

# Effects of serum amyloid A protein (SAA) on composition, size, and density of high density lipoproteins in subjects with myocardial infarction

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**Abstract** The acute phase reactant serum amyloid A protein (SAA) circulates in plasma as a constituent of high density lipoproteins (HDL). Advantage has been taken of the induction of SAA in human subjects with myocardial infarction to study the effect of SAA on the physical and chemical properties of HDL. HDL were isolated by sequential ultracentrifugation and assayed for chemical composition. Apolipoprotein composition was assessed by SDS polyacrylamide gel electrophoresis. Size distribution of HDL was determined by gradient gel electrophoresis and density distribution by density gradient ultracentrifugation. In studies of 18 subjects with myocardial infarction, SAA accounted for 8–87% (median 52%) of the HDL apolipoprotein. These SAA-enriched HDL had a density comparable to that of normal HDL subfraction-3 (HDL<sub>3</sub>). Their chemical composition differed from normal HDL<sub>3</sub>, however, with a reduced phospholipid (17% vs 24%) and an increased triglyceride (7.7% vs 1.6%) value. When separated by gradient gel electrophoresis, the SAA-enriched HDL were much larger than normal HDL<sub>3</sub>, having a radius of 4.5–5.3 nm that extended well into the size range of HDL<sub>2</sub>; particle size correlated with SAA content. This disassociation between particle density and particle size was also observed with the SAA-enriched HDL isolated from a subject with secondary amyloidosis and also with normal HDL that had been enriched with SAA during incubation *in vitro*. Thus, the presence of high levels of SAA has been found to be associated with phospholipid-depleted particles of a density comparable to HDL<sub>3</sub> but a size larger than normal HDL<sub>3</sub>. — Clifton, P. M., A. M. Mackinnon, and P. J. Barter. Effects of serum amyloid A protein (SAA) on composition, size, and density of high density lipoproteins in subjects with myocardial infarction. *J. Lipid Res.* 1985. 26: 1389–1398.

**Supplementary key words** HDL<sub>2</sub> • HDL<sub>3</sub> • gradient gel electrophoresis • density gradient ultracentrifugation • gel filtration

The serum amyloid protein (SAA) is an acute-phase protein that appears in the plasma in response to a variety of stimuli, including injections of bacterial endotoxin (1), trauma (2), and acute and chronic inflammation (3, 4). As was found in the present study, SAA is also observed

in the plasma of human subjects after acute myocardial infarction (4).

Within the plasma, SAA circulates as a component of high density lipoproteins (HDL) (5, 6) or, more specifically, as a component of the HDL subfraction-3 (HDL<sub>3</sub>) (5, 7, 8). The implications of this enrichment of HDL with SAA are uncertain. Studies in various animals have indicated that its presence has an effect on both the composition and metabolism of the lipoprotein. In mice, for example, it has been found that SAA-enriched HDL are depleted of phospholipids (7), raising the possibility that SAA may have actually displaced a proportion of the phospholipid from the surface monolayer of the lipoprotein. And in metabolic studies performed in both mice (9) and vervet monkeys (10), it has been shown that SAA-enriched HDL are catabolized more rapidly than normal particles. There is, however, virtually no information regarding the metabolism of SAA-enriched HDL in human subjects and surprisingly little information about the effects of SAA on the structure and composition of human HDL. Given both the amphipathic helical nature of SAA (11) and its high affinity for HDL (6, 12), it would be surprising if its presence in humans were not associated with significant changes to the composition and structure of HDL.

This issue has been addressed by examining the composition, density distribution, and size distribution of SAA-enriched HDL isolated either from the plasma of human subjects with acute myocardial infarction or after the incubation of normal plasma *in vitro* with the purified protein.

Abbreviations: SAA, serum amyloid A protein; HDL, high density lipoprotein.

## METHODS

### Subjects

Patients admitted to the coronary care unit at Flinders Medical Centre with a diagnosis of acute myocardial infarction and who had a serum concentration of creatine phosphokinase of 1500 units/l or greater were examined. Eighteen patients (thirteen male and five female) were studied between March and April, 1984. In addition three subjects with secondary amyloidosis were examined. Eight healthy laboratory workers, five male and three female, between the ages of 24 and 42 provided normal plasma.

Subjects were either fasting at the time of blood sampling or had recently eaten a light breakfast. In subsets of both patients and control subjects it was found that the eating of breakfast had no effect on any of the parameters of HDL measured in this study. Twenty ml of blood was collected in tubes containing either heparin or Na<sub>2</sub> EDTA (1 mg/ml). The plasma was separated by low speed centrifugation and stored at 4°C until further analyzed, usually within 1 week. There was no difference in results between samples in heparin or EDTA or between those in which HDL was isolated immediately or after storage at 4°C for up to 2 weeks. Further blood samples were collected from some subjects 10 days after admission.

### Lipoprotein separation

All ultracentrifugation was performed at 4°C and at 143,000 *g* using a 40.3 rotor in a Beckman L5-65 ultracentrifuge (Beckman Instruments Inc., Fullerton, CA). HDL were isolated in the density range 1.063–1.21 g/ml. The HDL subfraction-2 (HDL<sub>2</sub>) was isolated in the density range 1.063–1.125 g/ml and the HDL subfraction-3 (HDL<sub>3</sub>) in the density range 1.125–1.21 g/ml. Densities were adjusted by the addition of KBr (13) and centrifuged for 24 hr at 1.063 g/ml and for 48 hr at 1.125 and 1.21 g/ml. Fractions were washed by a second ultracentrifugation at the higher density. In some studies the 1.21 g/ml supernatant of plasma was isolated by a single 48-hr spin prior to further separation of lipoproteins by density gradient ultracentrifugation or gradient gel electrophoresis (see below). The lipoprotein preparations were exhaustively dialyzed against 0.05 M NH<sub>4</sub>CO<sub>3</sub>, pH 8.0, containing 1 mM Na<sub>2</sub> EDTA.

### Isolation of SAA

SAA was isolated from the serum of subjects with myocardial infarction by the method of Anders et al. (14). Briefly, serum was dialyzed against distilled water, and then formic acid was added to a final concentration of 10%. SAA was then isolated by gel filtration in 10% formic acid on Bio-Gel P60 and Sephadex G-75.

### Incubations

Incubations of normal plasma with pure isolated SAA were performed in a 37°C shaking water bath for 24 hr. Control samples were incubated without SAA. Two incubation schedules were used: 1 ml of plasma with 5 mg of SAA and 3 ml of plasma with 6 mg of SAA.

Incubations were also performed at room temperature for 10 min. One ml of HDL (1 mg/ml) was incubated with SAA in amounts varying from 1.3 mg to 3 mg. The incubation mixture was then subjected to density gradient ultracentrifugation.

### Electrophoretic methods

Apolipoproteins were separated using SDS polyacrylamide gel electrophoresis on 10% slab gels (15). Proteins were visualized by staining with Coomassie Blue R 250. After destaining, the gels were scanned in a laser densitometer (2202 Ultrascan, LKB, Bromma, Sweden) and quantitated using a Hewlett-Packard 3390A integrator. Assuming dye uptake to be proportional to apolipoprotein mass, the ratio of SAA to apoA-I was calculated from the integrated curves. However this method is only semi-quantitative and the ratio obtained was partially dependent on the amount of protein loaded on to the gel.

Urea-polyacrylamide gel electrophoresis was performed by the method of Davis (16) adapted for use with 7.5% slab gels in 8 M urea.

Polyacrylamide gradient gel electrophoresis of HDL was performed using Gradipore slab gradient gels (2.5–27% acrylamide, Gradient Labs Pty. Ltd., Sydney, Australia). Samples containing 30 µg of HDL protein were applied in a volume of 50 µl (40 µl of sample and 10 µl of a solution containing 40% sucrose and 0.01% bromophenol blue) and subjected to electrophoresis at 200 v for 17 hr in a Tris-borate buffer (pH 8.35). The gels were run to complete equilibrium. The Stokes radii of the HDL particles were calculated by reference to standards of thyroglobulin (8.50 nm), ferritin (6.10 nm), lactate dehydrogenase (4.08 nm), and bovine serum albumin (3.55 nm) from a High Molecular Weight Electrophoresis Calibration Kit (Pharmacia Fine Chemicals, Uppsala, Sweden). Gels were fixed in 10% sulfosalicylic acid for 1 hr, stained for 4 hr in 0.04% Coomassie G-250 in 3.5% perchloric acid, and then destained in 5% acetic acid (17). Gels were scanned as described above. Radii for a given preparation were reproducible to within 0.1 nm when subject to repeated electrophoresis.

### Density gradient ultracentrifugation

Lipoproteins in the plasma fraction of density < 1.21 g/ml were subjected to density gradient ultracentrifugation. This procedure separates subpopulations of HDL according to their various rates of flotation through a discontinuous NaBr gradient. The method uses the princi-

ples of rate zonal ultracentrifugation applied to a swinging bucket. The density gradient formed was a modified version of that described by Groot et al. (18) using a GP-250 gradient programmer (Pharmacia Fine Chemicals) and two Pharmacia high precision pumps (P-500). Prior to ultracentrifugation, the samples were adjusted to a density of 1.4 g/ml by the addition of NaBr, passed through a 0.2  $\mu\text{m}$  Acrodisc filter (Gelman Instrument Co., Ann Arbor, MI) and layered beneath the gradient. The samples were then ultracentrifuged at 205,000  $g$  for 22 hr using a swinging bucket rotor (Beckman SW41Ti) at 15°C. After ultracentrifugation the bottom of each tube was pierced using a Beckman fractional recovery system; NaBr solution, 1.4 g/ml, was pumped in and the displaced fractions were recovered from the top of the tube. A continuous profile of absorbance at 280 nm was obtained using a Pharmacia single path ultraviolet monitor (UV-1) and 0.5-ml fractions were collected on a Pharmacia fraction collector (FRAC 100).

### Gel-filtration chromatography

Lipoproteins in the plasma fraction of density < 1.21 g/ml were subjected to gel filtration chromatography which was performed at room temperature using Superose 6B and a K 16/70 chromatographic column (1.6  $\times$  56 cm) in conjunction with the Pharmacia fast protein liquid chromatography system. The equilibrating and eluting solution contained 0.15 M NaCl, 0.01% Na<sub>2</sub> EDTA, and 0.02% NaN<sub>3</sub>. This solution was degassed and filtered through a 0.22- $\mu\text{m}$  Millipore filter (Millipore Corp., Bedford, MA) before use. Fractions were eluted at a rate of 45 ml/hr and the absorbance of eluate was monitored continuously at 280 nm.

### Chemical analysis

Concentrations of total and free cholesterol were measured using enzymatic assays (CHOD-PAP method, Boehringer Mannheim) adapted for use in a centrifugal analyzer (Centrifichem System 400, Union Carbide Corp., New York, NY). The concentration of esterified cholesterol was determined as the difference between the concentrations of total and free cholesterol. Triglycerides were measured using an enzymatic kit (Boehringer) and the Centrifichem System. The mass of cholesteryl ester and triglyceride was calculated assuming respective molecular weights of 649 and 885.

Protein concentrations were measured by the method of Lowry et al. (19) using bovine serum albumin as standard. Phospholipids were measured by the method of Bartlett (20). The phospholipid phosphorus content was multiplied by 25 to estimate the original amount of phospholipid.

## RESULTS

### Apolipoprotein composition of HDL after acute myocardial infarction

Apolipoprotein composition was determined by SDS-PAGE. Fig. 1 shows laser densitometric scans of normal HDL (A) and HDL from a subject 2 days after a myocardial infarction (B). In the normal HDL there was a prominent peak of apoA-I and less prominent peaks of apoA-II and a C apolipoprotein. In the post-infarction subject, there was an additional very prominent peak of an apolipoprotein of apparent molecular weight of 14,000. SAA, purified by the method of Anders et al. (14) had a similar molecular weight on SDS polyacrylamide gel electrophoresis (PAGE) as shown in Fig. 1(C).

To further identify this newly appearing apolipoprotein, urea-PAGE was performed. Fig. 2 shows urea-PAGE of HDL from a post-infarction subject (A) and from a normal subject (B), and isolated SAA (C). In the post-infarction subject there were two major bands visible that were not present in the normal HDL, but were present in the isolated SAA. These bands were comparable in position to those previously described for SAA (21, 22). HDL from the subject with secondary amyloidosis showed identical bands on urea-PAGE (not shown). Thus, on the basis of both SDS and urea-PAGE, the newly appearing apolipoprotein appears to have an identity with SAA.

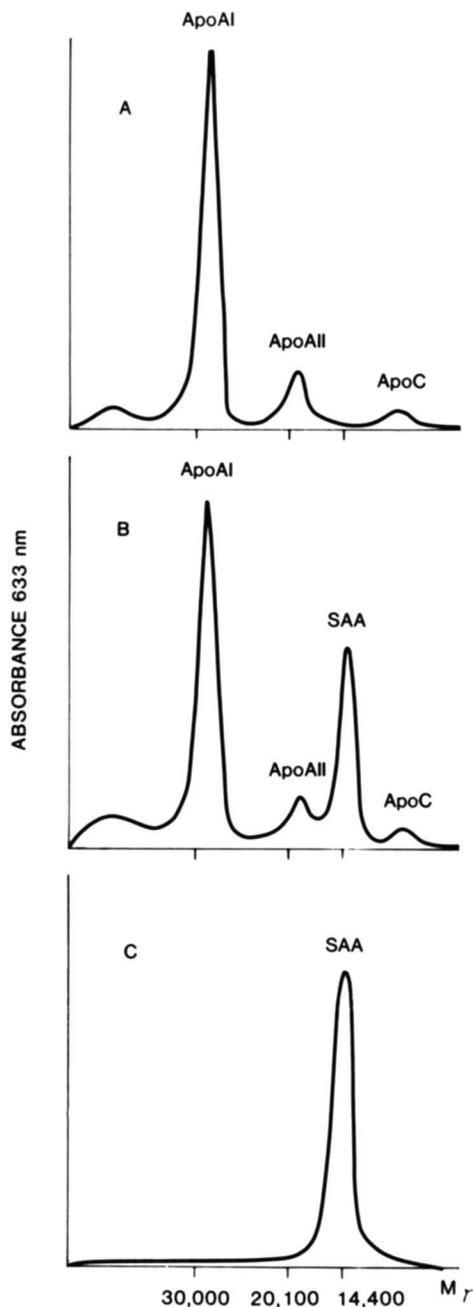
### Magnitude of the SAA enrichment of HDL after acute myocardial infarction

After SDS-PAGE of HDL, the gels were scanned and the peaks were integrated to provide a semi-quantitative estimate of the magnitude of the SAA response after myocardial infarction (Table 1). SAA was apparent in the HDL of all post-infarct subjects in an amount (in terms of dye-binding) ranging from 8–87% of the total protein in HDL (median 52%). There was no consistent time course for the appearance of SAA; in four subjects the level rose during the first week after presentation while in three subjects the level fell during this period (Table 1). Previous studies of four separate subjects had shown that SAA was not evident in HDL 1 month after discharge from hospital.

HDL from three patients with secondary amyloidosis were subjected to SDS-PAGE. SAA was detectable in only one of these three subjects (Patient 7, Table 1).

### Particle size of normal and SAA-enriched HDL

The particle size distribution of HDL was assessed by gradient gel electrophoresis (Fig. 3). The profiles from normal subjects were comparable to those reported elsewhere (17). Although five subpopulations have been reported in HDL, most subjects have only two or three of



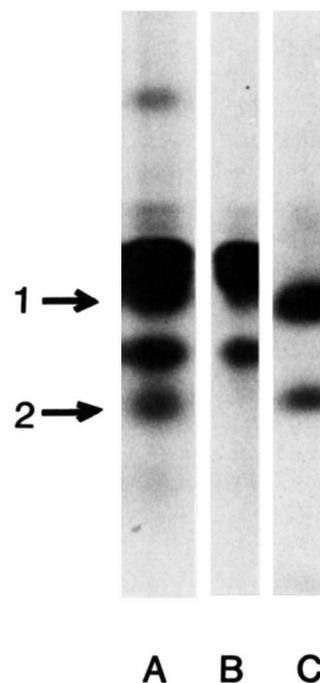
**Fig. 1.** SDS polyacrylamide gel electrophoresis of (A) normal HDL, (B) HDL from a subject after myocardial infarction, and (C) SAA isolated from the serum of a post-infarct subject by the method of Anders et al. (14). One hundred  $\mu\text{g}$  of protein was subjected to electrophoresis on 10% gels for 700 volt-hr. The gels were stained with Coomassie Blue R 250, destained, and then scanned at 633 nm with a laser densitometer. The profiles obtained from scanning are shown above: apoA-I, apoA-II, and the C apolipoproteins were identified by their position on the gel in relation to known standards. Note the appearance in (B) of a protein with an apparent molecular weight of 14,000. Purified SAA (C) runs in exactly the same position during SDS polyacrylamide gel electrophoresis.

these subpopulations. The HDL from the normal subject shown in Fig. 3 were representative of a large number of normal subjects, comprising two major peaks: one of particle radius 5.2 nm, corresponding to HDL<sub>2</sub>, and one

of particle radius 4.2 nm corresponding to HDL<sub>3</sub>. The SAA-enriched HDL from post-infarct patients had a quite different and distinctive profile. In 12 of the 25 scans, the HDL comprised only a single population of particles of radius (at the peak) of the order of 4.7–4.9 nm. In another five scans there were additional peaks visible, but particles larger than typical HDL<sub>3</sub> predominated. There was an apparent trend of increasing particle size with increasing SAA content of HDL in both male and female subjects (correlation coefficient  $r = 0.57$ ,  $P < 0.01$ ). In those subjects in whom two samples were available (numbers 1, 10, 12, 13, 15, 16, 18), the same trend was seen even more clearly (correlation coefficient  $r = 0.94$ ,  $P < 0.01$ ).

#### Density distribution of normal and SAA-enriched HDL

The density distribution of HDL was assessed by density gradient ultracentrifugation (Fig. 4). HDL from a normal subject eluted as typical peaks of HDL<sub>2</sub> (fraction 6) and HDL<sub>3</sub> (fraction 10). SAA-enriched HDL from subjects after myocardial infarction emerged as a single



**Fig. 2.** Urea polyacrylamide gel electrophoresis of (A) HDL from a post-infarct subject, (B) HDL from a normal subject, and (C) SAA isolated from the serum of a post-infarct subject by the method of Anders et al. (14). One hundred  $\mu\text{g}$  of protein was loaded onto a 7.5% polyacrylamide slab gel with a 1.66% stacking gel. Electrophoresis was performed in Tris-glycine (pH 8.8) containing 8 M urea for 700 volt-hr. The gels were stained with Coomassie Blue R 250, destained, scanned at 633 nm with a laser densitometer, and photographed. Two major bands not present in the normal subject were apparent in the post-infarct subject. The major band (band 1) is not clearly separable from the apoA-I band, but two bands are clearly seen in the SAA isolated from the post-infarct subject.

TABLE 1. Relationship between SAA content and particle size of HDL in patients with myocardial infarction

Subject	SAA Content (% of Apolipoprotein)	Mean Particle Radius of Major Peak	Number of Peaks
<i>nm</i>			
Male			
1	8 (32) <sup>a</sup>	4.1 (4.4) <sup>a</sup>	4 (3) <sup>a</sup>
2	21	4.2	2
3	23	4.4	1
4	37	4.3	2
5	48	4.3	2
6	52	4.5	1
7	52	4.7	1
8	52	5.3	1
9	54	4.5	1
10	62 (72)	4.7 (4.8)	1 (3)
11	69	4.9	2
12	77 (21)	4.8 (4.4)	1 (1)
13	78 (12)	4.6 (4.1)	1 (1)
Female			
14	26	4.9	1
15	26 (36)	4.9 (5.2)	2 (3)
16	32 (7)	4.7 (4.1)	1 (3)
17	56	4.6	1
18	60 (87)	4.7 (5.1)	1 (1)

The amount of SAA is shown as a percentage of HDL apolipoprotein estimated from laser densitometry of SDS gels. The mean particle radii were calculated by comparison with known standards on gradient gels. The major peak was defined as that peak occupying 50% or more of the integrated area on a laser densitometric scan of the gradient gel. The subjects are arranged in order of magnitude of SAA response. Samples were taken 24–48 hr after admission. There was a significant correlation ( $r = 0.57$ ,  $P < 0.01$ ) between SAA content and the mean particle radius of the major peak.

<sup>a</sup>The values in parentheses are of samples taken from the same subject 10 days after admission.

peak of particles eluting in fraction 9 and sometimes fraction 10, comparable in position to normal HDL<sub>3</sub>. Also shown in Fig. 4 is a population of particles of radius 4.6 nm which had been isolated from normal HDL by gel filtration and then subjected to density gradient ultracentrifugation. This population, despite being smaller than SAA-HDL (radius 4.7 nm) eluted in fraction 8.

Aliquots of fractions recovered after density gradient centrifugation were subjected to gradient gel electrophoresis. In the case of normal HDL, fraction 8 recovered after density gradient ultracentrifugation had a mean particle radius of 4.6 nm, fraction 9 had a radius of 4.4 nm, and fraction 10 had a radius of 4.2 nm. SAA-enriched HDL (fraction 9) had a mean particle radius of 4.7 nm in the subject illustrated in Fig. 4. In subjects with large amounts of SAA, the mean particle radius of material taken from this fraction varied between 4.6 nm and 5.2 nm, despite eluting after density gradient centrifugation very close to the position of normal HDL<sub>3</sub>.

#### Chemical composition of normal and SAA-enriched HDL

The chemical composition of HDL from post-myocardial infarction patients is shown in Table 2. The pro-

tein content of about 55% is typical of HDL<sub>3</sub> (23) and fits the density profile of these preparations (Fig. 4). The most striking difference between SAA-rich HDL and normal HDL<sub>3</sub> is the low phospholipid and elevated triglyceride concentration in the former. The higher triglyceride content may reflect the fact that ten of the subjects had a plasma triglyceride over 2 mmol/l, and may not be directly related to the presence of SAA. Total HDL protein levels in the post-infarct subjects are no different from those of normal subjects.

#### Particle size distribution of SAA-enriched HDL from subjects with secondary amyloidosis

As with the HDL from the post-myocardial infarct patients, the SAA-enriched HDL from a subject with secondary amyloidosis appeared as a single population of particles larger than normal HDL<sub>3</sub> (Fig. 3).

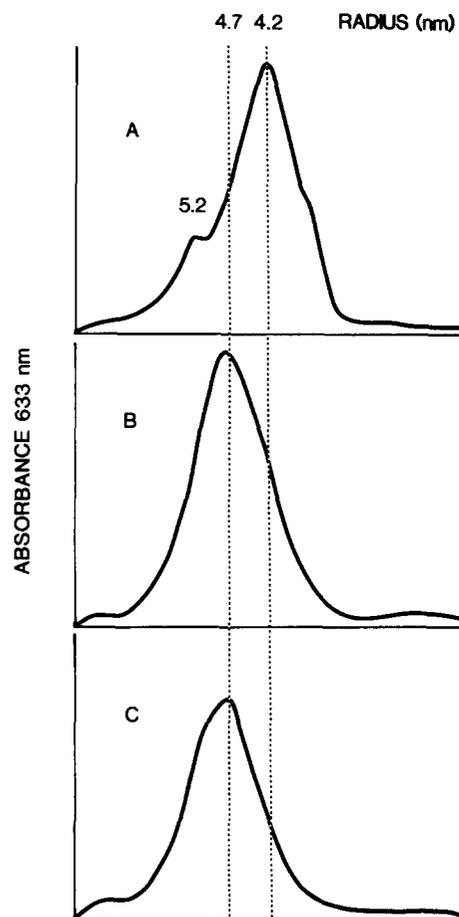
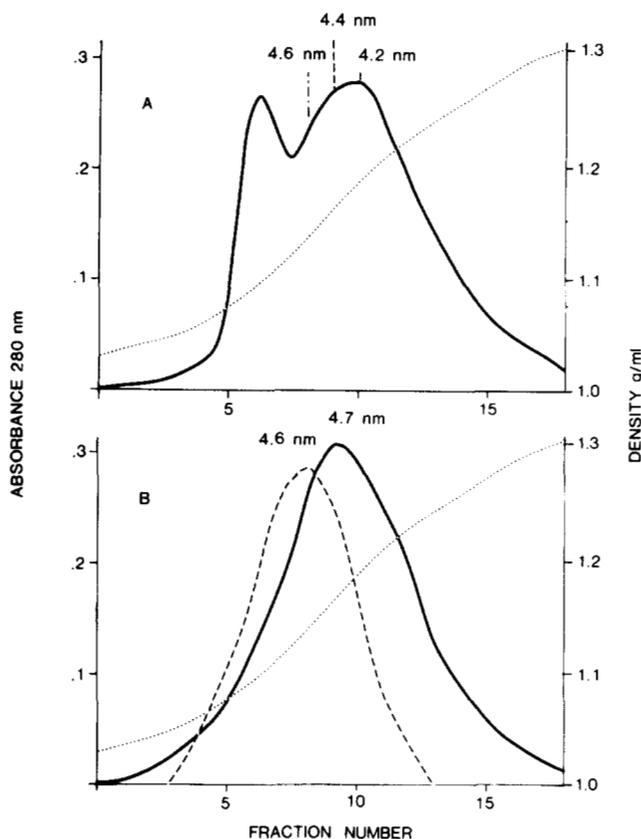


Fig. 3. Gradient gel electrophoresis of HDL from (A) a normal male subject, (B) a male subject 2 days after myocardial infarction, and (C) a male subject with secondary amyloidosis. Thirty  $\mu$ g of HDL protein was loaded onto each lane of a 2.5–27% polyacrylamide gradient gel and subjected to electrophoresis for 3,400 volt-hr in a Tris-borate buffer (pH 8.35). Gels were fixed in sulfosalicylic acid, stained with Coomassie G 250, and destained in acetic acid. Scanning was performed at 633 nm with a laser densitometer, and Stokes radii were calculated from the scan by reference to the position of high molecular weight standards.



**Fig. 4.** Density gradient ultracentrifugation. Lipoproteins isolated from 2 ml of plasma were layered beneath a discontinuous gradient of NaBr from 1.4 g/ml to 1.0 g/ml and spun at 205,000 *g* in a swinging bucket rotor for 22 hr. Tubes were then pierced and samples were eluted by upward displacement by a solution of density 1.4 g/ml. The absorbance at 280 nm was monitored continuously and 0.5-ml fractions were collected. The dotted line charts the density of the eluate measured on an Abbe refractometer. Profile (A) from a normal subject shows two peaks, HDL<sub>2</sub> in fractions 3-7 and HDL<sub>3</sub> in fractions 7-15. Profile (B) (solid line) is from a post-infarct subject and shows a single peak eluting at the same density as normal HDL<sub>3</sub>. The broken line profile in (B) is of an isolated subfraction of normal HDL with a mean particle radius of 4.6 nm. Aliquots of fractions 8, 9, and 10 of the normal HDL and an aliquot of fraction 9 of the post-infarct HDL were subjected to gradient gel electrophoresis. The mean HDL particle radii are indicated above the relevant fraction.

### Effect of enriching normal HDL with SAA during incubation in vitro

After incubation of normal plasma with purified SAA, the isolated HDL was found to be considerably enriched with SAA. HDL apolipoprotein isolated from 1 ml of plasma after incubation with 5 mg of purified SAA at 37°C for 24 hr contained approximately 45% SAA and was thus quite comparable to the HDL apolipoprotein of post-myocardial infarction subjects. Comparable results were obtained with lower concentrations of SAA.

The gradient gel electrophoretic profile of normal HDL before and after being enriched in vitro with SAA is shown in Fig. 5. Enrichment with SAA was associated with a loss of the discrete subpopulations present in the native HDL and the appearance of a single population of particles with a radius at the peak of 4.9 nm, similar to that in the SAA-enriched post-myocardial infarction patients. In control incubations performed in the absence of SAA, there was a change in the size profile with some particles being enlarged and others becoming reduced in size; these changes were comparable to those reported elsewhere (24, 25) and quite different from the single population emerging after incubation with SAA.

Fig. 6 shows the density gradient ultracentrifugal profiles of the same samples as shown in Fig. 5. The unmodified HDL eluted as clearly identifiable peaks of HDL<sub>2</sub> and HDL<sub>3</sub>, whereas the SAA-enriched sample eluted as a single peak in exactly the same position as HDL<sub>3</sub>. In the control situation of HDL incubated in the absence of SAA, there was a reduction in the density of HDL<sub>3</sub>, a pattern quite different from that in the presence of SAA.

Aliquots of fractions recovered after density gradient ultracentrifugation were subjected to gradient gel electrophoresis. Fraction 10 of normal HDL had a mean particle radius of 4.2 nm while fraction 10 of SAA-HDL had a mean particle radius of 4.9 nm.

Fig. 7 shows the density gradient centrifugation profile of normal HDL before and after incubation with purified

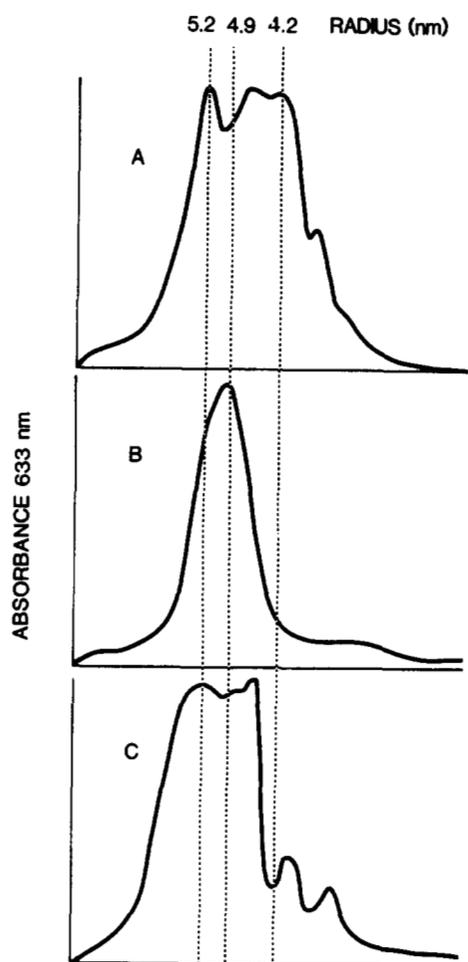
TABLE 2. Percentage composition of HDL

	Protein	Phospholipid	Cholesteryl ester	Free Cholesterol	Triglyceride
SAA-rich HDL <sup>a</sup>	55.4 ± 6.0 <sup>c</sup>	17.0 ± 3.7	17.1 ± 5.2	2.8 ± 1.2	7.7 ± 3.0
HDL <sub>2</sub> <sup>b</sup>	40.9 ± 4.0	28.1 ± 2.5	22.2 ± 3.1	5.4 ± 0.7	3.4 ± 1.3
HDL <sub>3</sub> <sup>b</sup>	53.2 ± 2.4	24.0 ± 1.3	18.5 ± 2.1	2.8 ± 0.7	1.6 ± 0.4

<sup>a</sup>Twenty-five preparations of HDL from 18 post-infarct subjects were analyzed. Mean HDL protein concentration was 159 ± 63 mg/dl.

<sup>b</sup>Eight healthy, non-fasting laboratory staff members provided normal plasma which was separated by sequential ultracentrifugation into HDL<sub>2</sub> (1.063-1.125 g/ml) and HDL<sub>3</sub> (1.125-1.21 g/ml). Mean HDL protein concentration was 156 ± 33 mg/dl.

<sup>c</sup>Mean ± SD.



**Fig. 5.** Gradient gel electrophoresis of (A) normal HDL, (B) HDL isolated from 1 ml of plasma that was incubated *in vitro* for 24 hr at 37°C with 5 mg of purified SAA, and (C) HDL isolated from 1 ml of plasma incubated as above but without SAA. Thirty  $\mu$ g of HDL protein was added to each track and electrophoresed as described in the legend to Fig. 3.

SAA at room temperature for 10 min. Both HDL<sub>2</sub> and HDL<sub>3</sub> appear to have become denser, eluting later than the normal subpopulations. Aliquots taken from the peaks of each subspecies were then subjected to gradient gel electrophoresis.

Normal HDL<sub>2</sub> had a mean particle radius of 5.2 nm, whereas SAA-enriched HDL<sub>2</sub> had a radius of 5.6 nm. Normal HDL<sub>3</sub> had a mean particle radius of 4.2 nm, while the SAA-enriched HDL<sub>3</sub> had a mean particle radius of 4.9 nm. This particular preparation contained 91% SAA, 6% apoA-I, and 3% apoA-II.

The alteration in density and particle size of HDL incubated with SAA was more marked with increasing amounts of SAA.

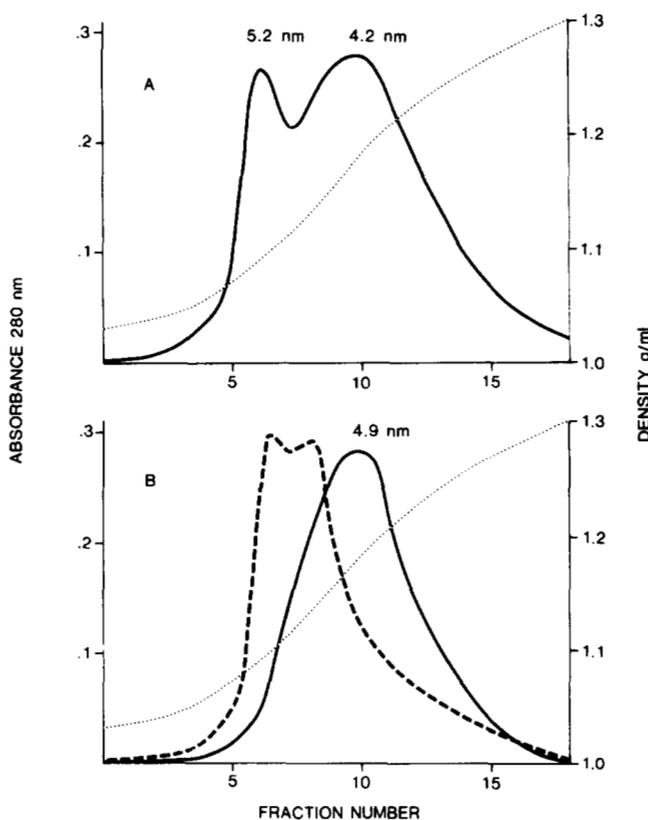
#### Particle size of ultracentrifugally separated HDL<sub>2</sub> and HDL<sub>3</sub>

HDL<sub>2</sub> (1.063–1.125 g/ml) and HDL<sub>3</sub> (1.125–1.21 g/ml)

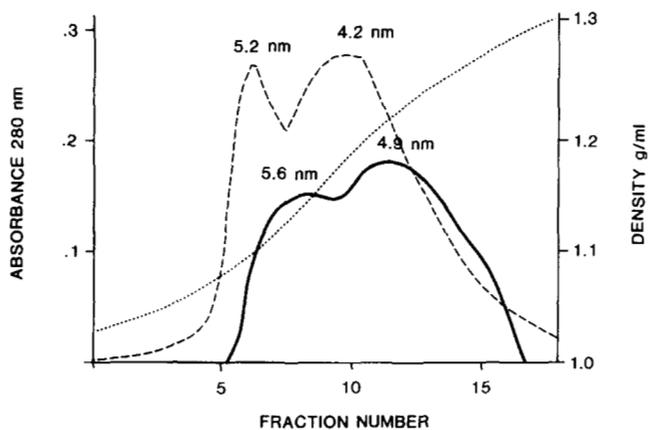
were isolated by sequential ultracentrifugation and then subjected to gradient gel electrophoresis. Normal HDL<sub>2</sub> comprised a single population of particles of radius 5.2 nm; normal HDL<sub>3</sub> comprised two populations of particle radius 4.2 nm and 3.9 nm (Fig. 8). The fractions of corresponding densities isolated from a post-myocardial infarct subject each comprised a single population of particles of mean radius 4.9 nm (Fig. 8). As there was often only a single population of particles as assessed by density gradient ultracentrifugation and gradient gel electrophoresis, routine subfraction of HDL into HDL<sub>2</sub> and HDL<sub>3</sub> was not performed in most subjects.

## DISCUSSION

Elevated levels of SAA have been reported in human subjects in a variety of acute and chronic inflammatory conditions (3, 4), after major surgery (26), and after trauma (2). Increased concentrations of SAA have also been observed in patients after myocardial infarction (4).



**Fig. 6.** Density gradient ultracentrifugation of (A) normal HDL; (B, solid line) HDL isolated from 1 ml of plasma that was incubated *in vitro* for 24 hr at 37°C with 5 mg of SAA; and (B, broken line) HDL isolated from 1 ml of plasma incubated as above but without SAA. The density gradient ultracentrifugation was performed as described in the legend to Fig. 4. Aliquots of fraction 6 and fraction 10 of normal HDL and fraction 10 of incubated HDL were subjected to gradient gel electrophoresis. The mean HDL particle radii are indicated above the relevant fraction.



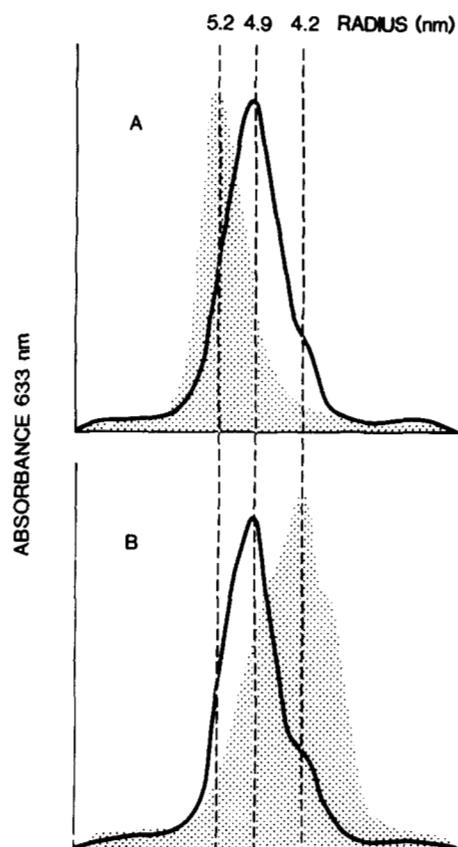
**Fig. 7.** Isolated SAA (2.8 mg) was incubated with 1 ml of HDL (1 mg/ml) at room temperature for 10 min. The incubation mixture was then subjected to density gradient ultracentrifugation as described in the legend to Fig. 4. The broken line shows the profile of normal HDL, and the solid line, the SAA-enriched HDL. Aliquots of fractions 6 and 10 of normal HDL and fractions 7 and 11 of SAA-HDL were subjected to gradient gel electrophoresis. The mean HDL particle radii are indicated above the relevant fraction.

In these studies the new apolipoprotein appearing in the HDL of subjects after myocardial infarction was presumed to be SAA on the basis of its behavior on SDS and urea polyacrylamide gels, where it was identical to SAA separated according to the method of Anders et al. (14) and SAA in the plasma of a patient with secondary amyloidosis. Although the amount of SAA was not quantitated by specific assay, laser densitometry of SDS gels provided a semiquantitative estimate of the amount of SAA in the HDL fraction. In some cases it was quite clear that SAA constituted a major proportion of the HDL apolipoprotein.

The association of SAA with HDL has been well documented (5, 6). More specifically, it has been reported that SAA associates preferentially with HDL<sub>3</sub> (5, 7, 8). An association of SAA with particles in the HDL<sub>3</sub> density range was also observed in the present studies. However, some of these particles of HDL<sub>3</sub> density were larger than expected for normal HDL<sub>3</sub>. In a number of the post-infarction subjects in whom SAA was prominent, the HDL comprised a single population of particles which on the basis of size were classified as HDL<sub>2</sub>, but on the basis of density were classified as HDL<sub>3</sub>. This paradoxical finding of particles larger than normal HDL<sub>3</sub> but with a density comparable to HDL<sub>3</sub> was not limited to the post-infarction subjects, being observed also in a subject with secondary amyloidosis and after normal HDL had been incubated in vitro with SAA. Indeed, the association of purified SAA with HDL in vitro produces particles denser and larger than the original HDL (Fig. 7). This capacity of SAA to associate with HDL during incubation in vitro has been well established (6, 12), although the effects on HDL particle size and density have not previously been reported.

In preparations of SAA-enriched HDL, the particle size of the predominant subpopulation increased in proportion to the percentage of SAA present (Table 1). In those preparations containing relatively little SAA, the major subpopulation tended to comprise particles of a size comparable to normal HDL<sub>3</sub>; with increasing amounts of SAA, however, the particle size of the major subpopulation became progressively closer to that of normal HDL<sub>2</sub>. A lack of concordance between size and density of these SAA-enriched HDL was further emphasized by finding that subfractions isolated by sequential ultracentrifugation in the density intervals 1.063–1.125 g/ml and 1.125–1.25 g/ml contained particles of identical size (Fig. 8).

One explanation for this effect of SAA on the physical properties of HDL is that the SAA is incorporated into the surface of pre-existing particles, with a consequent increase in protein content and thus an increase in particle density. In those subjects in whom SAA accounted for a major proportion of the HDL protein, however, there must also have been some displacement of



**Fig. 8.** Gradient gel electrophoresis of (A) HDL<sub>2</sub> from a normal subject (shaded profile) and from a post-infarct subject (solid line), and (B) HDL<sub>3</sub> from the same two subjects. HDL<sub>2</sub> (d 1.063–1.125 g/ml) and HDL<sub>3</sub> (d 1.125–1.21 g/ml) were separated by sequential ultracentrifugation. Thirty  $\mu$ g of HDL protein was added to each lane of the gel and electrophoresed as described in the legend to Fig. 3.

other apolipoproteins, since the percentage increase in HDL protein was much less than predicted by the amount of SAA incorporated, even allowing for potential errors in its quantification. If three SAA molecules displace one apoA-I molecule then the HDL particle could undergo up to a 40% increase in protein if all the apoA-I molecules are displaced. Thus an HDL<sub>2</sub> particle could acquire sufficient protein to appear as dense as normal HDL<sub>3</sub>, while still retaining its original size. On density gradient ultracentrifugation of HDL from post-infarct subjects, there is a marked reduction in the proportion of material that has the density of HDL<sub>2</sub> with an increase in the proportion of HDL<sub>3</sub>. However, no explanation is available for the apparent increase in size of both HDL<sub>2</sub> and HDL<sub>3</sub> when SAA binds in vitro. Given that SAA is an amphipathic helix-containing protein (11) and quite capable of forming an interface between water and the hydrophobic core, it is perhaps not surprising that its presence in the HDL particle surface would result in changes in the physical properties of the particle. When compared with normal HDL<sub>2</sub> of similar size, SAA-enriched HDL had an increased protein and a decreased phospholipid content. Thus, as has been suggested elsewhere (7), SAA may have taken over some of the role normally played by phospholipids in the lipoprotein surface. Regardless of the mechanism, however, it is apparent that the presence of SAA in HDL results in the formation of particles that are inappropriately large for their apparent density.

In the majority of the post-infarction subjects the SAA-enriched HDL comprised a single population of particles, rather than the multiple subpopulations observed in normal HDL. The explanation for this finding is not known. Given, however, that all HDL constituents are capable of exchange between different lipoprotein particles (27-31), it is apparent that the HDL fraction must exist in a state of dynamic equilibrium. It would appear that the presence of SAA in the HDL may change this equilibrium so as to convert a heterogeneous mixture into a single homogeneous population of particles.

There have been several recent reports of the particle size distribution of HDL as determined by gradient gel electrophoresis. A finding that the major subpopulations of HDL identified by analytic and zonal ultracentrifugation corresponded to certain of the subpopulations separated by gradient gel electrophoresis (17) has to be interpreted with some caution in the light of the present results. SAA-rich HDL particles are inappropriately large for their apparent density; thus the normal, predictable relationship between size and density is lost. A lack of concordance between the size and density of HDL has also been observed in the case of the discoidal HDL that appear in the plasma in states of LCAT deficiency (32). These discs have the density of HDL but on gel filtration they elute with the much larger particles in the lower density lipoprotein fractions (33).

In conclusion, these studies have confirmed an induction of high concentrations of SAA in the plasma of human subjects suffering myocardial infarctions. They have also defined effects of SAA on the chemical and physical properties of HDL. The physiological implications of these changes in terms of HDL metabolism remain to be determined. ■

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