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Plasma carboxyl ester lipase activity modulates apoB-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 (CE) of apoB-containing lipoproteins (apoB-Lp). To study the effect of CEL on metabolism of apoB-Lp and atherosclerosis *in vivo*, apoE-knockout (EKO) mice, which have high plasma levels of apoB-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 cDNA. Plasma CEL activity in EKO-CEL mice was comparable to that found in rats. Evidence of modification of apoB-Lp by plasma CEL *in vivo* was an increase in the free cholesterol:CE ratio of apoB-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 apoB-Lp fraction. Associated with the increase in steady-state apoB-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, in spite of the increase of apoB-lipoproteins, 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 apoB-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 first three substrates having the unusual dependence *in vitro* on the presence of millimolar concentrations of trihydroxy bile

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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 (i.e., 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). While the absorption of FC, TG, or RE was normal in one line of CEL-null mice (12), a 40% decrease of RE absorption, however, was observed in a different line (13). Notably, the lack of CEL does not appear to affect neonatal mouse growth (12).

These results indicate that the existence of additional digestive enzyme(s) that can function in roles previously attributed to CEL. Interestingly, while 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). CEL 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. CEL 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 apoB-containing lipoproteins (apoB-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 apoB-Lp metabolism and atherosclerosis *in vivo*. Towards this goal, we took apoE-knock out (EKO) mice, which have high plasma levels of apoB-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 apoB-Lp metabolism *in vivo*, which results in reduced 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 which had been modified previously to contain β -globin exon 2 and 3 and the intervening intron for greater expression of a transgene (21). The 324-bp hepatic-specific promoter for human apolipoprotein A-I was inserted into the plasmid multiple cloning site. The complete rat CEL cDNA (~ 2 kb) was inserted into the EcoRI 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 PCR with primer CE1US, 5' CTCAGTCTCTTGGGTGGTGACTCTG 3', and primer CE9LS, 5' GCTCAGTGCCATACCACTCTGAC 3'. Samples from transgenic mice (CEL mice) produced a 600 bp PCR product. No amplification could be detected in DNA obtained from non-transgenic mice. CEL mice were bred with EKO mice to produce EKO-CEL progeny.

EKO littermates were used as controls in all experiments. Mice were fed Purina mouse chow or a Western-type diet (23) and had access to water, *ad libitum*.

2.2. Determination of Plasma CEL Activity

Plasma CEL activity were determined radiometrically by using cholesteryl [1-¹⁴C] oleate as the substrate, with 50 mM cholate in the reaction (24). Some plasma samples were delipidated as described previously (25) before CEL activity was determined. CEL activity was expressed in units of nmole fatty acid (FA) released/h-ml plasma.

2.3. Analysis of plasma lipids and lipoproteins

Blood samples were obtained from mice in the fasted state through retro-orbital sinus. Total plasma cholesterol (TC) and triglycerides (TG) were determined enzymatically using commercial kits (no. 126012 and no. 236691, respectively, Boehringer Mannheim Corp., Indianapolis, IN). HDL-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-HDL-C (VLDL-cholesterol and LDL-cholesterol) 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 volumes of chloroform/methanol (2:1, vol/vol) and washed with 4 volumes of distilled water as described by Folch et al. (27). The phospholipids were separated by thin layer chromatography (TLC) on silica gel G (Analtech Inc., Newark, DE) with two 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 total cholesterol profiles were analyzed by fast protein liquid chromatography (FPLC) using two Suprose 6 columns in series (29). Plasma samples (200 μ l) 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 M NaCl containing 1 mM 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 (CETP) was obtained from human lipoprotein deficient plasma (LPDP) (25). Radiolabeled VLDL was then prepared *in vitro* by incorporating ³H-cholesteryl oleoyl ether into β -VLDL through CETP (30). Briefly, an aliquot of β -VLDL was added to an aliquot of LPDP, which contains 40 times more total protein than the β -VLDL aliquot. Then, 1/100 volume of 2% aprotinin solution was added, followed by gradually mixing in of 50 μ Ci ³H-cholesteryl oleoyl ether solution in ethanol (50 μ l). The mixture was capped under N₂ and incubated overnight at 37°C. Radiolabeled β -VLDL was isolated from the mixture by Sephadex G50 columns. Each mouse received radiolabeled VLDL (1.6×10^6 dpm) through the femoral vein. Clearance of radiolabeled β -VLDL was determined as described previously (31). The $t_{1/2}$ (half-life) 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 ethylenediamine-tetraacetic acid (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 two-tailed Student's *t* test (for normally distributed data), Mann-Whitney rank sum test (for non-normally distributed data), and repeated measures analysis of variance (ANOVA) (for VLDL turnover study). The SigmaStat software (SPSS Science, Chicago, IL) was used for statistical analyses. $P < 0.05$ was considered statistically significant.

3. Results

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

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 [1-¹⁴C] oleate as the substrate. EKO mice ($n = 11$; 6 male and 5 female) had very low to undetectable plasma CEL activity (0.5 ± 0.1 nm FA/h.ml plasma), whereas EKO-CEL mice ($n = 14$; 7 male and 7 female) had an activity of 50.1 ± 11.8 nmole FA/h.ml plasma, which is comparable to that found in rats (30 ~ 90 nmole FA/h.ml plasma) (16) and about four-fold higher than that in human plasma (~ 12 nmole FA/h.ml 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 total cholesterol (TC), HDL-cholesterol (HDL-C), and TG concentrations were determined enzymatically. Non-HDL-C was calculated as the difference between TC and HDL-C. Since 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 (apoB-Lp) fraction. No significant differences between the two 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-cholesterol from EKO-CEL mice was higher than that from EKO mice either on chow or Western diet. IDL- and LDL-cholesterol were not significantly different between EKO and EKO-CEL mice. No difference between the two groups was observed in the HDL fractions. VLDL and LDL fractions of the plasma were collected from the FPLC elution. To determine whether plasma CEL was active as a cholesteryl ester hydrolase using substrate carried by these lipoproteins, free cholesterol (FC) and CE were determined and the ratio of FC/CE 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 to those from EKO mice (Fig. 4). These data indicated that CEL in the plasma of EKO-CEL mice was active.

CEL is also a lysophospholipase under some conditions. Thus, pooled plasma samples were also subjected to phospholipid analysis. LysoPC and PC concentrations in the plasma were determined by lipid extraction, separation on TLC, and colorimetric measurement of inorganic phosphorus. LysoPC to PC ratios were calculated. No significant difference was found between two 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 apoB-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 to that in EKO mice (n=9) (Fig. 5). A regression analysis of the percentage recovery of labeled VLDL after injection showed that the plasma half life ($t_{1/2}$) of VLDL was increased by about 22% in EKO-CEL vs. EKO mice (152.3 ± 9.4 min vs. 124.5 ± 14.5 min). This degree of clearance delay was roughly comparable to the % increase in the steady-state levels of non-HDL-C (Fig. 2A).

3.4. Effect of circulating CEL on aortic atherosclerosis

EKO and EKO-CEL mice were fed the Western diet for 10 weeks after weaning, and then subjected to aortic root 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 both types of mice showing relatively large lesion areas ($>200,000 \mu\text{m}^2$).

4. Discussion

CEL 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 apoB-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 to those in the rat and about four-fold higher than those in human. With the availability of EKO mice, which accumulate apoB-Lp in its plasma and are susceptible to atherosclerosis (23), we were able to investigate the effect of CEL on apoB-Lp metabolism and atherosclerosis *in vivo* by the establishment of EKO-CEL mice.

Our results show that CEL modifies apoB-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 apoB while no interaction was found with apoA-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.

EKO-CEL mice on both types of diet had significantly higher plasma TC concentrations than EKO mice, with the differences found exclusively in the non-HDL-C fraction (Fig. 2 and Fig. 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 cholesterol levels in normolipidemic human subjects, suggesting elevated CEL may be contributory to increasing plasma cholesterol and LDL levels. How CEL increases apoB-Lp has not been elucidated. Our results from the VLDL-turnover studies in EKO-CEL mice indicated that an increase in the steady-state apoB-Lp levels associated with plasma CEL activity was caused by an increase in the plasma half life ($t_{1/2}$) 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 (LRP) pathway, and 3) the cell surface HSPG pathway. ApoE is the critical ligand responsible for remnant lipoprotein clearance (37). Lipases, such as lipoprotein lipase (LpL) and hepatic lipase (HL), may also serve as “bridging” ligands under some circumstances (38,39), primarily through interactions with the cell surface HSPG. Particularly, in the absence of apoE, 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 apoB-Lp and channeled apoB-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 apoB-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 apoB 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 millimolar 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 $\sim 10 \mu\text{M}$ (42) and in postprandial portal plasma is ~ 10 -fold greater (43). The concentration of bile salts in this range is well in excess of the nanomolar 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 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* (41).

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 (i.e., 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 anti-atherogenic 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 apoB-Lp (Fig. 4). This modification of apoB-Lp composition could be beneficial because a decrease of CE content in apoB-Lp is anti-atherogenic even in the presence of an elevated level of apoB-Lp (46-48) and could negate the atherogenicity of the elevated non-HDL-C levels in the EKO-CEL mice. Also, in the study of Aviram et al (41), cholesterol esterase-modified LDL was less well taken up by J774 macrophages, which could be another counter-balance 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 pro-atherogenic effect; we do not favor this explanation, however, because it was comparable to that in rats (16), and in excess of that observed in humans (15,33).

In conclusion, we have shown that circulating CEL can modify apoB-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 pro-atherogenic factors of delayed apoB-Lp clearance and increased total cholesterol levels were compensated for by less uptake of these lipoproteins in the arterial wall.

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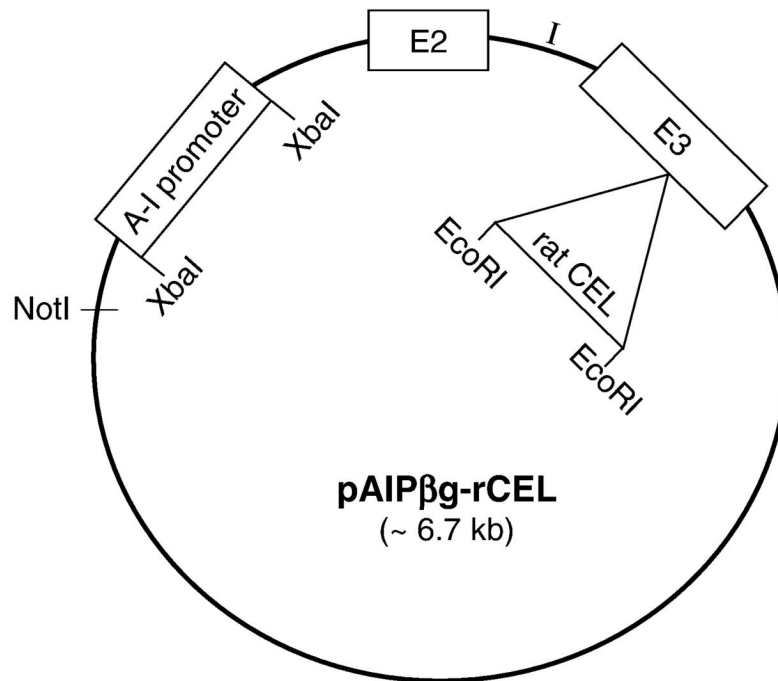


Fig. 1. Construction of rat CEL transgene

A Bluescript plasmid was previously modified to contain β -globin exons 2 and 3 (E2, E3) and the intervening intron (I). The 324 bp hepatic-specific promoter for apolipoprotein A-I (AI promoter) was inserted into the plasmid multiple cloning site and the complete rat CEL cDNA (~ 2 kb) inserted in the EcoRI site of exon 3 of β -globin. The plasmid was linearized by restriction digestion with NotI and purified for microinjection.

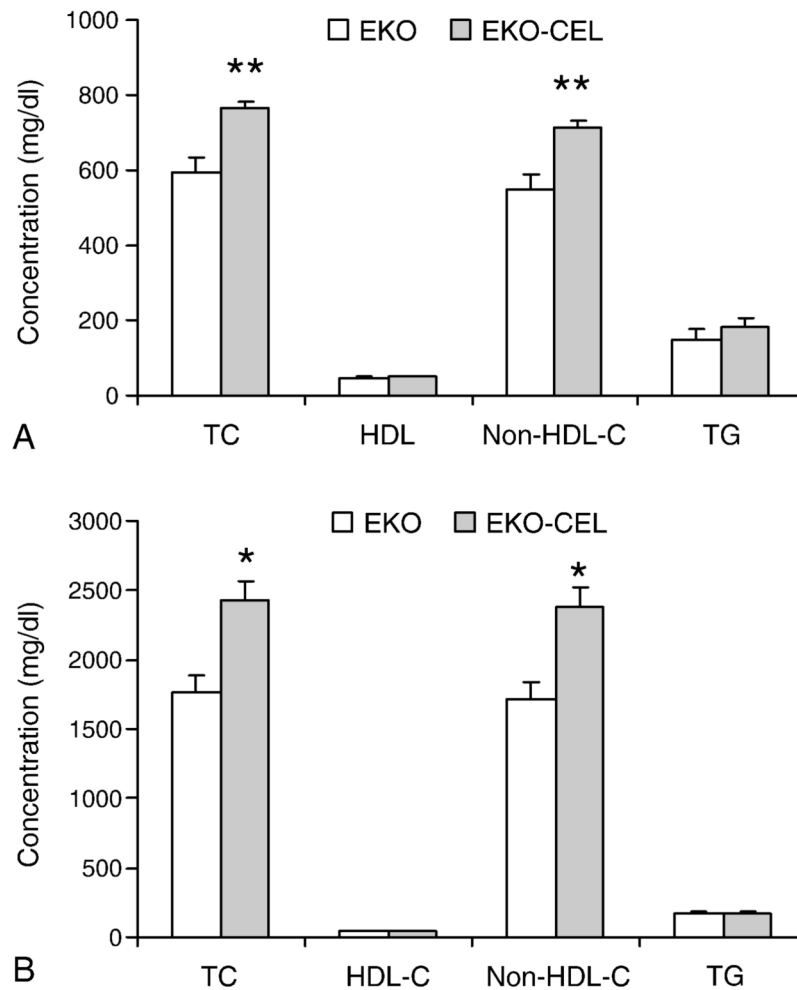


Fig. 2. Plasma lipid concentrations

Plasma samples were collected from 2 months old EKO mice (n=10) and EKO-CEL mice (n=10) on chow (A) or Western diet (B). Plasma total cholesterol (TC), HDL-cholesterol (HDL-C), and TG concentrations were determined enzymatically by using commercial kits. Non-HDL-C was calculated as the difference between TC and HDL-C. EKO-CEL mice had significantly higher plasma TC than EKO mice on either chow or Western diet. The difference was found exclusively in the non-HDL-C fraction. No significant difference between two groups was found in the plasma HDL and TG concentrations. * $P < 0.05$; ** $P < 0.01$.

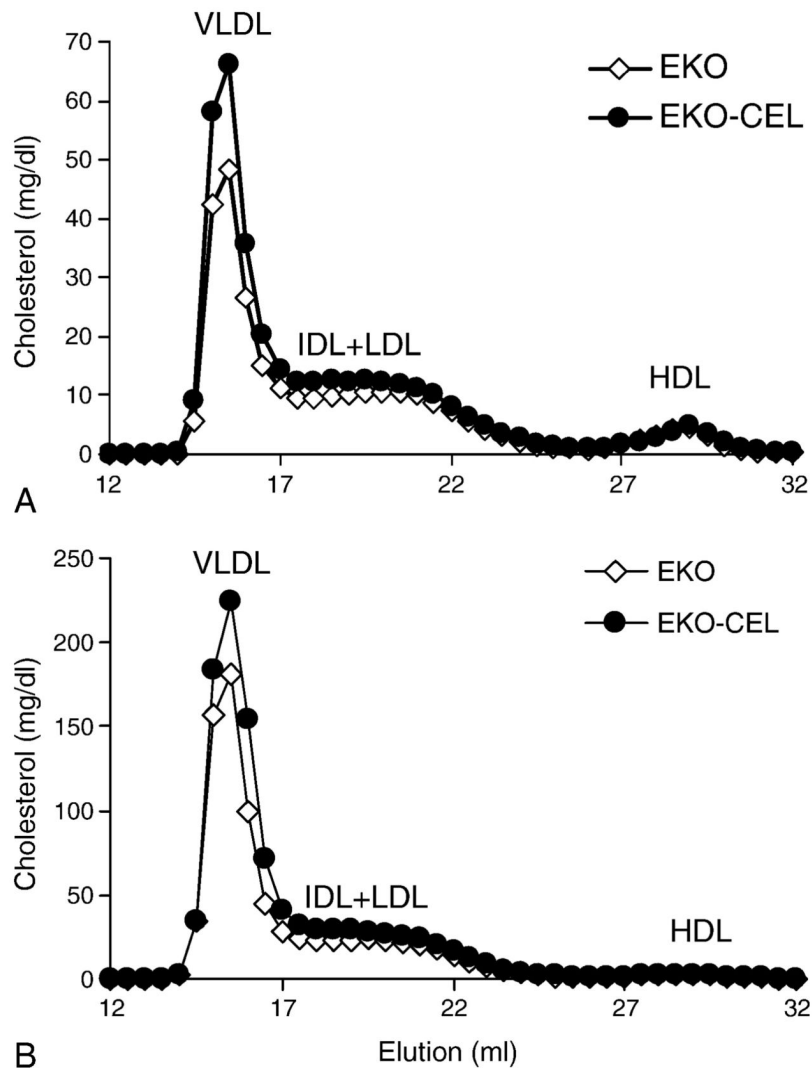


Fig. 3. Plasma lipoprotein cholesterol profiles

Plasma samples were pooled from EKO (n=10) and EKO-CEL mice (n=10), respectively, on chow (A) or Western diet (B). FPLC was used to separate plasma lipoproteins, and the total cholesterol profiles were determined. VLDL-cholesterol from EKO-CEL mice was significantly higher than that from EKO mice either on chow or Western diet. IDL- and LDL-cholesterol also tended to be higher in EKO-CEL mice. No difference between two groups was observed in HDL fraction.

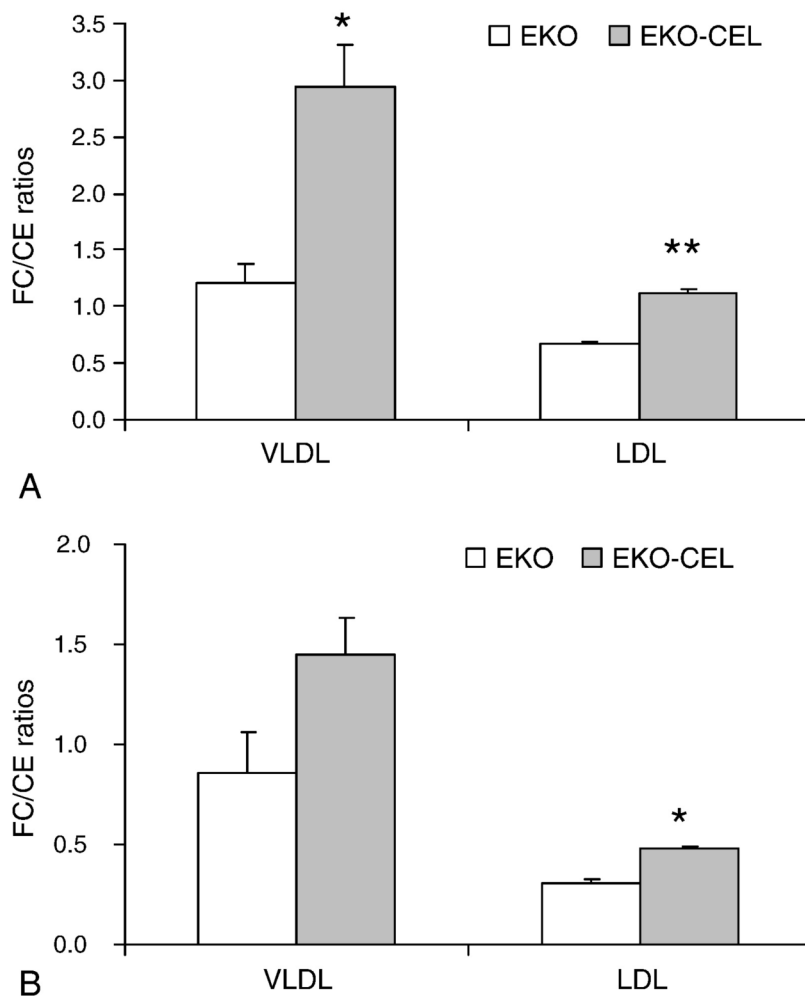


Fig. 4. Free cholesterol (FC) to cholesterol ester (CE) ratios in apoB-Lp VLDL and LDL fractions of the plasma were collected from the FPLC elution. FC and TC concentrations were determined enzymatically. Concentrations of CE were obtained by subtracting FC from TC. On chow diet (**A**), the FC/CE ratios were significantly higher in both VLDL and LDL from EKO-CEL mice compared to 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 ($P = 0.09$) compared to that in EKO mice. * $P < 0.05$; ** $P < 0.01$.

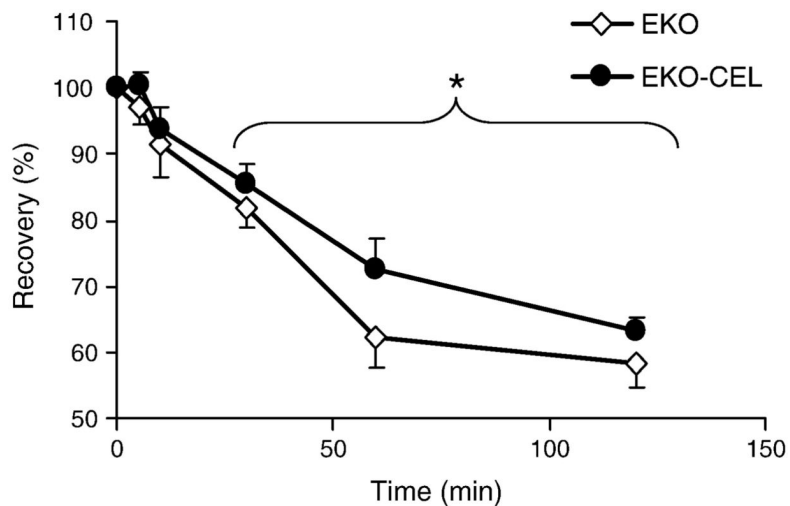


Fig. 5. Clearance of plasma VLDL on chow diet

The turnover rate of VLDL was measured by intravenous injection of a bolus radiolabeled VLDL. Percent 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 min after injection ($P = 0.045$ by repeated measures ANOVA). * $P < 0.05$.

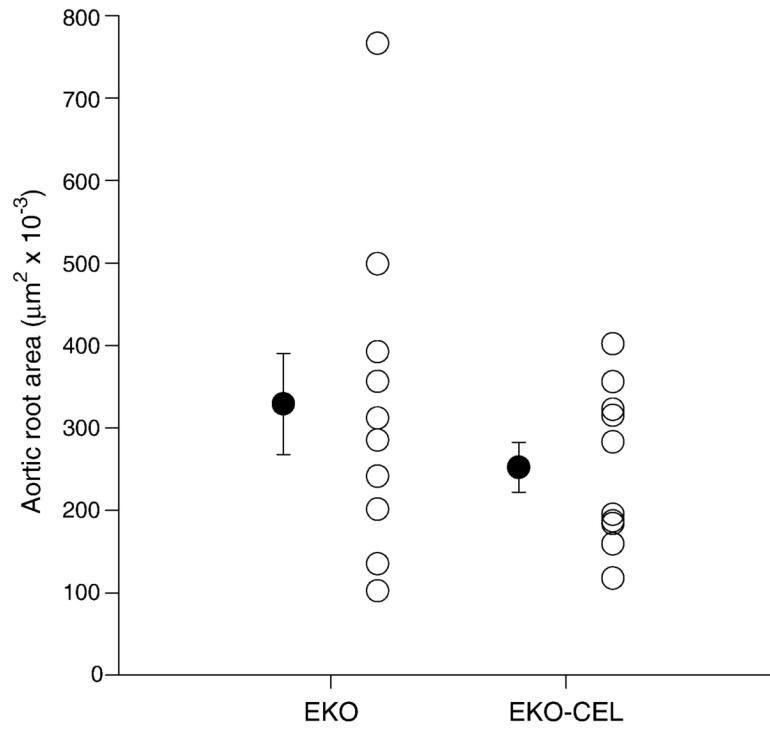


Fig. 6. Atherosclerotic lesion area in the aortic root of mice on Western diet for 10 weeks
No significant difference was observed between EKO (n=10) and EKO-CEL (n=10) mice.