Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins

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Abstract Longitudinal studies were carried out in the rabbit model to determine alterations in the concentration and density distribution of plasma lipids and apolipoproteins during the acute phase response (APR) characterized by elevated levels of C-reactive protein (CRP) and serum amyloid A (SAA). Twelve hr after the intramuscular injection of croton oil, SAA was detectable in high density lipoprotein (HDL). At the height of the response (72 hr), HDL decreased while SAA became the major HDL apoprotein, up to 80% of the proteins in the higher density fractions. The SAA-enriched particles became denser (density of HDL_3) but larger (size of HDL_2), had slower electrophoretic mobility, and were depleted in apoA-I, cholesterol, triglyceride, and phospholipid. HDL-cholesterol decreased and was redistributed to other fractions while apoA-I disappeared from the circulation. During this time plasma triglycerides increased 6- to 10-fold while plasma cholesterol and phospholipids showed minimal changes. ApoB increased 5- to 6-fold while the apoB-containing particles shifted to higher density resulting in elevated IDL and then LDL during recovery. VLDL ($d < 1.006$ g/ml) increased and acquired 30–40% of the plasma triglycerides, cholesterol, phospholipid, and apoB. SAA also increased in VLDL while apoE decreased.—**Cabana, V. G., J. N. Siegel, and S. M. Sabesin.** Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *J. Lipid Res.* 1989. **30:** 39–49.

Supplementary key words VLDL • LDL • HDL • apoB • apoE • apoA-I • inflammation • C-reactive protein • serum amyloid A

The acute phase response (APR) is a systemic reaction to infectious and noninfectious tissue destructive processes. Multiple physiologic adaptations occur including changes in the hepatic synthesis of a number of plasma proteins termed acute phase reactants (1). In man and rabbit, the two acute phase reactants, C-reactive protein (CRP) and serum amyloid A (SAA), increase greater than 1000-fold in concentration within 48 to 72 hr following inflammatory stimulation and the rapid, reversible changes of these proteins have been used to monitor the clinical course of certain diseases (2–4). Both CRP and SAA are known to interact with lipoproteins; CRP binds to triglyceride-rich and apoB-containing particles (5–7) while SAA circulates mostly with HDL_3 (8). Since plasma lipoproteins transport lipids, it may be expected that the interaction of acute phase proteins with plasma lipoproteins may have important metabolic consequences during acute illnesses when host-dependence on lipid for fuel is increased (9–11).

In our previous studies in the rabbit model (12) and in human plasma undergoing acute phase response (13, 14), marked changes in plasma lipoprotein patterns coincided with the elevations of CRP. Several studies have reported variations in the lipid and apolipoprotein composition of plasma lipoproteins during conditions that could promote APR such as microbial (15, 16) and parasitic (17) infections, myocardial infarction (18, 19), acute pancreatitis (19), burn injury (20), and other tissue destructive processes. Most of these studies were not conducted in the context of the APR; thus, differences in the time of sampling and the use of control subjects who were undergoing the APR from surgery, trauma, and other acute illnesses produced conflicting observations.

This report describes the sequential variations in the concentration and density distribution of plasma lipids and apolipoproteins occurring at different stages of the APR from induction to recovery. Importance of the time of sampling is discussed. The rabbit model has been chosen because its CRP and SAA structure and kinetics of response (3, 21), its lipoprotein structure (22), transfer

Abbreviations: APR, acute phase response; AP, acute phase; CH, cholesterol; CRP, C-reactive protein; IEF, isoelectric focusing; LPDF, lipoprotein-deficient plasma fraction; PAGE, polyacrylamide gel electrophoresis; SAA, serum amyloid A; TG, triglyceride; TMU, tetramethylurea; HDL, high density lipoprotein; LDL, low density lipoprotein; IDL, intermediate density lipoprotein; VLDL, very low density lipoprotein.

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protein activities (23, 24), and its ability to develop atherosclerosis (25, 26) are closer to the human characteristics than other laboratory animal models.

MATERIALS AND METHODS

Plasma samples

Rabbit plasma was obtained from young female (3–5 months old) New Zealand white rabbits obtained from commercial sources. The animals were housed in standard cages, and fed synthetic rabbit chow ad libitum. The acute phase was induced by the injection of 1% croton oil (v/v) (mineral oil, 2 ml/kg) at five intramuscular sites. Control animals were injected with pyrogen-free isotonic saline. Blood samples from nonfasting rabbits 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 enzyme inhibitor phenylmethylsulfonyl fluoride (0.001 M in methanol) and anti-bacterial agents (0.5 μg gentamycin sulfate, 50 μg sodium azide, 1 μg chloramphenicol per ml of plasma) and used within a week.

Lipoprotein fractionation

VLDL (d < 1.006 g/ml), IDL (d 1.006–1.019 g/ml), LDL (d 1.019–1.063 g/ml), and HDL (d 1.063–1.21 g/ml) were isolated by sequential ultracentrifugal flotation using procedures reviewed by Schumaker and Puppione (27). Alternatively, a single-spin density gradient ultracentrifugal flotation technique was used. In this procedure, 2 ml plasma was layered at the interface of a 3–20% NaBr gradient and centrifuged in a Beckman SW41 Ti rotor for 66 hr at 20°C. With this gradient the lipoproteins banded between d 1.006 and d 1.25 g/ml. The densities were determined from a solution blank included in the same run. Fractions were collected using an ISCO gradient collector with UV monitor (Instrument Specialties Co., Lincoln, NE), dialyzed in Tris-buffered saline (10 mM Tris, 150 mM NaCl, 0.01% EDTA, 20 mM NaN_3, pH 7.4) and used for the lipid and apoprotein analyses.

Lipoprotein and lipid assays

Lipoprotein electrophoresis was conducted on a Corning agarose electrophoretic system (Corning Medical and Scientific, Palo Alto, CA) for 30 min in 50 mM Tris-barbital buffer, pH 8.6. The films were dried and stained with Fat Red 7B.

Concentrations of TG, total and free CH, HDL-CH, and choline-containing phospholipids were assayed using enzymatic kits (Wako Pure Chemical Industries, Dallas, TX). A reference pool was included in each assay to monitor the inter- and intra-assay variations which were 1.4–2% and 4–8%, respectively.

Polyacrylamide gel electrophoresis

Isoelectric focusing (IEF) of gels prepared in 8 M urea with pH 4–6.5 ampholines (LKB, Sweden), either in tubes or slabs, was a modification of the procedure published by Kashyap et al. (28). Based on protein concentration, 250 μg of VLDL or IDL and 30 μg of HDL, mixed with an equal volume of tetramethylurea (TMU) were used. Electrophoresis was conducted for 1 hr at 110 V, then 3.5 hr 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 a Tris-buffered solution (10 mM Tris, 1% SDS, 10% sucrose, 1 mM EDTA, and 0.02 mg/ml bromphenol blue, pH 8.0) to be used for second dimension electrophoresis, or stained with Coomassie G250 (0.1% stain, 5% perchloric acid) and stored in 7% acetic acid.

Second dimension gels for molecular weight determinations were 12% acrylamide slabs prepared with 0.2% SDS based on the procedure of Weber and Osborne (29). Electrophoresis was conducted at 120 V with a Tris–acetate buffer, pH 7.4 (40 mM Tris base, 20 mM Na acetate, 2 mM EDTA), until the tracking dye moved to about 1 cm from the bottom. The gels were stained with Coomassie R250 (0.25% stain, 25% isopropanol), destained by diffusion into 25% isopropanol–10% acetic acid, and stored in 7% acetic acid.

To determine the particle size and radius of the lipoproteins, commercially purchased gradient gels (Pharmacia Fine Chemicals, Piscataway, NJ) were used, 2–16% gels for the larger fractions (LDL, IDL, VLDL) and 3–30% gels for HDL. Electrophoresis was carried out on a Pharmacia Electrophoresis Apparatus (GE 4-II) at 125 V, 10°C for at least 18 hr with a buffer containing 90 mM Tris base, 80 mM boric acid, 3 mM EDTA, and 3 mM NaN_3. Thirty μg protein was applied as a mixture, by volume, with three parts sample and one part solution of 40% sucrose with 0.01% bromphenol blue. A mixture of reference proteins (HMW Calibration Kit, Pharmacia) consisting of thyroglobulin (8.50 nm), ferritin (6.10 nm), catalase (5.20 mM), lactate dehydrogenase (4.08 nm), and bovine serum albumin (3.55 nm) was included in each gel. The gels were stained with Coomassie G250 in perchloric acid as described above.

Gel filtration chromatography

HDL prepared from normal or acute phase plasma were extensively dialyzed in 150 mM NaCl, 0.05% EDTA, pH 7.4, before delipidation in ice-cold ethanol–ether 3:2 according to the procedure of Scanu and Edelstein (30). The apoHDL was dissolved in Tris-buffered urea (8 M urea, 100 mM Tris, 0.02% NaN_3) pH 8.0, before chromatography on a Sephadex G-200 column. Usually, 50 mg protein was applied to two Sephadex G200 columns (2.5 × 90 cm) in tandem. Chromatography was performed at 4–7°C. The protein peaks monitored at 280 nm were pooled, dialyzed in 5 mM NH_4HCO_3, and stored lyophilized. In this proce-

dure the apoA-I, apoA-II, and SAA were resolved as discrete peaks. The SAA, however, was mixed with apoCs and needed further purification.

Antibody preparation

Antibody against rabbit apoA-I was prepared in goats using purified apoA-I as immunogen, following procedures we have previously published (31). Antibody against rabbit apoB was similarly prepared using twice-washed, narrow-cut LDL (d 1.030–1.050 g/ml) as immunogen (32).

Antibody against rabbit SAA was prepared using SAA purified by gel filtration chromatography as outlined above. After chromatography, the SAA was separated from the apoCs by IEF as described above. The protein bands were visualized with 4 M sodium acetate (33) and the two major isoforms of SAA were cut out, emulsified in adjuvant, and used as immunogen based on the protocol used for apoA-I. Specificity of the antisera was tested by Ouchterlony gel diffusion against purified apoproteins or by immunoblotting of proteins transferred from SDS or IEF gels.

Quantitative immunoassays

Quantitations of rabbit apoA-I and apoB were based on the procedures for human apoproteins as previously published (31, 32). Using purified rabbit apoA-I or LDL d 1.030 –1.050 g/ml, a plasma pool was calibrated, stored at – 80°C, and used as standard in the day-to-day assay to monitor the inter- and intra-assay variations (kept within 6%). A new pool was calibrated every 3 months to minimize the effect of storage upon the apoprotein concentrations. The lower limit of sensitivity of the assays was 25 μg/ml. CRP was similarly quantitated using purified CRP as a standard as described previously (6).

Protein assay

Concentration of proteins in the lipoprotein fractions were assayed by a modified Lowry procedure with SDS as outlined by Markwell et al. (34). A commercially prepared bovine serum albumin standard for protein quantitation was used to ensure reproducibility of the assay.

RESULTS

Lipid variations

Electrophoretic pattern. Confirming our previous results (12), agarose electrophoresis of homologous pairs of rabbit plasma (**Fig. 1A**) showed that distinct α, β, and pre-β mobility HDL, LDL, and VLDL, respectively, were evident in the 0-hr plasma. Forty eight hr after the injection of croton oil, the pre-β and β fractions fused into a broad, deeply staining band due to the increases in VLDL, d < 1.006 g/ml (Fig. 1B). The α mobility HDL disappeared and was replaced by particles with slower electrophoretic mobility (Fig. 1A,C). This pattern was consistently observed in all rabbits tested (n = > 50). Depending on the severity of the response, the patterns returned to baseline as early as 2 weeks when the acute phase resolved. These gross alterations of lipoprotein pattern were a reflection of the marked changes in the lipid concentrations of whole plasma and the different lipoprotein fractions.

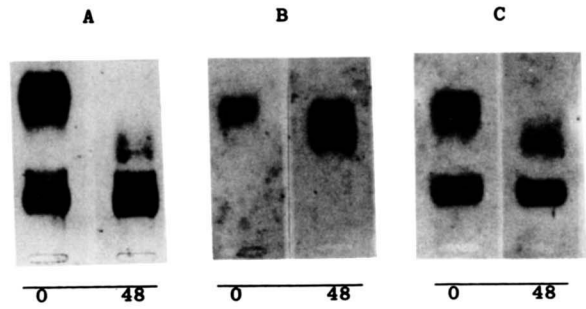

Fig. 1. Electrophoretic pattern of rabbit lipoproteins. Plasma (A), VLDL (B), and the LDL-HDL containing d > 1.006 g/ml fraction (C), obtained from the same rabbit before (0 hr) and after (48 hr) the induction of inflammation, were subjected to electrophoresis in agarose gel (barbital buffer, pH 8.6) and stained with Fat Red 7B. The pattern from the animal shown above was consistent in other rabbits analyzed (n = > 50).

Triglyceride, cholesterol, and phospholipid concentrations. During the development of the APR plasma, TG increased at least 8- to 10-fold and up to 15-fold in some animals (n = 68, **Fig. 2**). After an initial decrease at 6–12 hr, a steady increase followed until peak levels were reached at 48–72 hr. The distribution of TG in the different fractions varied during the development of the APR. Data from a single animal in which the lipid composition was analyzed at 12-hr intervals after the induction of inflammation are shown in **Table 1**. In the preinjection plasma, about 25% of plasma TG was carried by the VLDL, 15% by the IDL, < 10% by the LDL, and about 45% by the HDL. As the APR progressed, a major portion of plasma TG was carried by the VLDL (30–40%) with significant increases in the IDL and LDL. TG carried by the HDL decreased. Although the generation of multiple samples by density gradient ultracentrifugal flotation precluded the measurement of the lipid composition in the different lipoprotein fractions at all time points in all animals during the course of the APR, the distribution of lipids was consistent when samples were analyzed in paired 0 and 48/72-hr time periods (n = 15).

In contrast to TG, the CH level of whole plasma showed moderate increases during the APR (Fig. 2, n = 68). Analyses of the CH concentration in the different fractions (Table 1B) showed that in the preinjection plasma about 80% of CH was carried by the HDL, mainly by the HDL_2 fraction. As the APR progressed, HDL-CH decreased rapidly (**Fig. 3**), so that by 72 hr only about 10% of the plasma CH was in HDL (Table 1B). The rapid decrease in HDL-CH was observed in every acute phase rabbit analyzed in this study or in other projects (n = > 50) and was

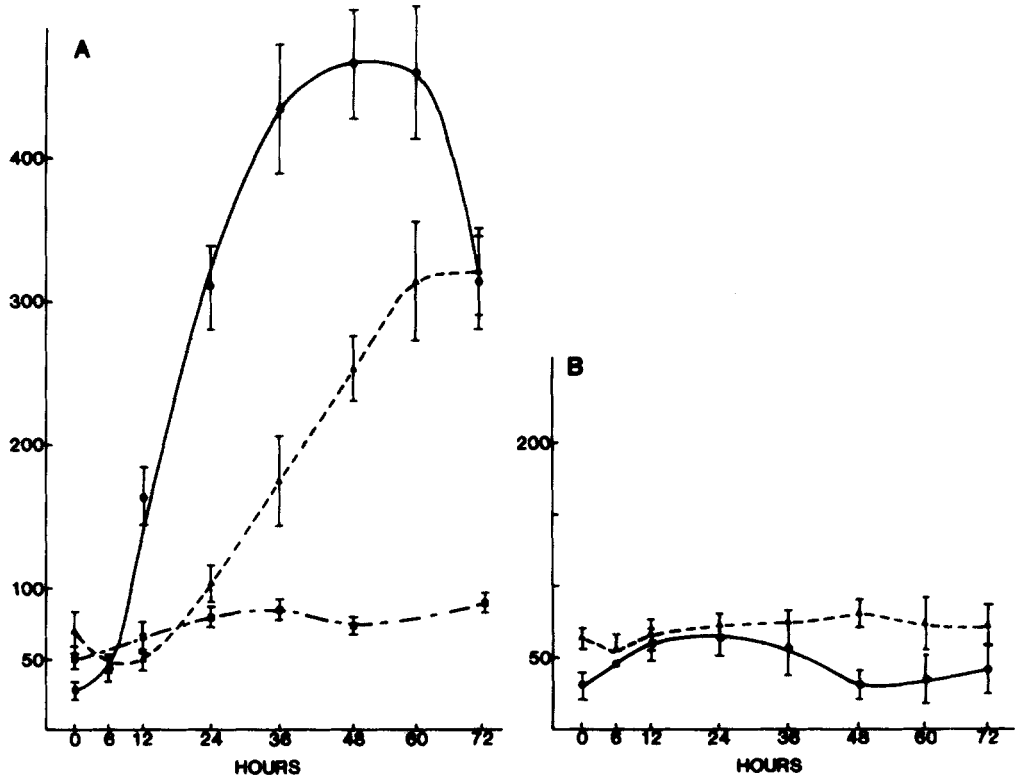

Fig. 2. CRP, TG, and CH levels during the acute phase response. Values, expressed as mean ± SEM, of CRP (●—●), TG(▲---▲), and CH (■- - -■) were from rabbits bled at the indicated time intervals following the injection of croton oil (A, n = 68) or saline (B, n = 10).

consistent whether measured by magnesium chloride–phosphotungstic acid precipitation of apoB-containing lipoproteins in whole plasma (HDL-CH kit) (Fig. 3) or by direct measurement of CH in the HDL fraction obtained by ultracentrifugal flotation (Table 1). While HDL-CH decreased, CH in the VLDL, IDL, and LDL increased with the major fraction (about 30%) carried by the VLDL (Table 1B).

Although the density distribution of CH changed, the ratio of free to esterified CH remained constant. Furthermore, LCAT activity was not significantly altered during the APR (data not shown) suggesting that esterification of CH was unaltered despite decreased HDL concentration.

Phospholipids increased 1- to 2-fold with kinetics similar to the TG concentrations, i.e., decrease at 6–12 hr before increasing to peak levels at 48–72 hr (data not shown). Analyses of the fractions (Table 1C) showed that in the preinjection plasma, about 60–70% of the choline-containing phospholipids was found in HDL. During the development of the APR, the phospholipids were equally distributed in the VLDL, IDL, and LDL representing a 5- to 6-fold increase in these fractions with a proportionate decrease in the HDL.

Apolipoprotein changes

Plasma apoA-I and apoB levels. Plasma concentrations of apoA-I and apoB were determined at various times after the induction of the APR (**Table 2**, n = 6). The level of apoA-I in preinjection plasma was 3-fold higher than apoB, indicating the high level of HDL in rabbits. During the development of the APR, apoA-I progressively decreased reaching the lowest level at 72 hr. The apoA-I slowly increased thereafter. In contrast to apoA-I, apoB progressively increased after a lag of 6–12 hr. By 72 hr apoB was 5-fold higher than apoA-I, resulting in a low apoA-I/apoB ratio. The apoB remained elevated and failed to return to baseline levels within the 6-week testing period in this group of six animals.

Density distribution of apoA-I and apoB. Analyses of apolipoproteins in the fractions obtained by density gradient ultracentrifugal flotation (**Fig. 4**, n = 6) showed that two pools of apoA-I were present in the preinjection plasma: one associated with HDL with peak levels at d 1.090 g/ml (about 90%) and one in the lipid-poor d > 1.21 g/ml bottom fraction. As the APR progressed, the HDL-associated pool decreased while shifting to the higher density region. Within 72 hr > 60% of the HDL-associated apoA-I had disappeared. During this time the remaining apoA-I-containing particles peaked at d > 1.15 g/ml. The lipid-poor pool remained constant. After 72 hr the HDL-associated apoA-I increased while shifting to a lower density until baseline conditions were reached.

In contrast to apoA-I, low levels of apoB were present

TABLE 1. Distribution of lipids in the different lipoprotein fractions

Lipid Stimulation	Time after Injection	Plasma	VLDL	IDL	LDL	HDL_2	HDL_3	LPDF
	hr	*mg/dl*			*% of plasma concentration*			
A: Triglycerides:								
Croton oil	0	66	22	15	7	45	9	3
	8	43	18	9	10	39	17	8
	12	69	19	18	17	25	14	7
	24	298	38	19	16	16	7	5
	48	643	29	22	23	19	7	5
	72	577	30	23	24	18	3	2
None								
	0	30	26	15	2	32	0	0
	8	28	25	12	12	39	8	4
	12	34	23	18	10	36	9	5
	24	35	28	15	9	40	5	3
	48	53	21	16	7	33	4	19
	72	56	14	14	20	38	9	16
B: Cholesterol:								
Croton oil								
	0	28	6	6	2	63	18	2
	8	25	7	1	1	46	21	3
	12	28	12	11	12	32	27	6
	24	113	31	24	16	19	7	4
	48	94	32	25	22	12	5	3
	72	94	33	26	26	9	9	2
None								
	0	30	3	4	6	73	11	3
	8	28	4	3	6	72	13	2
	12	34	5	8	8	62	14	3
	24	35	9	8	2	69	6	5
	48	53	9	14	5	63	5	5
	72	56	3	7	11	65	9	5
C: Choline-containing phospholipids:								
Croton oil	0	104	4	4	3	48	16	25
	8	85	4	2	3	44	22	25
	12	79	7	6	7	32	30	19
	24	153	22	14	11	21	12	20
	48	241	22	21	19	21	7	13
	72	224	23	20	20	20	6	11
None								
	0	94	3	3	3	65	8	19
	8	113	2	3	4	57	15	19
	12	92	3	4	3	60	13	18
	24	106	4	4	4	55	12	22
	48	130	4	5	3	55	8	25
	72	110	2	5	5	56	9	26

The values shown were from a single acute phase and control animal. Lipids were analyzed in the fractions obtained from single-spin ultracentrifugal flotation of whole plasma in a 3–20% NaBr gradient. Similar data were obtained in animals analyzed as paired 0- and 48/72-hr time periods ($n = 15$).

in the preinjection plasma and were found mostly in the d 1.019–1.063 g/ml LDL region. After a lag of about 6–12 hr, apoB progressively increased in concentration while shifting to lower density. At the peak of the response (72 hr), almost half of apoB was found in the VLDL d < 1.006 g/ml (Fig. 4 and Table 2). The remaining apoB-containing particles were distributed in a trailing peak between d 1.006 and 1.063 g/ml. The apoB remained elevated but shifted in position from VLDL to the higher density fractions resulting in an elevated IDL, and then LDL peaked later in the recovery phase.

Apoprotein composition of acute phase HDL. The apoprotein

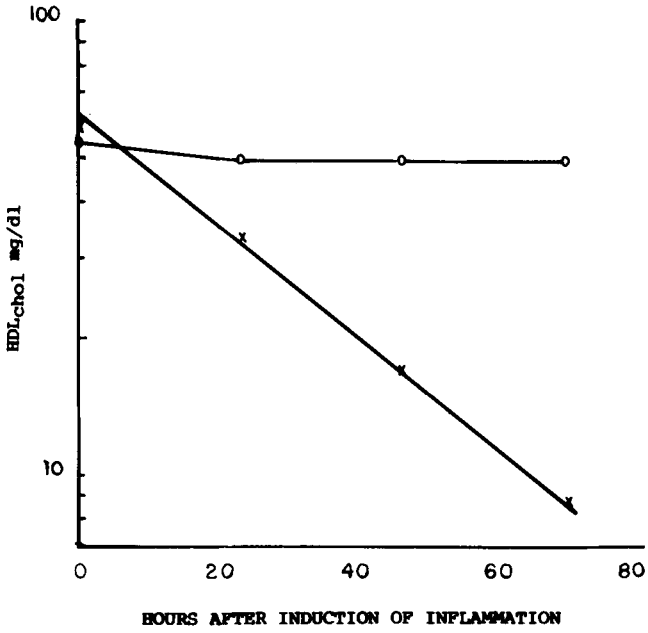

Fig. 3. Changes in HDL-CH concentration during the acute phase response. This figure shows the HDL-CH determined from a single rabbit injected with croton oil (x) or saline (O) obtained at 24-hr intervals. Other animals analyzed at 0 and 48/72 hr (n = > 50) showed up to 90% decrease in HDL-CH.

content of rabbit HDL was further analyzed by column chromatography. Results from a single control (solid line) and 72-hr acute phase (dotted line) plasma are shown in **Fig. 5**. Identical chromatography profiles were obtained from three APR (72 hr) and four pools of normal plasma used in apolipoprotein isolation. The compositions of five peaks (arrows) were identified as follows. Starting from the left, peaks 1 and 2 contained large molecular weight proteins and aggregates and were not further characterized; peaks 3,4, and 5 contained, respectively, apoA-I, apoA-II, and apoC/SAA with other low molecular weight proteins.

As expected, apoA-I was the major apoprotein of normal HDL (Fig. 5, solid line). Analyzed by computerized digitization of the protein peaks and expressed as percentage of the total HDL apoproteins, the normal HDL had 60% apoA-I (peak 3), 5% apoA-II (peak 4), and about 15% apoC/SAA (peak 5). Baseline levels of peak 5 differed depending on the acute phase status of the individual animals. The AP-HDL (Fig. 5, broken line) had only 17% apoA-I, 5% apoA-II, and 60% peak 5. The increase in peak 5 was due to SAA (Figs. 5 and 6) which was detectable by 12 hr (data not shown) and continued to increase as the APR progressed, becoming the major apoprotein constituent of HDL by 72 hr.

Although the total apoA-I concentration decreased, there were no differences in the proportion of apoA-I isoforms present in the control and the AP-HDL. **Fig. 6** shows a representative two-dimensional PAGE of a control and a

TABLE 2. ApoA-I and apoB levels in rabbits

Time after Croton Oil Injection	ApoA-I	ApoB[a] d > 1.006 g/ml	ApoB[a] VLDL	ApoA-I/ApoB[b]
	mg/dl	*mg/dl*		
0 hr	83	25	3	3.32
6 hr	76	21	2	3.62
24 hr	52	54	4	0.96
48 hr	21	88	40	0.24
72 hr	19	106	46	0.18
8 days	33	114	49	0.29
15 days	50	127	24	0.39
21 days	45	109	8	0.41
42 days	51	85	11	0.60

Values were determined from a plasma pool of six animals by radial immunodiffusion.

[a]ApoB was quantitated in the VLDL and the d > 1.006 g/ml fraction since at the height of the response (as shown in Fig. 4) the apoB produced a trailing peak with no discrete LDL peak.

[b]The apoA/-I/apoB ratios were determined from the total plasma values.

72-hr AP-HDL. Identical proportion of apoA-I isoforms was observed in every control or 48/72-hr AP HDL analyzed (n = 10). Based on the nomenclature used by Zannis, Breslow, and Katz (35) for human apoA-I and numbered from the most basic form, both the normal and the AP-HDL had isoform 5 as the most abundant, followed by iso-

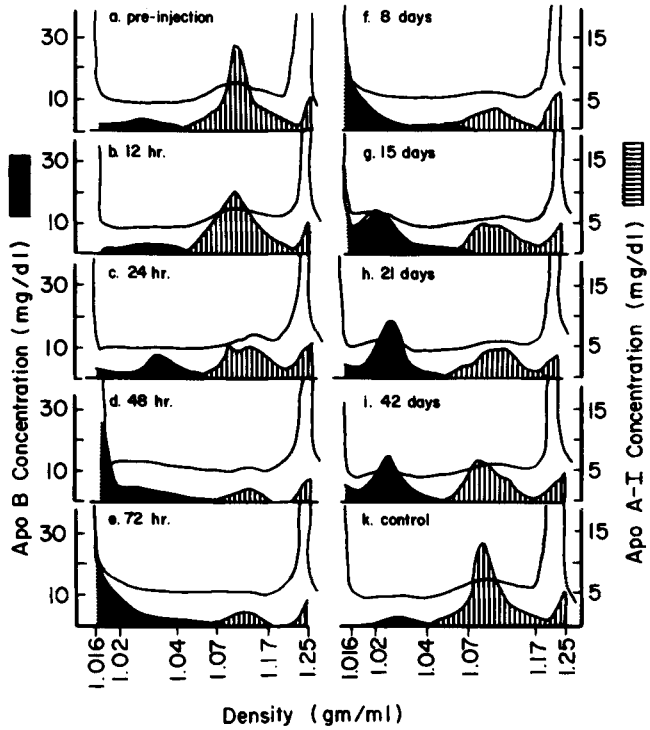

Fig. 4. Density distribution of apoA-I and apoB. ApoA-I and apoB were quantitated by radial immunodiffusion in the fractions obtained by density gradient ultracentrifugal flotation of plasma at the indicated time intervals (n = 6). The solid line represents absorbance at 280 nm.

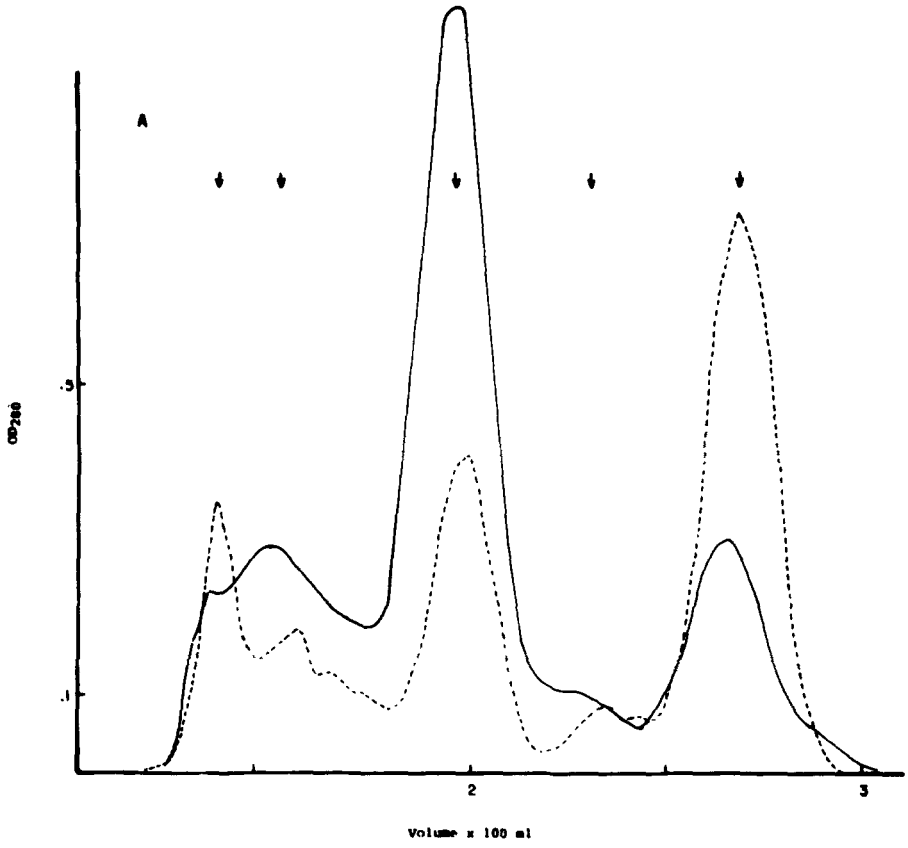

Fig. 5. Gel filtration chromatography profile of apoHDL. HDL from a representative control (solid line) or a 72-hr acute phase (broken line) rabbit plasma was delipidated by ether–ethanol extraction and chromatographed on Sephadex G-200 in Tris-buffered 8 M urea. The arrows correspond to the peaks pooled and analyzed for apoprotein content (left to right, peaks 1–5). Identical profiles were obtained from three acute phase response (72 hr) and four pools of normal plasma used in apolipoprotein isolation.

form 2. There was no increase in the immature apoA-I isoform in the AP-HDL.

Remodeling of HDL particles associated with elevated SAA. Gradient gel electrophoreses (4–30% acrylamide) of HDL fractions obtained by ultracentrifugal flotation in a 3–20% NaBr gradient showed that the higher density fractions of AP-HDL (d > 1.12 g/ml) (**Fig. 7B**, fractions 6 and 7) were larger (5.3–5.5 nm) than the corresponding fractions obtained before the induction of the APR (4.5 nm). The pattern was consistent in other control or 48/72-hr AP-HDL analyzed (n = 6). Thus, the size of the particles was comparable to the size of HDL_2 but had the density of HDL_3. Fraction 7 had the most abundant SAA content while the apoA-I level was below the limit of detection by the immunoassay. Estimates from the scanning of SDS gels showed that in fraction 7 up to 80% of the protein content was SAA, suggesting the existence of particles with SAA alone without apoA-I. Precise quantitation of SAA awaits the development of an immunoassay. Estimates of apoA-II in these fractions were not possible due to the low level of this apoprotein in rabbits.

VLDL apoproteins. VLDL apoproteins were analyzed by IEF and two-dimensional PAGE. At the height of the response, SAA became a major constituent of AP-VLDL. Depending on the severity of the response (n > 10), up to 60% of the TMU-soluble VLDL apoproteins were SAA. Three to four SAA isoforms were usually present. Some of the SAA isoforms overlapped in pI with the apoEs and could be resolved by two-dimensional PAGE (**Fig. 8**). Analyses of the other VLDL apoproteins showed that at the height of the response apoE decreased while the apoCs remained constant. Whether total apoE concentration was altered had not been determined, awaiting the development of immunoassay to rabbit apoE. Gradient gel electrophoresis of whole VLDL (2–16% acrylamide) showed abundant apoB-100 and undetectable apoB-48.

DISCUSSION

In conclusion, during the acute phase response in rabbit plasma, the HDL levels decreased while the particles became denser but larger, had slower electrophoretic mobility, and were depleted in lipids and apoA-I but enriched in SAA. The VLDL increased and became enriched with TG, CH, phospholipid, apoB, and SAA but depleted in apoE.

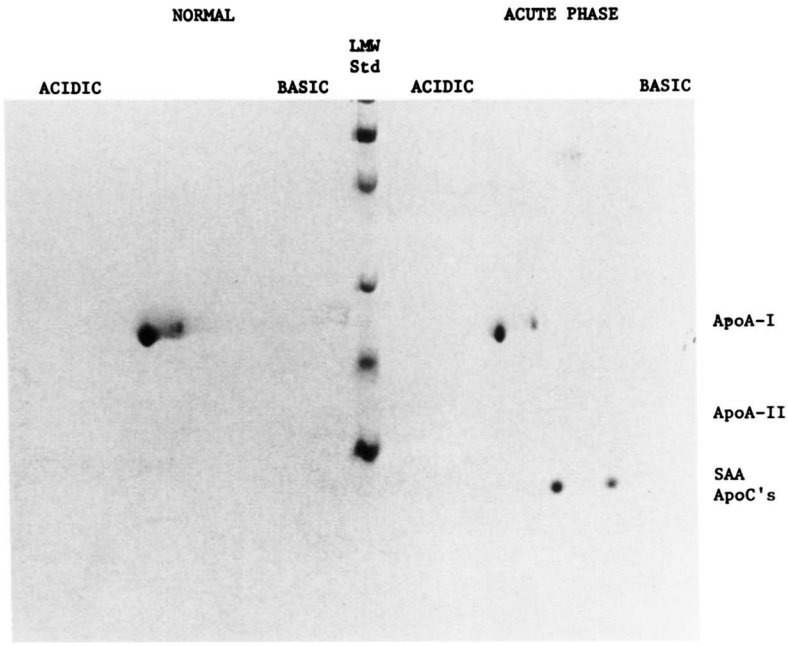

Fig. 6. Two-dimensional PAGE of rabbit apoHDL. First dimension IEF gels (pH 4–6.5) of a normal (left) and a 72-hr acute phase (right) apoHDL were applied to each half of a second dimension SDS-PAGE slab without reducing agent. Basic and acidic refer to the orientation of the first dimension gel. The low molecular weight standards (LMW std) applied between the IEF gels were, from top: 92,500, 66,200, 42,500, 31,000, 21,500, and 14,400. Identical patterns were observed in other control and 48/72-hr acute phase HDL analyzed (n = 10).

The apoB-containing particles shifted to lower density during the development of the acute phase and to higher density during recovery. These results show that, contrary to the suggestions by Pepys and Baltz (3), the proportions and absolute amounts of lipids and apolipoproteins in the different lipoprotein fractions varied during the course of the APR. In these studies, we used croton oil to induce a massive acute phase response. Other agents such as endotoxin

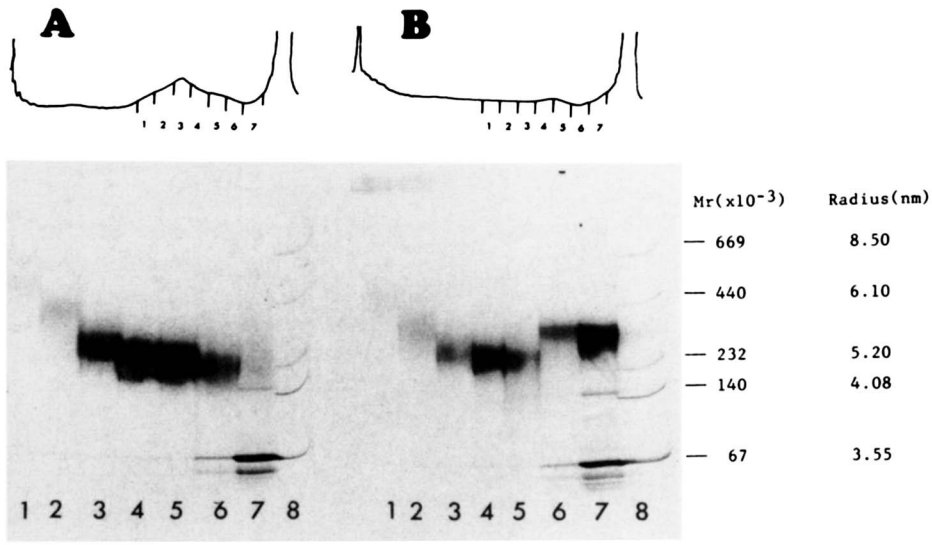

Fig. 7. Gradient gel electrophoresis of whole HDL. Gradient gels (4–30%) were subjected to electrophoresis without SDS. Lanes 1–7 contained the d 1.06–d 1.21 g/ml HDL fractions obtained from single-spin ultracentrifugal flotation runs (top figure) of plasma from a control (A) or a 72-hr acute phase (B) rabbit. Lane 8 contained the reference proteins with the molecular weights and particle size radii shown on the right. Note the larger size of the heavy HDL (fractions 6 and 7) from the acute phase animal. The patterns were consistent in four other rabbits analyzed.

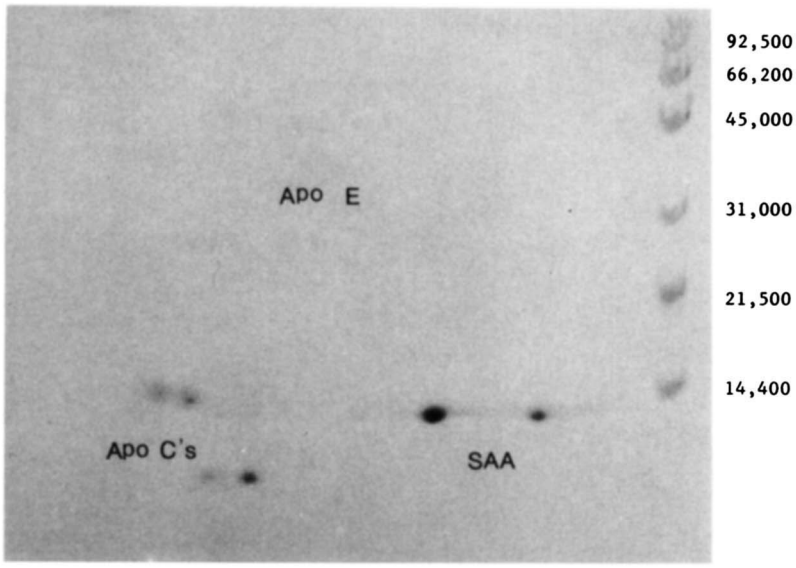

Fig. 8. Two-dimensional PAGE of acute phase rabbit VLDL. A first dimension IEF gel, pH 4–6.5, of rabbit VLDL obtained from a single 72-hr plasma was applied to a second dimension SDS-PAGE slab without reducing agent. The molecular weights of the reference proteins are shown on the right. Similar patterns were observed in other acute phase VLDL (n = 10).

and trauma by accidental bone fracture also induced both the APR and the lipoprotein alterations (Cabana, V. G., et al., unpublished results). Other investigators (36) have reported the induction of SAA and the alterations of the apolipoprotein composition of HDL by such mild stimuli as chair restraint in vervet monkeys. Changes in lipids and other apolipoproteins were not addressed by these authors. These observations and those of others (15–20) point to the participation of lipoproteins in host defense against infectious and noninfectious tissue destructive processes.

The mechanism of lipoprotein alterations during APR is still not known. The decrease in HDL-CH without decrease in the total plasma concentration indicated redistribution of the lipids to other fractions. This was not the case with apoA-I. At the height of the response, > 50% (up to 90% in some animals) of apoA-I disappeared from the circulation. Whether the decrease in apoA-I was due to decreased synthesis or increased catabolism was not determined. The proportion of the mature apoA-I isoforms did not change during the APR, suggesting unaltered maturation of the apoprotein regardless of the total plasma apoA-I concentration. Furthermore, apoA-I synthesis may not be altered during the APR as suggested by our preliminary results (Cabana, V. G., et al., unpublished observations) showing unchanged amounts of apoA-I mRNA in rabbit livers 72 hr after the induction of inflammation. Unaltered apoA-I mRNA levels were observed by Gou-Fen et al. (37) in turpentine-induced acute phase rat liver and intestine. Lowell, Stearman, and Morrow (38), on the other hand, reported a 2-fold decrease in apoA-I mRNA in murine liver 24 hr after the induction of inflammation.

Increased catabolism secondary to enrichment of the HDL with SAA and the remodeling of the particles could account for the low apoA-I levels. Up to 80% of the HDL apoproteins in the higher density fractions were SAA suggesting the existence of HDL particles with SAA alone. Coetzee et al. (39) have shown that SAA displaced apoA-I in vitro and could account for up to 80% of the total HDL apoproteins. Similar large but dense, phospholipid-depleted, triglyceride-rich HDL was isolated by Clifton, Mackinnon, and Barter (40) from myocardial infarction patients suggesting the remodeling of HDL particles in human plasma in vivo. Displacement of apoA-I by SAA in vivo could result in free apoA-I which is catabolized more rapidly. Accelerated catabolism of free apoA-I could be inferred from the results of Shepherd et al. (41) wherein exogenously labeled apoA-I has a shorter half-life than the endogenously labeled apoproteins. This concept is further supported by our preliminary results that show the shift in the density and the accelerated catabolism of ^{125}I-labeled HDL injected into acute phase rabbits; increased radioactivity in the d > 1.21 g/ml plasma fraction suggests increased nonHDL-bound apoA-I in these animals (Cabana, V. G., et al., unpublished observations).

Elevations of plasma TG were also consistently observed in the APR rabbits. As a consequence of the rapid elevations of TG, TG-rich particles of d < 1.006 g/ml increased while lipids and apoproteins including SAA accumulated in this fraction. The cause of the accumulation of TG-rich VLDL particles has not been determined. Hypertriglyceridemia is a commonly observed alteration associated with the APR induced by infection and other agents (17–20) and is usually

attributed to decreased clearance secondary to decreased activity of lipoprotein lipase (17, 42–44), although increased hepatic lipogenesis has also been suggested (45, 46).

Of special interest is the increase in plasma apoB level with a concomitant decrease in the LDL (d 1.019–1.063 g/ml) and increase in the VLDL apoB (d < 1.006 g/ml). While the apoB remained elevated, the apoB-containing particles shifted to higher density resulting in elevated IDL and then LDL during recovery. This shift in density may explain the inconsistent reports of apoB levels during conditions characterized by the APR. Thus, the time of sampling is an important variable in the analyses of lipoproteins during conditions associated with the APR. As a consequence of the elevated apoB, the apoA-I/apoB ratios remained low even during the recovery phase. Since low apoA-I/apoB ratios have been correlated with severity of cardiovascular diseases (47), the contribution of the APR to the level and density distribution of these apoproteins needs to be considered in the evaluation of patients recovering from infectious and noninfectious acute illnesses including myocardial infarction. Whether the increase in apoB is due to increased synthesis or decreased catabolism is not known. The accumulation of apoB-100 suggests hepatic origin, while the shifting density of the apoB-containing particles suggests alterations in the VLDL to LDL catabolic pathway.

An observation that has not been reported previously was the abundant amount of SAA in VLDL during the APR. Depending on the severity or stage of the response, up to 60% of the TMU-soluble VLDL apoproteins was SAA. Whether SAA affected the structural and functional integrity of VLDL has not been determined. Although lipids, apoB, and SAA accumulated in VLDL, the apoE concentration decreased in this fraction. The presence of apoE in other fractions was not observed. Werb and Chin (48) have shown that endotoxin, a potent APR inducer, suppressed apoE synthesis in murine macrophages.

The physiologic significance of lipoprotein alterations during the APR remains to be determined. VLDL transport endogenously produced TG and the increased VLDL-TG supports the concept of increased output to respond to the increased host dependence on lipid for fuel during acute illnesses. The changes in apoB, apoE, and HDL levels may be involved in the cholesterol homeostasis. ApoB binds to cellular receptors and thereby regulates the delivery of cholesterol to extrahepatic tissues. ApoE is also a recognition ligand for cellular receptors and is responsible for the clearance of remnant particles by the liver. HDL functions in the uptake of cholesterol from cells and transfers them via the cholesteryl ester transfer protein complex to the lower density fractions for excretion in the liver. All these changes are compatible with the idea that during acute illnesses delivery of cholesterol to the liver for excretion is altered to allow cholesterol to remain in the tissues where it is needed for repair and regeneration of damaged membranes. These concepts are presently speculative; nevertheless, the rapid but reversible alterations of plasma lipoproteins during the APR provide a novel way to study lipoprotein metabolism and its role in host response to tissue destructive processes.

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