LCAT facilitates transacylation of 17β-estradiol in the presence of HDL_3 subfraction

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Abstract **It has been shown that estrogens need to be metabolized to their hydrophobic estrogen ester derivatives to act as antioxidants in lipoproteins. Data suggest that 17β-estradiol (E_2) becomes esterified in LCAT-induced reactions and the esters are transported from HDL particles to LDL and VLDL particles by a CETP-dependent mechanism. In the present study we have further investigated the regulation of E_2 esterification by LCAT and focused on the importance of HDL structure and composition in the esterification process. Isolated LDL, HDL_2, HDL_3, and reconstituted discoidal HDL (rHDL) were incubated with labeled E_2, with and without purified LCAT, at 37°C for 24 h. After purification of the lipoprotein fractions, there was a significant peak of radioactivity representing esterified estradiol attached to HDL_3 and rHDL, but HDL_2 and LDL contained only trace amounts of labeled estradiol ester. TLC analysis confirmed that the radioactivity migrated in a position corresponding to that of 17β-E_2 17-monoester standard. The amount of radioactivity associated with HDL_3 and rHDL representing esterified E_2 was significantly increased by addition of purified LCAT. However, only limited increases of radioactivity were observed in HDL_2 and LDL. In conclusion, HDL subfractions differ in their potential to regulate estradiol esterification by LCAT.**—Höckerstedt, A., M. J. Tikkanen, and M. Jauhiainen. **LCAT facilitates transacylation of 17β-estradiol in the presence of HDL_3 subfraction.** ***J. Lipid Res.*** **2002.** 43: **392–397.**

Supplementary key words estrogen • estradiol ester • lecithin:cholesterol acyltransferase • high density lipoprotein 2 • high density lipoprotein 3 • reconstituted discoidal HDL

Epidemiological, clinical, and experimental studies have suggested that HDLs are protective against atherosclerosis. However, the respective effects of two main HDL subfractions, HDL_2 and HDL_3, on the atherosclerotic process are not yet clear (1). The association of low concentrations of HDL with atherosclerosis was reported already in the 1950s (2), and the inverse correlation between HDL cholesterol concentration and coronary heart disease has thereafter been confirmed in epidemiological studies (3). It has been suggested that one of the major antiatherogenic functions of HDL is its ability to remove cellular cholesterol from peripheral tissues (4) and to transfer it to the liver for excretion in a process called reverse cholesterol transport. Free cholesterol in HDL is esterified by LCAT (5) and the generated cholesteryl esters are incorporated into the HDL core. Following this, cholesteryl esters may be transported either to triglyceride-rich lipoproteins by a CETP-mediated mechanism or selectively taken up by the liver via a SR-BI mediated process (6) and converted to bile acids and eliminated via the bile.

The oxidation of LDL is considered to be as one of the initial steps in early atherosclerosis (7–9) and another atheroprotective mechanism of HDL may involve the inhibition of LDL oxidation (10–13). When HDL itself becomes oxidized, the efficacy of reverse cholesterol transport is decreased (14). Accordingly, both an increase in the HDL-LDL ratio and in the antioxidant capacities of both lipoproteins are considered advantageous. Estrogens decrease the plasma level of LDL cholesterol and increase that of HDL in vivo (15, 16), and they also inhibit oxidation of both lipoproteins in vitro (17, 18).

Recently, it has been shown that a prerequisite for estrogens to function as antioxidants is their transformation to lipophilic estrogen fatty acid esters (19). Furthermore, there is also indirect evidence that this important reaction is facilitated by LCAT (20, 21). Estrogen esters then become incorporated into lipoproteins (22) and may act as lipoprotein-associated antioxidants. Our recent results using human plasma have suggested that 17β-estradiol (E_2) becomes esterified in the HDL fraction and is transported to LDL by a CETP-mediated mechanism (21). The present study investigated the specific roles of HDL_2 and HDL_3 subclasses in E_2 esterification by LCAT in vitro. The findings suggested a preferential role for the HDL_3 subfraction.

Abbreviations: PC, phosphatidyl choline; rHDL, reconstituted HDL; 17β-E_2, 17β-estradiol.

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MATERIALS AND METHODS

Isolation of lipoproteins

Blood was drawn from male donors aged 21–35 years into EDTA-containing vacuum tubes. The lipoproteins were isolated by sequential ultracentrifugation (23) at the following cut-off densities: VLDL ($d < 1.006$ g ml^{-1}), LDL ($1.006 < d < 1.063$ g ml^{-1}), total HDL ($1.063 < d < 1.21$ g ml^{-1}), HDL_2 ($1.063 < d < 1.125$ g ml^{-1}), and HDL_3 ($1.125 < d < 1.21$ g ml^{-1}) using a Beckman Optima LE-80K ultracentrifuge and a Ti 50.4 rotor. Prior to the incubation with labeled E_2 (see incubation of 17β-estradiol with lipoproteins), ultracentrifugally isolated lipoprotein fractions were gel filtrated on Sephadex G-25 (column dimensions 1 × 20 cm; Pharmacia Biotech, Uppsala, Sweden) to remove EDTA and other small molecules. The sample was applied in 2.5 ml of PBS pH 7.4, which was also used as an elution buffer.

Preparation of reconstituted discoidal HDL

The sodium cholate dialysis method was used essentially as described (24) in the preparation of reconstituted discoidal HDL (rHDL) discs from human apolipoprotein A-I (apoA-I), egg phosphatidyl cholines (PC), and cholesterol. Purified human plasma apoA-I was kindly provided by Dr. Peter Lerch, Swiss Red Cross, Bern, Switzerland. Briefly, reaction mixture contained apoA-I-cholesterol-egg PC-sodium cholate in a molar ratio of 1:8:140:140. Total amount of apoA-I was 10 mg and the corresponding amounts of each lipid. ApoA-I was added into detergent-containing lipid dispersion and all the incubations were performed in 10 mM Tris-HCl, pH 7.4, containing 1 mM EDTA. First, the reaction mixture was incubated at 25°C for 20 min with slight shaking. Exhaustive dialysis to remove sodium cholate was carried out at 4°C against 5 liters of 10 mM Tris-HCl-1mM EDTA buffer, pH 7.4 (same volume of fresh buffer was changed four times during dialysis period). After dialysis, the discoidal rHDL preparation was purged with nitrogen and stored at 4°C for a maximum period of 4 weeks.

Purification and assay of human plasma LCAT

Human plasma LCAT was purified from fresh delipoproteinized $d > 1.21$ g/ml plasma fraction by combining Phenyl-Sepharose CL-4B, ion-exchange (a quaternary methylamine matrix, QMA), and hydroxyl-apatite chromatographies essentially as described previously (24, 25). LCAT activity was analyzed by a radiometric assay using a proteoliposome substrate (24). LCAT activity in purified preparations was 60–440 nmol cholesterol esterified/h/ml. Enzyme preparations did not express any CETP, PLTP, LPL, or HL activity. Endogenous LCAT activity in isolated lipoprotein fractions was determined prior to adding exogenous LCAT. The amount of added exogenous LCAT (10–73 nmol/h/ml) was the same within a single experiment in LDL, HDL_2, HDL_3, and rHDL incubations.

Incubation of 17β-estradiol with lipoproteins

Labeled 17β-estradiol [2,4,6,7-^{3}H(N)] (17β-E_2) (specific activity of 72 Ci/mmol, New England Nuclear, Boston, MA) in 0.5 M HEPES buffer (pH 7.4) was added to ultracentrifugally isolated and purified lipoproteins (1 mg or 2 mg protein in 3 ml of Tris-HCl-buffer, pH 7.4) to give a total radioactivity of 200,000 dpm (corresponding to 1.3 nmol of E_2). The mixture was incubated at 37°C for 24 h in the absence or presence of LCAT (activity 10–74 nmol/h/ml) as well as in the absence and presence of the LCAT inhibitor, dithionitrobenzoic acid (DTNB, Sigma, final concentration of 3 mM). Following incubation, the lipoproteins (sample volume 3 ml) were purified by a Sephadex G-25 gel filtration. Radioactivity in the eluted fractions was determined by liquid scintillation counting (Rack-beta, Wallac, Turku, Finland). Protein concentration was determined by the method of Lowry et al. (26). The fractions containing lipoproteins were pooled and stored at 4°C for further analysis.

Purification of esterified and unesterified [^{3}H]17β-estradiol

The lipoprotein fractions obtained by gel filtration on Sephadex G25 were pooled and extracted four times with ethylacetate-diethylether (1:1, v/v) (2.5 × sample volume). After a quick freezing of the water phase, the organic layer was removed and evaporated to dryness under N_2. The dry residues were dissolved in 0.3 ml of hexane-chloroform (1:1, v/v). In order to separate esterified E_2 from the free E_2, samples were chromatographed on a Sephadex LH-20 hydrophobic matrix (column dimensions 0.5 × 5 cm, Pharmacia Biotech, Uppsala, Sweden) using hexane-chloroform (1:1, v/v) as the eluting solvent at room temperature (27). Esterified E_2 was eluted first and, following 10 ml elution, the eluent was switched to methanol, which resulted in elution of unesterified E_2. All fractions were evaporated to dryness under N_2 and dissolved in 0.5 ml of hexane-chloroform, (1:1, v/v). The radioactivity was counted in each fraction. For certain experiments, Sephadex LH-20 chromatography was carried out using 9% methanol in toluene as eluting solvent, which separates unesterified estrone, 17β-estradiol, and estriol from each other (28).

Analysis of 17β-estradiol derivatives by TLC

Samples (~4,000 dpm or all radioactivity available) obtained from the 17β-estradiol "ester" fractions after chromatography on Sephadex LH-20 were applied to TLC plates (20 × 20 cm, Silica gel 60, Merck, Germany) and developed in a hexane-ethyl ether (1:7, v/v) solvent system. The following non-radioactive standards were used: 17β-E_2 (Steraloids, Newport, RI), 17β-E_2 17-stearate (Steraloids), 17β-E_2 3-oleate, and 17β-E_2 3,17-dioleate. The last two esters were prepared as described previously (27). TLC plates were dried in air, and the location of the standards was determined by visualization under UV light after rhodamine staining. The R_f values of the standards were: 17β-E_2 0.25, 17β-E_2 3-oleate 0.5, 17β-E_2 17-stearate 0.75, and 17β-E_2 3,17-dioleate 0.875. All bands were scraped from the TLC plates and counted directly for ^{3}H radioactivity. 17β-estradiol and 17β-estradiol 17-ester fractions of the samples were identified by comparing their R_f values with those of the standards.

Other methods

Concentrations of total and free cholesterol (Boehringer Mannheim, Germany), triglycerides (Roche, Switzerland), and phospholipids (Wako Chemicals, Germany) were measured with fully enzymatic tests. ApoA-I was measured with a turbidometric assay (29).

Statistical analysis

The statistical significance between the differences of two means was analyzed by Student's t-test. Paired t-test or, if appropriate, Wilcoxon signed-rank test, was used when comparing lipoprotein fractions before and after addition of exogenous LCAT or DTNB. When increasing amounts of LCAT were used in dose-response studies, ANOVA was used. If the ANOVA indicated significant differences ($P < 0.05$), it was followed by post-hoc statistical analysis using modified t-tests according to the Bonferroni method.

RESULTS

In order to investigate the formation of 17β-E_2 17-esters in lipoprotein fractions, we incubated isolated HDL and

major HDL subfractions HDL_2 and HDL_3 with [^{3}H]17β-E_2 in the presence and absence of purified LCAT (10–73 nmol/h/ml) as well as with and without DTNB (final concentration of 3 mM) at 37°C for 24 h. For comparison, incubations were carried out using LDL, and rHDL apoA-I-cholesterol-egg PC in a molar ratio of 1:8:140, v/v/v). After purification by gel filtration, the radioactivity and protein concentrations were measured in the eluted fractions.

Incubation of [^{3}H]17β-E_2 with total HDL

The radioactivity peaks illustrated in **Fig. 1** coincided with the protein peak (vertical arrows indicate the elution positions of proteins, fractions 7–11) suggesting that [^{3}H]17β-E_2 was associated with HDL. Addition of LCAT significantly increased the esterification and incorporation of [^{3}H]17β-E_2 into HDL ($P < 0.05$), whereas addition of DTNB significantly decreased this effect ($P < 0.005$). To separate esters from unesterified [^{3}H]17β-E_2, protein-containing fractions (fractions 7–11) were extracted with ethylacetate-diethylether (1:1, v/v) and subjected to hydrophobic interaction chromatography on Sephadex LH-20 (**Fig. 2A**). Most of the radioactivity was eluted in the "ester fraction" (fractions 2 and 3) and only a small peak was detected in the free 17β-estradiol fraction after switching the solvent to methanol (fraction 12). Further analysis of the "ester fraction" by TLC confirmed that the radioactivity comigrated with the 17-E_2 17-stearate standard, suggesting that they represent 17β-E_2 fatty acid 17-monoesters (R_f value, 0.75) (Fig. 2B). These findings indicated that almost all of the labeled 17β-E_2 which had been incorporated into HDL had been converted into esters, and the fatty acyl group was located at C-17.

When the incubations of HDL with ^{3}H-E_2 were carried out by adding increasing amounts of LCAT (0, 36, 73, and 110 nmol/h/ml), a clear dose-response in the esterification of 17β-estradiol after Sephadex G25 (**Fig. 3**) ($P < 0.05$) was observed. The esterification activity present in the absence of exogenous LCAT was probably due to endogenous enzyme activity in HDL, a finding observed previously (27). Endogenous LCAT activity ranged between 3 and 107 nmol/h/ml among the different HDL preparations used.

An additional Sephadex LH-20 chromatography (9% toluene in methanol) was performed using samples obtained from the "ester" fraction following hydrolysis by saponifica-

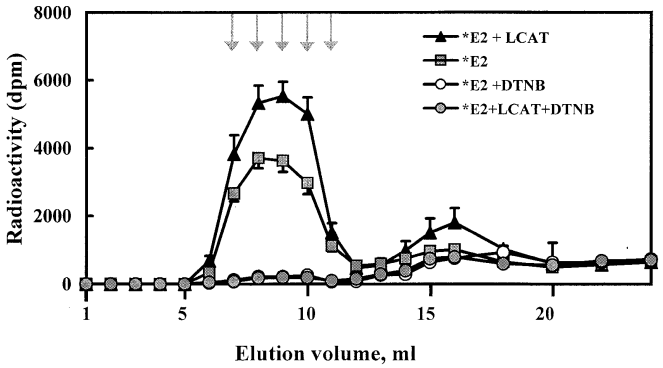

Fig. 1. Elution pattern of HDL-associated radioactivity after gel filtration on a Sephadex G25. Ultracentrifugally isolated HDL (1 or 2 mg protein in 3 ml of Tris-HCl-buffer, pH 7.4) was incubated with [^{3}H]17β-estradiol (E_2-17β) (total radioactivity 200,000 dpm) in the presence (closed triangle, n = 6) or absence (closed square, n = 6) of exogenous LCAT (10–73 nmol/h/ml) for 24 h. Dithionitrobenzoic acid (DTNB) (final concentration 3 mM) was also added to incubations with (closed circle, n = 3) and without (open circle, n = 3) purified LCAT. The amount of endogenous HDL_3-associated LCAT activity was 3–107 nmol/h/ml. After incubation, the HDL was isolated by gel filtration on a Sephadex G-25 column. Fractions were analyzed for radioactivity and protein concentrations (vertical arrows) ($P < 0.05$, HDL+LCAT vs. HDL−LCAT). Vertical bars indicate mean ± SEM.

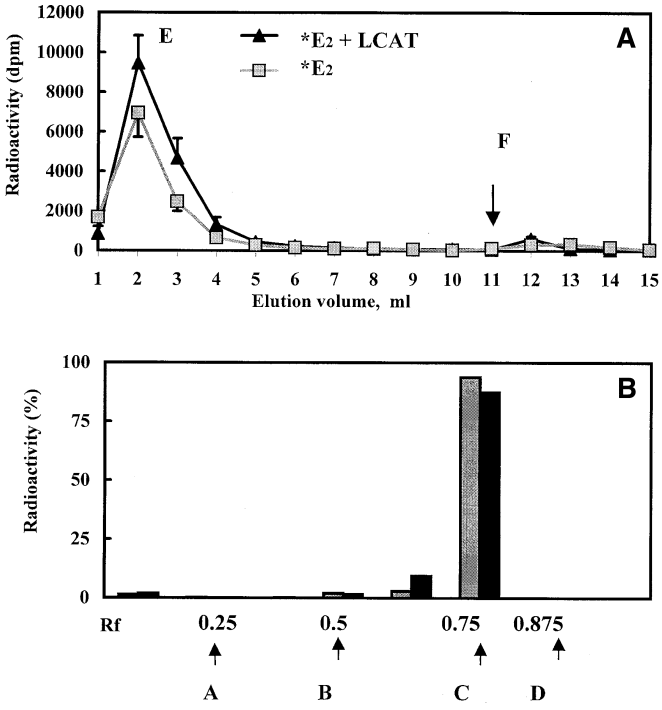

Fig. 2. Characterization of radioactivity associated with HDL. A: Elution pattern of HDL-associated radioactivity after chromatography on Sephadex LH-20. After incubation of HDL with [^{3}H]17β-E_2 in the presence (closed triangle, n = 6) or absence (closed square, n = 6) of purified LCAT and following purification by gel filtration on Sephadex G25, the protein containing fractions were pooled and extracted with ethyl acetate-diethylether (1:1, v/v) followed by evaporation to dryness under N_2. The dry residues were dissolved in hexane-chloroform (1:1, v/v) and chromatographed on a Sephadex LH-20 column using the same eluting solvent. B: Analysis of estradiol derivatives by TLC. Samples obtained from the "ester" fractions after chromatography on a Sephadex LH-20 were applied on a TLC plate in hexane-ethyl ether (3:7, v/v). Free 17β-E_2 (A), 17β-E_2 3-stearate (B), 17β-E_2 17-oleate (C), and 17β-E2 3,17-dioleate (D) were used as standards. R_f values of the standards are indicated by arrows. Samples were originally incubated in the presence (gray bar) or in the absence (black bar) of exogenous LCAT. Equal amounts of radioactivity obtained from the estradiol ester fractions after Sephadex LH 20 were applied on the plate. After development, TLC plates were dried, and the location of the standards was determined by visualization under UV light after rhodamine staining. The lane was divided into 1-cm strips which were scraped, and the material was transferred to bottles for liquid scintillation counting. E: Ester fraction, eluted with hexane-chloroform (1:1, v/v). F, Free fraction eluted with methanol (arrow). Vertical bars indicate mean ± SEM.

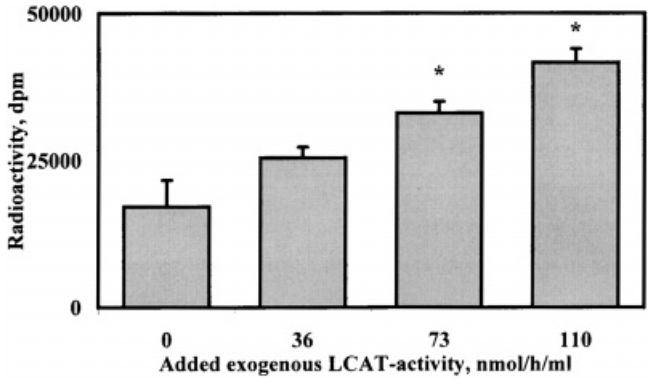

Fig. 3. Effect of exogenous, purified LCAT on 17β-estradiol esterification in HDL. [^{3}H]17β estradiol was incubated with HDL (2 mg protein) in the presence of increasing amounts of LCAT (36, 73, and 110 nmol/h/ml), and the labeled reaction products were isolated by gel filtration on Sephadex G25 as described under Materials and Methods. The protein-containing fractions were pooled together and their radioactivity was counted. Asterisks indicate significant differences versus baseline (0 = no addition of exogenous LCAT; $P < 0.05$), using ANOVA followed by pairwise comparison by *t*-test with Bonferroni correction. Vertical bars indicate mean ± SEM.

tion. The results indicated, as expected, that no conversion of E_2 into estrone or estriol had occurred (data not shown).

Incubation of [^{3}H]17β-E_2 with LDL, HDL_2, HDL_3, and rHDL

When experiments identical with those carried out with total HDL were performed using HDL subfractions, HDL_2 and HDL_3, as well as rHDL and LDL as substrates, a significant peak of HDL_3-associated radioactivity (**Fig. 4C**), as well as rHDL-associated radioactivity, (Fig. 4D), was recovered in the void volume after gel filtration on Sephadex G25. Only a trace amount of radioactivity, however, was attached to HDL_2 subfraction (Fig. 4B) and LDL (Fig. 4A). Further chromatography on Sephadex LH20 indicated that the radioactivity attached to HDL_3 and rHDL represented esterified [^{3}H]17β-E_2 when exogenous LCAT was added (data not shown). Presence of label in the HDL_3-fraction without addition of exogenous LCAT was due to endogenous HDL_3-associated LCAT activity, which ranged between 22 and 50 nmol/h/ml. When several batches of LDL and HDL_2 were analyzed for endogenous LCAT activity, the values were very low, ranging between 0.1 and 1.2 nmol/h/ml for LDL and between 0 and 4.2 nmol/h/ml for HDL_2. Addition of purified LCAT to the incubations enhanced the formation and incorporation of 17β-E_2 17-esters in the presence of HDL_3 ($P = 0.07$) and rHDL ($P < 0.05$). Addition of LCAT to HDL_2 or LDL in some experiments caused only a small increase in the radioactivity associated with these lipoproteins ($P < 0.00001$, HDL_2 vs. HDL_3 in the absence of exogenous LCAT; $P < 0.002$, HDL_2 vs. HDL_3 in the presence of exogenous LCAT; $P < 0.0005$, LDL vs. HDL_3 in the absence of exogenous LCAT; $P < 0.003$, LDL vs. HDL_3 in the presence of exogenous LCAT). This data suggests the importance of HDL particle composition and structure in the regulation of E_2 esterification by LCAT.

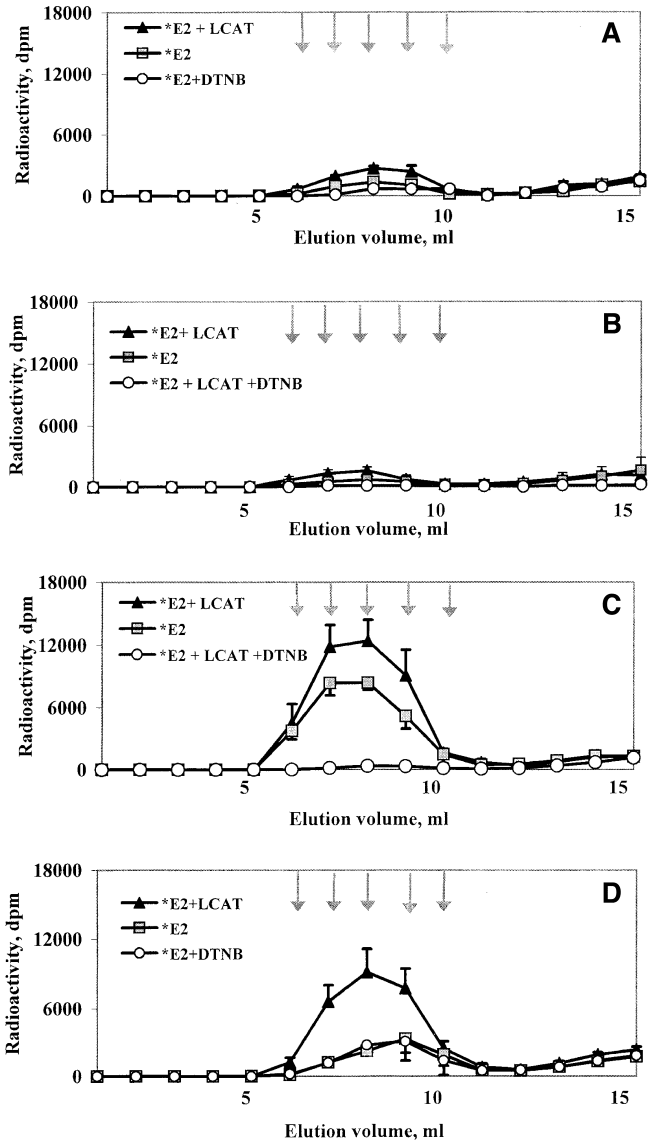

Fig. 4. Elution patterns of LDL, HDL_2, HDL_3, and rHDL-associated radioactivity after gel filtration on Sephadex G25. Ultracentrifugally isolated LDL (n = 3), HDL_2 (n = 4), and HDL_3 (n = 4) prepared from human plasma as well as rHDL (n = 7) were incubated with [^{3}H]17β-estradiol (17β-E2) (1.3 mg protein in 3 ml of Tris-HCl-buffer, pH 7.4, total radioactivity 200,000 dpm) in the presence (closed triangle) or in the absence (closed square) of exogenous LCAT (10–73 nmol/h/ml). The final total LCAT activities (i.e., exogenous + endogenous) in LDL, HDL_2, and rHDL incubations were similar, but the total LCAT activity in HDL_3 was ~1.1 to 1.4-fold compared with other lipoprotein subgroups because of the higher amount of endogenous LCAT. Incubations with DTNB (open circle) (final concentration 3 mM) are also shown. After incubation, lipoproteins were purified by gel filtration on a Sephadex G25 column to remove small-molecular-weight molecules not associated with lipoproteins. A: Incubation with LDL ($P < 0.0005$, LDL vs. HDL_3 in the absence of exogenous LCAT; $P < 0.003$, LDL vs. HDL_3 in the presence of exogenous LCAT). B: Incubation with HDL_2 ($P < 0.00001$, HDL_2 vs. HDL_3 in the absence of exogenous LCAT; $P < 0.002$, HDL_2 vs. HDL_3 in the presence of exogenous LCAT). C: Incubation with HDL_3 ($P = 0.07$, HDL_3+LCAT vs. HDL_3−LCAT). D: Incubation with rHDL ($P < 0.05$, rHDL+LCAT vs. rHDL−LCAT). Vertical bars indicate mean ± SEM.

DISCUSSION

In a recent study (21), we demonstrated that incubation of labeled 17β-estradiol (E_2) with human plasma E_2 generated 17-fatty acid esters which were incorporated in LDL and HDL particles. Further experiments in vitro indicated that these lipophilic E_2 17-fatty acid esters were transported from HDL to LDL in a process at least partially catalyzed by CETP. The present study was designed to clarify the specific roles of the HDL_2 and HDL_3 subfractions in the formation of E_2 17-fatty acid esters. Experiments using total HDL demonstrated a clear-cut dose-response when increasing amounts of LCAT were added to the incubations, and stepwise increases in E_2 17-fatty acid ester formations were observed. Separate incubations with HDL subfractions indicated that E_2 17-ester formation occurred almost exclusively in the small HDL_3 subfraction. Furthermore, in our preliminary experiments 17β-estradiol 17-ester formation was clearly enhanced when using apoA-I-egg phosphatidylcholine-cholesterol rHDL discs.

Current data available do not allow us to make detailed conclusions concerning the mechanism underlying the preferred role of HDL_3 and apoA-I-PC-chol discs as a substrate for LCAT-mediated formation of E_2 17-esters. However, certain important conclusions can be drawn from those results concerning the substrate specificity for LCAT-facilitated cholesterol esterification. Activation of LCAT by apoA-I, the major apolipoprotein component of HDL, has been clearly demonstrated and has been shown to depend directly upon the binding of apoA-I to the lipid surface (30, 31). The domain extending from 143 to 208 of the mature apoA-I protein seems to play a critical role in the overall LCAT reaction (32). The most important factor, apparently, is the nature of the HDL particle substrate, i.e., the detailed physicochemical characteristics of the particle surface where the interaction of LCAT with substrate takes place (33). It has been demonstrated that small HDL particles (HDL_{3bc}) as well as apoA-I-phospholipid discs provide the most active surface configuration for LCAT reaction (34–39), whereas large HDL (HDL_{2b}) particles may inhibit the enzymatic reaction (40, 41). It appears that the rate of estradiol esterification by LCAT is influenced by HDL surface configuration in the same way as cholesterol esterification.

It is evident that LCAT facilitates the esterification of 17β-estradiol and that the magnitude/rate of esterification is dependent on the LCAT substrate used. From a mechanistic point of view, it is interesting that LCAT, which normally esterifies the 3β-OH group of cholesterol and several hydroxysteroids, is able to facilitate the fatty acylation of the 17β-OH group of estradiol. The specificity of the LCAT enzyme toward various acyl acceptors has not been intensively investigated. However, it has been shown that β-sitosterol, cholestanol, and desmosterol are esterified in human plasma, which indicates that sterol molecules other than cholesterol can also function as the fatty acyl group acceptors (42, 43). The findings that several steroids that lack the side chain function as a substrate like cholesterol (44, 45) are interesting because 17β-estradiol also lacks the carbon side chain. This suggests that the hydrophobic side chain of sterols at carbon-17 is not a prerequisite for the acylation of the 3β-hydroxyl group. In the case of 17β-estradiol, the A-ring is aromatic, and the hydroxyl group at position C-3 is phenolic. This group is not fatty acylated because LCAT requires that the sterol acyl acceptor has a 3β-configuration for the hydroxyl group (37). TLC analysis in the present study clearly demonstrated that LCAT facilitated acylation of the 17β-OH group. Recently, Szedlacsek and co-workers (46) investigated the role of LCAT in forming fatty acid esters of oxysterols using discoidal bilayer particles. They discovered that following esterification of the preferred site (3β-hydroxyl group), the 27-hydroxyl group was esterified by LCAT in vitro. The data also suggested that the esterification at this less common site was dependent on particle size, i.e., the physical characteristics of the lipoprotein particle surface appeared to influence the LCAT reaction. Our present data suggests that although the 17β-OH group of estradiol is located at the other end of the molecule opposite the 3-OH group, it is well oriented at the enzyme active site, and the fatty acyl-group can be transferred from the acyl-enzyme intermediate. This is in line with the findings of Kanji et al. (22).

In summary, our results suggested that the smaller, less mature HDL_3 particles contributed almost all of the 17β-E_2 esterification both in the presence of only endogenous LCAT as well as after addition of exogenous LCAT, whereas HDL_2 exhibited only traces of esterification activity. Our previous finding indicated that esterified E_2 was transferred from HDL to LDL in a CETP-facilitated process (21). Our current results are compatible with the concept that, in vivo, this transfer may occur from HDL_3 directly. Another possibility is that the 17β-E_2 17-esters are retained in the HDL density range during the LCAT-facilitated maturation of HDL_3 to HDL_2 particles, and they are then transferred from HDL_2 to LDL mediated by CETP. Either way, LDL would receive powerful lipophilic antioxidant molecules which would, in theory, increase the oxidation resistance of LDL particles. This is supported by the reports of Shwaery et al. (47), indicating that incubation of physiologically relevant concentrations of 17β-E_2 with male plasma caused a significant prolongation of lag times in an in vitro LDL oxidation system. In theory, antioxidant protection of LDL would remain effective even if the particles would have penetrated the vascular endothelium and become sequestered from the water-soluble antioxidants in plasma. This would ultimately increase the subendothelial antioxidant potential and decrease foam cell formation.

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