Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

HHS Public Access
Author manuscript
Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:
Arterioscler Thromb Vasc Biol. 2018 August ; 38(8): 1890–1900. doi:10.1161/ATVBAHA.118.310979.

Serum Amyloid A is an exchangeable apolipoprotein

Patricia G Wilson[1,5,6], **Joel C Thompson**[1,5,6], **Preetha Shridas**[2,5,6], **Patrick J McNamara**[7], **Maria C de Beer**[4,5,6], **Frederick C de Beer**[2,5,6], **Nancy R Webb**[1,3,5,6], and **Lisa R Tannock**[1,2,5,6]

[1]Department of Veterans Affairs, Lexington, KY

[2]Department of Internal Medicine, College of Medicine, University of Kentucky

[3]Department of Pharmacology and Nutritional Sciences, College of Medicine, University of Kentucky

[4]Department of Physiology, College of Medicine, University of Kentucky

[5]Saha Cardiovascular Research Center, College of Medicine, University of Kentucky

[6]Barnstable Brown Diabetes Center, College of Medicine, University of Kentucky

[7]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky

Abstract

Objective—Serum Amyloid A (SAA) is a family of acute phase reactants that have pro-inflammatory and pro-atherogenic activities. SAA is more lipophilic than apoA-I and during an acute phase response <10% of plasma SAA is found lipid-free. In most reports SAA is found exclusively associated with HDL; however, we and others have reported SAA on apoB-containing lipoproteins in both mice and humans. The goal of this study was to determine if SAA is an exchangeable apolipoprotein.

Approach and Results—Delipidated human SAA was incubated with SAA-free human lipoproteins then samples were re-isolated by fast protein liquid chromatography and SAA analyzed by ELISA and immunoblot. Both *in vitro* and *in vivo* we show that SAA associates with any lipoprotein and does not remain in a lipid-free form. Although SAA is preferentially found on HDL, it can exchange between lipoproteins. In the presence of CETP there is greater exchange of SAA between lipoproteins. Subjects with diabetes, but not those with metabolic syndrome, showed altered SAA lipoprotein distribution post-prandially. Proteoglycan-mediated lipoprotein retention is thought to be an underlying mechanism for atherosclerosis development. SAA has a proteoglycan binding domain. Lipoproteins containing SAA had increased proteoglycan binding compared to SAA-free lipoproteins.

Conclusions—Thus, SAA is an exchangeable apolipoprotein and increases apoB-containing lipoproteins' proteoglycan binding. We and others have previously reported the presence of SAA on LDL in individuals with obesity, diabetes, and metabolic syndrome. We propose that the

Corresponding Author: Lisa R Tannock, MD, Department of Veterans Affairs, Department of Internal Medicine, University of Kentucky, 553 Wethington Building, Lexington, KY 40536-0200, Fax: (859) 257-3646, PH: (859) 218-1415, lisa.tannock@uky.edu.

Disclosures: There are no disclosures for any author.

Author Manuscript

presence of SAA on apoB-containing lipoproteins may contribute to cardiovascular disease development in these populations.

Keywords

animal models; HDL; apolipoprotein B; serum amyloid A; inflammation; atherosclerosis

Subject terms

animal models of human disease; biomarkers; inflammation; lipids and cholesterol; diabetes type 2; atherosclerosis

---

Introduction

Serum amyloid A (SAA) is a family of secreted proteins synthesized primarily in the liver. In humans, the SAA family is comprised of two acute phase isoforms (SAA1 and SAA2) as well as the constitutive isoform SAA4. SAA3 is an additional acute phase isoform expressed in mice that shares ~60% homology with SAA1 and SAA2 and is a pseudogene in humans. In healthy individuals SAA concentrations are < 5 mg/L but during an acute phase response SAA can increase up to 1000 mg/L or more for a few days, before rapidly returning to baseline levels. However, in chronic inflammatory states such as obesity, metabolic syndrome, diabetes, and rheumatoid arthritis, persistently and significantly elevated SAA concentrations of 30–100 mg/L or higher are seen. SAA is highly conserved through millions of years of evolution, implying that it must perform key functions affecting survival. SAA is proposed to play a major role in the response to injury and inflammation during the acute phase response by participating in cholesterol flux in injured tissues, recruiting inflammatory cells, and inducing factors that mediate tissue repair (for review see [1]). However, the chronic elevations of SAA now prevalent in modern society likely reflect a maladaptive response and numerous studies are now examining potential roles of SAA in disease pathology. Chronic elevations of SAA are predictive of increased cardiovascular events in humans[2–4]. Moreover, using murine models we and others have demonstrated that over-expression of SAA leads to increases in atherosclerosis development, whereas SAA deficiency results in reduced atherosclerosis[5–7].

A number of pro-atherogenic properties have been attributed to SAA. For example, SAA is chemotactic for neutrophils and monocytes[8]; SAA can stimulate the production of other pro-inflammatory cytokines, such as interleukin (IL)-1β and TNF-α [9] and thus exacerbate ongoing inflammation; SAA can induce the matrix-degrading enzymes matrix metalloproteinases[10] that may lead to the destabilization of an atherosclerotic plaque. Moreover, the presence of SAA on HDL is thought to convert athero-protective HDL to dysfunctional HDL[11].

The “response to retention” model of atherogenesis proposes that proteoglycan-mediated lipoprotein retention in the subendothelium is one of the earliest steps in the initiation of atherosclerosis[12, 13]. A wealth of data now supports this model (for review see[14]). There is accumulating evidence that SAA has pro-atherogenic activities via increasing lipoprotein-proteoglycan interactions. SAA increases vascular biglycan content and increases the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

binding capacity of vascular wall proteoglycans for LDL[15, 16], which could lead to increased LDL retention. SAA itself has proteoglycan binding domains[17, 18], and SAA carried on HDL has been suggested to increase HDL binding to proteoglycans in the vessel wall[19]. Thus, SAA can influence the proteoglycan content of the vasculature, interact directly with proteoglycans, or serve as a bridging molecule aiding in the retention of lipoproteins. Several mechanisms could lead to the presence of SAA in atherosclerotic lesions, including local synthesis of SAA, deposition of circulating SAA in a lipid-poor form, or retention of SAA-containing lipoproteins. Many cell types involved in atherosclerosis have been shown to express SAA, including macrophages, endothelial cells, vascular smooth muscle cells, and adventitial adipocytes, consistent with local production of SAA[20]. Immunohistochemical studies have demonstrated co-localization of SAA with apoE, apoB and apoA-I, and with proteoglycans in atherosclerotic plaques[16, 19, 21, 22], supporting the hypothesis that proteoglycans can mediate the retention of free or lipoprotein-associated SAA.

SAA is a lipophilic apolipoprotein and lipid-free SAA is generally not detected in plasma; our recent data demonstrate that essentially all of SAA1.1/2.1 in acute phase mouse plasma is bound to HDL, whereas ~15% of the less abundant acute phase isoform, SAA3, is lipid poor/free[23]. However, we and others have reported SAA on apoB-containing lipoproteins in both mice[19, 24] and humans[25, 26] under certain physiological conditions. Several studies have described a complex termed "SAA-LDL" that is associated with components of the metabolic syndrome[26], remnant like particle cholesterol[27], smoking status[28], lifestyle interventions[29], and statin treatment[30]. These studies suggest that SAA-LDL is a risk factor for CVD. However, little is known about the factors that influence the distribution of SAA on various lipoprotein classes. The goal of this study was to test the hypothesis that SAA is an exchangeable apolipoprotein, identify factors involved in promoting exchange, and to determine if the presence of SAA on apoB-lipoproteins affects their proteoglycan binding.

Materials and Methods

In vitro studies

The Lexington Veterans Affairs Medical Center Institutional Review Board approved all studies involving human samples. All animal studies were approved by the Lexington Veterans Affairs Institutional Animal Care and Use Committee and conducted in accordance with Public Health Service policy on humane care and use of laboratory animals. Native human acute phase HDL (containing a mixture of SAA1 and SAA2) was isolated from the plasma collected 24 hours after cardiac surgery as previously described[31]. Native murine acute phase HDL (AP-HDL, containing SAA1.1, SAA2.1, and to a lesser extent, SAA3) was isolated from lipopolysaccharide (LPS)-injected mice as previously described[32]. The SAA was purified from delipidated HDL by size exclusion chromatography as previously described[33]. Lipoproteins (VLDL d<1.019 g/ml; LDL d=1.019 to 1.063 g/ml; HDL d=1.063 to 1.210 g/ml) were isolated from pooled plasmas of normal healthy (non-obese) humans or from normal healthy chow-fed *apoE*$^{-/-}$ mice by density gradient ultracentrifugation. The concentration of SAA in healthy human and mouse plasmas was < 5 mg/L. All studies used human SAA in combination with human lipoproteins or murine SAA with murine

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

lipoproteins. Aliquots of SAA (10 or 200 μg) were added to 1mg of lipoprotein protein yielding final SAA concentrations of 3.8 mg/L or 77 mg/L (corresponding to concentrations seen in healthy individuals or individuals with chronic inflammation from obesity or diabetes, respectively[25]). SAA was incubated with lipoproteins at 37 °C for 3 hours in saline, prior to fast performance liquid chromatography (FPLC). SAA and cholesterol were measured in fractions using commercially available kits (human SAA ELISA cat. no. #EL10015, Anogen, Ontario, Canada; mouse SAA ELISA cat. no. #ab157723, Abcam, Cambridge, MA; total cholesterol E kit from Wako, Richmond VA). In each experiment the recovery of SAA was calculated as the sum of SAA in SAA-containing fractions as a percent of the total amount of SAA added in the experiment. In all experiments recovery ranged from 85–100% (not shown). In some experiments SAA and apolipoproteins in FPLC fractions were assessed by immunoblotting as previously described[25]. Lipoprotein-proteoglycan interactions were assessed by modified gel mobility shift assay as previously described[34]. HDL remodeling with CETP using SAA-free VLDL as a cholesteryl ester acceptor and triglyceride donor was performed as previously described[31]. Please see the Major Resources Table in the Supplemental Material.

Animal studies

ApoE$^{-/-}$ mice (both males and females) on the C57BL/6 background (Jackson Laboratories, stock #002052) were used for all studies. Mice deficient in SAA1.1 and SAA2.1 (*SAA1.1/2.1-DKO*) crossed to *apoE*$^{-/-}$ were generated as previously described[35]. A*poE*$^{-/-}$ *x SAA1.1/2.1-DKO* mice were injected with lipoprotein (60 μg protein) containing SAA in 100μl volume via tail vein. Mice were bled via cheek vein at 1, 3, and 6 hours then killed 24 hours after injections. Due to institutional policy on murine blood collection, not all mice were bled at all time-points. Aliquots of plasma were applied to an FPLC column and fractions were assayed for SAA. Total SAA in plasma was also measured at each time-point. SAA was below the limit of quantification 24 hours after injection, and in some lipoprotein fractions at earlier time-points. In some experiments mice were injected with 1×10e11 viral particles AdCETP, an adenovirus expressing cholesterol ester transfer protein (CETP), in 100μl buffer 72 hours prior to injection of lipoproteins (AdCETP kindly provided by the late Dr. van der Westhuyzen, University of Kentucky).

Human studies

Obese subjects without metabolic syndrome, with metabolic syndrome (meeting 3 or more of 5 criteria for metabolic syndrome) and subjects with a diagnosis of type 2 diabetes but who were not using insulin were invited to participate. Exclusion criteria included use of fibrates or niacin, anti-inflammatory drugs, estrogen, current smoking, acute illness, known chronic inflammatory diseases such as lupus, rheumatoid arthritis or psoriasis, and thyroid dysfunction. Statin therapy was not an exclusion criterion. Demographic information, including height, weight, blood pressure and waist circumference were measured. A lipid panel and comprehensive metabolic panel was performed using a fasting blood sample from all subjects; HbA1c levels were determined for diabetic subjects. After an overnight fast all subjects were given a high fat shake (Boost® with added cream and corn oil to comprise 40% of daily caloric requirements with 50% from fat[36]) and blood was collected hourly for 8 hours. Triglycerides and SAA were measured on an aliquot of each sample, then

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

lipoproteins were isolated by density gradient ultracentrifugation and FPLC as previously described[25, 31]. SAA was measured in lipoprotein fractions by ELISA and immunoblot. Proteoglycan binding was compared between fasting lipoprotein and the lipoprotein with peak SAA content within each individual subject using modified gel mobility shift assays.

Data analyses and statistics

Data is shown as mean±SEM. The clearance of SAA *in vivo* was analyzed as a simple monoexponential loss model. One way ANOVA was used to compare differences between 3 or more groups, two-tailed Student's paired t test was used to compare differences between two groups, and two way repeated measures ANOVA was used to compare groups over time. Binding curves using the Michaelis-Menten equation were compared between lipoprotein with and without SAA, or between baseline and peak samples from human studies. Binding and kinetic loss curves were calculated with GraphPad Prism software (GraphPad Software, San Diego, CA).

Results

In most published studies, virtually all circulating SAA is found associated with the HDL fraction. To determine if this reflects an inherent property of SAA to preferentially bind HDL, we investigated the extent to which SAA associates with VLDL, LDL, and HDL *in vitro*. Lipid-free human SAA (containing both SAA1 and SAA2) was incubated for 3 hours with isolated human VLDL, LDL or HDL shown to be devoid of SAA in a ratio of either 10 or 200 μg SAA to 1 mg lipoprotein protein. The mixtures were then subjected to FPLC and the elution profile of SAA was determined by ELISA and immunoblotting. When added to any single lipoprotein fraction, all of the SAA was found associated with that lipoprotein, with no evidence of lipid–free SAA. There was no difference in results between low dose (10 μg per mg; not shown) and high dose (200 μg per mg; Fig. 1) SAA. Thus, the finding that SAA is found primarily with the HDL fraction *in vivo* is not due to the lack of an ability to associate with apoB-containing lipoproteins.

We next incubated 10 or 200 μg of lipid-free human SAA for 3 hours with a mixture of human VLDL, LDL and HDL (1 mg protein for each lipoprotein). In the case of high dose SAA 24.4±1.8% was found on VLDL, 18.3±1.1% was found on LDL, and 57.3±2.9% was found on HDL (Fig. 2). Notably, there was no difference in lipoprotein association when SAA was added at a low dose (10 μg, or 3.8 mg/L) or a high dose (200 μg, or 77 mg/L) implying that the presence of SAA on apoB-containing lipoproteins was not due to "spill over" from HDL or that the ability of HDL to accommodate SAA was overwhelmed. As the lipoproteins were added based on equal protein, there was a non-physiological ratio of lipoprotein fractions in the mix, with VLDL relatively over-represented.

To determine if SAA can exchange between lipoprotein particles *in vitro* we first incubated human SAA with human VLDL, LDL, or HDL as described above. The lipoproteins with added SAA were then incubated separately with a mixture of the other two lipoproteins, each lacking SAA (equivalent amounts of protein for each lipoprotein). After 3 hours, samples were subjected to FPLC and the SAA content of eluted fractions was analyzed by ELISA (Table 1) and validated by immunoblotting (not shown). Results from these mixing

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

experiments demonstrated that more SAA ultimately associates with HDL compared to VLDL or LDL, regardless of the SAA concentration or the lipoprotein origin of the SAA. These experiments also provide evidence that SAA can shift between particles *in vitro*.

We next investigated whether SAA exchanges between lipoproteins *in vivo*. For these experiments lipoproteins were isolated from *apoE*$^{-/-}$ mice as this strain has abundant amounts of apoB-containing lipoproteins. Murine SAA (a mixture of SAA1.1, SAA2.1, and SAA3) was incubated with either VLDL, LDL, or HDL at a ratio of 40μg SAA to 1 mg lipoprotein protein. Aliquots (100μl) were injected via tail vein into *apoE*$^{-/-}$ *x SAA1.1/2.1-DKO* mice[35]. Thus, the only SAA1.1/2.1 present in these mice was derived from the injected lipoprotein (at time zero 100% of the SAA was associated with the injected lipoprotein). While *apoE*$^{-/-}$ *x SAA1.1/2.1-DKO* mice express SAA3, we previously determined this isoform is not detected by the ELISA used in this study and plasma SAA3 concentration is virtually undetectable in mice not injected with LPS[37]. Plasma was collected at 1, 3, 6, and 24 hours post-injection, and then subjected to FPLC to determine the lipoprotein distribution of SAA by ELISA (Fig. 3) and immunoblotting (not shown). Plasma SAA was measured at each time point, and the rate of disappearance of SAA from plasma was similar regardless which lipoprotein particle it originated on, with elimination half-lives of around 1.77h (Fig. 3A). In all mice at all time points, all of the SAA eluted with lipoprotein-containing fractions; none was found lipid-free. Like the *in vitro* experiments, these data found that SAA is an exchangeable lipoprotein *in vivo*, but prefers HDL. The elimination half-life of SAA was approximately 0.75h when it originated on VLDL or LDL, but was 2.21h when it originated on HDL (Fig. 3B,C,D). The SAA distribution to VLDL or LDL was limited when it originated on HDL (Fig. 3D).

We previously demonstrated that CETP remodeling of AP-HDL *ex vivo* leads to the release of small amounts of lipid-free SAA[31]. To investigate whether CETP facilitates the transfer of SAA between lipoproteins, we first incubated human SAA with human HDL as described above, and then incubated the HDL+SAA with SAA-free human VLDL in the presence of increasing concentrations of CETP (0–8 μg CETP/mg HDL). The mixtures were then subjected to non-denaturing gradient gel electrophoresis and immunoblotted for SAA. CETP caused enlargement of HDL, likely due to triglyceride enrichment. Even in the absence of added CETP some SAA shifted to VLDL (Fig. 4A, lane 1) as expected based on our data in Figs. 2A, B. With increasing CETP concentrations, there was increased transfer of SAA from HDL to VLDL and release of lipid-poor SAA (Fig. 4A, lanes 2–4). CETP activity is apparently required for this transfer; when experiments were repeated at 4°C there was minimal SAA exchange (not shown). Mice are naturally deficient in CETP. To investigate whether CETP enhances SAA transfer *in vivo*, *apoE*$^{-/-}$ *x SAA1.1/2.1-DKO* mice were injected with HDL containing SAA in the presence or absence of CETP expression. CETP expression was induced via adenoviral vector. To address the possibility that SAA added to lipoproteins *ex vivo* behaved differently from SAA incorporated into lipoproteins *in vivo*, SAA-enriched, acute phase HDL (AP-HDL) isolated from a separate group of mice 20 hours after injection of 100 μg LPS (as previously described[38]) was used for these experiments. The amount of SAA in AP-HDL was similar to that added to lipoproteins *ex vivo* in Fig. 3. The expression of CETP did not affect the rate of disappearance of SAA from plasma, with elimination half-lives of 1.42 h both in the absence and presence of CETP (Fig. 4B). In mice

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

lacking CETP the elimination half-life of SAA originating on AP-HDL was 1.92h, and SAA seemed to prefer HDL (Fig. 4C). However, in the presence of CETP SAA transfer from AP-HDL to VLDL was quite extensive (Fig. 4D), although this did not affect the elimination half life of total plasma SAA.

As SAA has a proteoglycan binding region[17, 18] we next sought to determine if the presence of SAA on apoB-containing lipoproteins increased their proteoglycan binding. SAA or saline were incubated with LDL or VLDL as described above. Increasing concentrations of lipoproteins were then mixed with fixed amounts of radiolabeled proteoglycans under physiological pH and temperature for 1 hour prior to modified gel shift assays. Proteoglycans that are bound by the lipoproteins are retained at the origin, whereas unbound proteoglycans migrate into the gel. The presence of SAA on either LDL or VLDL led to increased proteoglycan binding ($p<0.05$ for each; Fig. 5A, B, C).

To investigate whether SAA is exchangeable in humans, we recruited obese subjects without metabolic syndrome (n=4), with metabolic syndrome (n=7) or with type 2 diabetes (n=5), who would be expected to have elevated plasma SAA. Blood was collected fasting then hourly for 8 hours after consumption of a high-fat shake. There were no significant differences between groups in age, BMI, or blood pressure but there were significant differences between the 3 groups in HDL ($p=0.04$) and triglyceride levels ($p=0.03$; Table 2). All of the subjects with diabetes, 2 out of 4 subjects without diabetes and 3 out of 7 subjects with metabolic syndrome used statins. Statins are known to lower SAA levels, especially those with high baseline levels[30, 39, 40]. Plasma SAA was significantly higher in subjects with diabetes compared to non-diabetic subjects with or without metabolic syndrome (Fig. 6A, $p=0.02$); however, there was no significant change in SAA levels post-prandially for any of the groups. Plasma triglyceride levels demonstrated post-prandial excursions in all groups ($p<0.0001$) but were significantly higher in subjects with diabetes compared to those with or without metabolic syndrome (Fig. 6B, $p=0.03$). Lipoproteins were separated by density gradient ultracentrifugation (Fig. 6C, D, E) or FPLC (not shown) in the fasting and postprandial samples and SAA content analyzed by ELISA (Fig. 6C, D, E) and immunoblotting (not shown). There was a shift in the lipoprotein distribution of SAA in subjects with diabetes (Fig. 6E) but not those with or without metabolic syndrome (Fig. 6C, D) during the post-prandial period, with a relative enrichment of SAA in the VLDL/chylomicron remnant fraction and corresponding depletion of SAA in the HDL fraction. The peak of SAA on VLDL/remnant (~4 hours) appeared to occur ~2 hours after the post-prandial peak of triglycerides (compare Fig. 6B and E). There was a significant interaction between SAA lipoprotein distribution and time ($p=0.0009$) in subjects with diabetes.

To determine if the *in vivo* enrichment of SAA on apoB-containing particles increased their atherogenicity, proteoglycan binding assays were performed using lipoprotein fractions isolated from each individual with diabetes at fasting (with lowest SAA content) and at the post-prandial time point corresponding to maximal SAA content. Due to technical issues proteoglycan binding could only be assessed on 4 of the 5 subjects with diabetes. The presence of SAA on either VLDL/remants or LDL was associated with increased proteoglycan binding ($p<0.0005$ for each, Fig. 7).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Discussion

The major novel finding of the current study is that SAA is an exchangeable apoprotein and not restricted to HDL. Although the vast majority of studies evaluating SAA in humans and animals have found SAA predominantly associated with HDL, our studies clearly demonstrate that SAA can move between lipoprotein particles both *in vitro* and *in vivo*. Although SAA appears to associate preferentially with HDL, the transfer of SAA from HDL to apoB-containing lipoproteins, particularly VLDL/chylomicron remnants, is enhanced in the presence of CETP both *in vitro* and *in vivo*. Although additional studies are needed, our preliminary experiments suggest that the transfer of SAA from VLDL to CETP-remodeled HDL is reduced compared to normal HDL, suggesting that TG enrichment may impede the association of SAA to HDL. The lack of CETP in mice likely accounts for the numerous published reports concluding that SAA resides exclusively on HDL in mice, whereas we and others have found SAA on apoB-containing lipoproteins in a number of human studies[25, 26, 28–30]. Consistent with the murine studies, we saw a shift in the distribution of SAA from HDL to VLDL/remnants both at baseline and during the post-prandial period in subjects with diabetes. CETP activity is known to be increased in diabetes[41, 42]. Increased CETP activity combined with markedly increased postprandial hypertriglyceridemia may account for the shift in SAA lipoprotein distribution observed in subjects with diabetes. Notably, the presence of SAA on apoB-containing lipoproteins was associated with enhanced binding to proteoglycans, which could lead to increased retention in the vessel wall and augmented atherogenesis.

An important observation is that in the presence of any lipoprotein class, all SAA was found lipoprotein-associated and none was found lipid-free. It is important to note that most *in vitro* studies examining the pro-atherogenic and/or pro-inflammatory activities of SAA have used SAA in a lipid-free form. In the few studies that compared lipid-free SAA to HDL-associated SAA, substantial differences in activity were found[8, 43–45]. For example, we previously reported that only lipid-free SAA, but not SAA on AP-HDL, stimulated macrophages to express TNF-α[44]. Similarly, Badolato et al found that only lipid-free SAA but not HDL-associated SAA had chemoattractant activity for monocytes and polymorphonuclear cells[8]. The fact that SAA is not present in the circulation in a lipid-free form raises questions regarding the circumstances in which SAA might be liberated from lipoproteins to exert its biological activity. Our finding that CETP activity can liberate SAA from HDL (Fig. 4 and [31]) suggests one mechanism by which SAA may be released from lipoproteins. Gursky and colleagues have reported that mild oxidation of SAA-enriched HDL promotes the spontaneous release of SAA from the particle[46]. Whether other HDL-remodeling factors, including lipolytic enzymes, also stimulate the dissociation of SAA from AP-HDL merits further investigation.

It is possible that the majority of SAA in the circulation is bound to HDL and hence "neutralized", but in the milieu of an atherosclerotic plaque or inflammatory nidus SAA may be released and assume biological activity. Given that plasma SAA can increase >1000-fold during an acute inflammatory response, HDL binding may serve a key biological function. By blocking the indiscriminate activity of SAA, HDL may serve as a mechanism to avoid triggering unbridled systemic inflammation. However, during an acute phase response, SAA

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

at high concentrations on HDL may be susceptible to liberation when exposed to a particular micro-environment where SAA's biological activities might be beneficial. It is of great interest to determine if different lipoproteins have different effects on SAA's activities, particularly in light of several studies reporting that LDL-SAA is increased in humans at risk for CVD[25, 26, 28–30]. Given the finding that HDL, but not LDL or VLDL, attenuates the induction of TNF-α by SAA in THP-1 cells[43], it is possible that LDL and VLDL do not attenuate SAA's pro-inflammatory activities to the same extent as HDL. Thus, the shift of SAA from HDL to LDL or VLDL may be a mechanism allowing SAA to exert pro-inflammatory activities in the proper context. Interestingly, CETP mass and activity are decreased in humans during an acute phase response, providing a potential mechanism for negatively regulating SAA's impact during inflammation[47, 48].

SAA has a proteoglycan binding domain[17, 18] and SAA has been shown to mediate HDL binding to proteoglycans[22], which may impact HDL's functionality. For example, Chait and colleagues reported that HDL containing SAA binds proteoglycans whereas HDL without SAA does not[49]. This group went on to show that proteoglycan binding of SAA-containing HDL interferes with the anti-inflammatory actions of HDL in adipocyte cell cultures[11]. The current study provides the first direct evidence that SAA mediates proteoglycan binding of apoB-containing lipoproteins. SAA binding to proteoglycans may be involved in its liberation from lipoproteins in inflamed tissue, a process that is known to occur in SAA amyloidosis[50]. In addition, proteoglycan-mediated lipoprotein retention is thought to be one of the initiating steps in the formation of atherosclerosis[12, 13, 51]. Lipoproteins bind to proteoglycans via ionic interactions between positively charged residues on apolipoproteins and negatively charged sulfate and carboxylic groups on glycosaminoglycan chains of proteoglycans. We and others have shown that proteoglycan binding of lipoproteins precedes and contributes to atherosclerosis development[52–54]. We now demonstrate that the presence of SAA on human LDL or VLDL augments the proteoglycan binding of these particles. We previously reported that SAA is present on apoB-containing lipoproteins in humans with obesity/diabetes and that weight loss leads to a decrease in total plasma SAA and the selective depletion of SAA on apoB-lipoproteins[25]. Similarly, studies have found increased prevalence of SAA on LDL particles in humans with metabolic syndrome[26, 27], current smokers[28] and patients with known coronary artery disease[55]. The present study extends these observations and demonstrates that SAA appears to distribute dynamically between lipoproteins. The amount of SAA on apoB-containing lipoproteins peaked post-prandially in obese subjects with diabetes but not in obese non-diabetics with or without metabolic syndrome. Furthermore, the presence of SAA on these lipoproteins was associated with increased proteoglycan binding. Thus, the presence of SAA on apoB-containing particles may be both a biomarker and a contributing cause of increased cardiovascular disease in these populations. The fact that we did not find SAA on apoB-containing particles in metabolic syndrome subjects conflicts with previous reports[26, 27], perhaps due to differences in the populations studied and our fairly small sample size.

A potential limitation of our study is that we predominantly evaluated lipoprotein particles reconstituted with SAA *ex vivo*. The addition of SAA to lipoprotein particles *ex vivo* could result in a different conformation of SAA compared to what occurs when SAA is incorporated with lipoproteins *in vivo*. However, our *in vivo* studies involving mice injected

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

with HDL to which SAA had been added *ex vivo* provided similar results to mice injected with AP-HDL (in which SAA is incorporated *in vivo*). Furthermore, we found similar increases in proteoglycan binding between lipoproteins to which SAA was incorporated *ex vivo* and those isolated from post-prandial human samples (in which SAA was incorporated *in vivo*). In an acute phase reaction when SAA increases by up to 1000-fold changes in lipoprotein structure are expected. For example, high levels of SAA have been shown to displace apoA-I from HDL[56]. Although SAA does not appear to have any effect on the structural stability of HDL[57], it may lead to structural/conformational changes of other components of the particle. Moreover, these effects may be dose-dependent. However, we did not find any differences between our “low” and “high” concentrations of SAA implying that the presence of SAA on apoB-containing particles is not simply due to it exceeding HDL’s capacity to incorporate SAA. Although the mechanism by which SAA moves between lipoproteins was not completely delineated in our study, it seems unlikely that direct particle-collision is involved, given the low concentration of lipoprotein particles in our *in vitro* studies and in human plasma (VLDL, ~80 nmol/L; LDL, ~1.5 μmol/L)[58, 59]. Moreover, the preferential association of SAA to HDL does not appear to be due to differences in particle surface area. When taking into account the relative mass and particle size of the different lipoprotein fractions, we estimate that the relative surface area of VLDL and LDL was approximately 100-fold and 5-fold greater, respectively, than HDL under the experimental conditions performed in our *in vitro* studies. Further studies will be needed to investigate the structural/compositional features of lipoprotein particles, including TG content, that dictate SAA association.

In summary, we now demonstrate that SAA can shift between lipoproteins and that this shift is augmented in the presence of CETP. As mice do not express CETP this may account for the literature reporting that SAA is essentially exclusively an HDL apolipoprotein in mice. However, several studies have reported the presence of SAA on apoB-containing particles in humans, and the presence of CETP activity in humans may account for the discrepant findings between mice and humans. Our data suggests that CETP activity facilitates the exchange of SAA from HDL to apoB-containing lipoproteins. As the presence of SAA on apoB-containing particles augments their proteoglycan binding, SAA on apoB may be an underlying causal contributor to increased cardiovascular risk in certain populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of funding: These studies were supported by funding from the Department of Veterans Affairs CX000975 (to LRT), CX000773 (to NRW) and National Institutes of Health HL134731 (to NRW and FCdB) and used core facilities supported by P30 GM103527. The contents of the publication are solely the responsibility of the authors and do not represent the official views of the US Department of Veterans Affairs, the National Institutes of Health, or the US Government.

Abbreviations

AP-HDL	acute phase HDL

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

CETP	cholesterol ester transfer protein
FPLC	fast performance liquid chromatography
IL	interleukin
LPS	lipopolysaccharide
SAA	serum amyloid A

References

1. Kisilevsky R, Manley PN. Acute-phase serum amyloid a: Perspectives on its physiological and pathological roles. Amyloid. 2012; 19:5–14.
2. Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, Pepine CJ, Sharaf B, Bairey Merz CN, Sopko G, Olson MB, Reis SE. Serum amyloid a as a predictor of coronary artery disease and cardiovascular outcome in women: The national heart, lung, and blood institute-sponsored women's ischemia syndrome evaluation (wise). Circulation. 2004; 109:726–732. [PubMed: 14970107]
3. Kosuge M, Ebina T, Ishikawa T, Hibi K, Tsukahara K, Okuda J, Iwahashi N, Ozaki H, Yano H, Kusama I, Nakati T, Umemura S, Kimura K. Serum amyloid a is a better predictor of clinical outcomes than c-reactive protein in non-st-segment elevation acute coronary syndromes. Circ J. 2007; 71:186–190. [PubMed: 17251664]
4. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med. 2000; 342:836–843. [PubMed: 10733371]
5. Dong Z, Wu T, Qin W, An C, Wang Z, Zhang M, Zhang Y, Zhang C, An F. Serum amyloid a directly accelerates the progression of atherosclerosis in apolipoprotein e-deficient mice. Mol Med. 2011; 17:1357–1364. [PubMed: 21953420]
6. Thompson JC, Jayne C, Thompson J, Wilson PG, Yoder MH, Webb N, Tannock LR. A brief elevation of serum amyloid a is sufficient to increase atherosclerosis. J Lipid Res. 2015; 56:286–293. [PubMed: 25429103]
7. Thompson JC, Wilson PG, Shridas P, Ji A, De Beer MC, De Beer FC, Webb NR, Tannock LR. Serum amyloid a3 is pro-atherogenic. Atherosclerosis. 2017 In press.
8. Badolato R, Wang JM, Murphy WJ, Lloyd AR, Michiel DF, Bausserman LL, Kelvin DJ, Oppenheim JJ. Serum amyloid a is a chemoattractant: Induction of migration, adhesion, and tissue infiltration of monocytes and polymorphonuclear leukocytes. J Exp Med. 1994; 180:203–209. [PubMed: 7516407]
9. Furlaneto CJ, Campa A. A novel function of serum amyloid a: A potent stimulus for the release of tumor necrosis factor-alpha, interleukin-1beta, and interleukin-8 by human blood neutrophil. Biochem Biophys Res Commun. 2000; 268:405–408. [PubMed: 10679217]
10. O'Hara R, Murphy EP, Whitehead AS, FitzGerald O, Bresnihan B. Local expression of the serum amyloid a and formyl peptide receptor-like 1 genes in synovial tissue is associated with matrix metalloproteinase production in patients with inflammatory arthritis. Arthritis Rheum. 2004; 50:1788–1799. [PubMed: 15188355]
11. Han CY, Tang C, Guevara ME, Wei H, Wietecha T, Shao B, Subramanian S, Omer M, Wang S, O'Brien KD, Marcovina SM, Wight TN, Vaisar T, de Beer MC, de Beer FC, Osborne WR, Elkon KB, Chait A. Serum amyloid a impairs the antiinflammatory properties of hdl. J Clin Invest. 2016; 126:266–281. [PubMed: 26642365]
12. Williams KJ, Tabas I. The response-to-retention hypothesis of early atherogenesis. Arterioscler Thromb Vasc Biol. 1995; 15:551–561. [PubMed: 7749869]
13. Williams KJ, Tabas I. The response-to-retention hypothesis of atherogenesis reinforced. Curr Opin Lipidol. 1998; 9:471–474. [PubMed: 9812202]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

14. Boren J, Williams KJ. The central role of arterial retention of cholesterol-rich apolipoprotein-b-containing lipoproteins in the pathogenesis of atherosclerosis: A triumph of simplicity. Curr Opin Lipidol. 2016; 27:473–483. [PubMed: 27472409]
15. Elliott-Bryant R, Silbert JE, Sugumaran G. Serum amyloid a, an acute-phase protein, modulates proteoglycan synthesis in cultured murine peritoneal macrophages. Biochem Biophys Res Commun. 1999; 261:298–301. [PubMed: 10425181]
16. Wilson PG, Thompson JC, Webb NR, De Beer FC, King VL, Tannock LR. Saa, but not crp, stimulates vascular proteoglycan synthesis in a pro-atherogenic manner. Am J Pathol. 2008; 173:1902–1910. [PubMed: 18974302]
17. Malle E, De Beer FC. Human serum amyloid a (saa) protein: A prominent acute-phase reactant for clinical practice. Eur J Clin Invest. 1996; 26:427–435. [PubMed: 8817153]
18. Ancsin JB, Kisilevsky R. The heparin/heparan sulfate-binding site on apo-serum amyloid a. Implications for the therapeutic intervention of amyloidosis. J Biol Chem. 1999; 274:7172–7181. [PubMed: 10066777]
19. Lewis KE, Kirk EA, McDonald TO, Wang S, Wight TN, O'Brien KD, Chait A. Increase in serum amyloid a evoked by dietary cholesterol is associated with increased atherosclerosis in mice. Circulation. 2004; 110:540–545. [PubMed: 15277327]
20. Meek RL, Urieli-Shoval S, Benditt EP. Expression of apolipoprotein serum amyloid a mrna in human atherosclerotic lesions and cultured vascular cells: Implications for serum amyloid a function. Proc Natl Acad Sci U S A. 1994; 91:3186–3190. [PubMed: 8159722]
21. Yamada T, Kakihara T, Kamishima T, Fukuda T, Kawai T. Both acute phase and constitutive serum amyloid a are present in atherosclerotic lesions. Pathol Int. 1996; 46:797–800. [PubMed: 8916152]
22. O'Brien KD, McDonald TO, Kunjathoor V, Eng K, Knopp EA, Lewis K, Lopez R, Kirk EA, Chait A, Wight TN, deBeer FC, LeBoeuf RC. Serum amyloid a and lipoprotein retention in murine models of atherosclerosis. Arterioscler Thromb Vasc Biol. 2005; 25:785–790. [PubMed: 15692094]
23. Tannock LR, De Beer MC, Ji A, Shridas P, Noffsinger VP, den Hartigh L, Chait A, De Beer FC, Webb NR. Serum amyloid a3 is a high density lipoprotein-associated acute-phase protein. J Lipid Res. 2018; 59:339–347. [PubMed: 29247043]
24. King VL, Hatch NW, Chan HW, de Beer MC, de Beer FC, Tannock LR. A murine model of obesity with accelerated atherosclerosis. Obesity (Silver Spring, Md. 2010; 18:35–41.
25. Jahangiri A, Wilson PG, Hou T, Brown A, King VL, Tannock LR. Saa is found on apob-containing lipoproteins in obese diabetic humans. Obesity. 2013; 21:993–996. [PubMed: 23784902]
26. Kotani K, Satoh N, Kato Y, Araki R, Koyama K, Okajima T, Tanabe M, Oishi M, Yamakage H, Yamada K, Hattori M, Shimatsu A. A novel oxidized low-density lipoprotein marker, serum amyloid a-ldl, is associated with obesity and the metabolic syndrome. Atherosclerosis. 2009; 204:526–531. [PubMed: 19007930]
27. Kotani K, Asahara-Satoh N, Kato Y, Araki R, Himeno A, Yamakage H, Koyama K, Tanabe M, Oishi M, Okajima T, Shimatsu A, Japan O. Metabolic Syndrome Study G. Remnant-like particle cholesterol and serum amyloid a-low-density lipoprotein levels in obese subjects with metabolic syndrome. J Clin Lipidol. 2011; 5:395–400. [PubMed: 21981841]
28. Kotani K, Satoh-Asahara N, Kato Y, Araki R, Himeno A, Yamakage H, Koyama K, Tanabe M, Oishi M, Okajima T, Shimatsu A, Japan O. Metabolic Syndrome Study G. Serum amyloid a low-density lipoprotein levels and smoking status in obese japanese patients. The Journal of international medical research. 2011; 39:1917–1922. [PubMed: 22117994]
29. Kotani K, Koibuchi H, Yamada T, Taniguchi N. The effects of lifestyle modification on a new oxidized low-density lipoprotein marker, serum amyloid a-ldl, in subjects with primary lipid disorder. Clin Chim Acta. 2009; 409:67–69. [PubMed: 19723514]
30. Kotani K, Yamada T, Miyamoto M, Ishibashi S, Taniguchi N, Gugliucci A. Influence of atorvastatin on serum amyloid a-low density lipoprotein complex in hypercholesterolemic patients. Pharmacological reports : PR. 2012; 64:212–216. [PubMed: 22580538]
31. Jahangiri A, de Beer MC, Noffsinger V, Tannock LR, Ramaiah C, Webb NR, van der Westhuyzen DR, de Beer FC. Hdl remodeling during the acute phase response. Arterioscler Thromb Vasc Biol. 2009; 29:261–267. [PubMed: 19008529]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

32. Strachan AF, Shephard EG, Bellstedt DU, Coetzee GA, van der Westhuyzen DR, de Beer FC. Human serum amyloid a protein. Behaviour in aqueous and urea-containing solutions and antibody production. Biochem J. 1989; 263:365–370. [PubMed: 2597108]
33. Kindy MS, King AR, Yu J, Gerardot C, Whitley J, de Beer FC. Adenoviral expression of murine serum amyloid a proteins to study amyloid fibrillogenesis. Biochem J. 1998; 332(Pt 3):721–728. [PubMed: 9620875]
34. Tannock LR, Little PJ, Wight TN, Chait A. Arterial smooth muscle cell proteoglycans synthesized in the presence of glucosamine demonstrate reduced binding to ldl. J Lipid Res. 2002; 43:149–157. [PubMed: 11792734]
35. De Beer MC, Wroblewski JM, Noffsinger VP, Rateri DL, Howatt DA, Balakrishnan A, Ji A, Shridas P, Thompson JC, van der Westhuyzen DR, Tannock LR, Daugherty A, Webb NR, De Beer FC. Deficiency of endogenous acute phase serum amyloid a does not affect atherosclerotic lesions in apolipoprotein e-deficient mice. Arterioscler Thromb Vasc Biol. 2014; 34:255–261. [PubMed: 24265416]
36. Kern PA, Finlin BS, Ross D, Boyechko T, Zhu B, Grayson N, Sims R, Bland JS. Effects of kdt501 on metabolic parameters in insulin-resistant prediabetic humans. J Endocr Soc. 2017; 1:650–659. [PubMed: 29264518]
37. Tannock LR, de Beer MC, Ji A, Shridas P, Noffsinger V, Den Hartigh LJ, Chait A, De Beer FC, Webb NR. Serum amyloid a3 is a high-density lipoprotein-associated acute phase protein. J Lipid Res. 2017 in press.
38. de Beer MC, Webb NR, Wroblewski JM, Noffsinger VP, Rateri DL, Ji A, van der Westhuyzen DR, de Beer FC. Impact of serum amyloid a on high density lipoprotein composition and levels. J Lipid Res. 2010; 51:3117–3125. [PubMed: 20667817]
39. Wiklund O, Mattsson-Hulten L, Hurt-Camejo E, Oscarsson J. Effects of simvastatin and atorvastatin on inflammation markers in plasma. Journal of internal medicine. 2002; 251:338–347. [PubMed: 11952885]
40. Horiuchi Y, Hirayama S, Soda S, Seino U, Kon M, Ueno T, Idei M, Hanyu O, Tsuda T, Ohmura H, Miida T. Statin therapy reduces inflammatory markers in hypercholesterolemic patients with high baseline levels. J Atheroscler Thromb. 2010; 17:722–729. [PubMed: 20523010]
41. Colhoun HM, Scheek LM, Rubens MB, Van Gent T, Underwood SR, Fuller JH, Van Tol A. Lipid transfer protein activities in type 1 diabetic patients without renal failure and nondiabetic control subjects and their association with coronary artery calcification. Diabetes. 2001; 50:652–659. [PubMed: 11246887]
42. Riemens S, van Tol A, Sluiter W, Dullaart R. Elevated plasma cholesteryl ester transfer in niddm: Relationships with apolipoprotein b-containing lipoproteins and phospholipid transfer protein. Atherosclerosis. 1998; 140:71–79. [PubMed: 9733217]
43. Franco AG, Sandri S, Campa A. High-density lipoprotein prevents saa-induced production of tnf-alpha in thp-1 monocytic cells and peripheral blood mononuclear cells. Mem Inst Oswaldo Cruz. 2011; 106:986–992. [PubMed: 22241121]
44. Kim MH, de Beer MC, Wroblewski JM, Webb NR, de Beer FC. Saa does not induce cytokine production in physiological conditions. Cytokine. 2013; 61:506–512. [PubMed: 23165195]
45. Zhu S, Wang Y, Chen W, Li W, Wang A, Wong S, Bao G, Li J, Yang H, Tracey KJ, D'Angelo J, Wang H. High-density lipoprotein (hdl) counter-regulates serum amyloid a (saa)-induced spla2-iie and spla2-v expression in macrophages. PLoS One. 2016; 11:e0167468. [PubMed: 27898742]
46. Jayaraman S, Haupt C, Gursky O. Paradoxical effects of saa on lipoprotein oxidation suggest a new antioxidant function for saa. J Lipid Res. 2016; 57:2138–2149. [PubMed: 27744369]
47. Ye D, Kraaijeveld AO, Grauss RW, Willems SM, van Vark-van der Zee LC, de Jager SC, Jauhiainen M, Kuivenhoven JA, Dallinga-Thie GM, Atsma DE, Hogendoorn PC, Biessen EA, Van Berkel TJ, Jukema JW, van Eck M. Reduced leucocyte cholesteryl ester transfer protein expression in acute coronary syndromes. Journal of internal medicine. 2008; 264:571–585. [PubMed: 18783479]
48. Carvalho LS, Virginio VW, Panzoldo NB, Figueiredo VN, Santos SN, Modolo RG, Andrade JM, Quinaglia ESJC, Nadruz-Junior W, de Faria EC, Sposito AC. Brasilia Heart Study G. Elevated cetp activity during acute phase of myocardial infarction is independently associated with endothelial

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

dysfunction and adverse clinical outcome. Atherosclerosis. 2014; 237:777–783. [PubMed: 25463120]

49. Chiba T, Chang MY, Wang S, Wight TN, McMillen TS, Oram JF, Vaisar T, Heinecke JW, De Beer FC, De Beer MC, Chait A. Serum amyloid a facilitates the binding of high-density lipoprotein from mice injected with lipopolysaccharide to vascular proteoglycans. Arterioscler Thromb Vasc Biol. 2011; 31:1326–1332. [PubMed: 21474830]
50. Noborn F, Ancsin JB, Ubhayasekera W, Kisilevsky R, Li JP. Heparan sulfate dissociates serum amyloid a (saa) from acute-phase high-density lipoprotein, promoting saa aggregation. J Biol Chem. 2012; 287:25669–25677. [PubMed: 22654109]
51. Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: Update and therapeutic implications. Circulation. 2007; 116:1832–1844. [PubMed: 17938300]
52. Huang F, Thompson JC, Wilson PG, Aung HH, Rutledge JC, Tannock LR. Angiotensin ii increases vascular proteoglycan content preceding and contributing to atherosclerosis development. J Lipid Res. 2008; 49:521–530. [PubMed: 18033753]
53. Thompson JC, Tang T, Wilson PG, Yoder MH, Tannock LR. Increased atherosclerosis in mice with increased vascular biglycan content. Atherosclerosis. 2014; 235:71–75. [PubMed: 24816040]
54. Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL, Boren J. Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature. 2002; 417:750–754. [PubMed: 12066187]
55. Ogasawara K, Mashiba S, Wada Y, Sahara M, Uchida K, Aizawa T, Kodama T. A serum amyloid a and ldl complex as a new prognostic marker in stable coronary artery disease. Atherosclerosis. 2004; 174:349–356. [PubMed: 15136066]
56. Artl A, Marsche G, Lestavel S, Sattler W, Malle E. Role of serum amyloid a during metabolism of acute-phase hdl by macrophages. Arterioscler Thromb Vasc Biol. 2000; 20:763–772. [PubMed: 10712402]
57. Jayaraman S, Haupt C, Gursky O. Thermal transitions in serum amyloid a in solution and on the lipid: Implications for structure and stability of acute-phase hdl. J Lipid Res. 2015; 56:1531–1542. [PubMed: 26022803]
58. Cromwell WC, Otvos JD, Keyes MJ, Pencina MJ, Sullivan L, Vasan RS, Wilson PW, D'Agostino RB. Ldl particle number and risk of future cardiovascular disease in the framingham offspring study - implications for ldl management. J Clin Lipidol. 2007; 1:583–592. [PubMed: 19657464]
59. Matyus SP, Braun PJ, Wolak-Dinsmore J, Saenger AK, Jeyarajah EJ, Shalaurova I, Warner SM, Fischer TJ, Connelly MA. Hdl particle number measured on the vantera(r), the first clinical nmr analyzer. Clin Biochem. 2015; 48:148–155. [PubMed: 25438074]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- SAA is an exchangeable apoprotein
- CETP facilitates SAA exchange from HDL to apoB-containing lipoproteins
- The presence of SAA on apoB-containing lipoproteins increases their proteoglycan binding

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

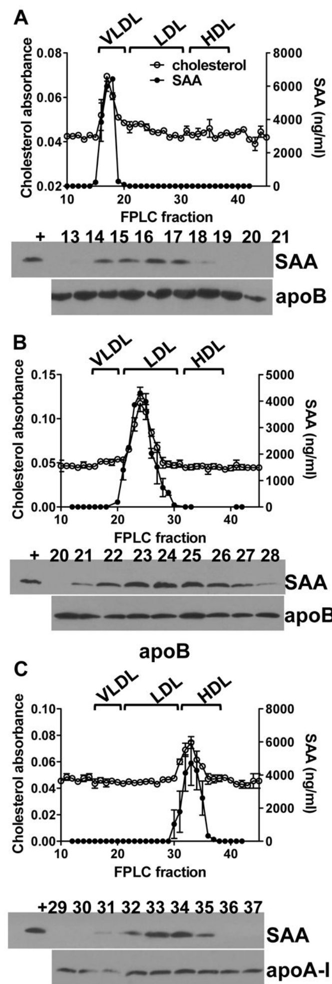

Fig. 1. SAA does not remain lipid free

Lipid-free human SAA was incubated for 3 hours with SAA-free human VLDL (A), LDL (B), or HDL (C) (each at a ratio of 200 μg SAA to 1 mg lipoprotein protein) then subjected to FPLC. SAA in individual fractions was assessed by ELISA (upper panels) or immunoblotting (lower panels). FPLC fractions corresponding to each lane are indicated above the immunoblot. Shown is the mean±SEM of the cholesterol absorbance (left Y axis) and SAA found in each FPLC fraction (right Y axis). Data shown is mean±SEM from n=3 separate experiments for each.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

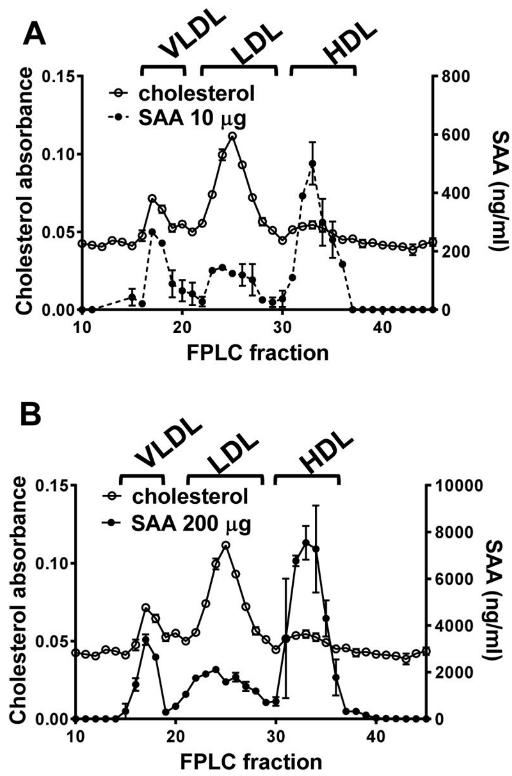

Fig. 2. SAA associates with all lipoproteins

Lipid-free human SAA was incubated for 3 hours with a mixture of human VLDL, LDL and HDL [at a ratio of 10 μg (panel A) or 200 μg (panel B) SAA to 1 mg protein for each lipoprotein] then subjected to FPLC. SAA in individual fractions was assessed by ELISA. Shown is the mean±SEM of the cholesterol absorbance (left Y axis) and SAA found in each FPLC fraction (right Y axis). Data shown is mean±SEM from n=3 separate experiments for each.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

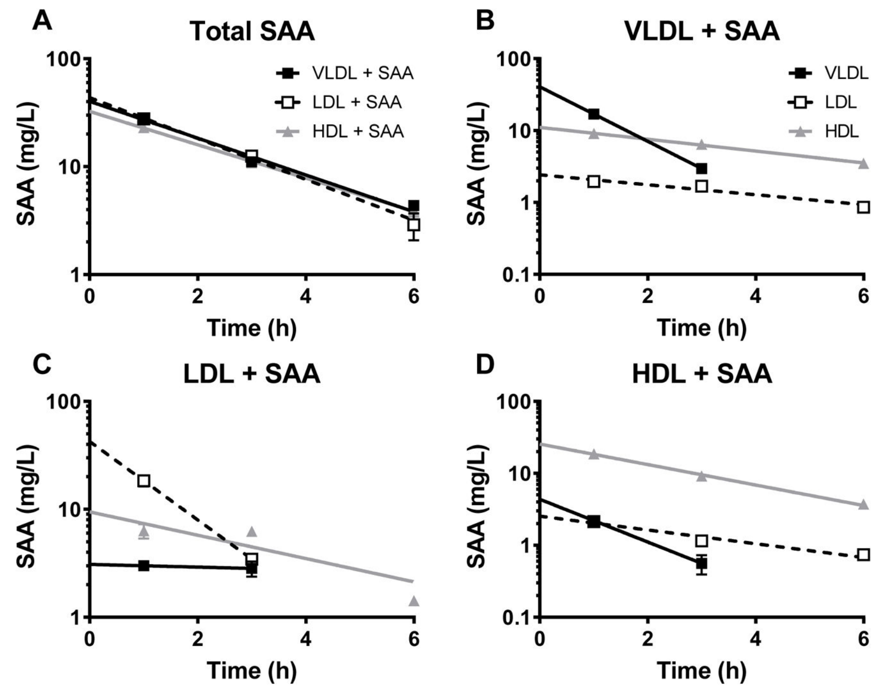

Fig. 3. SAA moves between lipoproteins *in vivo*

ApoE$^{-/-}$ *x SAA1.1/2.1-DKO* mice were injected with VLDL+ SAA (panel B, n=15), LDL +SAA (panel C, n=15), or HDL+SAA (panel D, n=12) and blood was collected at the indicated times. Not every mouse was bled at each time-point (n=3–6/data point). SAA was measured in an aliquot of plasma at each time point and is presented as mean±SEM (panel A). Samples were subjected to FPLC and SAA in individual fractions was assessed by ELISA. Shown is the mean±SEM of the SAA in each fraction (VLDL: black squares; LDL: open squares; HDL, gray triangles).

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

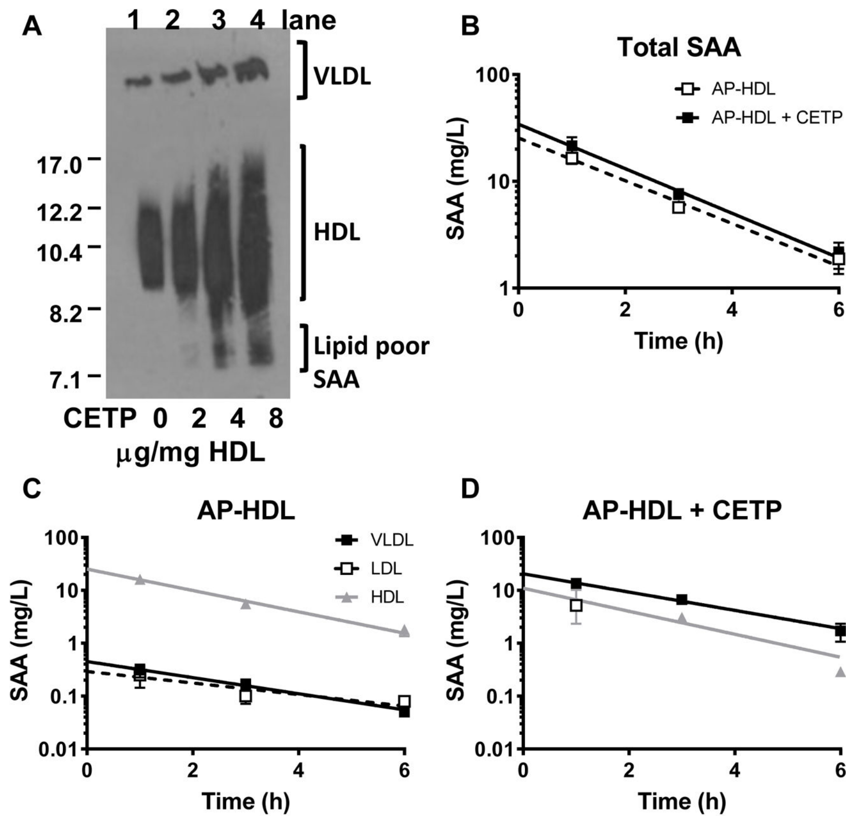

Fig. 4. CETP facilitates the transfer of SAA from HDL to VLDL

A. HDL containing SAA was incubated with increasing concentrations of CETP in the presence of SAA-free VLDL, then subjected to non-denaturing gradient gel electrophoresis, and immunoblotted for SAA. The migration of standards with known radii (nm) are indicated on the left. Gel shown is representative of 3 separate experiments. B,C,D. *ApoE*$^{-/-}$ *x SAA1.1/2.1-DKO* mice were injected with murine AP-HDL in the absence (n=15, open squares) or presence of CETP expression (n=12, black squares) and blood was collected at the indicated times. AdCETP was injected 72 hours prior to injection with AP-HDL to induce CETP expression. Not every mouse was bled at each time-point (n=3–6/data point). B. SAA was measured in an aliquot of plasma at each time point and is presented as mean ±SEM. C,D. Samples were subjected to FPLC and SAA in individual fractions was assessed by ELISA. Shown is the mean±SEM of the SAA in each fraction (VLDL: black squares; LDL: open squares; HDL, gray triangles).

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

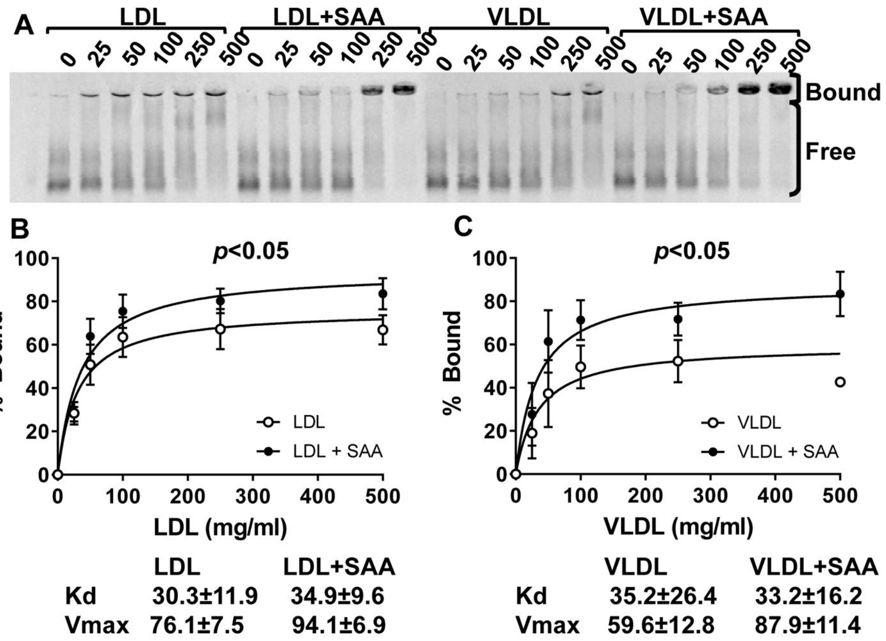

Fig. 5. The presence of SAA on LDL or VLDL increases their proteoglycan binding

Human SAA (closed circles) or saline (open circles) was incubated with human LDL or VLDL for 3 hours (at a ratio of 200 μg SAA to 1 mg lipoprotein protein). Increasing concentrations of LDL or VLDL (0–500 μg/mL lipoprotein as indicated) were then mixed with fixed amounts of radiolabeled proteoglycans for 1h under physiological pH and temperature before electrophoresis in agarose gels. Proteoglycans that are bound by lipoproteins are retained near the origin while free proteoglycans migrate into the gel. A. Gel shown is representative of n=5. B,C: Binding curves shown are mean±SEM from 5 separate experiments. Kd and Vmax (mean±SD) for curve fit are shown on the figures.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

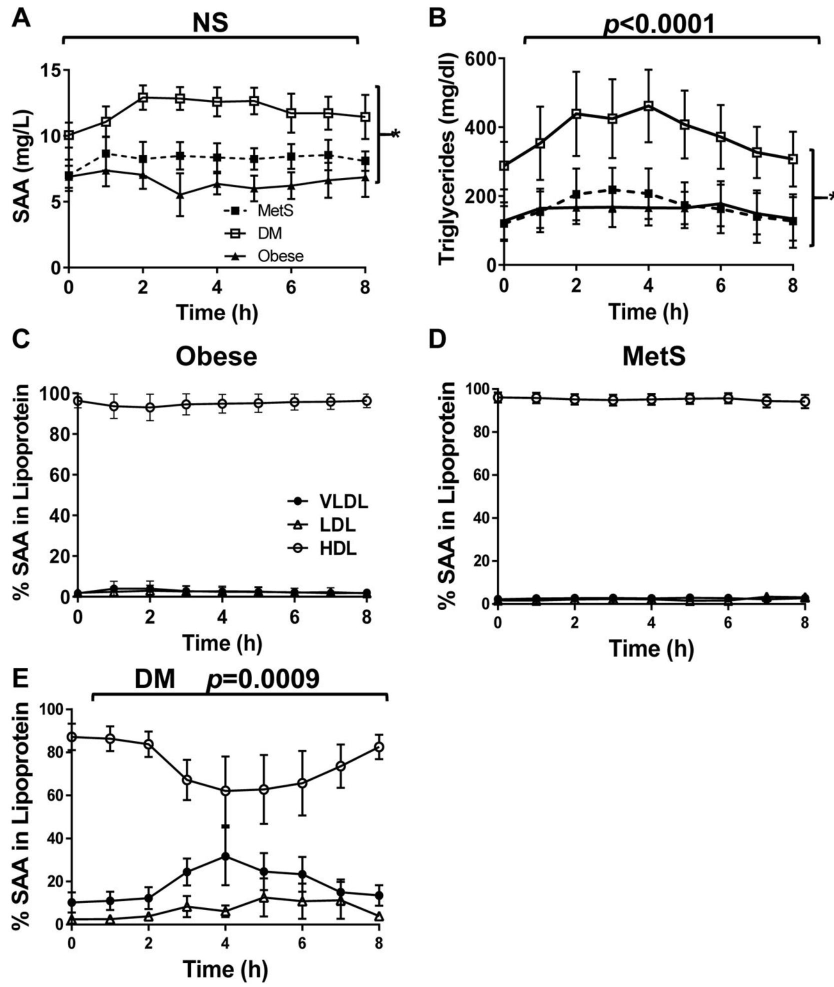

Fig. 6. SAA lipoprotein distribution is significantly altered post-prandially in obese subjects with diabetes

Blood was collected from obese humans with or without metabolic syndrome (Obese, N=4, closed triangles, MetS, N=7, closed squares) or type 2 diabetes (DM, n=5, open squares) in the fasted state (time 0), and hourly for 8 hours after consumption of a high fat shake. A. Plasma SAA. B. Plasma triglycerides. C, D, E: VLDL/remnants (closed circles), LDL (open triangles), and HDL (open circles) were isolated by sequential density gradient ultracentrifugation and SAA content determined by ELISA. C. Obese subjects; D. MetS subjects; E. DM subjects. Data shown are mean±SEM from each individual subject.* $p<0.05$ between group comparison.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

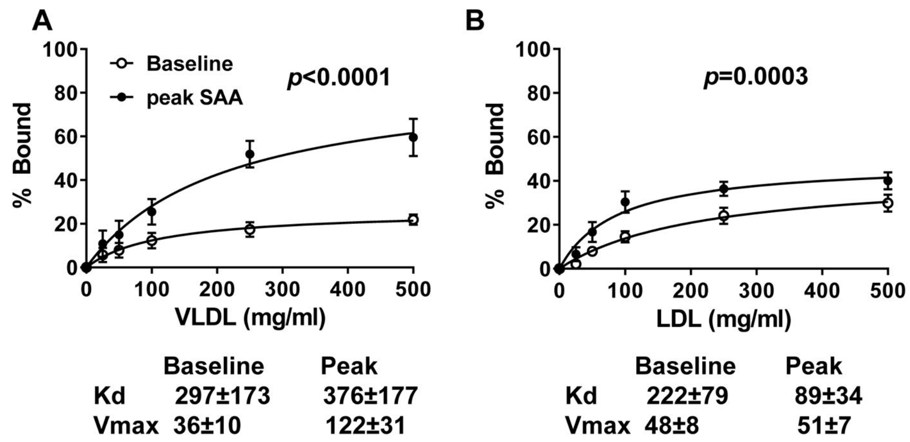

Fig. 7. Endogenous enrichment of SAA on LDL or VLDL/remnants from diabetic subjects is associated with increased proteoglycan binding

Proteoglycan binding of lipoproteins collected fasting (with lowest SAA content, open circles) and at time of peak SAA content (closed circles) were compared within each individual with diabetes as described in legend to Fig. 5. Binding curves shown are mean ±SEM from n=4 diabetic subjects. Kd and Vmax (mean±SD) for curve fit are shown on the figures.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

SAA transfers between lipoprotein fractions *in vitro*.

Starting Complex	Added to	% SAA on VLDL	% SAA on LDL	% SAA on HDL
10 μg SAA				
VLDL+SAA	LDL & HDL	26.1±2.9	17.9±15.6	56±11.4
LDL+SAA	VLDL & HDL	21.5±5.8	22.8±4.9	55.7±0.9
HDL+SAA	VLDL & LDL	12.6±0.2	10.9±5.2	76.5±5.4
200 μg SAA				
VLDL+SAA	LDL & HDL	18.4±10.1	11.1±0.0	70.5±10.1
LDL+SAA	VLDL & HDL	20.1±0.4	11.7±4.4	68.2±4.8
HDL+SAA	VLDL & LDL	8.5±1.5	5.1±2.4	86.4±3.9

Lipid-free human SAA was incubated for 3 hours with either human VLDL, LDL or HDL (at a ratio of 10 μg or 200 μg SAA to 1 mg lipoprotein protein), and then mixed separately with the other two lipoprotein fractions, each lacking SAA. After a 3 hour incubation, mixtures were separated by FPLC and SAA in individual fractions was assessed by ELISA. Data shown are the % of added SAA recovered on each lipoprotein fraction, and are mean±SEM from n=3 separate experiments.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Subject Characteristics

	Obese (n=4)	Metabolic Syndrome (n=7)	Diabetes (n=5)	*p*
Age (years)	64 ±11	65 ± 4	59 ± 4	NS
BMI (kg/m^2)	31.9 ± 2.9	33.7 ± 2.0	36.2 ± 2.2	NS
BP (mm Hg)	126±6/79±5	125±3/80±2	127±7/75±3	NS
Cholesterol (mmol/L)	4.4 ± 0.5	3.8 ± 0.4	4.6 ± 0.3	NS
LDL-c (mmol/L)	2.6 ± 0.4	3.4 ±1.2	2.4 ± 0.2	NS
HDL-c (mmol/L)	1.2 ± 0.2	0.8 ± 0.0	1.1 ± 0.1	0.04
Triglycerides (mmol/L)	1.6 ± 0.3	1.4 ± 0.2	3.3 ± 0.8	0.03
HbA1c (%)	ND	ND	7.1 ± 0.4	NS
Statin use (y/n)	2/2	3/4	5/0	

Data shown is mean ± SEM. BMI: body mass index. BP: blood pressure. ND: not determined