HDL content and composition in acute phase response in three species: triglyceride enrichment of HDL a factor in its decrease

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Abstract High density lipoprotein (HDL) levels decrease during the acute phase response (APR). We have used the APR model of rabbit, baboon, and mouse to study the factors that influence HDL level. In the baboons and rabbits there was massive hypertriglyceridemia, triglyceride enrichment of HDL (60–80% of core lipids), decreases of HDL-cholesterol and apolipoprotein (apo)A-I (to 10% of baseline), and increases of apoA-I in the non-lipoprotein bottom fraction suggesting dissociation of apoA-I from the particles. Detailed analyses of serum amyloid A (SAA)-rich HDL done in the rabbit revealed large, triglyceride-enriched (>60% of core lipids) particles containing >95% SAA. These particles had a high surface to core ratio (13.4 ± 1.94, control = 3.0 ± 0.12) and a very high protein (79.71 ± 5.25 weight %, control = 37.2 ± 0.43) proportion, large (r = 5.95 nm) when examined by non-denaturing gradient electrophoresis but small when examined by electron microscopy (r = 4.2 nm). In the mouse there was no hypertriglyceridemia, no triglyceride enrichment of HDL, no decrease of HDL cholesterol. ApoA-I decreased to about 61.4% of baseline but did not increase in the bottom fraction although large but dense SAA-enriched HDL particles were also produced. These results suggest that hypertriglyceridemia, triglyceride-enrichment of HDL, and dissociation of apoA-I from the particles, possibly by displacement of apoA-I by SAA, are important factors in the decline of HDL during the APR. Whether differences in triglyceride metabolism account for the differences in the HDL response in the species studied requires further experimentation.—**Cabana, V. G., J. R. Lukens, K. S. Rice, T. J. Hawkins, and G. S. Getz.** HDL content and composition in acute phase response in three species: triglyceride enrichment of HDL a factor in its decrease. *J. Lipid Res.* 1996. **37:** 2662–2674.

Supplementary key words apoA-I • HDL • HDL-cholesterol • hypertriglyceridemia • triglyceride-rich HDL • serum amyloid A • lipoproteins • baboon acute phase response • mouse acute phase response • rabbit acute phase response

Reduced plasma high density lipoprotein (HDL) is a well-recognized risk factor in the development of atherosclerosis. The mechanisms responsible for reduced HDL levels are frequently unclear. In humans the acute phase response (APR) is associated with an often precipitous decline in plasma HDL cholesterol and apoprotein A-I (1–4). In this report we present data on the comparison of the HDL changes following the APR in three non-human species, namely, rabbit, baboon, and mouse. Our aim is to set the stage for studies of the factors that control HDL level by describing the APR-associated alterations in lipoproteins in those species most frequently used for studies of atherosclerosis. It is our goal to define the differences in the HDL response during the APR in the expectation that this might point to the mechanisms that account for these changes. Of particular interest is the relationship between the hypertriglyceridemia and the associated changes in HDL that accompanies the APR in some species. In non-acute phase conditions in humans, there is a close relationship between hypertriglyceridemia and low HDL (5).

The APR is a systemic reaction to infectious and non-infectious tissue destructive processes characterized by rapid changes in the level of plasma proteins termed acute phase reactants. The two proteins that are unique in their magnitude of response (>1000-fold within 48–72 h) are C-reactive protein and serum amyloid A (SAA) (6). Despite years of study, the biological significance of their rapid increases remains unknown. Both interact with lipoproteins, i.e., C-reactive protein is known to

Abbreviations: APR, acute phase response; CETP, cholesteryl ester transfer protein; HDL, high density lipoprotein; LPS, lipopolysaccharide; SAA, serum amyloid A; TG, triglyceride.

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bind triglyceride-rich apoB-containing particles in vitro (6,7), while SAA circulates with HDL_3 (8). The significance of these interactions with lipoproteins remains to be elucidated.

Alterations of lipoproteins have been reported during conditions that precipitate the APR, which in humans include microbial infection (9, 10), Kawasaki disease (11, 12), myocardial infarction (13), and trauma (14). The most commonly observed changes are elevations of plasma triglyceride and triglyceride-rich VLDL, and decreases in HDL cholesterol and apoA-I levels. In our study of a group of human plasmas (1, 2) analyzed using C-reactive protein as a monitor of the APR, apoA-I and HDL-cholesterol levels were inversely correlated with C-reactive protein ($r = -0.77$, n = 326). The changes were reversible and the levels returned to baseline when the acute phase resolved. A similar dramatic reversible reduction in HDL was observed in our study of a subject with lymphoma (3). In a group of 12 children with Kawasaki disease (4) tested during the acute illness and at 1, 2, and 12 months after recovery, the presence of SAA was correlated with severity of the disease and decreased levels of apoA-I and HDL-cholesterol. Recently, elevated levels of C-reactive protein and SAA, indicators of acute inflammation, have been implicated as markers of a poor prognosis in unstable angina (15).

Similar alterations in lipoprotein metabolism occur in laboratory animals. Our previous studies in the rabbit showed that within 48 h of the induction of the acute phase by croton oil (16, 17) while plasma triglyceride and triglyceride-rich apoB-containing lipoproteins of d $<$ 1.006 g/ml accumulated, apoA-I and HDL-cholesterol decreased to $<$10% of baseline. The alpha mobility HDL disappeared and was replaced by a band faintly stained for lipid and with slower electrophoretic mobility suggestive of new species of HDL.

This present study characterizes the changes that occur in HDL in the three animal models. Two of these species express plasma cholesteryl ester transfer protein while the mouse does not express this plasma activity (18). The role of this difference remains to be fully elucidated. Detailed analyses of changes in HDL were obtained from the rabbit, which is easier to maintain and to bleed sequentially after induction of the APR. The baboon was used to take advantage of its similarity to humans and of the availability of cross-reactive human reagents (i.e., antibody, apoA-I c-DNA probe). Mice have been studied preliminary to detailed analyses of the mechanisms of HDL decline in genetically defined mouse strains. Furthermore, the lack of hypertriglyceridemia in the APR of mice provides an opportunity for the examination of the role of triglyceride changes in modulating HDL metabolism.

MATERIALS AND METHODS

Plasma and tissue samples

Rabbit plasma samples were obtained from young female (3–5 months old) New Zealand White rabbits purchased from commercial sources. The animals were housed in standard cages, and fed synthetic rabbit pellet ad libitum. The acute phase was induced by the injection of 1% croton oil (v/v mineral oil, 2 ml/kg body weight) at multiple intramuscular sites. Blood samples were drawn from the central ear vessels into tubes containing 0.1% EDTA, pH 7.4, before the injection of croton oil and at various times thereafter. The plasma recovered after centrifugation was stored at 4°C in the presence of the protease inhibitor phenylmethylsulfonyl fluoride (0.001 M in methanol) and anti-bacterial agents (per ml plasma: 0.5 μg gentamicin sulfate, 50 μg NaN_3, 1 μg chloramphenicol) and used within a week. At termination of the study the rabbits were killed by cardiac puncture under anesthesia (ketamine hydrochloride, 0.5 ml/kg).

Baboons used in this study were maintained at the Southwest Foundation for Biomedical Research at San Antonio, Texas. Throughout the experiment they were fed a commercially prepared monkey chow low in fat and containing only trace amounts of cholesterol. At the start of the experiment a dummy tether was planted. On the second week a health check was performed and fasting plasma samples were obtained and sent to the University of Chicago for analysis. On the third week additional fasting plasma samples were taken after which a jugular catheter was inserted to allow sequential bleeding. Five hundred μg/kg of lipopolysaccharide (LPS) (*E. coli* serotype 0127:88, Sigma) was infused intravenously through the catheter in a single bolus. Plasma samples were obtained at timed intervals until 72 h as follows. The animals were fasted overnight before the start of the experiment. After the 6-h bleed the animals were given a single meal; then food was withdrawn for another overnight fast for the 24-h sample. The overnight fast was repeated for the 48- and 72-h samples. All plasma samples were collected in the same "cocktail" as described for rabbit plasma and transported to Chicago in wet ice immediately after the bleeding and separation of the plasma. The animals were allowed to recover at the termination of the study.

Mouse plasma samples were obtained from 8–10-week-old C57BL/6 animals purchased from commercial sources. The animals were housed in standard cages, 4 mice per cage, and fed synthetic mice pellet ad libitum, and were allowed to rest 2–3 weeks before the start of the study. The acute phase was induced by the intraperitoneal injection of 50 μg of the same LPS used in baboons. Blood samples were drawn by retroorbital

puncture of mice anesthetized by inhalation of methoxyflurane (Methofane, Pitman-Moore). The blood was dripped into tubes containing the "cocktail" described above and processed as for the other species. The mice were terminally bled for each time point. Due to the small volume of blood, plasma from each set of 4 animals in a cage was pooled. Zero h samples were obtained from control non-injected mice; each time point represents the plasma levels obtained from a group of 4 mice bled at various times after injection of LPS.

HDL isolation

HDL was isolated by density gradient ultracentrifugal flotation as described previously (16). In this procedure, 1 or 2 ml of plasma was layered in the interface of a 3–20% discontinuous NaBr gradient (4.6 ml 20% NaBr at the bottom, plasma at the interface, filled to the top of the tube with 3% NaBr) and centrifuged to equilibrium for 66 h, 38,000 rpm, SW41Ti rotor. After centrifugation, 0.4-ml fractions were collected using a gradient fractionator (ISCO, Lincoln, NE) with UV monitor. Density of the fractions was determined as refractive indices based on the refractive index of known salt solutions. The fractions were dialyzed against Tris-buffered saline (10 mM Tris, 150 mM NaCl, 10 mM NaN_3, pH 7.4) and used for analyses.

For detailed analyses of the particles, HDL was isolated from terminal-bleed rabbit plasma by sequential flotation at d 1.063–1.25 g/ml following standard techniques (19) to remove most of the plasma proteins. The isolated HDL was dialyzed in Tris-buffered saline and 2 mg of HDL protein/tube was re-isolated by density gradient ultracentrifugation (as described above) to separate the different peaks. The HDL fractions were dialyzed in Tris-buffered saline before use in the analyses.

Lipoprotein particles were also isolated from plasma or HDL fractions by fast phase liquid chromatography (FPLC) using two Superose 6 columns (Pharmacia) in tandem. Five hundred µl of plasma or 1 mg (protein) HDL was run at 0.4 ml/min per fraction in 0.02 M phosphate-buffered saline, pH 7.2, with 0.02% NaN_3 as preservative.

Lipid and protein quantitation

Triglycerides and cholesterol were analyzed using commercially purchased enzymatic kits (Lancer). HDL cholesterol was determined after precipitation of apoB-containing lipoproteins by magnesium chloride and phosphotungstic acid (Lancer). Phospholipids were quantitated based on the Bartlett phosphorus assay (20). Protein was quantitated by a modified Lowry procedure with SDS to disrupt the lipid micelles (21) using bovine serum albumin as standard. ApoA-I was quantitated by radial immunodiffusion as described previously (22) using the appropriate antisera (goat antibody to rabbit apoA-I, and rabbit antibody to human apoA-I) with purified apoA-I as standard.

Polyacrylamide gel electrophoresis (PAGE)

Molecular weight was estimated from 10–20% SDS-PAGE slab electrophoresis (0.2% SDS) based on the procedure of Weber and Osborne (23). One µl of plasma or 2 to 5 µg HDL protein was loaded per lane, reduced with 5% β-mercaptoethanol, and heated to 100°C for 3 min in a Tris-buffered solution (sample buffer) (10 mM Tris, 1% SDS, 10% sucrose, 1 mM EDTA, and 0.02 mg/ml bromphenol blue, pH 8.0). Electrophoresis was conducted at 200 V with a Tris-glycine buffer (25 mM Tris, 192 mM glycine, 1% SDS), until the tracking dye moved to about 1 cm from the bottom. The gels were either used for electrotransfer of the proteins or stained with Coomassie blue R250 (0.25% stain, 25% isopropanol), destained by diffusion into 25% isopropanol–10% acetic acid solution, and stored in 7% acetic acid.

Isoelectric focusing (IEF) on gels prepared in 8 M urea with pH 4–6.5 ampholines (Pharmacia), either in tubes or slabs, was by a modification of the procedure published by Kashyap et al. (24). Thirty µg (protein) of HDL mixed with an equal volume of tetramethylurea was used. Electrophoresis was conducted for 1 h at 110 V, then overnight at 400 V, with 20 mM NaOH and 0.01% H_3PO_4 as anodic and cathodic electrolytes, respectively. After electrophoresis, the gels were either stored frozen in the sample buffer described above to be used for second dimension electrophoresis by SDS PAGE, or stained with Coomassie blue G250 (25) (0.1% stain, 5% perchloric acid) and stored in 7% acetic acid.

Size of the HDL particles was estimated from nondenaturing 4–30% gradient gel electrophoresis (GGE) system as described by Blanche et al. (26) using commercially available gels. A mixture of reference proteins (HMW Standard, Pharmacia) consisting of thyroglobulin (r = 8.5 nm), ferritin (r = 6.1 nm), catalase (r = 5.2 nm), lactate dehydrogenase (r = 4.08 nm), and bovine serum albumin (r = 3.55 nm) was included in each gel. The gels were stained with Coomassie G250 in perchloric acid and stored in 7% acetic acid.

Detection of serum amyloid A

In the rabbit, in the absence of a specific antibody, the presence of SAA was verified by 2-dimensional electrophoresis with isoelectric focusing (pH 4–6.5) in the first dimension and SDS PAGE in the second dimension. Serum amyloid A was detected as a 12,000 kD spot in this system. Serum amyloid A in baboon plasma or

TABLE 1. Plasma lipid and apolipoprotein levels of rabbits after the intramuscular injection of croton oil

Hours Post Injection	Plasma Level					d > 1.21 g/ml
	Triglyceride	HDL-Chol	Total Chol	ApoA-I	SAA	ApoA-I
			mg/dl			*% of total*
Pre-inj. (n = 11)	63.4 ± 3.9	28.3 ± 2.7	51.1 ± 6.6	119.6 ± 21.3	–	10.7 ± 0.92 (n = 3)
24 (n = 7)	83.9 ± 18.5	20.3 ± 2.9[a]	75.8 ± 8.5[b]	nd	+++	nd
48 (n = 11)	221.4 ± 35.2[c]	10.1 ± 1.06[d]	74.1 ± 4.7[c]	nd	+++	nd
72 (n = 10)	199.3 ± 28.7[c]	5.6 ± 0.76[c]	71.3 ± 9.3[a]	10.5 ± 1.5[c]	+++	23.2 ± 2.02 (n = 3)

[a]$P < 0.05$; [b]$P < 0.025$; [c]$P < 0.0001$; [d]$P < 0.0005$; and [e]$P < 0.01$ versus 0 hour; nd, not determined.

plasma fractions was verified by western immunoblotting of proteins transferred from IEF or SDS PAGE based on the procedure of Towbin and Gordon (27). Electrotransfer to membrane (Immubilon, Millipore) was conducted at 360 mA/gel (Transphor Electrophoresis Unit, Hoeffer Scientific Instruments) for 1 h and with cooling using a circulating water bath (Forma Scientific) at 10°C. Blotting was done using antibody against human SAA obtained from commercial sources (Calbiochem) or our recently produced anti-human SAA prepared by using recombinant human SAA as immunogen. The specific bands were detected with a chemiluminescence detection system (ECL, Amersham) following the protocol outlined by the manufacturer. Murine SAA was similarly detected using anti-murine SAA prepared against recombinant murine SAA2 (V. G. Cabana, C. A. Reardon, B. Wei, J. R. Lukens, and G. S. Getz, unpublished results).

Electron microscopy

HDL isolated by gradient centrifugation or FPLC were examined in the Philips CM10 at the Cummings Laboratory of the University of Chicago after staining with sodium phosphotungstate as previously described (28). Size of the particles was estimated as the mean of 120 particles measured from enlarged prints of the micrographs.

RESULTS

Acute phase changes in rabbits

Plasma lipids. The results for rabbits are summarized in **Table 1.** In response to induction of the APR, plasma triglyceride began to increase at 24 h and reached peak levels of 221.4 ± 35.2 mg/dl at 48 h (n = 11). The levels declined slightly by 72 h. Total plasma cholesterol showed a modest but significant increase. In contrast, HDL cholesterol declined substantially from 28.3 ± 2.7 mg/dl before injection to 5.6 ± 0.76 mg/dl at 72 h. Over the same period apoA-I decreased from 119.6 ± 21.3 mg/dl to 10.5 ± 1.5 mg/dl. Non-lipoprotein-associated apoA-I increased from 10.7% of total apoA-I at 0 h to 23.2% at 72 h. Serum amyloid A was readily detectable in plasma at 24 h, and increased to become a prominent apolipoprotein by 48 and 72 h.

The time course of lipid changes was followed in four rabbits (**Fig. 1**). HDL-cholesterol started to decrease and HDL core triglyceride started to increase even before there were significant increases in plasma triglyceride. In fact, there was an early fall in plasma triglyceride that reached statistical significance by 4 h. ApoA-I decreased exponentially with an estimated apparent half-life of about 11.5 h (**Fig. 2,** n = 3).

HDL density. The induction of the APR resulted in major changes in the plasma lipoproteins. Repeated analysis (n = 15) by density gradient ultracentrifugal flotation of plasma obtained at various times after induction of the acute phase revealed that the changes occur mostly in the HDL region. Before induction of the acute phase a single peak of HDL was evident at d 1.096 g/ml. After croton oil administration the HDL profile was more complex with the peak of HDL shifting to higher density.

Detailed analyses were performed using HDL isolated from plasma by a two-step procedure: sequential flotation of total HDL at d 1.063–1.25 g/ml to remove most of the plasma proteins, followed by ultracentrifugal flotation in a 3–20% NaBr gradient. Results of a typical density gradient run are shown in **Fig. 3** and summarized in **Table 2.** At baseline (Fig. 3A, top), the HDL had a single major peak at d 1.09 g/ml corresponding to human HDL_2. Seventy two h after injection of croton oil (Fig. 3A, bottom), three HDL peaks were evident. Peak 1 (d 1.088 g/ml) corresponds in equilibrium density to the major peak of the baseline HDL. Peak 2 (d 1.127 g/ml) was detectable as early as 8 h (Fig. 3A, middle). Peak 3 had the highest density (d 1.19 g/ml) and became clearly evident only after most of the plasma proteins were removed by the first step centrifugation. During centrifugation of whole plasma, this peak merged with proteins at the bottom (d > 1.21) fraction.

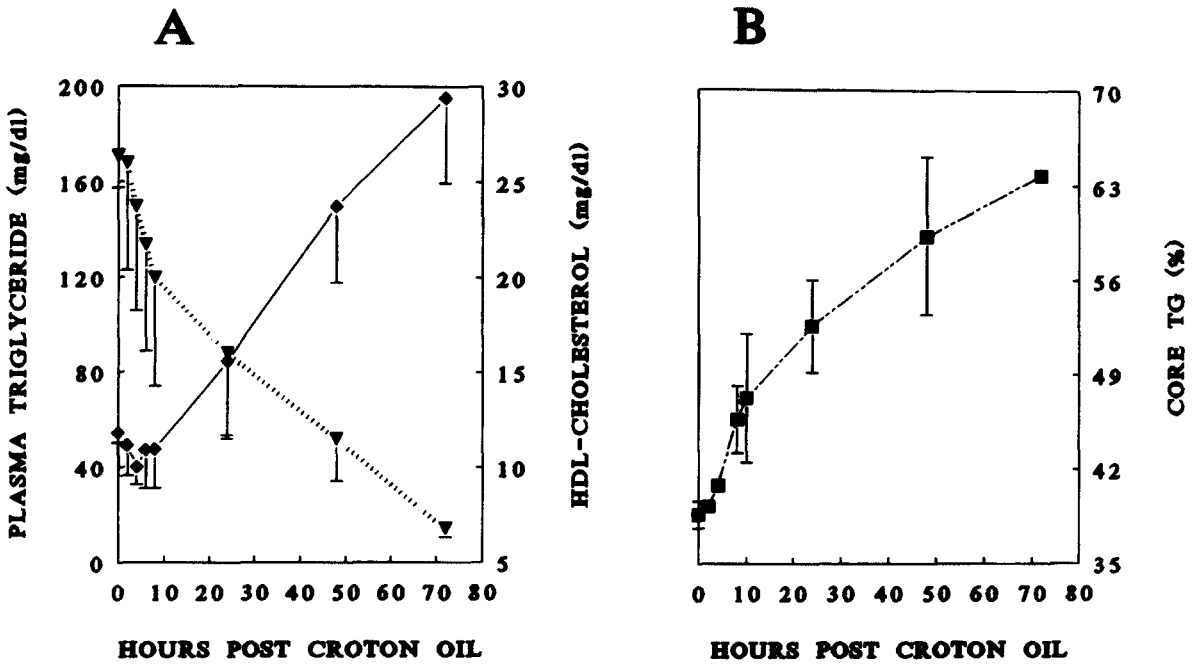

Fig. 1. Changes in rabbit (n = 4) plasma and HDL lipids after induction of the acute phase response by croton oil. The same animals were sampled at each time point. The results are the mean ± SEM of the 4 rabbits. A: (◆---◆) plasma triglycerides; (▼---▼) HDL cholesterol; B: (■---■) HDL core triglycerides.

The size of peak 3 varied with the severity of the response of the individual animal, often becoming the major fraction of acute phase HDL (Fig. 3B).

HDL apoprotein composition. The apoprotein composition of the three acute phase HDL peaks was analyzed by densitometric scans of the SDS PAGE profile. Peak 1 was composed mostly of apoA-I with small amounts of the low molecular weight apoproteins which may include apoCs and SAA. Peak 2 was composed of apoA-I and SAA in roughly equal proportions. SAA was identified by its staining and electrophoretic mobility and by the fact that it was absent from baseline HDL. SAA was detectable in this peak by about 10 h after induction of the acute phase. By 24 h both peak 2 and peak 3 were fully developed. Peak 3 was composed of mostly SAA with a minor contribution from apoA-I. This SAA-containing peak was found in all rabbits tested. Fast phase liquid chromatography (FPLC) of a prominent peak 3 (Fig. 3B) furnished a fraction that contained >95% SAA.

HDL lipid composition. The lipid composition of HDL was determined at baseline for the control HDL and for the HDL remaining in plasma 72 h after induction of the acute phase (Table 2). Peak 1 of the acute phase HDL compared to the corresponding peak of control HDL contained significantly more triglyceride, more protein, less cholesteryl ester, and slightly lower phospholipid. Peaks 2 and 3 were absent in control HDL.

The density of each of the three peaks of acute phase HDL increased along with the increased protein content. All three peaks of the acute phase HDL had a triglyceride-enriched core (exceeding 60% of core lipids, Table 2) and lower cholesteryl ester and phospholipid contents. These changes are reflected in the higher surface (FC, PL, PR percentage) to core (CE, TG percentage) ratio of the SAA-rich HDL subfractions (peaks 2 and 3). While peak 1 had the same surface to core ratio as the control HDL, both peak 2, which contained half of its apoproteins as SAA, and peak 3, which contained

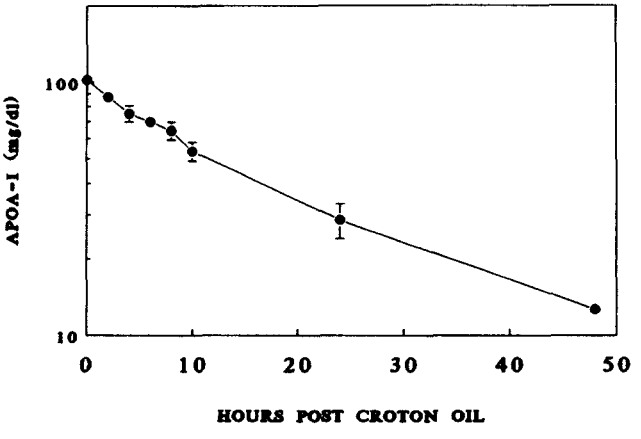

Fig. 2. Decrease of apoA-I in plasma quantitated by radial immunodiffusion assay. The results are mean ± SEM of 3 rabbits sampled at the different time points after induction of the acute phase response.

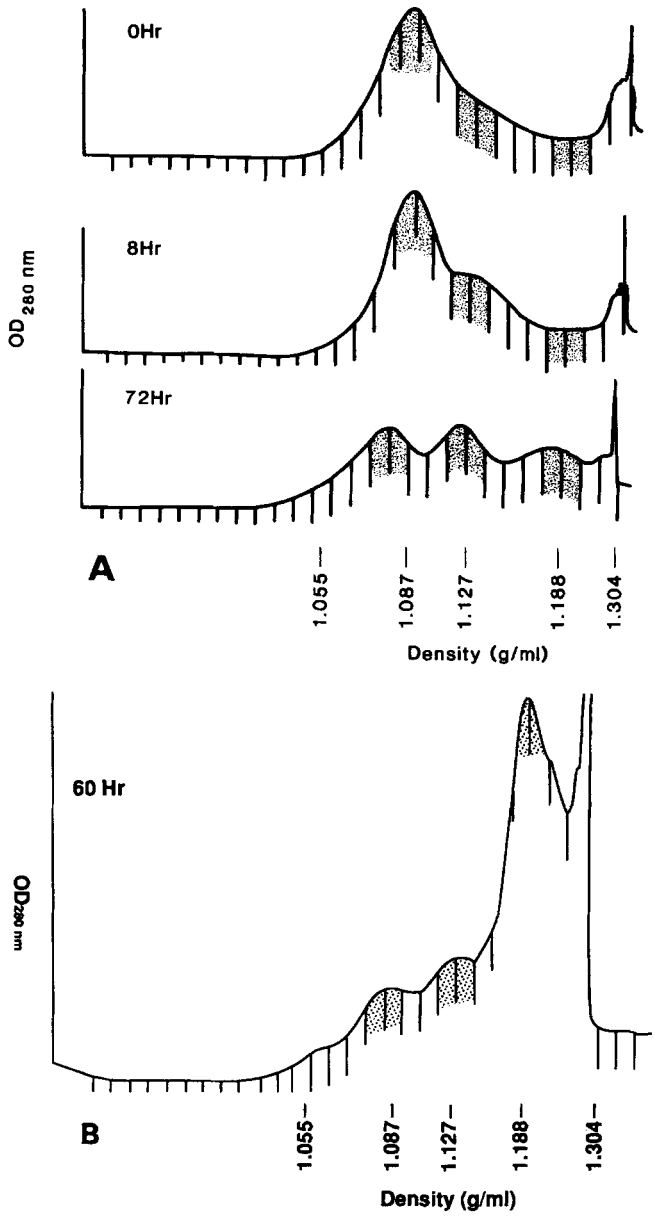

Fig. 3. Density profile of HDL in rabbits at different times after induction of the acute phase response. HDL was first isolated by sequential flotation at d 1.063–1.25 g/ml. Two mg (protein) of the isolated HDL was subjected to equilibrium density ultracentrifugation in the 3–20% NaBr gradient. A: HDL from one rabbit sampled at times 0, 8, and 72 h; B: HDL from another rabbit sampled at 60 h. Density of the fractions is indicated on the ordinate.

mostly SAA, had higher surface to core ratio, peak 3 reaching a ratio of 13.43 ± 1.94, more than four times the surface to core ratio of baseline and peak 1 HDL. This suggests that the SAA-containing particles had a small, triglyceride-rich core and protein-rich surface, most obvious in the case of peak 3.

HDL particle size. Particle radii of the acute phase HDL were analyzed by non-denaturing gradient gel electrophoresis in 4–30% gels. Results of a typical run are shown in **Fig. 4.** Fifteen μg of protein from corresponding fractions collected during density gradient centrifugation was applied to each lane. The single peak of the control (lane 1) and the corresponding fraction of acute phase HDL (lane 4) showed particles with radii of 5.6 nm comparable to human HDL_2. The fraction corresponding to peak 2 showed smaller particles in the control HDL (lane 2, 5.3 nm) than the acute phase HDL (lane 5, 5.5 nm). Fractions corresponding to peak 3 were significantly different. Whereas the fraction from the control HDL (lane 3) had very little material representing the trailing end of the major HDL peak (albumin and free proteins that run off the gel composed the rest of the proteins in this fraction), peak 3 of acute phase HDL (lane 6) was composed of particles which, despite their density, were significantly larger (r = 5.95 nm) than any of the control or other acute phase HDL subfractions. The large size of the SAA-containing particles was anomalous when related to the composition data.

To verify the size of the particles, fractions corresponding to peak 3 from the density gradient centrifugation were further separated by FPLC, then examined under an electron microscope. The acute phase HDL particles that were larger than control HDL in non-denaturing gradient gel electrophoresis (Fig. 4) were much smaller than the peak fraction of control HDL when examined by electron microscopy (**Fig. 5**). Actual measurements of the particles from enlarged electron micrographs showed that the radius of the acute phase HDL was 4.2 nm while the control HDL was 5.9 nm.

Lipid and apoprotein changes in baboons

Plasma lipids. The plasma lipid changes in baboons responding to intravenous infusion of LPS are shown in **Table 3.** The results, presented separately for each animal, were similar to the results obtained in rabbits with the peak time of changes evident at 48 h. During this time plasma triglyceride increased 10-fold. There was a slight decrease in total cholesterol by 24 h. The HDL-cholesterol declined progressively from 0 h to the nadir of 4 and 9 mg/dl 48 h post-injection of LPS. ApoA-I decreased from by about 50% by 48 h. A significant percentage of the plasma apoA-I was found in the lipoprotein-deficient bottom fraction which increased 2- to 3-fold by 48 h. As with the data in rabbits, HDL cholesterol and apoA-I appeared to decrease before there was any significant increase in plasma triglyceride. Indeed, as with the rabbit, there seemed to be a modest initial decline in plasma triglyceride at the time when HDL was enriched in triglyceride.

Density distribution of plasma lipoproteins. Similar to the observations in rabbits, the density distributions of plasma lipoproteins in baboons were altered after the induction of the APR. A new peak with a higher density

TABLE 2. Size, apparent density, and composition of rabbit HDL before (n = 12) and 72 h after (n = 8) the injection of croton oil

	Density	r	TG	PR	FC	CE	PL	Core TG	Surface/Core Ratio
	g/ml	*nm*			*weight %*			*% core lipids*	
Peak 1, 0 h	1.0921 ± 0.007	5.51 ± 0.11	10.2 ± 0.60	37.2 ± 0.43	2.80 ± 0.21	14.08 ± 0.70	36.33 ± 0.569	41.25 ± 2.61	3.0 ± 0.12
Peak 1, 72 h	1.0866 ± 0.007	5.49 ± 0.12	17.63 ± 2.20	41.13 ± 1.50	2.86 ± 0.34	8.63 ± 0.91	29.50 ± 2.02	64.25 ± 4.71	3.0 ± 0.113
Peak 2, 72 h	1.1269 ± 0.007	5.07 ± 0.12	14.13 ± 2.33	53.25 ± 2.29	1.86 ± 0.26	8.13 ± 0.79	22.38 ± 2.99	61.75 ± 3.38	4.0 ± 0.5
Peak 3, 72 h	1.2015 ± 0.032	5.96 ± 0.18	5.71 ± 1.04	79.71 ± 15.35	0.86 ± 0.26	2.57 ± 0.48	11.29 ± 4.09	67.00 ± 3.68	13.4 ± 1.94

(1.128 g/ml) was detectable at 6 h and became the major peak by 24 and 48 h after injection with LPS (data not shown). Unfortunately, due to the limited sample volume obtained, detailed analyses of HDL after removal of plasma proteins were not done.

Serum amyloid A was detected in plasma by western immunoblotting with antibody to human SAA. At the early time points to 6 h no SAA-reactive protein was observed. SAA was present at high levels at 24 h and 48 h (**Fig. 6**) and also at 72 h (blot not shown).

HDL composition. The HDL composition analyzed from the fractions obtained by density gradient centrifugation of plasma is summarized in Table 3. As the HDL cholesterol decreased, the HDL became significantly triglyceride-enriched (>75% at 48 h). It is notable that the proportion of HDL triglyceride increased in the 24-h sample, before there was any increase in total plasma triglycerides. There were no significant changes in the other lipids of HDL (data not shown).

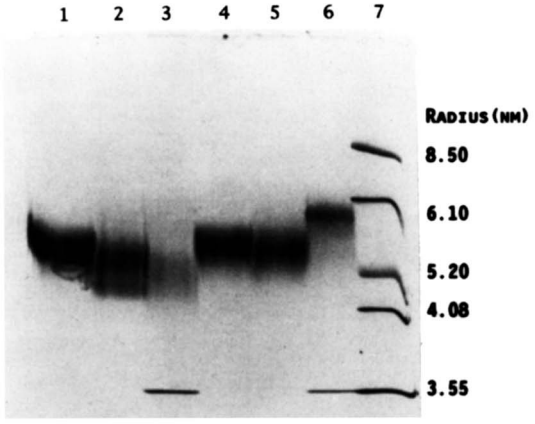

Fig. 4. Non-denaturing gradient gel (4–30%) electrophoresis of rabbit HDL. Fifteen μg (protein) of corresponding fractions from control (left half) and 48-h acute phase (right half) HDL fractions were loaded to the lanes: lanes 1 and 4, peak 1; lanes 2 and 5, peak 2; lanes 3 and 6, peak 3. The faint band in lane 3 suggests that only a few HDL particles were present in the fraction, the rest of the proteins were albumin and other smaller plasma proteins that ran off the bottom of the gel. Reference proteins are shown on the right.

Acute phase response in mice

Plasma lipids. The lipoprotein changes in the mouse in response to the APR are shown in **Table 4.** Despite the very modest apparent increase in plasma triglyceride in the mouse, this was not statistically significant. Total cholesterol increased significantly at 24 h ($P <$ 0.01 vs. 0 h). There was no decrease in HDL cholesterol. However, apoA-I decreased significantly from 103.7 ± 10.3 mg/dl at 0 h to 67.5 ± 5.5 mg/dl at 12 h and 81.0 ± 4.5 mg/dl at 24 h. There was no apparent increase of apoA-I in the non-lipoprotein fraction. These changes were all modest compared to those seen in rabbits or baboons. Three different time course experiments were performed with three sets of C57BL/6 mice. While baseline levels varied somewhat from set to set, there was no consistent change in plasma triglycerides, HDL cholesterol, or HDL triglyceride through the course of an APR (data not shown).

Density, size, and composition of HDL. Although there were no significant changes in plasma lipid composition, the density profile changes (**Fig. 7**) were similar to those seen in the rabbit and baboon. Before induction of the acute phase, there was a monodisperse HDL peak at density 1.10959 ± 0.005 g/ml. After induction of the acute phase, a new peak at density 1.1306 ± 0.002 g/ml appeared. This new peak, which was evident by 6–8 h, progressively increased until it became a major peak by 16 h. Western immunoblotting of plasma revealed that this fraction contained the most SAA (data not shown). Its development was parallel to the increase of SAA. SAA was detectable in mouse plasma earlier (at 2 h) than in the baboon and reached maximal levels by 24 h (**Fig. 8**).

Analyses of the apoprotein composition of the two peaks of acute phase HDL obtained by density gradient centrifugation of plasma revealed that the lower density peak (d 1.10959 g/ml) corresponding to the major peak of control HDL contained predominantly apoA-I (HDL_{A-I}) while the new higher density peak (d 1.1306 g/ml) contained predominantly SAA (HDL_{SAA}), >60% SAA at the height of the response. As in the other spe-

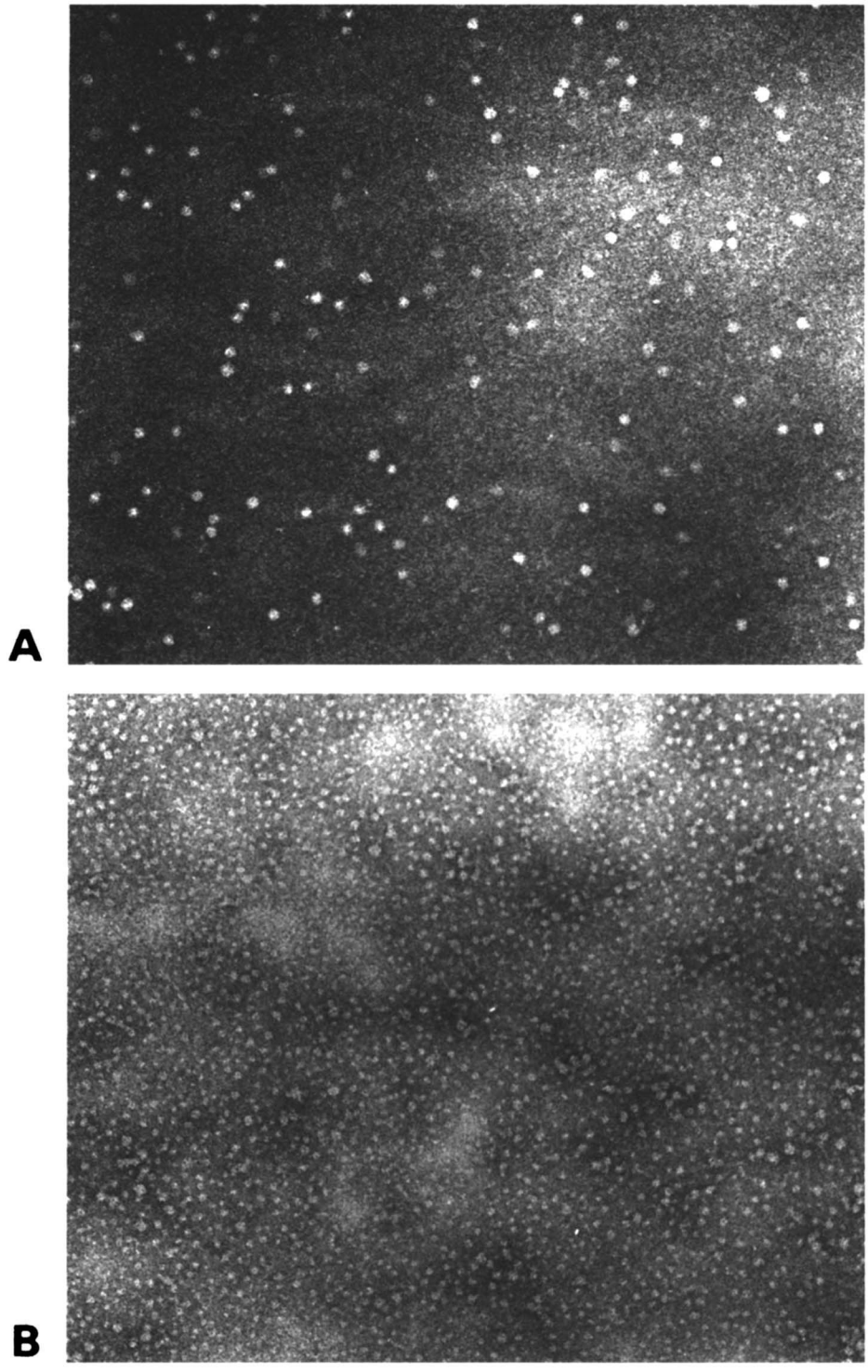

Fig. 5. Electron photomicrographs of negatively stained particles from rabbit HDL. Both are shown at 116 K magnification. The HDL were first isolated from plasma by sequential flotation of lipoproteins at d > 1.25 g/ml. The isolated HDL was further fractionated by density gradient centrifugation. A: the major peak of control HDL, fraction 19, corresponding to lane 1 of Fig. 4; B: peak 3 HDL, fraction 27, corresponding to lane 6 of Fig. 4.

TABLE 3. Lipid and apolipoprotein levels of baboon after the intravenous injection of bacterial lipopolysaccharide (n = 2)

Hours Post Injection	Plasma										HDL		d > 1.21 g/ml	
	TG		HDL-C		TC		ApoA-I		SAA		TG		ApoA-I	
	Bb 1	Bb 2	Bb 1	Bb 2	Bb 1	Bb 2	Bb 1	Bb 2	Bb 1	Bb 2	Bb 1	Bb 2	Bb 1	Bb 2
	mg/dl										*% core lipids*		*%*[a]	
0	32	21	35	53	73	90	141	183	−	−	21	15	10.8	8.6
2	40	24	28	44	73	88	131	170	−	−	28	nd	10.1	nd
4	36	35	26	42	70	89	122	160	−	−	28	18	12.1	11.0
6	39	36	21	34	60	76	118	150	−	−	31	23	11.2	9.9
24	24	16	14	30	47	54	107	131	+++	+++	45	29	13.5	12.2
48	349	215	4	9	109	79	69	99	+++	+++	79	76	32.2	23.8
72	250	107	7	14	117	100	73	109	+++	+++	66	48	18.1	14.7

Values are given separately for each of the two baboons, Bb 1 and Bb 2.
[a]Percent of total plasma apoA-I in the d > 1.21 g/ml fraction.

cies, the presence of SAA produced enlarged particles as examined by non-denaturing gradient gel electrophoresis (data not shown). The increase in size paralleled the content of SAA. The fractions without SAA were not enlarged.

DISCUSSION

This study compared the lipoprotein responses during the APR in three species. Increases of SAA in plasma and the appearance of SAA-rich, dense but large HDL particles occurred in all three species. In rabbit and baboon, despite the use of different agents (croton oil or LPS) to induce the APR, very similar changes in plasma lipids and lipoproteins occurred: hypertriglyceridemia and triglyceride enrichment of HDL, reduction in HDL cholesterol and apoA-I, and an increase in the proportion of apoA-I in the lipid-poor fraction. In the mouse, despite induction of the APR with an agent similar to that used in baboon (LPS), no change was observed in the plasma lipids. While there was a substantial increment in plasma SAA and a refashioning of HDL with the accretion of SAA, there was only a modest decline in plasma apoA-I.

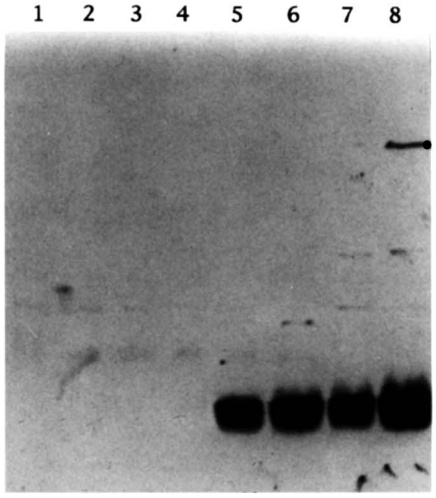

Fig. 6. Western immunoblot of baboon plasma against human SAA. One-μl samples of plasma obtained at various times after injection of LPS were loaded into each lane of a 10–20% gradient SDS PAGE. Lane 1, 0 h; lane 2, 2 h; lane 3, 4 h; lane 4, 6 h; lane 5, 24 h; lane 6, 48 h. For comparison, acute phase human plasma samples obtained from the serology laboratory of the University of Chicago were included in lanes 7 and 8. These plasma samples contained high SAA levels as shown.

Triglyceride changes in acute phase response

There is much evidence in the literature for a relationship between hypertriglyceridemia and low HDL (5). The present study shows that the species (rabbit, baboon) that displayed the most profound decreases in HDL cholesterol and apoA-I were also the species that displayed profound hypertriglyceridemia during the APR. Similar changes occur in human subjects undergoing the APR secondary to Kawasaki disease. Triglyceride enrichment of human HDL occurs even without elevations of plasma triglyceride concentrations (V. G. Cabana, S. S. Giddings, G. S. Getz, J. Chapman, and S. Shulman, unpublished results).

The early time course in both rabbits and baboons indicates that the triglyceride enrichment of HDL occurs before any plasma hypertriglyceridemia is evident. The mechanism for this is not clear. In the baboon, there may be a cytokine-mediated inhibition of the hepatic lipase limiting hydrolysis of HDL triglyceride. It is tempting to speculate that there may be an up-regulation of cholesteryl ester transfer protein (CETP) expressed in these two species mediating more rapid ex-

TABLE 4. Lipid and apolipoprotein levels of C57BL/6 mice after the intraperitoneal injection of bacterial lipopolysaccharide

Hours Post Injection	Plasma					HDL	d > 1.21 g/ml
	TG	HDL-C	TC	ApoA-I	SAA	TG	ApoA-I
	mg/dl					*% core lipids*	*%*[a]
Control (n = 5)	61.6 ± 19.6	21.0 ± 6.1	60.0 ± 6.4	109.3 ± 12.2	–	22.7 ± 2.6	7.5 ± 0.5 (n = 3)
12 (n = 2)	39.0 ± 5.0	19.0 ± 8	56.3 ± 8.4	67.5 ± 5.5[b]	+++	24.0 ± 1.0	3.8 (n = 1)
24 (n = 5)	95.4 ± 15.7	26.4 ± 3.2	91.7 ± 7.7[c]	81.0 ± 4.5[b]	+++	20.0 ± 2.0	5.0 ± 0.0 (n = 3)

Number of pools (n), 4 mice per pool.
[a]Percent of total plasma apoA-I in the d > 1.21 g/ml fraction.
[b]$P < 0.05$; [c]$P < 0.01$ versus 0 h.

change of TG, although recent data in mice transgenic for the human CETP gene show that hepatic CETP mRNA and plasma CETP activity decreased after LPS administration (29).

The mechanisms that account for the plasma hypertriglyceridemia in the APR are not fully elucidated. The cytokines, IL-1, TNF, and IL-6, that are the major mediators of the APR, have been shown to inhibit lipoprotein lipase (30). These cytokines may also activate the transcription of apoC-III, which has an active NFκB site in its promoter (31), and this may retard the clearance of VLDL from the plasma perhaps by displacing apoE, as evidenced in transgenic mice overexpressing apoC-III (32). These cytokines may also induce lipid synthesis and secretion and de novo fatty acid synthesis (33).

The absence of hypertriglyceridemia in the APR of mice is not well accounted for. Modest (about 2-fold) increases in plasma triglyceride were observed in C57BL/6 mice after injection with LPS and interleukin 1 (34), a very blunted response compared to other species.

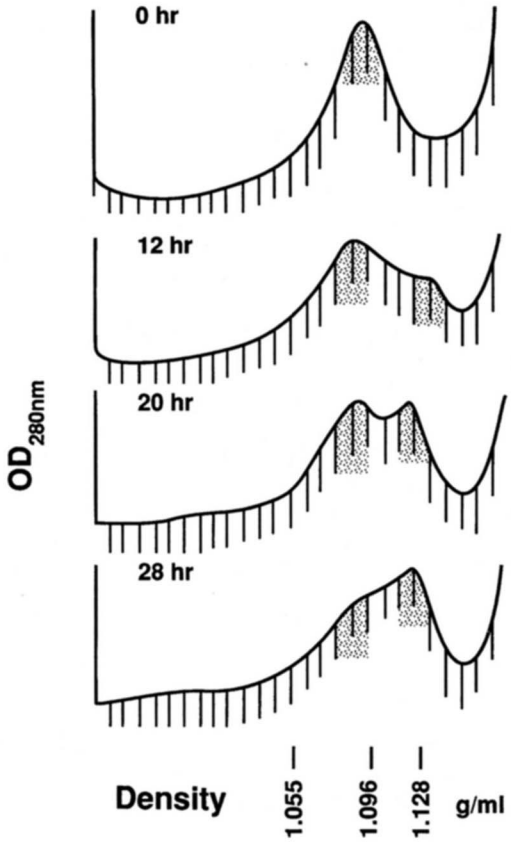

Fig. 7. Density profile of mouse lipoproteins at different time points after the induction of the acute phase response. One-ml samples of plasma pooled from 5 mice at each time point were subjected to equilibrium density gradient ultracentrifugal flotation in a 3–20% NaBr gradient. Density of the fractions is indicated on the ordinate.

Possible mechanisms for decline in plasma HDL

The decline in plasma HDL cholesterol and apoA-I during the APR could result from a decline in the synthesis of apoA-I, from an increase in catabolism of HDL

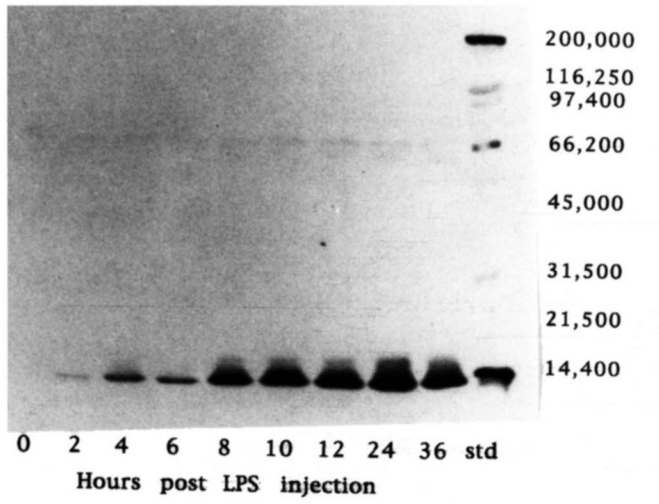

Fig. 8. Western immunoblot of mouse plasma with antibody against recombinant mouse SAA. One-μl samples of plasma pooled from 5 mice at each time point was loaded to a 10–20% gradient SDS PAGE.

and apoA-I, or from a combination of both. From the early time course it is clear that there is an initial decline in HDL cholesterol and apoA-I before any hypertriglyceridemia or SAA is evident in the plasma, suggesting that these two factors may not participate in the early decline of HDL. This raises the possibility that an early reduction in apoA-I synthesis could account for the decline in plasma HDL during the APR, and possibly be responsible for the modest reduction in plasma apoA-I concentration in the mice, though this is not directly assessed in this study. Our preliminary results in baboons indicated there is a reduction in baboon hepatic apoA-I mRNA within 6 h of the induction of the APR. Decrease of apoA-I mRNA had been reported by Morrone et al. (35) in Hep3B cells cultured in the presence of macrophage-conditioned medium, an acute phase inducer.

On the other hand, as the APR evolves, both the hypertriglyceridemia and increasing concentrations of SAA could result in changes in the surface conformation of the HDL and in labilization of apoA-I, with increased catabolism of the latter. Triglyceride enrichment of HDL and labilization of apoA-I have been suggested as the responsible mechanism for low plasma HDL in mice transgenic for the simian CETP gene (36). A shift in the equilibrium between HDL apoA-I and the "free" apoA-I may result in an increased clearance of the latter in the kidney (37). In cynomolgus monkeys the interference with the action of lipoprotein lipase by antibody infusion results in a rapid decline in plasma apoA-I, much of which is cleared in the kidney (38). It has been reported that smaller HDLs are removed from the plasma more rapidly than larger apoA-I-containing HDL (39). Preliminary evidence in our laboratory suggests that SAA-containing HDL is rapidly cleared from the plasma. It is not yet clear whether SAA-containing HDL is recognized physiologically as a large HDL, as detected by non-denaturing gradient gel electrophoresis, or as small HDL as detected by electron microscopy. The reduction in the size of HDL as a result of the action of hepatic lipase on HDL triglyceride is probably a minor factor in the rabbit, which is hepatic lipase-deficient (40). It is notable that the rabbit control HDL has a relatively high triglyceride content (Table 2).

The labilization of apoA-I in triglyceride-enriched HDL of baboons and rabbits may play an important role in the catabolism of HDL. This is suggested by the significant increment in lipoprotein-poor apoA-I in the plasma of rabbits and baboons at the peak of the APR. Both the triglyceride enrichment of HDL and, perhaps more significantly, the displacement of apoA-I by the accumulating SAA may contribute to the apoA-I labilization. As the mouse lacks CETP, HDL triglyceride enrichment would not be expected even were there to be an increase in plasma triglyceride. When hypertriglyceridemia is induced in mice by overexpression of apoC-III, plasma HDL cholesterol is only dramatically decreased when CETP and human apoA-I are co-expressed at elevated levels (41), implying a role for triglyceride enrichment of HDL in its decline. The role of triglyceride enrichment of HDL, absent in mice, in facilitating the displacement of apoA-I by SAA is yet to be determined. It remains to be established whether murine SAA has the same propensity to displace murine apoA-I as is the case in the rabbit, baboon, or human. This is the subject of current investigation.

Formation and significance of the SAA-HDL

The SAA-HDL (peak 3) is an unusual lipoprotein with a very modest core (only 15% of particle components) and unusually high protein content. As expected, it behaves like a protein-rich, relatively dense lipoprotein and, although it travels in an equilibrium density gradient like an HDL_3 type particle, its size on non-denaturing gradient gel electrophoresis is much larger (r = 5.95 nm), more like an HDL_2 particle. Paradoxically, its size as determined by electron microscopy is small (r = 4.2 nm). Using the composition data of peak 3 (Table 2) and radius as determined by gel electrophoresis, there appear to be more than 20 SAA molecules per particles. We cannot explain the small size noted by negative staining electron microscopy. This paradox could be accounted for by a spoke-like arrangement of SAA molecules on the surface of HDL radiating out from the core. It has been reported that the first 11 amino acid terminal sequences of SAA are important for binding to HDL (42, and B. Kluve-Beckerman, personal communication), so that only the terminal amino acid segments may be associated with the HDL surface. The spokes could be hydrodynamically active in the gel electrophoresis separation systems while the phosphotungstic acid used in the negative staining may permeate to the lipoprotein core surface.

Recent data on acute phase HDL have pointed to the importance of the remodeling of HDL. Acute phase HDL has essentially no anti-oxidant effect on the monocyte transmigration assayed in reconstructed model endothelial matrix system (43). Whereas normal HDL containing paraoxonase and the platelet activating factor (PAF) acetyl hydrolase functions as an antioxidant preventing the cell-mediated oxidation of added LDL, acute phase HDL has lowered levels of paraoxonase and PAF acetyl hydrolase. Whether the SAA of the APR HDL or the hypertriglyceridemia of the APR and the associated triglyceride enrichment of HDL contribute to the loss of paraoxonase and PAF acetyl hydrolase is yet to be established. Hypertriglyceridemia could be a risk factor in atherogenesis by labilizing the paraoxonase and PAF acetyl hydrolase of HDL, either as a constitutive

destabilization or by accentuating the sporadic influence associated with short term APR through the course of a lifetime.

The authors wish to thank Miss Yimei Chen of the Laboratory for Image Analysis and Electron Microscopy of the University of Chicago for technical assistance in the preparation of the electron micrographs. This work is supported by HL-15062 (Specialized Center of Research in Atherosclerosis), AHA-96015880 (Formation and Interaction of Acute Phase HDL), and HV-53030 (Nonhuman Primate Models of Arteriosclerosis and Hypertension) and HL-28972 (Diet and Genotype in Primate Atherosclerosis) for the baboon work.

Manuscript received 25 July 1996 and in revised form 30 September 1996.

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