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Estrogen Decreases Atherosclerosis In Part By Reducing Hepatic Acyl-CoA:Cholesterol Acyltransferase 2 (ACAT2) In Monkeys

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Abstract

Objective—Estrogens decrease atherosclerosis progression, mediated in part through changes in plasma lipids and lipoproteins. This study aimed to determine estrogen-induced changes in hepatic cholesterol metabolism, plasma lipoproteins, and the relationship of these changes to atherosclerosis extent.

Methods and Results—Ovariectomized monkeys (n=34) consumed atherogenic diets for 30 months which contained either no hormones (control, n=17) or conjugated equine estrogens (CEE, n=17) at a human dose equivalent of 0.625 mg/d. Hepatic cholesterol content, low-density lipoprotein (LDL) receptor expression, cholesterol 7 α -hydroxylase and acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity and expression levels were determined. CEE treatment resulted in lower plasma concentrations of very-low- and intermediate density lipoprotein cholesterol (V+IDL; p=0.01), smaller LDL particles (p=0.002) and 50% lower hepatic cholesterol content (total, free and esterified; p<0.05 for all). Total ACAT activity was significantly lower (p=0.01), explained primarily by reductions in the activity of ACAT2. Estrogen regulation of enzymatic activity was at the protein level as both ACAT1 and 2 protein, but not mRNA levels, were lower (p=0.02 and <0.0001, respectively). ACAT2 activity was significantly associated with hepatic total cholesterol, plasma V+IDL cholesterol, and atherosclerosis.

Conclusions—Atheroprotective effects of estrogen therapy may be related to reduced hepatic secretion of ACAT2-derived cholesteryl esters in plasma lipoproteins.

Condensed Abstract—Estrogen inhibits atherogenesis. We demonstrate in ovariectomized monkeys that estrogen therapy led to lower hepatic and circulating lipoprotein cholesterol, and lower ACAT2 protein and associated activity levels as compared to controls. Hepatic ACAT2 activity was highly correlated with, and was an independent predictor of coronary artery atherosclerosis extent.

Keywords

ACAT2; coronary artery atherosclerosis; estrogen; hepatic cholesterol; lipoproteins

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Kavanagh; Estrogen decreases ACAT2 and atherosclerosis

Estrogens have beneficial effects on plasma lipid and lipoprotein concentrations and reduce atherosclerosis extent in a number of animal models including mice, rabbits and monkeys ^{1, 2}. In postmenopausal women, hormone therapy improves lipoprotein concentrations and reduces atherosclerosis (as determined by carotid-intimal medial thickness) in primary prevention studies ³ but not when cardiovascular disease (CVD) is present ⁴. In the Women's Health Initiative (WHI), an increased number of CVD events in the first year of therapy with combined conjugated equine estrogens (CEE) plus progestin (medroxyprogesterone acetate) was reported in 2003 ⁵. In 2004, the results from WHI-estrogen-only trial showed no increase in overall CVD events and potential benefit for heart disease in women 50 to 59 years old ⁶. More recently, a subsequent report from the WHI showed that women 50 to 59 years of age had 42% reduced coronary calcification with estrogen therapy, which was even greater (61%) in those women adherent to treatment ⁷ indicative of reduced or less complicated atherosclerotic lesions. Hypothesized mechanisms underlying these discrepancies include pro-inflammatory effects which may be particularly important when advanced lesions are present ^{8, 9}.

The mechanisms for atheroprotection with estrogens are complex and have been shown to be due, in part, to beneficial effects on plasma lipids and lipoproteins ^{2, 8} mediated primarily by changes in hepatic cholesterol metabolism ^{8, 10}. The effects of exogenous estrogens on plasma lipoproteins vary with dose, route of administration, and formulation but generally are characterized by a decrease in total and low-density-lipoprotein cholesterol (TPC and LDLC, respectively) and an increase in high-density-lipoprotein cholesterol (HDL) and triglycerides (TG). Oral estrogens dose-dependently lower plasma LDLC concentrations due to a first-pass effect that results in increased hepatic LDL catabolism ^{11, 12}. Studies in animal models and cell culture systems suggest this is a result of estrogen-induced hepatic LDL receptor upregulation; however our previous results in monkeys treated with clinically relevant estrogen doses found no increase in LDL receptor mRNA abundance despite decreased hepatic cholesterol content ¹³. This suggests that changes in LDL receptor may be a secondary response to estrogen-related changes in hepatic cholesterol metabolism.

The purpose of this study was to expand upon our previous studies and assess other known steps in hepatic cholesterol metabolism that may be estrogen-sensitive, specifically hepatic cholesterol 7 α -hydroxylase (C7H) and acyl-coenzyme A:cholesterol acyltransferase (ACAT) activities. C7H is the rate-limiting step in hepatocyte bile acid synthesis in the primary pathway for cholesterol catabolism and elimination from the body ¹⁴. Two ACAT isoforms (ACAT1 and ACAT2) are responsible for cholesteryl ester formation in tissues. ACAT2 is found in hepatocytes and facilitates cholesteryl ester incorporation into apoprotein (apo) B-containing lipoproteins and represents the majority of hepatic ACAT activity ¹⁵. ACAT2 is also the dominant cholesterol esterifying enzyme in the enterocyte ¹⁶. By contrast, ACAT1 is found in liver Kupffer cells and macrophages of many tissues ¹⁶ and, in these locations, is assumed to be responsible for cellular cholesterol homeostasis ¹⁷. Previous studies in monkeys have shown a high correlation between hepatic ACAT activity, VLDL secretion, LDL particle size, and coronary atherosclerosis ^{18, 19}. In this study, we extend our previously published findings that estrogens decrease progression of coronary artery atherosclerosis in ovariectomized (OVX) monkeys ¹ by documenting associated effects on hepatic cholesterol metabolism and reporting a high and significant correlation between estrogen-induced alterations in hepatic cholesterol metabolism and coronary artery atherosclerosis extent.

Methods

Study Design

Monkeys evaluated were OVX cynomolgus macaques (n=34) fed a moderately atherogenic diet (40% of calories supplied as fat supplemented with 0.28 mg cholesterol/kcal) for 30 months

¹. Diet was fed with either no hormones (control, n=17) or with conjugated equine estrogens (CEE, n=17) at a human dose equivalent of 0.625 mg/day. At necropsy, the liver was weighed, and portions of liver were minced prior to immediate freezing in liquid nitrogen for storage at -70° C until analysis.

Plasma lipoprotein cholesterol and LDL molecular weight

Plasma collected at study end was measured for TPC, HDLC, TG and average LDL molecular weight as previously described ¹. The cholesterol distribution among lipoproteins was determined after isolation by ultracentrifugation and separation of lipoprotein classes with high performance liquid chromatography (HPLC) ¹.

Hepatic Cholesterol Concentrations

Lipids were extracted ²⁰ with total and free cholesterol concentrations (TC and FC) determined enzymatically ²¹. Esterified cholesterol (EC) concentration was calculated as the difference between TC and FC. TG content was measured as previously described ²².

Hepatic C7H and ACAT Activity

Hepatic microsomes were prepared from frozen tissue after homogenization and centrifugation to remove membranes and cell debris. The supernatant was recentrifuged and the resulting microsomal pellet was resuspended in Tris buffer whereupon protein concentration was measured ²³. C7H activity was determined as described by Rudel ²⁴ and co-workers adapted from the method of Ogishima and Okuda ²⁵. Total ACAT activity was determined as described previously ^{17, 18}. Microsomes were first pre-incubated for 30 minutes with a cholesterol-saturated solution of β -hydroxypropyl cyclodextrin and the ACAT reaction was then initiated in the presence or absence of a specific ACAT2 inhibitor ²⁶, pyripyropene A, at a concentration of 5 μ mol/l to separately identify ACAT1 (uninhibited) and ACAT2 (total - ACAT1) activities ¹⁵. ACAT and C7H activities are expressed as nmol/min/mg and pmol/min/mg microsomal protein, respectively.

Immunoblotting for LDL receptor, C7H, ACAT1 and 2

LDL receptor was quantified in membrane proteins, while microsomal protein was used to quantify ACAT and C7H. Protein samples were electrophoresed on Novex pre-cast Tris-Glycine gels (Invitrogen, Carlsbad CA) prior to transfer to polyvinylidene fluoride membranes for LDL receptor, or nitrocellulose for ACAT and C7H. Nonspecific binding was blocked by incubation in 5% non-fat dried milk in Tris-buffered saline with Tween-20. Monospecific fusion protein antibodies to the N-terminal 100-110 amino acids of ACAT1 and ACAT2 were prepared in rabbits and purified as described by Lee et al. ¹⁷. C7H antibodies are fusion protein antibodies prepared in chickens against the sequence from AA353-436 of African green monkey C7H. The primary ACAT and C7H antibodies were applied to the nitrocellulose membrane in blocking solution at 3 μ g/ml for ACAT1 and 2, and 1 μ g/ml for C7H. A donkey anti-rabbit secondary antibody conjugated to horseradish peroxidase in a 1:20000 dilution was applied to membranes for detection of ACAT1 and 2, whereas a goat anti-chicken secondary antibody conjugated to horseradish peroxidase was used for C7H at a 1:15 000 dilution (Amersham Pharmacia Biotech, Buckinghamshire, UK). The peroxidase signal was obtained using chemiluminescence reagents (ECL+ Western Blotting Detection, Amersham Biosciences UK, Buckinghamshire, UK) and detected using phosphorimaging (Storm 860, Molecular Dynamics, Sunnyvale, CA). The membranes were stripped of antibody and then re-probed for actin (Oncogene Research Products, San Diego, CA) according to manufacturer instructions. Quantification was determined using ImageQuant 5.2 software (Molecular Dynamics, Sunnyvale, CA) and results expressed as the ratio to actin densities.

RNA preparation and C7H and ACAT mRNA determination

Total RNA was extracted with Trizol reagent (Invitrogen) according to the manufacturer's instructions. Quantification of mRNAs was performed by the SYBR Green real-time polymerase chain reaction (PCR) in an ABI PRISM 7000 thermocycler (Applied Biosystems, Foster City, CA) using primer sequences for ACAT1, ACAT2, ABCG5 and C7H (CYP 7A) as previously published¹⁵. Primer sequences used for HMG-CoA reductase were forward 5'-GACGCAACCTTTATATCCGTTT-3' and reverse 5'-TTGAAAGTGCTTTCTCTGTACCC-3'; HMG-CoA synthase (cytoplasmic) forward 5'-TTCGTGGCTCACTCCCTTT-3' and reverse 5'-CTGTCACTGTTTCCTCCTTGG-3'; HMG-CoA synthase (mitochondrial) forward 5'-CTCGTTCTGTCCCACCA-3' and reverse 5'-AAGAGAAGGCACCAATCCTG-3'. Data are expressed in arbitrary units normalized by the qRT-PCR signal obtained in the same cDNA preparation for glyceraldehyde 3-phosphate dehydrogenase mRNA.

Statistical Methods

Data are presented as mean \pm standard error (SEM) for each group. Data were analyzed for normality and logarithmically transformed where necessary prior to comparisons by unpaired Student's t-tests. Pair-wise associations between variables were evaluated by Pearson's correlation coefficient. To assess associations between atherosclerosis and other variables, multiple regression analysis was performed with backward stepwise evaluation of independent variables. Significant predictors of atherosclerosis extent determined by the multiple regression model were used as covariates in ANCOVA to assess the contribution of these variables to group differences in atherosclerosis extent. Statistical analysis was performed using Statistica 6 (StatSoft Inc., Tulsa, OK) with significance set at $\alpha < 0.05$.

Results

Table 1 details body and liver weights. Liver weight was not different despite the 60% lower TG content in the CEE group. Plasma lipid and lipoprotein cholesterol concentrations are also reported in Table 1. CEE-treatment resulted in a statistically significant lowering of cholesterol concentration in very-low and intermediate-density lipoproteins (V+IDL; $p=0.01$). Total, LDLC and HDLC were not changed. Plasma TG concentrations were increased ($p<0.001$) and LDL particle size, as estimated by molecular weight, was decreased ($p=0.002$) in the CEE-treated animals compared to controls.

Hepatic TC was reduced approximately 50% by CEE-treatment ($p<0.001$, Figure 1) compared to control. This was due to equivalent reductions in both FC and CE concentrations ($p<0.05$ for both). There were no differences in total hepatic protein concentrations (mg/g wet wt) between groups (data not shown; $p=0.33$).

Hepatic total ACAT activity was significantly less in CEE-treated monkeys (1.54 ± 0.13 nmol/min/mg vs. 1.03 ± 0.13 nmol/min/mg, $p=0.01$). ACAT2 represents the majority (>95%) of hepatic ACAT enzymatic activity and ACAT2 activity was about a third lower with CEE treatment (1.49 ± 0.13 vs. 0.99 ± 0.13 nmol/min/mg, $p=0.01$), depicted in Figure 2A. CEE treatment resulted in lower ACAT2 protein concentrations by more than two thirds (0.089 ± 0.014 vs. 0.028 ± 0.003 AU, $p<0.001$), while ACAT2 mRNA abundance was not statistically different (19.7 ± 1.4 vs. 16.4 ± 1.6 AU, $p=0.13$) (Figure 2A). For ACAT1, CEE treatment resulted in about 20% lower activity (0.048 ± 0.003 vs. 0.039 ± 0.003 nmol/min/mg, $p<0.03$), and similarly decreased ACAT1 protein (0.052 ± 0.005 vs. 0.040 ± 0.009 AU, $p<0.02$) while not affecting ACAT1 mRNA (70.9 ± 6.3 vs. $59.3\pm 6.0\pm 0.04$ AU, $p=0.20$) (Figure 2B). Since ACAT2 is expressed in hepatocytes while ACAT1 is expressed primarily in Kupffer cells, these results suggest that estrogen effects on protein and activity are present in both cell types through

mechanisms that are not mediated principally through gene expression. The majority of estrogen-mediated reduction in ACAT activity was on the ACAT2 isoform present in hepatocytes, however.

Only ACAT2 protein was associated with total ACAT activity and hepatic TC content ($R=0.64$, $p<0.001$; $R=0.48$, $p<0.01$ respectively). Additionally, ACAT2 activity correlated highly with ACAT2 protein ($R=0.64$, $p<0.001$), ACAT2 mRNA ($R=0.56$, $p=0.002$), TPC ($R=0.38$, $p=0.03$), V+IDL ($R=0.45$, $p=0.01$), and LDL size ($R=0.55$, $p=0.001$). ACAT1 protein also was associated with V+IDL ($R=0.38$, $p=0.03$) and LDL size ($R=0.48$, $p=0.006$).

CEE-treatment resulted in a significant 85% higher C7H protein mass estimated by Western blotting ($p=0.005$, Table 2) which paralleled C7H activity and mRNA expression though the latter were not statistically different from control ($p=0.23$, and 0.22 , respectively; Table 2). There was no significant effect of CEE on expression of LDL receptor protein ($p=0.15$) or mRNA levels of several other proteins commonly associated with cholesterol efflux and synthesis (Table 2).

As previously reported, coronary artery atherosclerosis extent, determined as intimal area (mm^2) [defined as the cross-sectional area occupied by intimal lesion (plaque size)] was significantly lower with CEE-treatment (0.255 ± 0.08 vs. 0.09 ± 0.02 mm^2)³. Correlation and multiple regression analyses were done to evaluate CEE-treatment effects on cholesterol metabolism and atherosclerosis. Coronary artery intimal area (mm^2) was positively associated with ACAT2 activity ($R=0.44$, $p=0.01$, Figure 3A) with LDL size ($R=0.58$, $p<0.001$, Figure 3B). In regression analysis using all study outcome variables as independent variables, ACAT2 protein and LDLR were significant positive predictors while HDLC and C7H protein were significant negative predictors of atherosclerosis cumulatively accounting for 67% of variability in lesion size (Table 3). ACAT2 activity was then used as a covariate in ANCOVA which eliminated group differences in hepatic FC and CE content, providing evidence that CEE mediates effects on hepatic cholesterol metabolism through reduction in ACAT2 activity.

Discussion

The major finding from this study is that estrogens (CEE) decrease total hepatic ACAT activity in ovariectomized monkeys, due primarily to reductions in ACAT2, the isoform of the enzyme found in hepatocytes. ACAT2 activity was assayed utilizing the selective inhibition by pyripyropene A and found to be responsible for over 95% of hepatic microsomal activity, a finding consistent with our previous report²⁶. CEE treatment resulted in a greater reduction of ACAT2 protein levels compared to that of ACAT1. Further, ACAT2 activity was significantly and independently associated with atherosclerosis extent. This suggests that a primary mechanism for the atheroprotective effects of estrogens could be through a reduction in the ACAT2 enzyme activity in hepatocytes and the associated reduction in the product cholesteryl esters that get secreted into apoB-containing lipoproteins^{18, 19}.

Elevation in plasma concentrations of apoB-containing lipoproteins is a known risk factor for atherosclerosis development. Both the concentration of these lipoproteins and the size of the particles are considered important in atherogenesis²⁷. The incorporation of cholesteryl esters into apoB-containing lipoprotein particles is regulated by ACAT2. Higher ACAT2 activity results in increased hepatic secretion of VLDL particles that are enriched in cholesteryl oleate (as oleoyl-CoA is a primary substrate for ACAT). Resulting LDL particles are larger and more cholesteryl oleate-enriched¹⁹. ACAT activity has been associated with increased atherosclerosis extent in African green monkeys¹⁸ and deletion of the ACAT2 gene has been shown to protect against atherosclerosis in mice^{22, 28}. The present studies suggest that estrogen

can act to limit ACAT2-derived cholesteryl oleate enrichment of apoB-containing lipoproteins, thus providing an additional mechanism underlying the atheroprotective effects of estrogen.

The effect of CEE treatment on reducing hepatic total ACAT activity was determined to be primarily the result of decreased activity and protein levels of the ACAT2 isoform. This enzyme is expressed in the hepatocytes of liver and in the enterocytes of the intestine of human and nonhuman primates, the two cell types in the body where apoB-containing lipoprotein production, assembly and secretion occurs^{15, 17}. CEE treatment did not result in significantly lower ACAT2 mRNA although the trend was in the same direction as the protein, suggesting that ACAT2 protein and activity is primarily modified post-transcriptionally and may be related to hepatic free cholesterol content as shown previously²⁹. This is consistent with the significant correlation that was found between hepatic cholesterol content and ACAT2 protein and ACAT activity.

Findings related to the effects of estrogen on ACAT have been inconsistent, with studies of rats reporting no effect on total ACAT activity³⁰ and baboons showing a 40% reduction that did not reach statistical significance³¹. However recent studies have shown that tamoxifen (a selective estrogen receptor modulator) and other estrogen receptor ligands (including estradiol) display three-dimensional structural homology with SAH 58-035, shown *in vitro* to be a prototypical competitive inhibitor of the ACAT enzyme³². Estradiol directly inhibits ACAT only at supraphysiologic concentrations ($IC_{50} \approx 20 \mu M$)³² whereas estrogen concentrations measured in premenopausal women and CEE-treated primates are in the nanomolar range¹, suggesting significant direct inhibition of ACAT2 *in vivo* is unlikely. Estrogens appear more likely to exert effects on ACAT through estrogen's pleiotropic nuclear receptor action and regulation of ACAT synthesis, or through reductions in hepatic cholesterol content.

Lower hepatic cholesterol content may result from estrogen enhancement of biliary secretion of both free cholesterol and bile acids. The increase in the cholesterol to bile acid ratio can result in gallstone formation, which is a relatively common occurrence with hormone therapy³³. One mechanism for increased biliary sterol secretion involves C7H, the rate limiting enzyme in bile acid production. The increases in C7H protein expression and trend toward increased activity and mRNA with estrogen treatment is consistent with our prior report¹³ and those of others^{34, 35} on C7H message levels in monkeys. Hepatic sterol 27 hydroxylase is involved in another pathway for increased biliary sterol secretion. It catalyzes the first step in the "alternative" bile acid biosynthetic pathway and has also been found to be increased with estrogens³¹. While a large portion (50-60%) of the secreted biliary cholesterol is reabsorbed in the GI tract, the loss of fecal cholesterol still represents the primary mechanism for cholesterol removal from the body. An increased conversion of cholesterol into bile acids via increased C7H would appear to be consistent with the decrease in hepatic cholesterol accumulation.

Our finding of reduced hepatic free cholesterol with CEE treatment, and reduced ACAT2 activity, is additionally supported by recent elucidation of the 'alternative' pathway for cholesterol excretion in ACAT2-deficient mice³⁶. In the alternative pathway, cholesterol is transported to proximal segments of the small intestine and is excreted directly and independently of bile. Evidence for this non-dietary sterol loss through the feces was also seen in dogs and people^{37, 38} with a net reduction in enterohepatic recirculation of biliary cholesterol. Free cholesterol also was apparently not shunted to HDL biogenesis as HDL-associated cholesterol and ABCA1 levels were comparable between groups. Increased fecal cholesterol excretion is seen with ACAT2 gene disruption³⁶ and the use of bile acid sequestrants. Both interventions also result in higher plasma TG and greater fecal sterol loss, however ACAT2 gene disruption did not increase biliary sterol secretion. The similarity in

clinical profile suggests that CEE-related reductions in ACAT2 activity may be coupled with free cholesterol loss through the 'alternative' pathway³⁶.

Reduction of cholesterol absorption from the intestine is another mechanism for reducing hepatic cholesterol concentration. While we did not determine estrogen effects on intestinal ACAT, it is possible that estrogen reduces intestinal ACAT2 (the predominant isoform in enterocytes) in a manner similar to hepatic ACAT2. CEE was not shown to have an effect on cholesterol absorption in a previous report using this same model³⁹, but decreases of 18% and 9% following oral and transdermal estrogen therapy, respectively, have been noted in women⁴⁰. Lower intestinal ACAT2 activity does lead to reduced cholesterol absorption from the intestine^{41, 42} but regulation of intestinal and hepatic ACAT2 activity can be independent, as has been demonstrated in diabetes⁴³, allowing the possibility that ACAT2 activity reductions in liver may not be seen concurrently in the intestine. This is supported by studies in primates where similar plasma lipid improvements were documented following CEE treatment, although cholesterol absorption was not significantly altered³⁹.

In this study there were no significant changes in hepatic LDL receptor protein with CEE treatment, as has been reported previously in the same model¹³ and in rats³⁵. However we have seen increases in hepatic LDL fractional degradation rates with the same estrogen dose¹⁰ although this would include both LDL receptor- and nonreceptor-mediated uptake. Increased catabolism of VLDL and LDL cholesterol, via receptor dependent and independent mechanisms, may factor into the observed predominance of smaller plasma LDL particles. Studies in women found that estrogen increases both the production and clearance of large LDL and small LDL, however the greater effect was on clearance of large LDL, resulting in an overall increase in concentration of small LDL. The notion that smaller particles are inherently more atherogenic has been questioned⁴⁴. A decreased CE secretion associated with the estrogen-related reduction in hepatic ACAT2 activity could result in plasma very low density LDL, intermediate density LDL and LDL particles with few CE molecules; such a compositional shift may indicate a situation where these lipoproteins are unable to result in the deposition of as much cholesterol in the arterial intima during atherogenesis.

Elevated plasma TG is seen commonly with oral estrogen therapy. Similarly, TG elevation is a consistent feature of ACAT2 disruption in mouse models^{28, 41, 45, 46}. The mechanisms are unknown, however unpublished data from our group demonstrates higher hepatic TG secretion rates in ACAT deficiency. One hypothetical mechanism underlying this effect is that hepatic TG is more efficiently mobilized when hepatic CE depletion is present. Hepatic lipase is generally thought to be decreased by oral estrogen treatment⁴⁷ and thus the contribution of liver TG and hepatic lipase activity on plasma lipids and atherosclerosis is controversial⁴⁸. Hepatic lipase activity was 40% lower with CEE treatment in the primate model, however the lipid measures in plasma and liver were of the same magnitude as reported here¹⁰.

In conclusion, CEE-treatment of ovariectomized monkeys decreased hepatic ACAT activity with reductions of both ACAT1 and ACAT2 protein and activity, with the major reduction seen in hepatocyte-associated ACAT2. Increased bile acid secretion, following C7H upregulation, may contribute to the decrease in hepatic cholesterol content which is important in preventing down-regulation of hepatic LDL receptors. If ACAT2 inhibition by estrogen is not liver specific, reductions in dietary and endogenous cholesterol absorption from the intestinal tract could further contribute to the reductions in hepatic cholesterol. Of great interest, regression analysis showed that ACAT2 was significantly and independently associated with coronary atherosclerosis extent. This suggests that suppression of ACAT2 activity and the associated decrease in V+ILDL CE secretion could play an important role in estrogen-induced inhibition of the progression of atherosclerosis.

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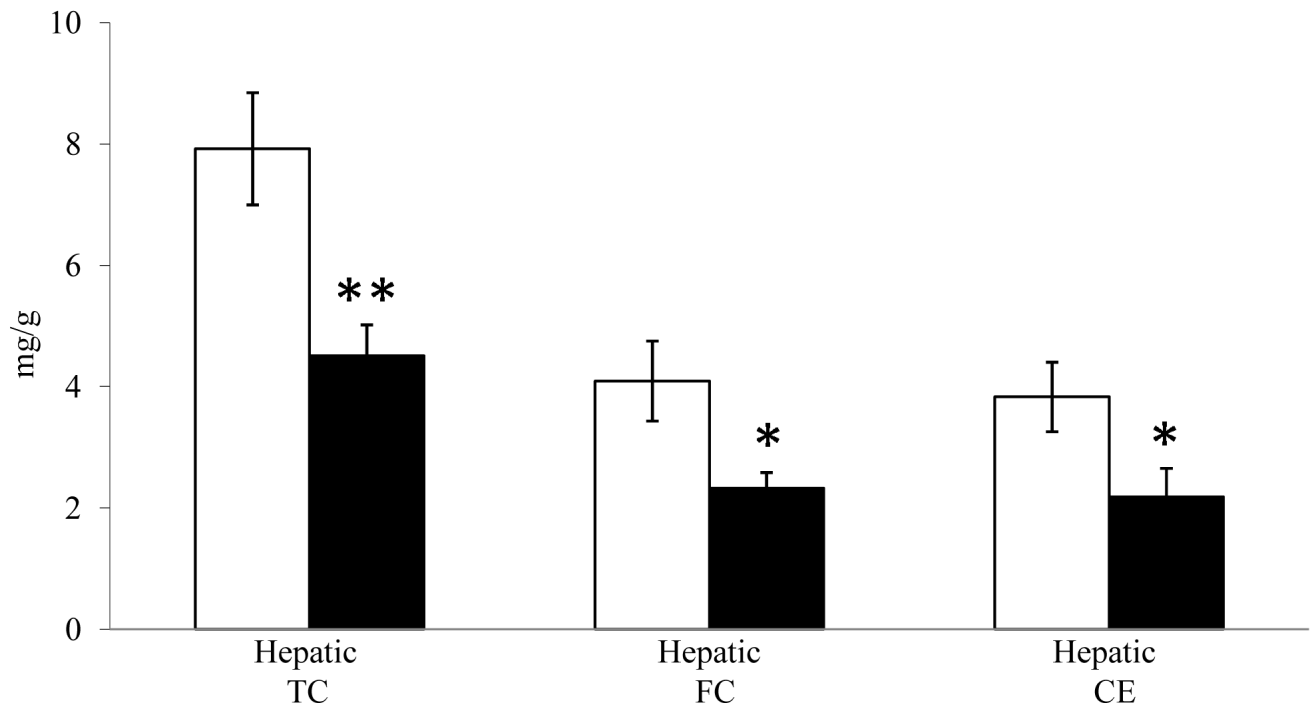
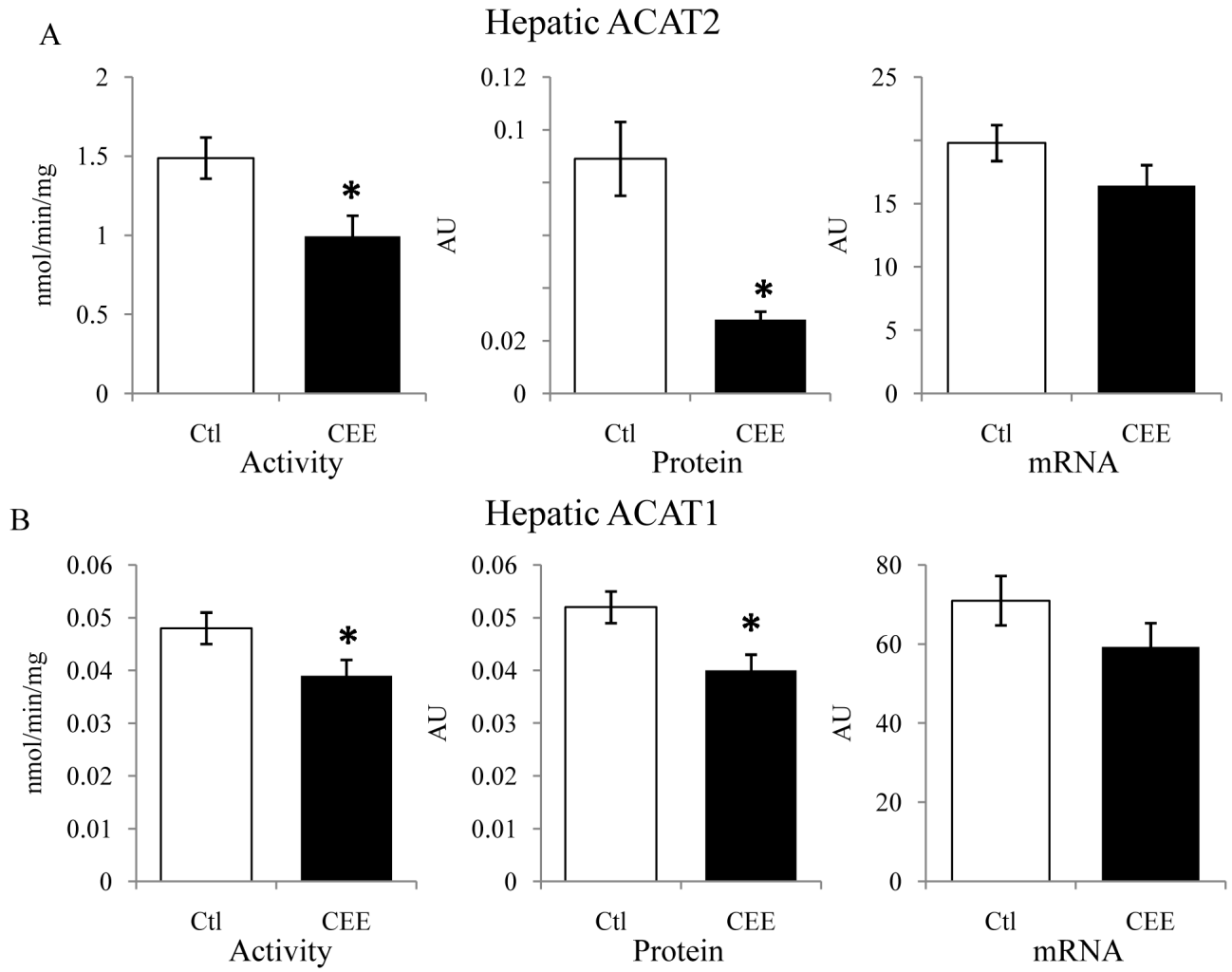


Figure 1. Mean (\pm SEM) hepatic cholesterol content (total, free and esterified: TC, FC, and CE respectively) in control (\square) and CEE-treated (\blacksquare) monkeys (n=17/group). ** p<0.01; * p<0.05.

**Figure 2.**

Mean (\pm SEM) activity (ACT), protein (Pro), and mRNA comparisons for ACAT2 and ACAT1 in livers of control (\square) and CEE-treated (\blacksquare) monkeys (n=17/group). * p<0.05.

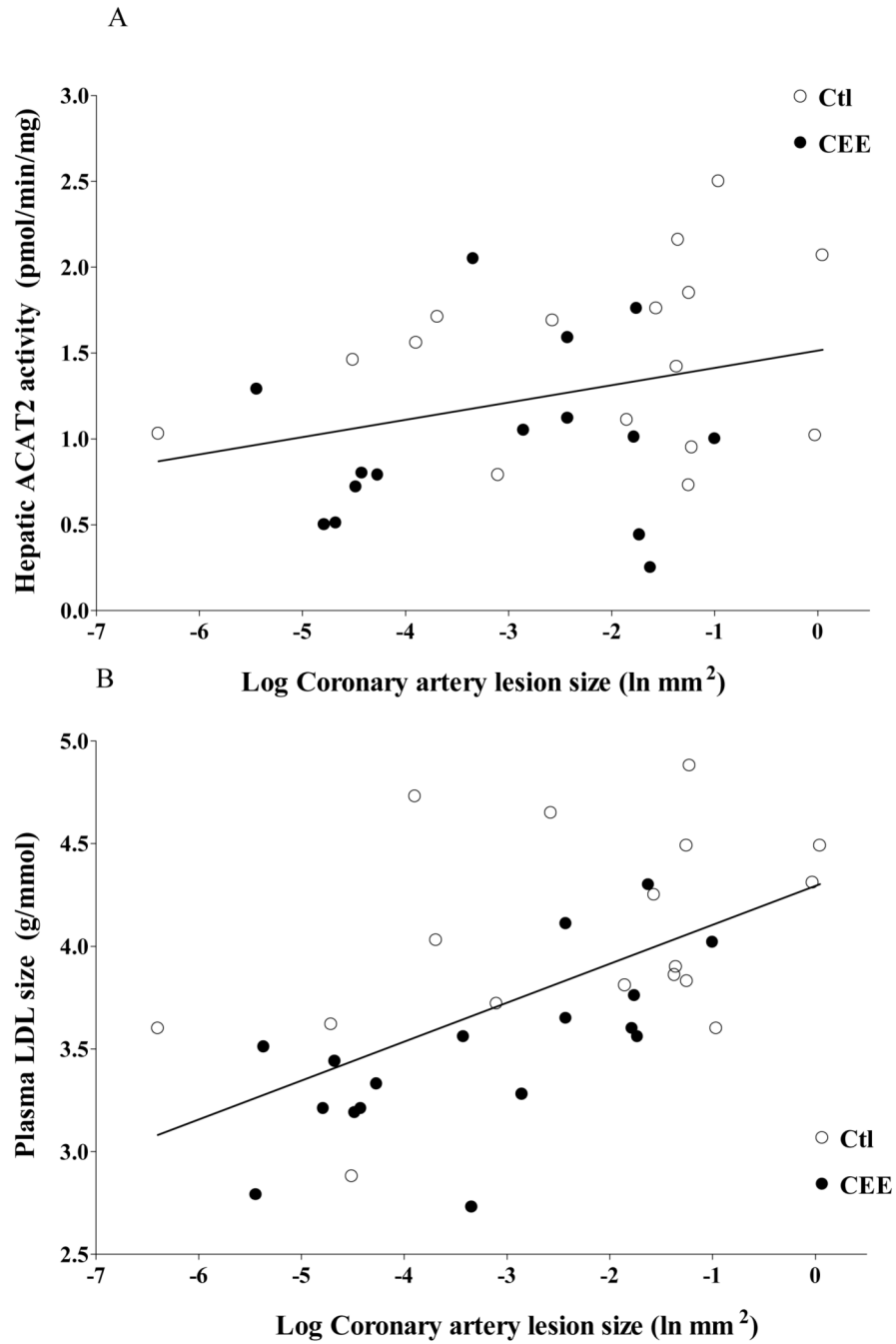


Figure 3. Scatterplot (n=34) demonstrating the relationship between coronary artery atherosclerosis extent (depicted on the x-axis as the logarithmic transformation of intimal area (mm²) and **A**) Total hepatic ACAT2 activity (R=0.44; p=0.01) and **B**) plasma LDL molecular weight (R=0.58; p<0.001) in control (○) and CEE (●) treated monkeys.

Table 1

Bodyweight (BW), liver actual and relative weight, total liver triglyceride (TG) (Mean \pm SEM, n=13-17/group), plasma cholesterol (TPC), very-low + intermediate-density lipoprotein cholesterol (V+IDL), low- and high-density lipoprotein cholesterol (LDLC and HDLC) and triglycerides (TG) concentrations and LDL molecular weight (LDL mol.wt.) in control and CEE-treated OVX monkeys (Mean \pm SEM, n=17/group). Conversion to mmol/L cholesterol and TG by multiplying by 0.0259 and 0.0113, respectively

		CTL	CEE	p-value
<i>BW</i>	(kg)	3.13 (0.27)	2.80 (0.098)	0.3
<i>Liver Wt</i>	(g)	72.74 (5.80)	68.22 (2.74)	0.6
<i>Relative Liver Wt</i>	(%)	2.34 (0.12)	2.46 (0.090)	0.41
<i>Liver TG</i>	(mg/g)	74.96 (13.95)	41.86 (7.15)	0.04
<i>TPC</i>	(mg/dL)	419.69 (23.55)	384.56 (26.25)	0.32
<i>V+IDL</i>	(mg/dL)	49.03 (6.18)	29.31 (4.31)	0.01
<i>LDLC</i>	(mg/dL)	322.21 (21.81)	314.32 (25.70)	0.81
<i>HDLC</i>	(mg/dL)	49.76 (5.19)	41.24 (2.88)	0.13
<i>TG</i>	(mg/dL)	34.41 (6.88)	65.76 (19.22)	<0.001
<i>LDL mol.wt.</i>	(g/mmol)	4.0 (0.12)	3.5 (0.10)	0.002

Table 2

Hepatic C7H activity, protein, and mRNA and LDLR protein and selected mRNA of genes that modulate hepatic cholesterol levels (cholesterol efflux: ABCA1, ABCG8 and synthesis: HMG-CoA reductase and synthases) in control and CEE-treated monkeys (Mean \pm SEM, n=17/group)

		CTL	CEE	p-value
<i>LDLR protein</i>	(AU)	6.57 (0.5)	7.64 (0.52)	0.15
<i>C7H activity</i>	(pmol/min/mg)	1.17 (0.20)	2.48 (0.58)	0.23
<i>C7H protein</i>	(AU)	3.48 (0.50)	6.50 (0.88)	0.005
<i>C7H mRNA</i>	(AU)	35.86 (9.61)	61.24 (16.88)	0.22
<i>ABCA1 mRNA</i>	(AU)	9.33 (0.62)	9.41 (0.75)	0.60
<i>ABCG8 mRNA</i>	(AU)	142.38 (8.70)	151.23 (10.29)	0.52
<i>HMG-CoA Reductase mRNA</i>	(AU)	6.19 (0.69)	5.98 (0.47)	0.80
<i>HMG-CoA Synthase (cytoplasmic) mRNA</i>	(AU)	0.30 (0.06)	0.55 (0.11)	0.061
<i>HMG-CoA Synthase (mitochondrial) mRNA</i>	(AU)	1612.30 (176.32)	1709.16 (200.89)	0.72

Table 3

Multiple regression analysis (overall model $R^2 = 0.67$; $p < 0.0001$) with parameter estimates of β (slope), the standard error (SE) of β , squared semi-partial correlation coefficients (Sr_{II}^2 ; percent of variance uniquely accounted for by the model parameter) and statistical results for significant predictors of coronary artery atherosclerosis

	β -coefficient	SE of β	Sr_{II}^2	p-value
<i>HDLC</i>	-0.407	0.127	11.94%	0.004
<i>ACAT2 protein</i>	0.397	0.133	10.23%	0.007
<i>LDLR</i>	0.385	0.124	11.16%	0.005
<i>C7H protein</i>	-0.302	0.118	7.61%	0.02