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HDL remodeling during the acute phase response

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Abstract

Objective— To study the interactive action of serum amyloid A (SAA), group IIA secretory phospholipase A_2 (sPLA$_2$-IIA) and cholesteryl ester transfer protein (CETP) on HDL remodeling and cholesterol efflux during the acute phase (AP) response elicited in humans following cardiac surgery.

Methods and Results—Plasma was collected from patients prior to (pre-AP), 24 hours after (AP-1d) and 5 days after cardiac surgery (AP- 5d). SAA levels were increased 16-fold in AP- 1d samples. The activity of sPLA$_2$-IIA was increased from 77.7 ± 38.3 U/ml (pre-AP) to 281.4 ± 57.1 U/ml (AP-1d; $p < 0.001$). CETP mass and activity reduction was commensurate to the reduction of HDL cholesterol levels. The combined action of SAA, sPLA$_2$-IIA and CETP *in vitro* markedly remodeled HDL with the generation of lipid-poor apoA-I from both pre-AP and AP- 1d HDL. The net result of this remodeling was a relative preservation of ABCA1 and ABCG1-dependent cholesterol efflux during the acute phase response.

Conclusions—Our results show that the many and complex changes in plasma proteins during the acute phase response markedly remodel HDL with functional implications, particularly the relative retention of cholesterol efflux capacity.

Keywords

SAA; HDL; CETP; apoA-I; inflammation

Inflammation induces major changes in HDL levels and composition. Mediators of inflammation such as TNF-α and IL-6 induce expression of serum amyloid A[1] and group IIA secretory phospholipase A_2 (sPLA$_2$-IIA)[2] which dramatically alter HDL apolipoprotein content and levels respectively. Acute phase SAA in the plasma is associated with HDL, where it can comprise the major apolipoprotein[3]. The increase in sPLA$_2$-IIA activity results in

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hydrolysis of HDL surface phospholipids and a decrease in HDL particle size[4]. The plasma cholesteryl ester transfer protein (CETP) is an integral component of reverse cholesterol transport and regulates HDL cholesterol concentrations. By promoting the transfer of cholesteryl esters (CE) from HDL to apoB-containing lipoprotein particles, HDL-derived CE is taken up via the LDL receptor and cleared by the liver[5]. An additional result of CETP action is the generation of lipid-poor apoA-I[6], a key acceptor in ATP-binding cassette transporter AI (ABCA1)-mediated lipid efflux[7]. The presence of SAA on HDL holds the potential to impact both the CE transfer and the apoA-I liberating ability of CETP. $sPLA_2$-IIA could also impact the latter action of CETP as apoA-I was shown to dissociate more readily from CETP-remodeled reconstituted HDL after hydrolysis by bee venom phospholipase A_2[8].

Given the centrality of inflammation in atherogenesis, there is a paucity of information regarding CETP function when acute phase HDL is the "substrate". In the present study, we used plasma from patients undergoing cardiac surgery with cardiopulmonary bypass as a "standardized" insult where the oxygenator membrane activates macrophages to produce cytokines[9]. We characterized the SAA-containing AP HDL during the acute phase to define the polydisperse HDL "substrate" that CETP would encounter. We further investigated CETP function in the acute phase, particularly as it relates to the presence of SAA and $sPLA_2$ on AP HDL, with respect to its CE transfer and apoA-I liberating functions.

Teleologically, the dramatic changes in HDL composition and metabolism during inflammation must serve a short term purpose to allow the organism to survive a noxious assault. Acute tissue injury results in cell death with large quantities of cell membranes rich in phospholipids and cholesterol generated. Macrophages are mobilized to such sites, ingest these fragments and acquire considerable lipid load [10]. We thus examined the influence of the AP response on the ability of serum to promote cholesterol efflux as a removal mechanism to mobilize this cholesterol in an ABCA1 and ABCG1 dependent manner.

Methods

Human subjects

Patients undergoing cardiac surgery donated plasma prior to (pre-acute phase, pre-AP), 24 hr post-operatively (acute phase, AP- 1d), and at discharge, 5 days after surgery (AP- 5d) as outlined in the online data supplement. This study was approved by the University of Kentucky Medical Institutional Review Board (IRB). For the full descriptions of the methods used, please see the supplemental materials.

Statistical analyses

Data are presented as mean ± SEM. Differences between pre-AP and AP parameters were tested by paired t-test (SigmaStat 3.5). Statistical analyses between pre-AP, AP- 1d and AP-5d were performed using repeated measures one-way ANOVA with the Holm-Sidak multiple comparisons test. Significance was set at $p < 0.05$. A Wilcoxon signed rank test was used for post-test of CETP mass. The power in all tests was > 0.9.

Results

SAA, $sPLA_2$, CETP and HDL in Acute Phase (AP) plasma

As expected, plasma SAA levels increased from pre-AP levels of 47.2 ± 19.9 μg/ml to 785.6 ± 66.4 μg/ml in AP- 1d samples ($p < 0.05$) and were still elevated at discharge (AP- 5d; 567.2 ± 50.2 μg/ml; $p < 0.05$; Fig 1A). The activity of $sPLA_2$-IIA followed a similar pattern: 77.7 ± 38.3 U/ml (pre-AP), 281.4 ± 57.1 U/ml (AP- 1d) and 250.5 ± 43 U/ml (AP- 5d) ($p < 0.05$; Fig 1B). Quantitative immunoblot analysis showed a 2.9 fold reduction in CETP mass from 2.6 ±

0.6 μg/ml in pre-AP plasma to 0.9 ± 0.2 μg/ml in AP- 1d plasma, (Fig 2A $p < 0.01$). Consistent with this, CETP activity was 2.2-fold lower in AP compared to pre-AP plasma (16.9 ± 3.0 nmol/ml/h and 37.9 ± 4.1 nmol/ml/h respectively, $p < 0.001$ (Fig 2B)). HDL-C levels were reduced 1.9 fold, from 372.2 ± 44.6 μg/ml to 195.0 ± 22.2 μg/ml ($p < 0.001$; Fig 2C) and apoA-I dropped from 1590.0 ± 84.2 μg/ml to 771.4 ± 138.7 μg/ml ($p < 0.001$; Fig 2D). The decrease in CETP activity was commensurate with the reduction in HDL-C and apoA-I, hence the activity of CETP normalized to HDL-C or apoA-I levels was not different between pre-AP and AP plasma (not shown).

Characterization of pre-AP and AP HDL by immunoaffinity chromatography

It was reported that the presence of apoA-II on reconstituted HDL particles inhibits the CETP-mediated dissociation of apoA-I[11]. In order to assess the influence of SAA, we studied pre-AP and AP- 1d HDL as well as HDL_2 enriched *in vitro* with SAA (SAA-HDL). We subjected ^{125}I-HDL to immunoaffinity chromatography to determine the proportion of the LpAI fraction (ie HDL particles lacking both apoA-II and SAA, containing only apoA-I) in AP HDL. Fig 3A is a Coomassie stained SDS-PAGE gel showing the major apolipoproteins present in these HDL. Whereas SAA is virtually undetectable in pre-AP HDL, it is a major component of AP- 1d HDL (27.2% of total protein by mass). In SAA-HDL, SAA comprised a major apolipoprotein. Fig 3B shows the autoradiograph (ARG) of the SDS-PAGE analysis of the fractions when pre-AP HDL was passed through an anti-apoA-II column so that the FT comprises only LpAI particles. ApoA-II containing particles were eluted from the column with sequential chaotropic washes (E1 and E2). In pre-AP HDL, 84% of the total apoA-I counts were in LpAI particles, indicating abundant CETP substrate.

When AP- 1d HDL was passed through an anti-SAA column, 20% of the total apoA-I counts and approximately 5% of the apoA-II counts were in the FT, the remainder associated with SAA-containing particles (Fig 3C). Thus, 80% of apoA-I in AP HDL was present on particles that also contained SAA or both SAA and apoA-II. This indicates that less than 20% of apoA-I in AP HDL is present on LpA-I particles that contain neither apoA-II nor SAA. Specificity of the column was verified with pre-AP HDL passed through the same column. This resulted in 97% of the counts being retrieved in the FT indicating negligible non-specific binding.

In the case of AP HDL passed through an anti-apoA-II column (Fig 3D), 66% of the apoA-I counts and 52% of the SAA counts in AP HDL were in the FT fraction. This indicated that SAA was distributed relatively equally between particles that contain only apoA-I and those that contain both apoA-I and apoA-II. The majority of apoA-I in AP HDL (66%) was present in particles containing no apoA-II, as in the case of pre-AP HDL. However, the majority of such particles contained additional SAA (Fig 3C&D) reducing the LpAI fraction in AP HDL.

Comparative displacement of apoA-I in pre-AP and AP HDL by CETP

Since ultracentrifugation displaces CETP from HDL,[12] recombinant CETP was exogenously added in the remodeling experiments. The CETP activity in the incubations corresponded to the activity of CETP in normal human plasma. When the total pre-AP and AP HDL were incubated with CETP *in vitro* for 24 hr, a dose-dependent dissociation of apoA-I was observed (Fig 4A). The dissociation of apoA-I from pre-AP and AP HDL was comparable. Although it appears that 50% or more of the apoA-I is in the lipid-poor state, the enhanced immunoreactivity of dissociated apoA-I precludes exact quantification. In addition to generating lipid-poor apoA-I, CETP action also resulted in the formation of larger HDL particles which are likely TG-enriched due to CE/TG exchange. CETP action on AP HDL liberated a very limited amount of “lipid-poor” SAA (Fig 4B). Recombinant SAA exhibited slower mobility than apoA-I on gradient gels, likely due to increased aggregation under the

non-denaturing conditions (Fig 4B). SAA is dispersed on a broader spectrum of particle sizes, some likely containing little apoA-I (compare Fig 4A lane 8 and Fig 4B lane 4).

Comparative displacement of apoA-I in pre-AP and AP HDL by concomitant action of CETP and $sPLA_2$-IIA

During the acute phase, dissociation of apoA-I from HDL could be influenced by acute phase secretory phospholipase (ie $sPLA_2$-IIA) which is present in plasma and associates with HDL[13]. We thus used SAA-HDL (Fig. 1A) to study the remodeling of HDL by CETP and $sPLA_2$-IIA. Treatment of SAA-HDL with CETP alone resulted in the generation of comparable amounts of lipid-poor apoA-I (Fig 5B, lane 3) as observed with control HDL_2 (Fig 5A, lane 3). CETP treatment did not result in the displacement of significant amounts of lipid-poor SAA from SAA-HDL_2 (Fig 5C, lane 3). Surface hydrolysis of HDL phospholipids by $sPLA_2$ action alone resulted in a reduction in HDL particle size not accompanied by the dissociation of apoA-I or SAA (Fig 5A, B, C- lane 2). However the combined action of $sPLA_2$ and CETP converted the majority of the apoA-I from the HDL-bound to the lipid-poor form in both HDL_2 and SAA-HDL (Fig 5A & B-lane 4). This combined action may also result in the dissociation of limited amounts of lipid-poor SAA from SAA-HDL(Fig 5C- lane 4). We conclude that CETP action on the core, and $sPLA_2$-IIA hydrolysis on the HDL surface, synergize to liberate lipid-poor apoA-I.

ABCA1 and ABCG1-dependent cholesterol efflux

To test the effects of the acute phase response on HDL function, efflux assays were carried out using pre-AP, AP- 1d and AP- 5d serum diluted to 2.5%. When compared to pre-AP serum, ABCA1-dependent efflux was not significantly decreased at AP- 1d despite a highly significant 53% fall in plasma apoA-I concentrations (Table 1). At AP- 5d, ABCA1 efflux was still maintained despite apoA-I remaining 1.5 fold lower than pre-AP levels. Similarly, ABCG1 efflux was modestly reduced at AP- 1d whilst HDL-C decreased by 48.1%. When efflux was normalized to HDL-C concentrations, there was a doubling in the ABCG1-dependent cholesterol efflux efficiency of AP- 1d serum compared to pre-AP serum, and this was maintained at AP-5d. Thus cholesterol efflux appears to be relatively well maintained in the acute phase response despite large reductions in HDL-C and apoA-I.

Discussion

Data presented here indicate the following. (i) The reduction in CETP concentration and activity during the acute phase response is commensurate with the reduction in HDL levels. (ii) Despite the striking alterations in HDL composition during the acute phase, CETP activity was maintained both with respect to its cholesteryl ester transfer function and its capacity to liberate apoA-I. (iii) $sPLA_2$-IIA enhances CETP's ability to liberate apoA-I and this is not impaired by the presence of SAA on the HDL. (iv) Although SAA has been shown to be an effective acceptor in lipid efflux, CETP action liberates it to a limited extent in a lipid-poor form. (v) The interplay between the numerous acute phase proteins impacting HDL remodeling result in the relative preservation of ABCA1 and ABCG1- dependent cholesterol efflux.

There is an inverse relationship between CETP activity and HDL concentrations in the normal state[14]. This was the basis for developing CETP inhibitors to increase HDL levels for potential therapeutic benefit. During inflammation this inverse relationship does not hold as both CETP and HDL levels are reduced. Studies have shown that inflammatory cytokines reduce CETP transcription and levels[15,16]. This could result in increased HDL levels. However our data shows a commensurate decrease in HDL and CETP in the acute phase. This suggests that during inflammation factors operate to reduce HDL despite the normal tendency of CETP to increase HDL. A number of mechanisms could operate to decrease plasma HDL during the acute phase.

It was originally assumed that SAA enrichment of HDL was the basis for decreased apoA-I and HDL levels. However, this is unlikely since the decrease in plasma HDL during inflammation occurs rapidly, before SAA accumulation[17]. An alternative explanation for decreased HDL levels during inflammation could be remodeling by acute phase group II phospholipases, particularly $sPLA_2$-IIA that increase HDL catabolism[18, 19]. SAA itself can also enhance the activity of $sPLA_2$[20]. The proinflammatory cytokines that induce SAA and $sPLA_2$-IIA, simultaneously decrease apoA-I expression[21]. Thus the decreased levels of apoA-I and increased levels of SAA on HDL during inflammation are due at least in part to reciprocal coordinated regulation. Finally, the combined remodeling action of $sPLA_2$-IIA and CETP during the acute phase response (Fig. 5) may also result in the increased catabolism of HDL. Given the numerous factors that operate during inflammation it is notable that the ratio of CETP to HDL is maintained. CETP reduction during inflammation could be viewed as a defensive adaptation to prevent “excessive” HDL reduction mediated by the mechanisms discussed.

It was reported that apoA-II abrogates the CETP-mediated liberation of apoA-I from reconstituted HDL containing both apoA-I and apoA-II[11]. In pre-AP HDL, we demonstrated that approximately 80% of apoA-I is present in LpAI particles (Fig. 3B). Lipid-poor apoA-I dissociating from pre-AP HDL in our study is thus likely derived from these LpAI particles. In contrast, in AP HDL, less than 20% of the apoA-I was present as LpAI, the remainder being associated with SAA and/or apoA-II (Fig. 3C). However, CETP action resulted in the liberation of the bulk of apoA-I from AP HDL. This establishes that SAA, in contrast to apoA-II, does not interfere with the liberation of apoA-I induced by CETP remodeling.

Our data indicate that SAA is present on most AP HDL particles (Fig. 3C). Unlike SAA, CETP is present as a dynamic exchange protein rather than a structural protein and undergoes rapid bidirectional transfer between HDL particles and acceptors. At any given time, only ~1 in 1,000 HDL particles carry a CETP molecule. The fact that the activity of CETP when normalized to HDL-C is unaltered during the acute phase suggests that CETP dynamics are also unchanged during the APR. Our results strongly suggest that any decrease in total cholesteryl ester transfer from HDL during the acute phase is the result of the concomitant reduction of plasma CETP and HDL-C rather than the reduced functionality of the HDL/CETP interaction.

It is notable that CETP action results in an apparent increase in size of the HDL particles (Fig 4 and 5). In addition to liberating apoA-I, CETP-mediated remodeling has been reported to result in particle fusion[22]. The increased particle size of CETP-remodeled HDL may also relate to the exchange of CE for TG by CETP action. As TG molecules are larger than CE molecules, HDL size would increase as TG content increases[23]. CETP action on HDL results in core/surface disequilibrium that is alleviated by the dissociation of lipid-poor apoAI[24]. Notably, in our study, CETP-remodeling of HDL resulted in both an increase in size of the particles, as well as the dissociation of lipid-poor apoA-I. Increased $sPLA_2$-IIA activity during the acute-phase response may further potentiate the generation of lipid-poor apoA-I.

The increased $sPLA_2$ activity in acute phase plasma (Fig 1B) is likely due to an increase in $sPLA_2$-IIA[25]. $sPLA_2$-IIA was shown to be present in atherosclerotic plaques bound to heparan-sulfate proteoglycans of the subendothelial extracellular matrix[2]. Proteoglycan binding of $sPLA_2$-IIA serves to “concentrate” the enzyme and it is more active in the bound form[26]. Thus the effect of $sPLA_2$-IIA on HDL remodeling may be more pronounced in the intima of a vessel. Furthermore, $sPLA_2$ is more active when SAA is present on HDL.[20] It may also be relevant that like $sPLA_2$, SAA is also bound by proteoglycans in lesions[27]. Although efflux of cholesterol from macrophages at this site represents only a small fraction of overall cellular cholesterol efflux, it is critically protective in the context of atherosclerosis[7]. The interaction of $sPLA_2$ and SAA might constitute a defensive mechanism against lipid accumulation. SAA-HDL is particularly enriched in SAA compared to AP HDL, with SAA present on 80% of

particles[3]. In our study, CETP action on SAA-HDL liberated significant amounts of lipid-poor apoA-I. This confirms that the presence of SAA does not impair the dissociation of apoA-I. In our study, there was no clear evidence that SAA was significantly displaced in a lipid-poor form by either CETP or $sPLA_2$-IIA (Figs. 4 & 5), though one has to recognize the limitations of analyzing SAA on non-denaturing gels as its tendency to self-aggregate and associate with the acrylamide matrix is well established.

Given the extensive remodeling of HDL during the acute phase that affects both the polydisperse particles themselves as well as the equilibrium between bound and free apolipoproteins, we evaluated the integral of all these actions on ABCA1 and ABCG1-dependent cholesterol efflux. We show an overall preservation of cholesterol efflux capacity of serum during the acute phase response. A large body of evidence suggested that HDL is part of the innate immune system[28], and that acute phase HDL remodeling could impact the unique cargo of proteins on HDL[29] reducing its anti-inflammatory functions. For example, the incorporation of SAA and $sPLA_2$-IIA onto AP HDL particles results in the loss of paraoxonase activity of HDL[30]. During acute injury when macrophages accumulate lipid, relative preservation of efflux mechanisms could be more beneficial than the impairment of the anti-inflammatory properties of HDL. However during chronic inflammatory conditions, the latter might constitute a much more important risk factor for atherogenesis.

Inhibition of CETP results in elevated HDL levels[14], but this could abrogate its two anti-atherogenic functions. The recent cessation of a human clinical trial testing such an inhibitor[31] illustrates the need to better understand CETP function, not only on circulating lipoproteins, but also on apoA-I liberation at the level of the atherosclerotic lesion in the vessel wall. One can imagine a scenario where CETP inhibition could increase plasma HDL but also alter the equilibrium between HDL and lipid-poor apoA-I and consequently efflux potential. Our results indicate that AP HDL is not impaired in its ability to liberate apoA-I following CETP remodeling. This combined with the potentiation of apoA-I release by the combined action of CETP and the acute phase $sPLA_2$-IIA, supports our results showing that during the acute phase, cholesterol effluxing capacity may be preserved despite a reduction of plasma HDL. However, prolonged inflammation and continual HDL remodeling may eventually lead to pro-atherogenic conditions by limiting the levels of HDL and apolipoprotein cholesterol acceptors and the pro-inflammatory nature of AP HDL having an impact.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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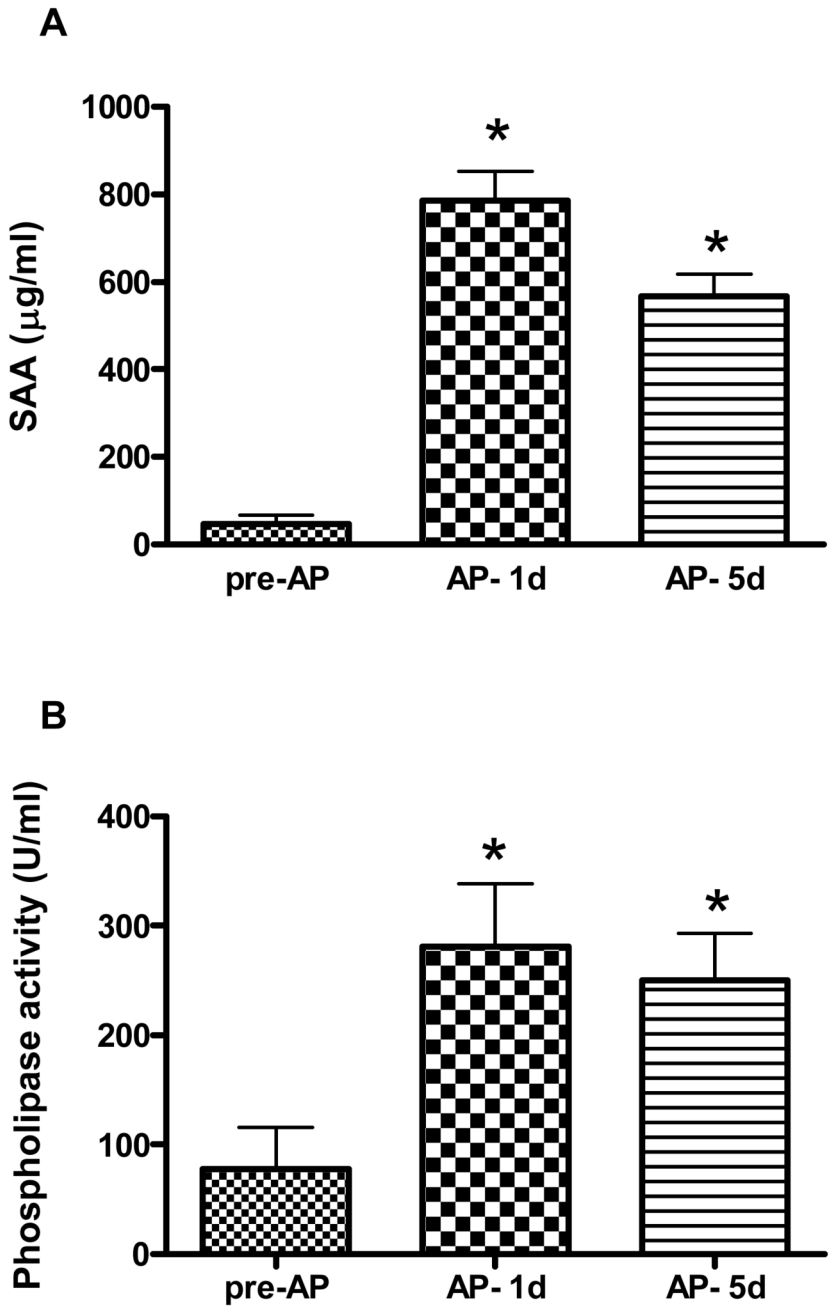

Figure 1.
SAA (A) and $sPLA_2$ (B) concentrations in pre-AP, AP- 1d and AP- 5d plasma. Data is presented as mean ± SEM. n=12 (SAA); n=6 ($sPLA_2$); * $p < 0.05$ versus pre-AP by one way repeated measures ANOVA.

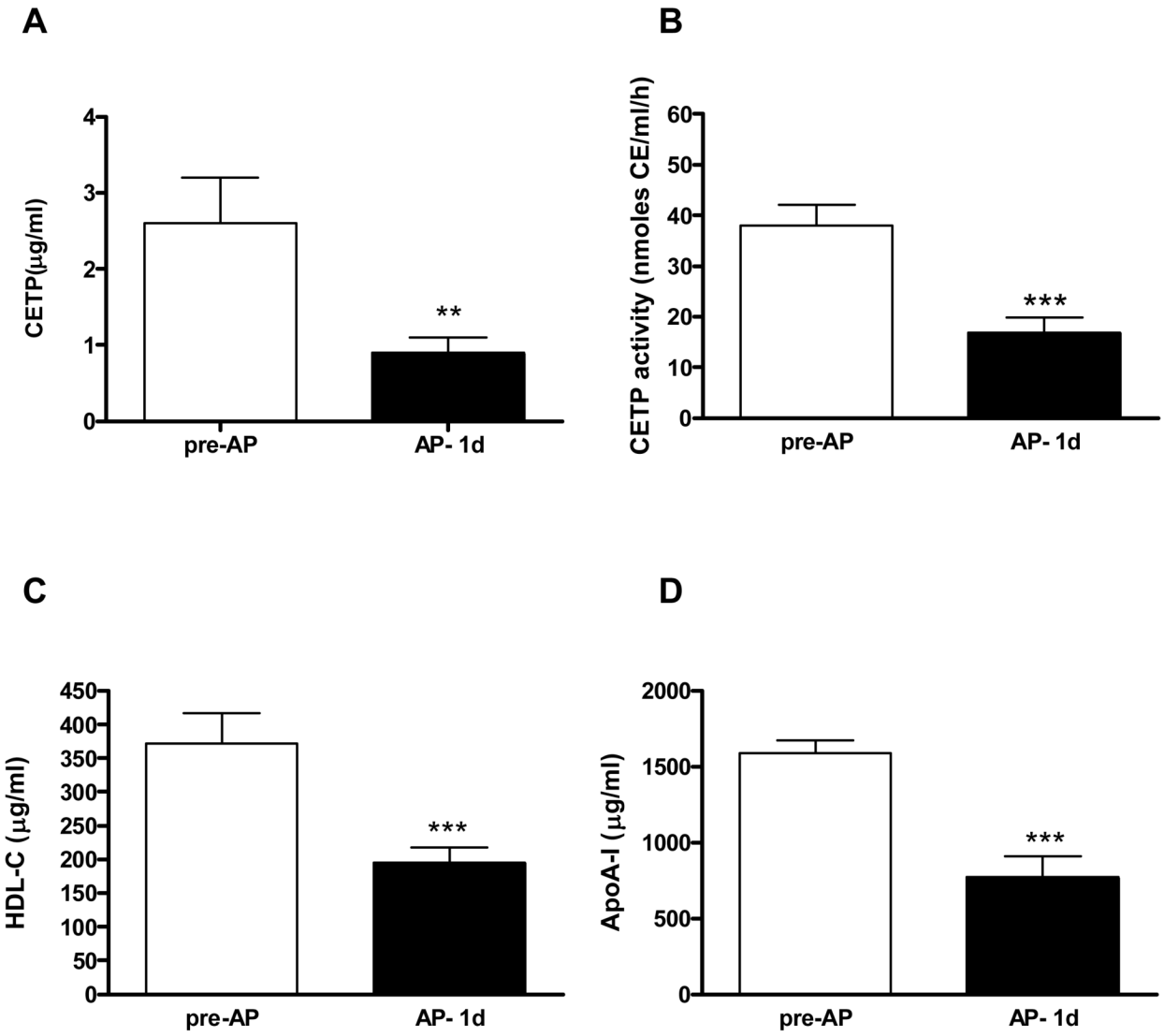

Figure 2.

CETP, HDL cholesterol and apoA-I are reduced in AP- 1d plasma. (A) Plasma CETP concentrations were quantified by densitometric analysis of Western blots. (B) CETP activity (C) HDL-C concentrations and (D) apoA-I concentrations in pre-AP and AP- 1d plasma. ** $p < 0.01$, *** $p < 0.001$ by paired t-test.

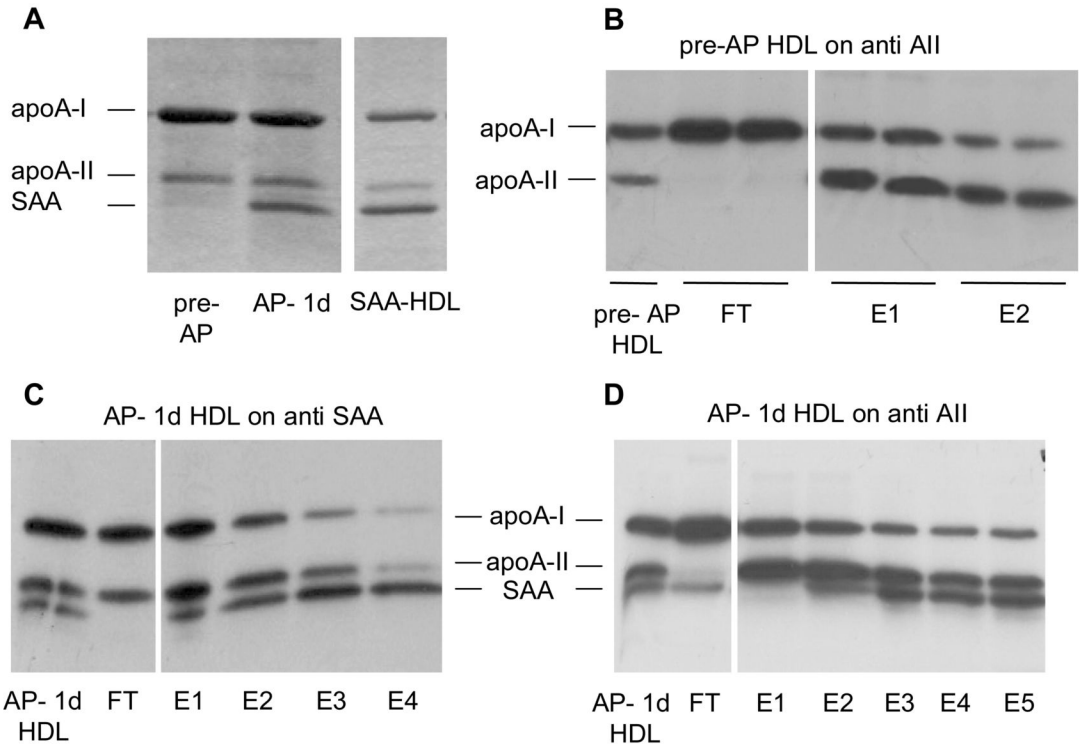

Figure 3.

Apolipoprotein characterization of pre-AP HDL, AP- 1d HDL and SAA-HDL. (A) SDS gel of HDL (5 μg total protein). (B) ^{125}I-pre-AP HDL (5 μg) was passed through an anti-apoA-II immunoaffinity column and fractions were electrophoresed and autoradiographed as outlined in the methods (ARG). (C) ^{125}I-AP HDL (10 μg) was passed through an anti-SAA immunoaffinity column with subsequent electrophoresis and ARG as described in (B). (D) ^{125}I-AP HDL (10 μg) was passed through an anti-apoA-II immunoaffinity column with subsequent electrophoresis and ARG as described in (B). Note: the gels in B–D were loaded on the basis of 2000 cpm per lane and since the majority of counts were present in E1 and E2, E3–E5 quantitatively represent a smaller percentage of total protein mass.

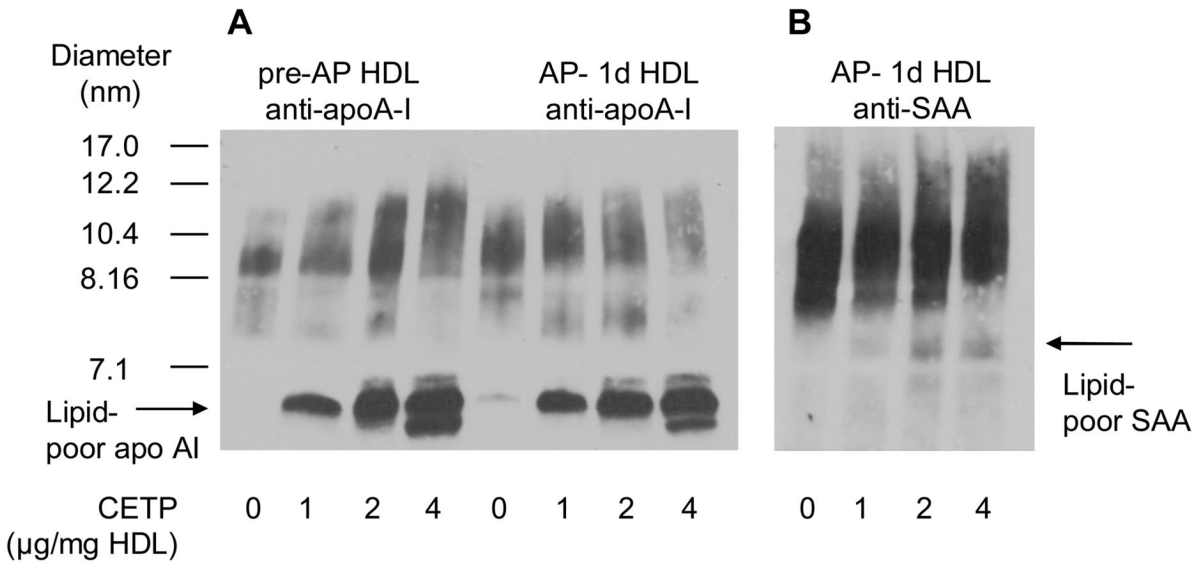

Figure 4.

Dissociation of lipid-poor apoA-I from pre-AP and AP- 1d HDL following remodeling by CETP. HDLs were incubated with CETP in the presence of VLDL for 24 hr at 37°C as outlined in the methods. Reactions were analyzed by Western blot for (A) apoA-I and (B) SAA. The migration of lipid-poor apoA-I and SAA are marked with arrows.

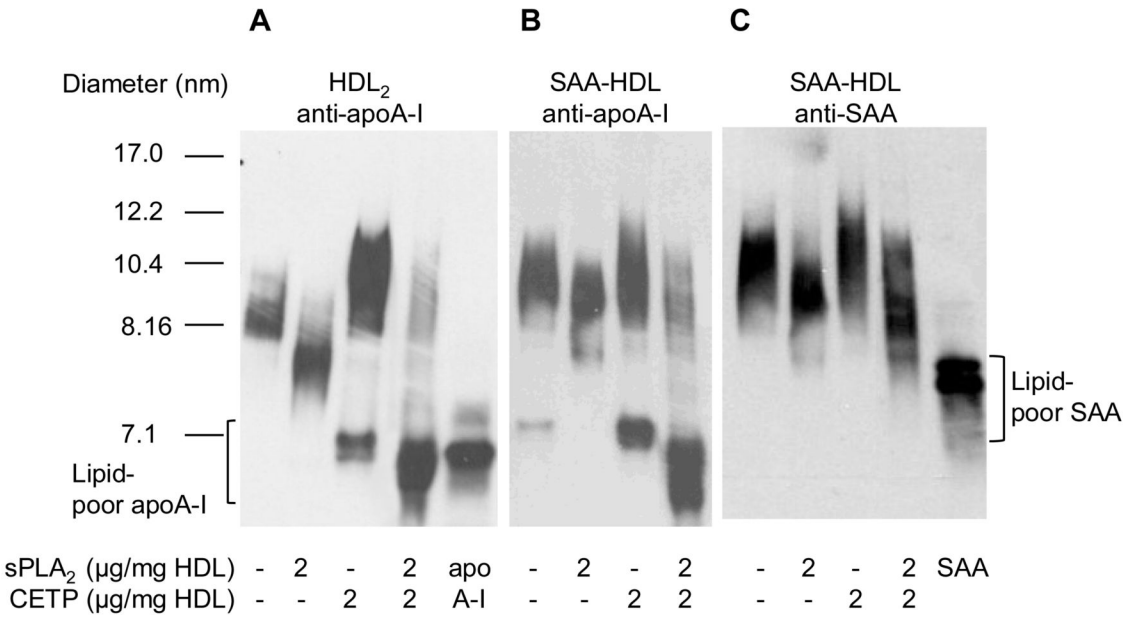

Figure 5.

The combined action of $sPLA_2$-IIA and CETP on HDL_2 and SAA-HDL. HDL were incubated with $sPLA_2$-IIA and CETP as set out in the methods. Reactions were analyzed by Western blot for apoA-I in (A) HDL_2 and (B) SAA-HDL and (C) SAA blot of SAA-HDL.

Table 1

ABCA1- and ABCG1- dependent cholesterol efflux was determined as outlined in the methods. Efflux experiments were performed at 37 °C by incubating cells with serum (diluted to 2.5%) from patients pre-AP, AP1- d and AP- 5d for 16 h in DMEM containing 0.2% BSA. Values represent the average of triplicate determinations of n=6–8 patients. Results are presented as a percentage of pre-AP serum efflux. ApoA-I was measured using an automated turbidimetric immunoassay (Mayo Medical Laboratories, Rochester, MN). HDL was quantitated using a commercial kit.

	pre-AP	AP- 1d	AP- 5d
ABCA1-efflux (% of pre-AP)	100	82.5 ± 3.5	99.2 ± 2.9
ABCG1- efflux (% of pre-AP)	100	70.0 ± 14.3[a]	108.5 ± 16.7
plasma apoA-I (μg/ml)	1161.7 ± 117.6[b]	545.0 ± 62.4	785.0 ± 36.6
plasma HDL-C (μg/ml)	389.7 ± 60.5	196.6 ± 39.1[c]	238.7 ± 27.9

[a] $p<0.05$ vs pre-AP and AP- 5d;

[b] $p<0.001$ vs AP- 1d and AP- 5d;

[c] $p < 0.5$ versus pre-AP.