Alteration of high density lipoprotein subfraction distribution with induction of serum amyloid A protein (SAA) in the nonhuman primate

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Abstract Overnight chair restraint results in a dramatic increase in serum amyloid A protein (apoSAA) of nonhuman primate high density lipoprotein (HDL). To determine whether apoSAA induction resulted in a displacement of indigenous HDL protein or a change in the subfraction distribution of HDL, we analyzed the characteristics of HDL subfractions in eight vervet monkeys before and 24 hr after apoSAA induction. Blood was taken from each animal before and after chair restraint to induce apoSAA. HDL was isolated from the plasma by ultracentrifugation and agarose column chromatography. The isolated HDL was subfractionated by density gradient centrifugation and five resulting subfractions were analyzed for protein and lipid content. With apoSAA induction there was a significant increase in d < 1.09 g/ml protein, phospholipid, and free and esterified cholesterol which resulted in a 44% increase in the total mass of this subfraction. Concomitantly, there was a significant decrease in d 1.10–1.11 g/ml protein, total cholesterol, and cholesteryl ester, which resulted in a 16% decrease in the total mass of the subfraction. The response of the d 1.10–1.11 and d > 1.12 g/ml subfraction protein, cholesterol, and phospholipid concentrations to chair restraint for individual animals was directly proportional to their plasma HDL concentrations. Although there was a change in the HDL subfraction concentrations after chair restraint, there was no change in the lipid composition of the HDL subfractions nor in the total amount of HDL protein. However, the apoSAA/A-I ratio was significantly increased with induction while the apoA-II + C's/A-I ratio remained unchanged. The apoSAA/A-I ratio progressively increased with the density of the HDL subfraction. The protein composition of the d > 1.12 g/ml subfraction was changed from an average of three apoA-I and two apoA-II (or C's) molecules per particle to an average of two apoA-I, one apoA-II (or C's), and three or four apoSAA molecules per particle after chair restraint. Thus, apoSAA was predominantly associated with the denser HDL subfractions even though the lighter HDL subfractions were the most responsive in terms of changes in concentration. These data suggest that chair restraint of nonhuman primates induces apoSAA which displaces apoA-I and apoA-II or C's from HDL without altering the overall lipid and protein composition of the particle. In addition, chair restraint alters the concentration of HDL subfractions in ways that may be independent of apoSAA induction.—**Parks, J. S., and L. L. Rudel.** Alteration of high density lipoprotein subfraction distribution with induction of serum amyloid A protein (SAA) in the nonhuman primate. *J. Lipid Res.* 1985. **26:** 82–91.

Supplementary key words apoA-I • apoA-II • C-apoproteins

Serum amyloid A protein (SAA) is an acute phase reactant protein and has been shown to be an apoprotein of human (1), mouse (2), and monkey HDL (3, 4) and monkey lymph chylomicrons (4). ApoSAA is the presumed precursor of amyloid A protein, which can deposit in various tissues leading to secondary amyloidosis (5, 6). ApoSAA synthesis can be rapidly induced by a number of different stimuli and the liver is thought to be the major synthetic site for the protein (7, 8). The catabolic rate of apoSAA is much more rapid than that of the other two apoproteins of HDL, apoA-I and apoA-II, suggesting that the apoproteins are metabolized individually or that a population of apoSAA-rich particles exist which are catabolized more rapidly than other HDL particles (4). Recently Hoffman and Benditt (2) reported that endotoxin treatment of mice resulted in a subfraction of HDL that was enriched in apoSAA relative to apoA-I. The HDL of these endotoxin-treated animals had significantly more protein and less phospholipid than the control group, suggesting that apoSAA addition to the particles may displace phospholipid.

The association of apoSAA with HDL could have at least two consequences which could influence HDL composition, distribution, or catabolism. First, the

Abbreviations: HDL, high density lipoproteins; apoSAA, serum amyloid A protein; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); IEF, isoelectric focusing; SDS, sodium dodecylsulfate; PAGE, polyacrylamide gel electrophoresis; LDL, low density lipoproteins; TG, triglyceride; PL, phospholipid; FC, free cholesterol; CE, cholesteryl ester; FCR, fractional catabolic rate; TPC, total plasma cholesterol.

apoSAA could add to pre-existing HDL particles resulting in an increase in the particle density. Or the apoSAA could displace existing HDL apoproteins which may or may not change the subfraction composition and distribution of the HDL particles. Either one of these possibilities might lead to alterations in the catabolism of HDL subfractions. The purpose of this study was to explore these possibilities by characterizing HDL subfractions from individual animals before and after apoSAA induction. We have used vervet monkeys as experimental animals for this study because the characterization and metabolism of apoSAA has been detailed previously in this species (3, 4).

METHODS

Animals

Eight adult male African green monkeys (*Cercopithecus aethiops;* vervet subspecies) were used for the SAA induction studies. In order to maximize the range of HDL responses, the animals were fed diets containing 40% of calories as either polyunsaturated fat (safflower oil) or saturated fat (lard) and either 0.16 or 0.75 mg/Kcal of cholesterol. The experimental animals had HDL cholesterol concentrations ranging from 31 to 70 mg/dl.

SAA was induced in the experimental animals by chair restraint as previously described (4). At 8 AM, after an overnight fast, the animals were immobilized with ketamine (Ketalar®, 10 mg/kg I.M.) and removed from their cages. A 20-ml blood sample was collected and the animals were then placed in the restraining chair. The animals were routinely monitored during chair restraint by the clinical veterinarian staff and no adverse effect on the health of the animals was noted. The animals were fed ad libitum throughout the day but were fasted again overnight. The following morning at 8 AM, the animals were again given ketamine, removed from the chair, and another 20-ml blood sample was collected. Blood was collected into tubes which had a final concentration of 0.1% EDTA, 0.02% azide, and 0.04% DTNB, pH 7.4. The blood samples were placed immediately on ice and the plasma was isolated within 30 min.

HDL isolation and subfractionation

High density lipoproteins were isolated by the combined ultracentrifugation and agarose column chromatography method described by Rudel et al. (9). Plasma was adjusted to d 1.225 g/ml with solid KBr and spun in a SW40 rotor for 40 hr at 40,000 rpm (15°C). The floating lipoprotein layer was isolated after tube slicing, resuspended in saline, and applied to an agarose column. The HDL-containing fractions eluted from the column were pooled for further analysis.

HDL was subfractionated by a discontinuous density gradient in the VTi50 vertical rotor (Beckman Instruments). The HDL fraction derived from the agarose column was dialyzed to a density of 1.11 g/ml. Twenty-one ml of the dialyzed sample was layered into a VTi50 vertical rotor tube between a bottom layer of d 1.15 g/ml (9 ml) and a top solution of d 1.060 g/ml (9 ml). The tubes were spun at 50,000 rpm for 18 hr at 20°C. After puncturing the bottom of the tube, Fluorinert (d 1.9 g/ml; 3M Company) was pumped in, forcing the contents of the tube to drain from the top of the tube through a UV monitor into a fraction collector. The density of each fraction collected was determined by reading the refractive index of the solution. A standard curve of density versus refractive index was determined by pycnometry on fractions from a blank gradient tube. Tubes were pooled within defined density regions and analyzed as described below. The details of this density gradient procedure are published elsewhere (10). The pooled HDL subfractions were dialyzed against 0.01% EDTA, 0.01% azide, pH 7.4, to remove the KBr prior to chemical analyses.

Chemical analyses

Protein determinations were performed by the method of Lowry et al. (11) using bovine serum albumin, fraction V (Sigma Chemical Co.) as the standard. Cholesterol determinations were done according to the procedure of Rudel and Morris (12). Phospholipids were quantitated by the method of Fiske and SubbaRow (13), while the triglyceride assays were done according to the method of Sardesai and Manning (14). Free and esterified cholesterol and triglycerides were quantitated after extraction (15) and thin-layer chromatography of the HDL lipids as has been detailed previously (16). All HDL subfraction chemical constituents were converted back to concentrations in whole plasma (mg/dl) using recovery of total cholesterol to correct for any losses. In a separate study, molecular weights were determined by analytical ultracentrifugation of HDL subfractions isolated from the density gradient runs. HDL molecular weights were generously measured by sedimentation equilibrium analysis (17) by Dr. Charles Nelson of the University of Arkansas Medical Center.

Gel electrophoresis

SDS polyacrylamide gradient gels were run to analyze HDL apoproteins. A separating gel from 12 to 30% polyacrylamide with a 4% acrylamide stacking gel was used. The separating gel buffer consisted of a final concentration of 380 mM Tris-HCl, 2 mM EDTA, and 0.1% SDS, pH 8.8. The stacking gel buffer consisted of 125 mM Tris-HCl, 2 mM EDTA, and 0.1%

SDS, pH 6.8. The electrode buffer consisted of 383 mM glycine, 16 mM Tris base, 1.57 mM EDTA, and 0.1% SDS, pH 8.3. The sample buffer consisted of 0.025 M barbital (pH 8.6), 2% SDS, and 20% glycerol. Individual HDL subfractions were dialyzed against 0.01% EDTA (pH 7.4), 0.01% azide, and an aliquot equivalent to 40 μg of protein was lyophilized for SDS gels. The lyophilized protein was dissolved with sample buffer; no organic solvent delipidation was performed. The gels were run in a Bethesda Research Labs gel electrophoresis apparatus at 100 volts constant voltage for 18 hr at room temperature. The gels were stained with 0.1% Coomassie Blue, 50% methanol, 10% acetic acid, and destained in 50% methanol, 10% acetic acid. Isoelectric focusing was done as previously described (3).

SDS PAGE gels were scanned using a Zeineh laser scanning densitometer (Biomed Instruments, Fullerton, CA) interfaced with a microcomputer. Peak areas were determined by digital integration. A linear response was found for peak area units versus μg of HDL protein applied to the gel using dilutions of an induced HDL sample.

Statistics

Since each animal served as its own control in the experiment, the paired *t*-test was used for statistical analyses. All reported values are the mean ± standard error of the mean (SEM).

RESULTS

The diets fed to the eight animals of this experiment resulted in a relatively wide range of plasma (84–284 mg/dl) and HDL (31–70 mg/dl) cholesterol concentrations. The mean plasma cholesterol concentrations for the group was 163 ± 23 mg/dl before SAA induction. After overnight chair restraint to induce apoSAA, the total plasma cholesterol (TPC) concentrations for the group were 17% lower (136 ± 18 mg/dl; $P < 0.05$). Since total HDL cholesterol concentrations were not affected by chair restraint (see below) and these animals had minimal amounts of VLDL, the lower TPC presumably was the result of lower LDL cholesterol concentrations. However, there was no significant change in the size of LDL before versus after chair restraint (LDL particle weight = 3.26 ± 0.17 vs. 3.09 ± 0.10 g/μmol).

HDL isolated from plasma of animals before and after SAA induction by chair restraint were subfractionated. In **Fig. 1** are shown the density gradient profiles of two animals before and after chair restraint, one with low and one with high initial HDL cholesterol concentrations. The animal with high initial HDL concentration (#267) had no change in HDL concentration with chair restraint while animal #265 had an 11 mg/dl increase in HDL cholesterol. In general, three populations of particles were seen which correspond approxi-

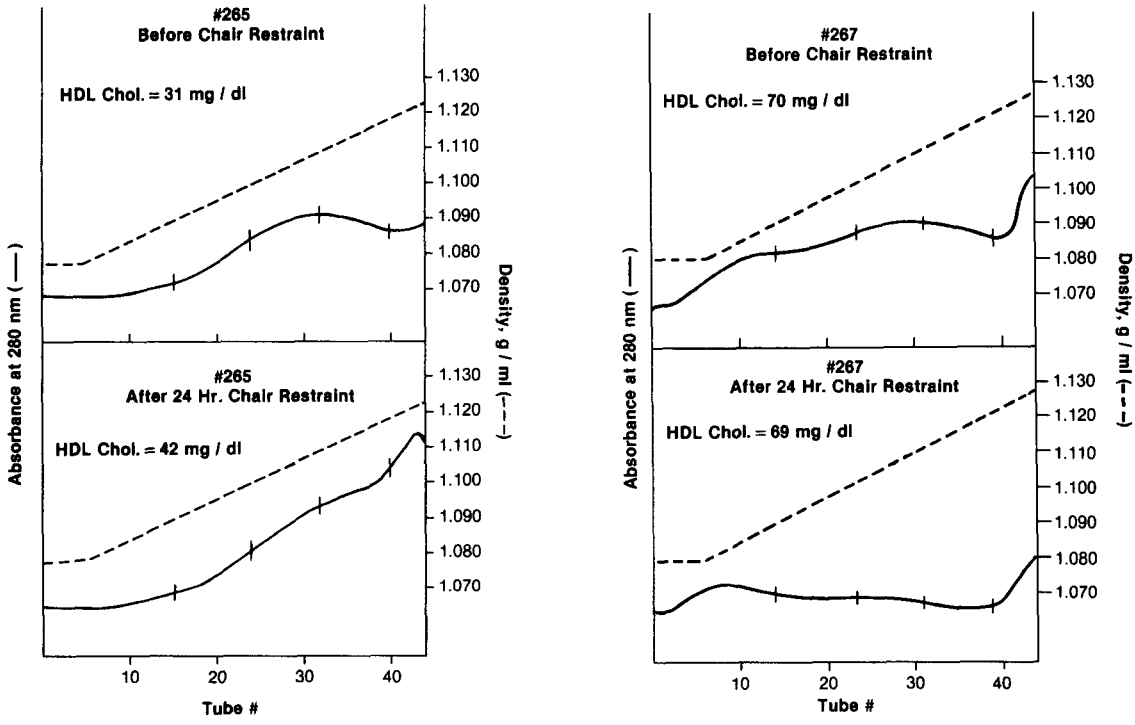

Fig. 1 HDL density gradient profiles from animals with high (#267) and low (#265) total HDL cholesterol concentrations before chair restraint. HDL was isolated from plasma of animals before and after 24 hr of chair restraint to induce apoSAA. Isolated HDL was subfractionated by density gradient centrifugation as outlined in the Methods section. Tubes were combined for analysis at the indicated vertical marks on the absorbance profile. Each tube contained 0.86 ml.

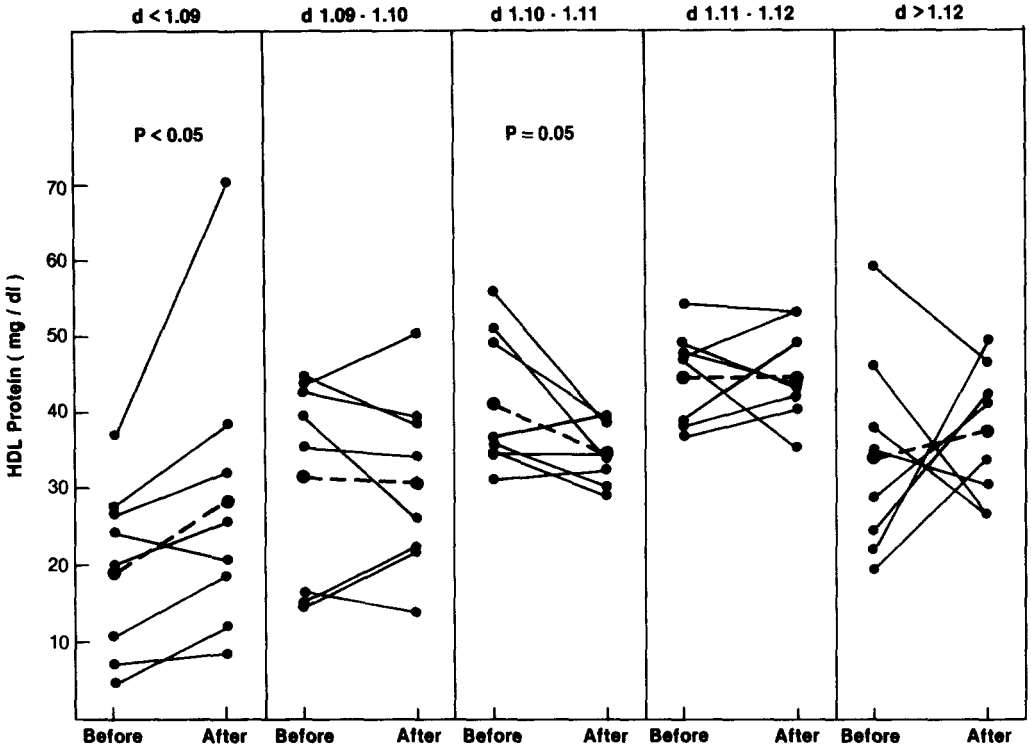

Fig. 2 A plot of each individual animal's HDL protein concentration before and 24 hr after chair restraint for the HDL density gradient subfractions (g/ml). HDL protein concentrations are corrected back to mg/dl protein in whole plasma. The points in each panel connected by a dashed line are the mean values for the group. Significant differences before versus after chair restraint for the group were seen for the d < 1.09 and d 1.10–1.11 g/ml subfraction.

mately to tubes 1–15, 16–40, and 41–45. Five density cuts were made such that the middle population of particles (tubes 16–40) was split into thirds. The corresponding density cuts are given in subsequent figures and tables.

In order to quantitate the changes among the HDL density subfractions, protein concentrations were measured and plotted for each individual animal. The results are shown in **Fig. 2.** Seven of eight animals had an increase in protein concentration of the d < 1.09 g/ml subfraction after chair restraint. The mean HDL protein for the group (indicated by the dashed line) was 19.8 ± 4.0 versus 28.4 ± 6.9 mg/dl for before versus after chair restraint, respectively ($P < 0.05$). The response of the HDL protein to chair restraint was more variable among individual animals in the d 1.09–1.10, d 1.11–1.12, and d > 1.12 g/ml subfractions and, because of the variability, none of the mean HDL protein changes after SAA induction were significant (see Table 2 for numerical data). The mean protein concentration in the d 1.10–1.11 g/ml subfraction was lower (41.4 ± 3.3 vs. 34.9 ± 1.4 mg/dl; $P = 0.05$) after chair restraint with five of eight animals having lower HDL protein concentrations in that subfraction.

The data in Fig. 2 suggested that an individual animal's HDL protein response was dependent on the initial concentration of protein in the HDL subfractions. Animals with high initial HDL protein concentrations in many of the subfractions had lower HDL protein concentrations after chair restraint, while animals with low initial protein concentrations had increased HDL protein concentrations. To explore this possibility further, HDL protein concentration for each animal before chair restraint was plotted against the change in protein concentration of each subfraction after chair restraint (before minus after concentration). The results are shown in **Fig. 3.** The graph for total HDL protein is also shown for comparison. The correlation coefficient for the d 1.10–1.11 and d > 1.12 g/ml subfractions was statistically significant. The points plotting below zero on the ordinate indicate increases in protein concentration with chair restraint, while points above zero indicate decreases. Thus the initial HDL concentrations of some HDL subfractions not only reflect the magnitude but the direction of HDL protein response to apoSAA induction by chair restraint. In general, animals with high initial HDL subfraction protein exhibited a decrease in protein with

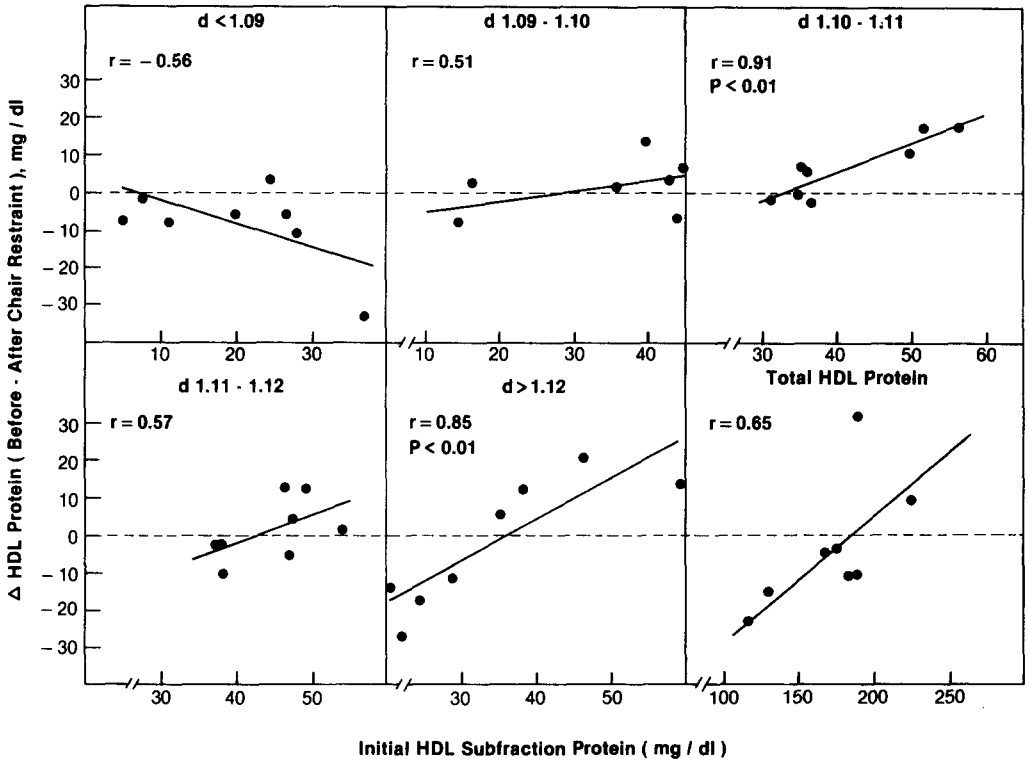

Fig. 3 The relationship between initial HDL subfraction protein (before chair restraint) and the change in HDL subfraction protein with chair restraint (before minus after chair restraint). The line drawn for each plot is the line of best fit determined by linear regression. The correlation coefficient is given for each line along with the *P* value if the correlation was significant. The total HDL protein plot is also shown for reference. Note that points that plot below zero indicate that there was an increase in HDL subfraction protein with chair restraint.

chair restraint, while animals with low initial protein concentrations had increased HDL protein.

Similar plots, comparing the initial subfraction concentration to the change resulting from chair restraint, were made for total cholesterol and phospholipid concentrations. The correlation coefficients are given in **Table 1.** For all three constituents there was a significant correlation between the initial concentrations and the change in concentration for the d 1.10–1.11 and d > 1.12 g/ml subfractions. For total cholesterol concentrations there was a statistically significant correlation coefficient for three of five subfractions and for total HDL.

The effect of total HDL protein concentration on the distribution of protein among the five HDL subfractions before SAA induction was examined, also (**Fig. 4**). As total HDL protein increased there was a significant decrease in the percentage protein in the d 1.11–1.12 g/ml subfraction with a corresponding increase in the d < 1.09 g/ml subfraction protein. No significant cor-

TABLE 1. Correlation coefficients of the change in concentration of HDL constituents versus initial concentration of HDL constituent[a]

	Density (g/ml)					
	<1.09	1.09–1.10	1.10–1.11	1.11–1.12	>1.12	Total HDL
Protein	−0.56	0.51	0.91[b]	0.57	0.85[b]	0.65
Total cholesterol	0.47	0.72[c]	0.83[c]	0.64	0.91[b]	0.84[c]
Phospholipid	0.49	0.48	0.91[b]	0.49	0.80[c]	0.30

[a]Correlation coefficients were determined for each subfraction constituent by plotting the initial HDL subfraction constituent concentration versus the change in concentration (before–after) with chair restraint.
[b]$P < 0.01$.
[c]$P < 0.05$.

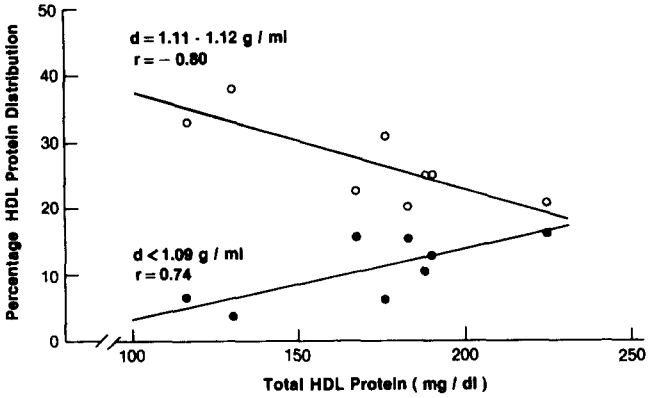

Fig. 4 Relationship between total protein before SAA induction and percentage of HDL protein distributed in the d < 1.09 and d 1.11–1.12 g/ml subfractions. The lines were determined by linear regression and the correlation coefficient is given for each line. Both lines had statistically significant correlation coefficients ($P < 0.05$). The equations for the d < 1.09 and d 1.11–1.12 g/ml subfractions are $y = 0.106x \pm 7.45$ and $y = -0.146x \pm 52.1$, respectively. The correlation coefficients for the other three HDL subfractions were not significant so the lines are not shown. The equations for those lines are: d 1.09–1.10 g/ml, $y = 0.086x \pm 2.96$, $r = 0.52$; d 1.10–1.11 g/ml, $y = -0.061x \pm 35$, $r = -0.44$, and d > 1.12 g/ml, $y = 0.015x \pm 17.2$, $r = 0.08$.

relations between percentage HDL protein distribution and total HDL protein were found for the other HDL subfractions.

Chemical compositions of the HDL subfractions obtained from the animals before and after chair restraint were determined and the results are shown in **Table 2.** Because a limited amount of material was available from the two animals with the lowest HDL concentrations, free and esterified cholesterol and triglyceride values were not determined for those animals. There was a significant increase in all constituents except triglyceride in the d < 1.09 g/ml HDL subfraction. No significant changes after chair restraint were noted for any chemical constituent in the d 1.09–1.10 g/ml or d > 1.12 g/ml subfractions or total HDL. There was a significant lowering of total cholesterol, cholesteryl ester, and protein in the d 1.10–1.11 g/ml subfraction after SAA induction. Free cholesterol had a similar trend but was not significantly lower.

The within-particle compositions of the HDL subfractions before and after induction by chair restraint were calculated and are shown in **Table 3.** Since the amount of individual apoproteins was not known for the subfractions, the number of 10,000 dalton units of protein was calculated. This molecular weight was used because apoSAA, A-II, and C-apoproteins are close to 10,000 in molecular weight (3). ApoA-I has a molecular weight of 28,000 (3), so that approximately three protein units would equal one apoA-I molecule. The total number of protein units did not change for

TABLE 2. Plasma concentration (mg/dl) of HDL subfraction constituents before and after SAA induction[a]

Density	Before Pro	After Pro	Before PL	After PL	Before TC	After TC	Before FC	After FC	Before CE	After CE	Before TG	After TG
g/ml												
<1.09	19.8 ± 4.0	28.4 ± 6.9[b]	20.0 ± 4.1	29.9 ± 6.8[b]	11.1 ± 2.3	15.2 ± 3.1[b]	4.5 ± 0.7	6.4 ± 1.2[b]	15.9 ± 2.2	20.5 ± 3.2[b]	1.1 ± 0.2	3.1 ± 1.7
1.09–1.10	31.6 ± 4.9	30.8 ± 4.2	27.7 ± 4.3	28.5 ± 3.9	13.4 ± 2.4	13.2 ± 1.9	4.6 ± 0.7	4.7 ± 0.6	19.3 ± 2.9	17.6 ± 2.3	1.6 ± 0.3	1.6 ± 0.6
1.10–1.11	41.4 ± 3.3	34.9 ± 1.4[b]	30.4 ± 2.6	26.0 ± 1.1	13.4 ± 1.0	11.5 ± 0.6[b]	4.1 ± 0.4	3.5 ± 0.2	17.7 ± 0.9	13.8 ± 0.8[b]	1.8 ± 0.6	1.5 ± 0.3
1.11–1.12	44.9 ± 2.2	44.7 ± 2.4	27.0 ± 1.3	27.4 ± 1.4	12.9 ± 1.1	11.8 ± 0.9	3.5 ± 0.3	3.3 ± 0.3	16.7 ± 1.6	14.6 ± 1.4[b]	1.9 ± 0.4	1.7 ± 0.3
>1.12	34.3 ± 4.8	37.5 ± 3.2	14.4 ± 2.1	16.5 ± 1.6	7.0 ± 1.2	6.8 ± 0.6	1.8 ± 0.3	1.8 ± 0.3	10.2 ± 2.0	8.2 ± 1.0	1.8 ± 0.4	1.5 ± 0.4
Total HDL[c]	171.9 ± 12.3	176.1 ± 9.6	119.6 ± 8.9	128.3 ± 8.9	54.9 ± 5.3	57.0 ± 3.9	17.8 ± 1.4	19.8 ± 1.0	76.4 ± 3.4	75.2 ± 2.7	7.6 ± 1.4	9.3 ± 2.7

[a]Plasma samples were taken from individual animals before and after 24 hr of SAA induction by chair restraint. HDL was isolated and subfractionated and chemical constituents were measured. Values represent the mean ± SEM for eight (Pro, PL, TC) or six (TC, CE, FC) animals.
[b]Values before versus after SAA induction by chair restraint that are significantly different ($P < 0.05$).
[c]The mean ± SEM of the sum of the five individual subfractions.

TABLE 3. Within particle compositions of HDL subfraction constituents before and after SAA induction

Density	HDL MW	Molecules/Particle									
		Before Pro	After Pro	Before PL	After PL	Before TG	After TG	Before FC	After FC	Before CE	After CE
g/ml	*g/mmol*										
<1.09	408	14 ± 0.5[a]	14 ± 0.5	183 ± 4	190 ± 5	8 ± 2	10 ± 3	66 ± 2	68 ± 2	141 ± 5	131 ± 6
1.09–1.10	328	13 ± 0.4	13 ± 0.5	145 ± 3	151 ± 4	6 ± 1	6 ± 2	41 ± 2	44 ± 2[b]	101 ± 5	95 ± 5
1.10–1.11	283	13 ± 0.2	12 ± 0.3	118 ± 3	120 ± 3	5 ± 1	5 ± 1	30 ± 2	32 ± 1	76 ± 4	76 ± 5
1.11–1.12	231	11 ± 0.3	11 ± 0.3	86 ± 3	90 ± 2	5 ± 1	5 ± 1	22 ± 1	21 ± 1	62 ± 3	55 ± 3[b]
<1.12	204	12 ± 0.3	12 ± 0.3	60 ± 3	65 ± 1[b]	6 ± 2	5 ± 1	15 ± 1	15 ± 1	45 ± 2	39 ± 2

[a]Values are the mean ± SEM for six animals. Values used for molecular weight (MW) are as follows: Pro (10,000), PL (775), TG (900), FC (387) and CE (667).
[b]Values before versus after SAA induction by chair restraint that are significantly different ($P < 0.05$).

any HDL subfraction with SAA induction. There was a mean increase of five PL molecules/particle with SAA induction among the five HDL subfractions but the increase was only significant in the d > 1.12 g/ml subfraction, where it represented an 8% increase. There was little change in the number of TG, FC, or CE molecules/particle before versus after SAA induction. The increase in FC in the d 1.09–1.10 g/ml subfraction was significant because five of six animals had an increase while one animal remained the same. Five of six animals had a decrease in the number of CE molecules/particle in the d 1.11–1.12 g/ml subfraction, which resulted in a significant mean decrease of 11% for the group.

The apoprotein pattern of the HDL subfractions isolated before and after chair restraint was analyzed by SDS-PAGE and IEF gels. A representative SDS polyacrylamide gradient gel is shown in **Fig. 5.** The left side of the figure shows a gel of purified apoSAA, A-II, and A-I along with a subfraction of HDL; the right side of the figure shows all five HDL subfractions for an animal before and after apoSAA induction. The predominant apoprotein in all subfractions was apoA-I. Only small amounts of apoSAA-size material were seen in any of the HDL subfractions before chair restraint. However, after 24 hr of chair restraint more apoSAA was apparent in all of the HDL subfractions. In addition, after chair restraint there was a progressive increase in the amount of apoSAA as the density of the HDL subfraction increased. The same trend was confirmed with IEF gels of the subfractions (data not shown).

The SDS-PAGE gels were scanned with a laser densitometer and the apoA-II + C's/A-I and apoSAA/A-I area ratios were determined (**Table 4**). The use of area ratios obviates the need for assumptions regarding the dye binding potential of individual apoproteins. The A-II + C's/A-I ratio was unchanged with apoSAA induction but the apoSAA/A-I ratio was 3- to 10-fold higher ($P < 0.001$). The apoSAA/A-I ratio increased progressively with the density of the HDL subfraction. In the d > 1.12 g/ml subfraction the amount of apoSAA was 80% of that of apoA-I compared to 8% before induction.

DISCUSSION

Chair restraint of nonhuman primates results in a rapid induction of HDL apoSAA (4). We have hypothesized that the direct addition of apoSAA to HDL particles may result in a shift in the density distribution of HDL subfractions or alternatively, may result in the displacement of other apoproteins and/or lipid from

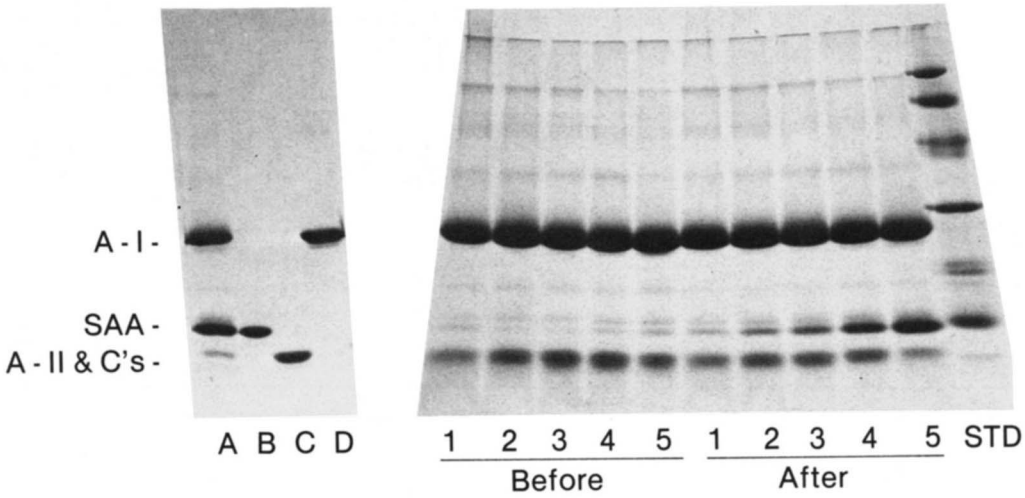

Fig. 5 SDS polyacrylamide gradient gels (12 to 30%) of purified HDL apoproteins and density gradient fractions. Details of sample and gel preparation are given in the Methods section. Left: HDL subfraction d > 1.12 g/ml of animal #281 (A), purified apoSAA (B), purified apoA-II (C), and purified apoA-I (D). Right: HDL density gradient subfractions from animal #239 before and after apoSAA induction by chair restraint. A molecular weight standard mixture was run for reference; the standards were phosphorylase b(mol wt 94,000), albumin (mol wt 67,000), ovalbumin (mol wt 43,000), carbonic anhydrase (mol wt 30,000), trypsin inhibitor (mol wt 20,100), and lactalbumin (mol wt 14,400). The HDL density subfractions (g/ml) before (left) and after (right) induction are indicated by the numbers. Legend: 1, (d < 1.09); 2, (d 1.09–1.10); 3, (d 1.10–1.11); 4, (d 1.11–1.12); and 5 (d > 1.12).

the particle surface. In our study apoSAA was associated with all HDL subfractions but was found predominantly associated with the denser HDL subfractions (Fig. 5). A similar finding has been reported for mouse HDL where apoSAA was induced by injection of endotoxin (2). In that study there was a significant increase in the percentage protein mass in total HDL after apoSAA induction (46 vs. 50%). Our findings for apoSAA induction in the monkey were different because within particle compositions for the HDL subfractions were unchanged with induction of apoSAA (Table 3). However, the amount of apoSAA relative to apoA-I was significantly increased with induction (Table 4). Since the total number of units of protein per particle was unchanged and the relative amount of apoSAA in HDL subfractions increased with induction, apoSAA must have displaced other apoproteins from the particle surface. If the data in Table 4 are used with the data in Table 3, an estimate of the number of apoA-I and apoA-II or apoC molecules displaced by apoSAA can be made.[1] The d > 1.12 g/ml subfraction contained an average of three molecules of apoA-I and two molecules of apoA-II (or C)/particle before induction. After induction an average of two apoA-I, one apoA-II (or C's) and three or four apoSAA molecules per particle were found for the same subfraction. Thus three or four molecules of apoSAA displaced one molecule of apoA-I and one molecule of apoA-II or C per HDL particle in the d > 1.12 g/ml subfraction. We conclude from these data that apoSAA can be added to HDL particles resulting in the displacement of indigenous apoA-I and apoA-II or C molecules with no overall change in the total per particle protein composition.

Hoffman and Benditt (2) reported that apoSAA induction in the mouse resulted in a significant percentage decrease in the phospholipid composition of HDL (35.9 vs. 31.4%). No such decrease was found in our study. In fact there was a slight average increase of 4.5% in the number of phospholipid molecules per particle among all the HDL subfractions, with the largest increase resulting in the d > 1.12 g/ml subfraction (8.3%; five molecules PL/particle). The explanation for the disparity between our results and those of Hoffman and Benditt is not known but the method of apoSAA induction and the animal model used were both different, as were many methodologic details. Our data establish the possibility that apoSAA can alter the apoprotein composition of HDL particles without dramatically altering other characteristics of the particle.

[1] A percentage protein distribution was calculated from the peak area ratio assuming that the chromogenicity of each individual apoprotein was similar. The percentage protein distribution was then multiplied by the total units of protein in Table 3 and corrected for differences in molecular weight. For example, the mean d > 1.12 g/ml subfraction protein distribution after induction was 50.5% apoA-I, 40.4% apoSAA, and 9.1% apoA-II. This is equal to 2.2 molecules of apoA-I/particle (0.505 × 12 units ÷ 2.8), 3.5 molecules of apoSAA/particles (0.404 × 12 units ÷ 1.4), and 1.1 molecules of apoA-II/particle (0.091 × 12).

TABLE 4. Area ratio of HDL subfraction apoproteins before and after apoSAA induction[a]

Density	A-II + C's/A-I (× 100)		SAA/A-I (× 100)	
	Before	After	Before	After
g/ml				
<1.09	19.0 ± 2.9[b]	22.2 ± 5.1	4.3 ± 2.3	13.2 ± 6.8
1.09–1.10	30.4 ± 5.7	29.6 ± 3.7	2.4 ± 0.8	20.5 ± 6.7
1.10–1.11	33.2 ± 4.6	36.0 ± 5.5	3.2 ± 0.9	29.4 ± 10.0
1.11–1.12	32.6 ± 4.4	28.0 ± 3.4	5.5 ± 2.2	40.3 ± 14.0
>1.12	23.3 ± 6.8	18.0 ± 4.0	7.8 ± 2.9	80.1 ± 27.1
Significance, before versus after[c]	$P = 0.603$		$P < 0.001$	

[a]Area ratio derived from laser densitometry of Coomassie Blue-stained SDS polyacrylamide gradient gels of HDL subfractions.
[b]Mean ± standard error of the mean (n = 7).
[c]Determined by paired *t*-test for all seven samples in each density interval.

Although there was no significant change in chemical composition of the HDL subfractions with apoSAA induction, there was a shift in the density distribution. After apoSAA induction there was a decrease in the mass of the d 1.10–1.11 g/ml subfraction and an increase in the d < 1.09 g/ml subfraction (Table 2). There are at least two hypothetical explanations for this trend. With chair restraint there may be a redistribution of d 1.10–1.11 g/ml material to the d < 1.09 g/ml subfraction. For this to occur there would have to be extensive remodeling of the lipoprotein particle since there was almost twice the number of lipid molecules per particle for the same amount of protein in the d < 1.09 versus d 1.10–1.11 g/ml subfraction. An alternative explanation could be that selective anabolism and/or catabolism of HDL subfractions leads to the observed changes in subfraction distribution.

Induction of apoSAA by chair restraint resulted in the displacement of apoA-I and apoA-II with no net change in the lipid composition of the HDL particles. However, the density distribution of HDL subfractions does change and the change is related to initial HDL concentrations (Fig. 3). These results suggest that chair restraint has effects on HDL subfraction distribution that are independent of apoSAA induction. These effects may be related to a general increase in stress experienced by the restrained animals. The more classical methods of apoSAA induction may also result in changes in the HDL characteristics independent of apoSAA induction, although this possibility has not been examined in other studies.

We have previously reported that the metabolism of nonhuman primate HDL apoSAA is rapid compared to that of apoA-I and apoA-II (4, 18). In addition, we have found that HDL apoA-I and apoA-II are metabolized slightly faster when HDL have increased amounts of apoSAA (FCR = 0.558 ± 0.027 vs. 0.485 ± 0.022 for apoA-I and 0.798 ± 0.094 vs. 0.637 ± 0.081 for apoA-II) (J. S. Parks and L. L. Rudel, unpublished data). If apoA-I and apoA-II are catabolized faster when displaced from HDL, then displacement of these apoproteins from HDL by apoSAA may explain the faster turnover rate with apoSAA induction. Alternatively, apoSAA may increase the turnover rate of a subfraction of HDL, resulting in a faster catabolism of the indigenous apoA-I and apoA-II of that subfraction.

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