclusively in the non–HDL-C fraction (Figs. 2 and 3). This result is consistent with what Brodt-Eppley et al [35] found in a human study. They reported that high plasma CEL activity correlated with plasma TC and LDL-C levels in normolipidemic human subjects, suggesting that elevated CEL may be contributory to increasing plasma cholesterol and LDL levels. How CEL increases apo B-Lp has not been elucidated. Our results from the VLDL turnover studies in EKO-CEL mice indicated that an increase in the steady-state apo B-Lp levels associated with plasma CEL activity was caused by an increase in the plasma $t_{1/2}$ of VLDL. The average increase in the $t_{1/2}$ was 22%, which kinetically accounted for the steady-state data (Fig. 2A).

The decrease of VLDL clearance in EKO-CEL mice may have been caused by several different mechanisms. Normally, remnant lipoproteins are cleared from plasma by the liver through 3 major pathways [36,37]: (1) the LDL receptor pathway, (2) the LDL receptor–related protein pathway, and (3) the cell surface heparan sulfate proteoglycan pathway. Apolipoprotein E is the critical ligand responsible for remnant lipoprotein clearance [37]. Lipases, such as lipoprotein lipase and hepatic lipase (HL), may also serve as "bridging" ligands under some circumstances [38,39], primarily through interactions with the cell surface heparan sulfate proteoglycan. Particularly, in the absence of apo E, the role of HL in remnant lipoprotein removal becomes evident. In EKO mice, HL deficiency causes a further increase in the accumulation of β-VLDL resulting from an impairment in HL-facilitated remnant removal [40]. We speculate that, in EKO-CEL mice, circulating CEL may have competed with HL for binding apo B-Lp and channeled apo B-Lp to a less efficient clearance pathway. Clearly, further studies are needed to confirm this speculation.

In addition, CEL-mediated modifications of the composition of apo B-Lp in EKO-CEL mice may also have impaired clearance. Aviram et al [41] has reported that hydrolysis of LDL-CE by a bacterial cholesterol esterase leads to a significant reduction in cellular lipoprotein binding, presumably by a change in apo B conformation because of a reduction in the core CE content. In EKO-CEL mice, the FC/CE ratios were significantly increased in the fraction of VLDL and LDL (Fig. 4) as a result of CEL activity in the plasma and/or in the liver sinusoids. Thus, the relative reduction of CE content in the VLDL of EKO-CEL mice may have contributed to decreased VLDL clearance from plasma.

Although CEL requires millimoles-per-liter concentrations of bile salts for its maximal activity in vitro, it is important to note that plasma CEL was active in vivo. In plasma, the concentration of bile acid is ~10 μmol/L [42]; and in postprandial portal plasma, it is ~10-fold greater [43]. The concentration of bile salts in this range is well in excess of the nanomoles-per-liter levels shown to affect the conformation of CEL [44]. Our data suggest that the amounts of circulating bile salts are sufficient for the activity of CEL to hydrolyze lipoprotein-associated CE. Aviram et al [41] also found cholesterol esterase activity toward LDL in plasma and in cells of the arterial wall such as macrophages and endothelial cells and concluded, in agreement with our results, that cholesterol esterase modification of LDL may take place in vivo.

Human aortic homogenate contains CEL activity [17]. Consistent with this, human macrophages and endothelial cells have been shown to synthesize and secrete CEL [6,7]. In vitro, CEL has also been shown to stimulate smooth muscle cell proliferation [45]. These findings suggest a potential role of CEL in atherogenesis. Our present study, however, did not find significant effects of circulating CEL on aortic atherosclerosis in Western diet–fed EKO mice. Interestingly, a recent study has demonstrated that CEL expression in macrophages promotes atherosclerosis in EKO mice [18]. These results are not necessarily conflicting, for a number of reasons. First, in the previous study [18], the Western-type dietary period was only for 8 weeks; and the resulting lesion was much smaller than the lesion in the present study. It could be that if the atherogenic stimulus had been longer as in our study, the difference related to CEL expression would have narrowed or disappeared. Second, local (ie, arterial wall) vs systemic effects of CEL may not be equivalent. Indeed, CEL, like lipoprotein lipase, for example, could be envisioned to be pro- or antiatherogenic under different circumstances. For example, inside macrophages, CEL is not likely to be a CE hydrolyzing enzyme. Indeed, CEL in macrophages was found to function as a hydrolase for ceramide and lysoPC [18]. Lower levels of ceramide and lysoPC promoted the accumulation of CE and decreased cholesterol efflux in CEL-expressing macrophages, which lead to more atherosclerosis [18]. In contrast, in the circulation, as we have shown in this study, CEL hydrolyzes CE and increases FC/CE ratios in atherogenic apo B-Lp (Fig. 4). This modification of apo B-Lp composition could be beneficial because a decrease of CE content in apo B-Lp is antiatherogenic even in the presence of an elevated level of apo B-Lp [46-48] and could negate the atherogenicity of the elevated non–HDL-C levels in the EKO-CEL mice. Furthermore, in the study of Aviram et al [41], cholesterol esterase–modified LDL was less well taken up by J774

macrophages, which could be another counterbalance to the atherogenic potential of CEL.

Yet another possibility is that the level of circulating CEL in EKO-CEL mice achieved in our study was too low to exert a putative proatherogenic effect; we do not favor this explanation, however, because it was comparable with that in rats [16] and in excess of that observed in humans [15,33].

In conclusion, we have shown that circulating CEL can modify apo B-Lp composition and turnover in vivo, resulting in their decreased clearance and elevated plasma cholesterol levels. These changes, however, were not sufficient to affect the development of atherosclerosis in Western diet–fed EKO mice, perhaps because the normally proatherogenic factors of delayed apo B-Lp clearance and increased TC levels were compensated for by less uptake of these lipoproteins in the arterial wall.

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