Lipoprotein Abnormalities Associated with Lipopolysaccharide-induced Lecithin:Cholesterol Acyltransferase and Lipase Deficiency*

(Received for publication, August 3, 1988)

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Density gradient ultracentrifugation was used to isolate and characterize the plasma lipoproteins from African green monkeys before and 24 and 48 h after subcutaneous injection of 300 μg/kg lipopolysaccharide (LPS) to induce an acute phase response. Compared with 0 h values, reductions occurred in plasma cholesterol (39%), high density lipoprotein (HDL) cholesterol (54%), lecithin:cholesterol acyltransferase (LCAT) activity (55%), and post-heparin plasma lipase activity (68%) 48 h after LPS injection while plasma triglyceride concentrations increased 700%. Cholesterol distribution among lipoproteins shifted from 7 to 41% in very low density lipoproteins (VLDL), 65 to 38% in low density lipoproteins (LDL), and 28 to 21% in HDL after LPS injection. At 48 h after LPS injection, all lipoprotein classes were relatively enriched in phospholipid and triglyceride and depleted of cholesteryl ester. The plasma concentration of all chemical constituents in VLDL was increased 3-9-fold within 48 h after LPS injection. By negative stain electron microscopy, HDL were discoidal in shape while VLDL and LDL appeared to have excess surface material present. Even though total HDL protein concentration in plasma was unaffected, the plasma mass of the smallest HDL subfractions (HDL3s) doubled while the mass of intermediate-sized subfractions (HDL3a) was dramatically decreased within 24 h after treatment. HDL became enriched in apoE, acquired apoSAA, and became depleted of apoA-I, A-II, and Cs by 48 h after LPS injection while apoB-100 remained the major apoprotein of VLDL and LDL. We conclude that administration of LPS to monkeys prevents normal intravascular metabolism of lipoproteins and results in the accumulation of relatively rasant forms of lipoproteins in plasma. These immature lipoproteins resemble those isolated from the recirculating perfusion of African green monkey livers, which are relatively deficient of LCAT activity and those isolated from the plasma of patients with familial LCAT deficiency.

Recent studies have demonstrated an interrelationship be-

between acute and chronic inflammatory events and plasma lipid concentrations (1–5). Changes in plasma lipids have been associated with bacterial, viral, and protozoal infections as well as cancer (1, 4, 6–8). The most typical response to infection is elevated plasma triglycerides with a decrease in total plasma and HDL cholesterol concentrations. The acquired hypocholesterolemia that accompanies infections or malignant illness has even been suggested as an indicator of poor prognostic outcome (9).

Probably the best studied experimental procedure of the interrelationship between infection and host response has been the injection of lipopolysaccharide (LPS) in experimental animals. LPS is a complex macromolecule, containing lipid and polysaccharides, that is present in the cell wall of Gram-negative bacteria (10). When LPS is injected into experimental animals or humans in sublethal doses, it results in a complicated series of pathological responses including fever, leukocytosis, disseminated intravascular coagulation, and the increased synthesis and secretion into plasma of a class of proteins collectively referred to as acute phase reactant proteins (10, 11). When macrophages encounter LPS they are stimulated to secrete a number of monokines including interleukin-1 and cachectin that cause many of the observed systemic effects of infection. With regard to the effects on lipid concentrations, both interleukin-1 and cachectin inhibit lipoprotein lipase activity in vitro, and cachectin has been shown to inhibit this enzyme in vivo (12–14). Lipoprotein lipase is a key enzyme in the hydrolysis of plasma triglyceride, and its inhibition is thought to lead to the observed hypertriglyceridemia when LPS is given to experimental animals. The cause of the hypocholesterolemia seen with infections or observed after LPS administration is poorly understood.

Although the effects of LPS on plasma lipid concentrations are well known, there is little information regarding the effect of LPS on plasma lipoprotein composition and distribution. This study was undertaken to characterize the changes in lipoprotein composition, distribution, and concentration when LPS was given to African green monkeys. We have used this species in the past to study the effects of environment factors on lipoprotein characteristics (15, 16). African green monkeys fed diets typical of those consumed by North Americans have lipoprotein profiles more similar to that of man than many other nonhuman primate species (17). We found that LPS administration to monkeys appears to prevent normal intravascular metabolism of lipoproteins and results in the accumulation of immature forms of lipoproteins that

*This research was supported by National Heart, Lung, and Blood Institute Grants HL-14184 (Specialized Center of Research in Arteriosclerosis), HL-24736, HL-38066, and HL-07115. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡This work was carried out as partial requirement for the Master of Science degree in Comparative and Experimental Pathology from the Bowman Gray School of Medicine of Wake Forest University.

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†The abbreviations used are: HDL, high density lipoprotein; LCAT, lecithin; cholesterol acyltransferase; LPS, lipopolysaccharide; VLDL, very low density lipoprotein; SDS, sodium dodecyl sulfate; LPL, lipoprotein lipase; LDL, low density lipoprotein; apoSAA, serum amyloid A protein.
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resemble those isolated from monkey liver perfusates or the plasma of human beings with familial LCAT deficiency.

**Materials and Methods**

**Experimental Animals—**Eight adult (4 male/4 female) African green monkeys (Cercopithecus aethiops), weighing 2.8-7.5 kg, were used in this study. The animals were maintained on a diet containing 0.78% of cholesterol/kcal and 40% of calories as fat (butter or safflower oil) for a period of at least 3 years before the study began (18). The animals were generously supplied by Dr. Lawrence L. Rudel, Department of Comparative Medicine of The Bowman Gray School of Medicine.

Lipoprotein Isolations—All animals were fasted at least 18 h prior to blood samplings or administration of LPS. A solution of LPS (2.5 mg/ml) from *Escherichia coli*, serotype O55:B5 (Sigma) was prepared and injected subcutaneously in the abdomen at a dose of 300 μg/kg body weight into animals sedated with 10 μg of Ketamine HCL (Bristol Laboratories, NY)/kg body weight. Control animals were given injections of saline.

At the time of blood sampling, the fasted animals were sedated with Ketamine, and blood was collected in tubes containing a final concentration of 0.1% EDTA, 0.04% 5,5'-dithiobis(2-nitrobenzoic acid), and 0.01% sodium azide, pH 7.4. Red blood cells were removed by centrifugation at 39,000 × g/min. Lipoproteins were then isolated from the plasma by adjusting the plasma solvent density to 1.225 g/ml by the addition of solid KBr, followed by centrifugation of plasma for 20 h at 50,000 × g at 4 °C in a Ti-50 rotor (Beckman Instruments, Inc., Palo Alto, CA). The lipoproteins were removed by slicing the top 1 cm of the tube and aspirating the supernatant.

Density gradient centrifugation was used for lipoprotein class fractionation. A discontinuous gradient was established by overlaying 4.5 ml of 1.060, 5.5 ml of 1.000, and 2.5 ml of 1.060 g/ml KBr solutions in SW 40 rotor tubes (Beckman Instruments). The KBr solutions were made by addition of solid KBr to 0.9% NaCl, 0.01% EDTA, 0.01% sodium azide. The lipoprotein samples were dialyzed to a density of 1.030 g/ml and added to the = 1.030 g/ml segment of the gradient. The tubes were centrifuged at 40,000 rpm for 4 h at 4 °C in an SW 40 rotor. The gradients were observed using a laser interferometer (Electrophoresis-Prepoured Preparative Electrophoresis, Fisher Scientific, Springfield, NJ) underlayed with 1.060 KBr. The tubes were run through a UV monitor after piercing the bottom of the centrifuge tube and pumping in a dense (1.9 g/ml) solution of Fluorinert (3M Company, St. Paul, MN). Fractions of 0.35 ml were collected using a fraction collector. The density profile of the samples was determined by a standard curve generated by reading the refractive index of solutions of known densities that were prepared by the addition of solid KBr to saline. Fractions containing discrete peaks were pooled for further analysis. Fractions pooled from the density gradient run on the 24- and 48-h plasma samples were chosen using the analysis of variance for each animal, such that, for each animal, equivalent density gradient fractions of 0.24, and 48-h plasma samples were taken for analysis. Plasma HDL cholesterol concentrations were also determined using the heparin-manganese precipitation procedure (19).

Electron Microscopy—Isolated lipoprotein fractions, at a concentration of 2 mg of total mass/ml, were negatively stained with 2% potassium phosphotungstate, pH 6.5, on Formvar-carbon-coated grids. The negatively stained lipoproteins were observed with a Philips 400 transmission electron microscope (Philips Electronic Instruments, Mahwah, NJ).

**Chemical Analyses—**Chemical composition of the isolated lipoproteins was determined as follows. Aliquots of lipoproteins were lyophilized and were extracted with chloroform/methanol (2:1). The extracted lipids were dried under nitrogen and were dissolved with chloroform. Approximately 300 μg of total lipid were applied to activated silica gel plates, which were developed using a solvent system of hexane/ethyl ether/acidic acid (80:20:2 v/v/v). The separated lipids were scrapped from the plate and eluted from the silica gel with either chloroform (free cholesterol, and cholesteryl ester) or chloroform/methanol (2:1 for triglyceride). Concentrations of free cholesterol were measured using the method of Zlatkis et al. (20). Plasma cholesterol and total cholesterol concentrations of aqueous aliquots of isolated lipoproteins were also measured by an enzymatic method (Gilford Single Vial Reagent System, Gilford Diagnostics, Cleveland, OH). Protein content was determined using the method of Lowry et al. (21), with the method of examiner color development and extraction of the samples with hexane. Phospholipid phosphorus was measured by the method of Fiske and SubbaRow (22). Triglycerides isolated by thin layer chromatography were quantitated by the method of Sardessai and Manning (23). Plasma triglycerides were also quantitated by this method after adsorption of plasma phospholipids with activated Foliiari (24).

**Gradient Gel Electrophoresis—**Prepared gradient gels (+3%) (Pharmacia LKB Biotechnology Inc.) were used to investigate lipoprotein subclass size heterogeneity as described previously (18, 25). Bovine plasma (d < 1.255 g/ml) was dialyzed in water, and 1/20 vol of 25% glycerol mixed with 1/20 vol of 0.1% sodium azide. A 19-20 μl aliquot containing 10 μg of protein was applied to the gels. Gels were subjected to electrophoresis for 18 h at 125 V (10 °C) and after electrophoresis gels were stained with Coomassie Blue G-250. After destaining the gels were scanned using a laser densitometer. The relative migration distance (Rf) for an individual band for each sample was calculated by taking the ratio of the migration distance of the band relative to the migration distance of bovine serum albumin in the standard lane of the same gel. Each peak was designated as either HDL, (Rf = 0.445-0.711), HDL2 (Rf = 0.711-0.781) or HDL3 (Rf = 0.781-0.962) based on the nomenclature of Blanche et al. (25) and the area under each peak was calculated by dropping vertical lines from the trough of each subfraction peak to the abscissa. Plasma concentration of individual HDL subfractions was calculated by multiplying the percentage protein distribution derived by the densitometry by the concentration of the HDL protein in plasma. The latter was calculated from the Lowry protein concentration of the density gradient isolated HDL and was corrected back to whole plasma concentration by cholesterol recovery.

**SOD-Gradient Gel Electrophoresis—**Qualitative analysis of apoprotein B was done using the method of Tuppy et al. (26). SDS-gradient gel electrophoresis. Preparative gels (4-35%) were equilibrated with SDS by prerunning the gels for 2 h at 100 V (15 °C) with an electrode buffer consisting of 50 mM Tris-HCl, 20 mM sodium acetate, 2 mM EDTA, 0.2% SDS, pH 6.8. Samples were dialyzed against 0.01% EDTA, 0.01% NaNO3, pH 7.4, and then lyophilized. A solution containing 1% SDS, 8% sucrose, and 0.002% bromphenol blue was added to the sample to give a final protein concentration of 1 mg/ml. Aliquots of samples containing 5-10 μg of protein were heated to 100 °C for 2 min and were subjected to electrophoresis of 4 h at 100 V (10 °C). After electrophoresis, gels were stained overnight with 0.25% Coomassie Blue R-250 and stained overnight with 25% methanol, 10% acetic acid and were destained consecutively with 50% methanol, 10% acetic acid and 10% methanol, 10% acetic acid.

**Enzyme Assays—**Plasma LCAT activity was measured by the addition of a radiolabeled artificial substrate to plasma using a modification (27) of the procedure of Chen and Albers (28). Briefly, recombinant particles consisting of egg yolk lecithin, [14C]cholesterol, and human apo-A-I (258:80.8 molar ratio) were made by the cholate dialysis method. Each assay contained 30 μl of plasma, 3 μg of substrate cholesterol, 2% human serum albumin, and 5 mM β-mercaptoethanol. The samples were incubated in a shaking water bath at 37 °C for 30 min. The incubation was stopped by the addition of 495 μl of ethanol. The lipids were extracted twice with 5 and 3 ml of hexane containing 20 μg/ml each of cholesteryl oleate and free cholesterol as carriers. The extract was then dried under a stream of nitrogen and redissolved in chloroform. The lipids were then separated by thin layer chromatography as described previously. CE spot was scraped, and the radioactivity was measured in a liquid scintillation counter. LCAT activity was reported as nanomoles of cholesteryl ester formed/milliliter of plasma/hour.

Post-heparin plasma triglyceride lipase activity was measured using Intralipid (Cutter Laboratories, Berkeley, CA) containing 9:10-3Holeoylglycerol as the substrate (46). Post-heparin plasma was obtained 20 min after the intravenous injection of 100 units of heparin/kg body weight. Ten μl of post-heparin plasma was added to 90 μl of the substrate solution containing the labeled Intralipid and 1% albumin in 0.2 M Tris buffer, pH 8.5. HDL was added to provide a source of apo-C1, an LPL activator. The mixture was incubated at 37 °C for 60 min, and the reaction was stopped with the addition of 1.5 ml of benzene/chloroform/methanol (1:0.5:1.2, v/v/v) containing 0.1 mM oleic acid as carrier. A 0.5-ml aliquot of 0.3 N NaOH was then added and the mixture was vortexed to completely release free fatty acids. The solution was then separated by low speed centrifugation. An aliquot of the upper phase was taken, and the radioactivity was determined by liquid scintillation counting. One unit of activity was equal to 1.0 μmol of oleic acid hydrolyzed/h.

**Statistical Analyses—**All values are given as mean ± S.E. Statistical comparisons of base-line values to 24 and 48 h post-injection values were made using repeated measures analysis of variance and the Fisher’s least significant difference test (29).
RESULTS

Plasma Cholesterol, Triglyceride, and HDL Concentrations—Bacterial endotoxin was injected subcutaneously in the abdomen of eight African green monkeys at a dose of 300 µg/kg body weight to study its effect on plasma lipid concentrations. During preliminary studies, this dose did not result in a measurable increase in body temperature at the indicated time points nor in any clinical signs of illness of the animals. Fig. 1 shows the concentrations of total plasma and HDL cholesterol and plasma triglyceride at 24 and 48 h after injection. The total plasma cholesterol concentrations decreased 33% (p < 0.001), HDL concentrations (HDL-C) fell 21% (p < 0.04), and triglyceride concentrations were slightly higher 24 h after LPS injection. Forty-eight h after injection total plasma cholesterol and HDL-C concentrations were still significantly (p < 0.02) lower (29 and 54%, respectively) than preinjection concentrations while plasma triglycerides were 7-fold higher (20 ± 1 versus 136 ± 14 mg/dl; p < 0.015). Two animals were followed for a longer period of time; within 72 h after LPS injection the total plasma cholesterol, triglyceride, and HDL concentrations were approaching base-line values, but 6 days were required for all values to return to normal in these two animals. In control studies two animals had mean total plasma cholesterol values of 429, 406, and 431 mg/dl at 0, 24, and 48 h after injection of saline. The corresponding mean HDL-C values after normal saline injection were 48, 56, and 48 mg/dl. Triglyceride concentrations were not determined for the control animals.

To determine the effect of LPS administration on lipoprotein distribution, plasma lipoproteins were fractionated for six of the eight animals using density gradient centrifugation and the cholesterol distribution was measured. Generally, three distinct regions were apparent that corresponded to VLDL, LDL, and HDL based on chemical compositions (Tables II–IV), apoprotein composition (Fig. 2), agarose gel mobility (data not shown), and size on gradient gels of these isolated fractions. In four of the animals, the LDL region contained two peaks (designated as light and heavy LDL) that were pooled separately and analyzed. The cholesterol distribution of each region from the density gradient analysis is given in Table I. At base-line LDL contained much of the cholesterol mass (65%) with very little in VLDL (6.5%). After LPS administration the proportion of cholesterol distributed in VLDL increased 6-fold relative to base line while the proportion of cholesterol in LDL decreased to 60% of the preinjection value.

Lipoprotein Compositions—Chemical compositions were measured on the lipoprotein fractions isolated by density gradient ultracentrifugation. Tables II–IV show the results for VLDL, LDL, and HDL, respectively. All of the chemical constituents of VLDL showed significant (p < 0.05) mass increases 48 h after LPS injection (Table II). However, when analyzed as percentage composition, the major changes in VLDL composition involved an enrichment in triglyceride and PL and a decrease in CE content. The relative depletion of VLDL cholesteryl ester was nearly equal to the enrichment of VLDL triglyceride such that the percentage of core neutral lipid was similar between 0 versus 48 h time points.

The chemical compositions of LDL are shown in Table III. Twenty-four h after LPS injection there was a 53% decrease in plasma concentration of LDL protein (p < 0.05) and a 70% decrease in LDL cholesteryl ester (p < 0.05). Large changes in the percentage of LDL phospholipid (21 versus 31%) and cholesteryl ester (46 versus 27%) were also observed between base-line and 24-h plasma samples. Two days after LPS injection there was a 3-fold increase in LDL triglyceride concentration and a 74% reduction in LDL cholesteryl ester such that the two neutral lipids were comparable in concen-
The percentage of LDL protein did not change at either time point after LPS treatment.

The chemical compositions of HDL are shown in Table IV. There were significant increases in plasma HDL phospholipid and free cholesterol and a 44% decrease in HDL cholesteryl ester concentration 24 h after LPS injection. Forty-eight h after injection the HDL cholesteryl ester concentration was 67% lower than base-line values, and there was nearly a 3-fold increase in HDL triglyceride. HDL protein, phospholipid, and free cholesterol were similar to preinjection concentrations.

The apoprotein profile of the density gradient regions was examined by SDS-polyacrylamide gradient gels. Aliquots of VLDL, light LDL, heavy LDL, and HDL were pooled separately from the gradients of four individual animals and analyzed (Fig. 2). Before LPS injection the major apoprotein of VLDL and LDL was apoB and HDL (lane e) contained apoa-I, A-II, and Ca. After LPS injection the major apoprotein of VLDL and light LDL (lanes f, g, j, and k) was still apoB, but there was an additional band at M, = 14,000 that we have previously identified as apoSAA (15). Heavy LDL (lanes h and i) contained very little apoB and apoA-I and apoE were the predominant apoproteins. This may represent contamination of heavy LDL with HDL particles since LDL protein concentration decreased (Table III) while HDL protein concentration was unchanged (Table IV). HDL fractions contained apoSAA as well as apoA-I after LPS injection (lanes i and m).

Lipoprotein Morphology—The changes in lipoprotein morphology associated with endotoxin administration were examined by electron microscopy (Fig. 3). Prior to LPS administration, the VLDL were round in appearance and electronlucent when negatively stained and viewed by electron microscopy (Fig. 3). After LPS treatment the VLDL were no longer uniformly round and some of the VLDL particles had surface projections. The LDL and HDL had a typical round appearance before LPS administration, after which, both fractions contained many particles that were discoidal in shape. The HDL particles following LPS administration also tended to form rouleaux upon negative staining.

HDL Size Heterogeneity—The size distribution of HDL subfractions was examined by gradient gel electrophoresis in a subset of the animals. Peaks were classified as HDL, HDL or HDL based on Rf values of the HDL subfractions relative to bovine serum albumin according to the nomenclature of Blanche et al. (25). It has previously been shown that the results of this analysis correspond well with results from density gradient centrifugation and analytical ultracentrifugation for monkey HDL subfractions (26).

Peak areas were quantitated and are shown in Table V. Before LPS treatment half of the HDL protein mass was distributed in HDL; and the distribution did not change significantly after LPS injection. HDL was initially 30% of the protein mass, but 24 h after LPS injection this subfraction was dramatically reduced. Although there were no detectable peaks in this size range, there was some protein mass which could have been HDL material or could have been incomplete separation of HDL and HDL subfractions. The percentage protein in HDL increased at 24 h after LPS injection, and this was associated in a doubling of protein mass in that subfraction (37 ± 3 versus 70 ± 6 mg/dl). Forty-eight h after injection the distribution of HDL subfraction protein was similar to base-line values.

LCAT and Lipase Activities—Plasma LCAT activity was measured using an exogenous substrate. The results are shown in Fig. 4 which illustrates a precipitous drop in plasma LCAT.

| Table II | Chemical composition of plasma VLDL before and after LPS administration |
|----------|---------------------------------------------------------------|
| Time*    | Protein | Phospholipid | Free cholesterol | Triglyceride | Cholesteryl ester |
| h        | mg/dl   |            |               |             |                  |
| 0        | 5.6±1.4 | 8.9        | 3.8           | 9.8         | 19.0             |
| (11.9%)  | (18.9%) | (8.1%)     | (23.9%)       | (40.3%)     |
| 24       | 9.9±3.4 | 18.4       | 7.5           | 16.6        | 24.3             |
| (13.0%)  | (24.0%) | (9.7%)     | (21.7%)       | (31.7%)     |
| 48       | 39.3±10.1 | 78.4±5.8 | 24.7±5.8      | 111.0±44.6 | 62.6±13.7        |

*Time after LPS injection.

| Table III | Chemical composition of plasma LDL before and after LPS administration |
|-----------|---------------------------------------------------------------|
| Time*    | Protein | Phospholipid | Free cholesterol | Triglyceride | Cholesteryl ester |
| h        | mg/dl   |            |               |             |                  |
| 0        | 87.9±10.6 | 90.2  | 36.5          | 13.9        | 192.4           |
| (20.9%)  | (21.4%) | (8.7%)     | (3.3%)        | (45.7%)     |
| 24       | 42.8±8.4 | 64.6    | 25.4          | 15.1        | 56.4±4.4        |
| (21.0%)  | (31.6%) | (12.4%)   | (7.4%)        | (27.6%)     |
| 48       | 61.2±13.0 | 97.7   | 30.3          | 45.8±4.9    | 49.9±2.1        |
| (21.5%)  | (34.3%) | (10.6%)   | (16.1%)       | (17.5%)     |

*Time after LPS injection.

| Table IV | Chemical composition of plasma HDL before and after LPS administration |
|-----------|---------------------------------------------------------------|
| Time*    | Protein | Phospholipid | Free cholesterol | Triglyceride | Cholesteryl ester |
| h        | mg/dl   |            |               |             |                  |
| 0        | 165.2±33.0 | 123.6 | 14.3          | 5.6         | 80.2             |
| (42.5%)  | (31.8%) | (3.7%)     | (1.5%)        | (20.6%)     |
| 24       | 161.6±29.1 | 144.5±7.4 | 27.6±4.3      | 9.5         | 45.0±14.3       |
| (41.6%)  | (37.2%) | (7.1%)     | (2.4%)        | (11.6%)     |
| 48       | 157.0±34.9 | 131.8 | 15.3          | 14.6±4.9    | 26.5±11.7       |
| (45.6%)  | (39.2%) | (4.4%)     | (4.2%)        | (7.7%)      |

*Time after LPS injection.

*Mean ± S.E. (n = 6).

*Mean % composition.

0 h versus 48 h; p < 0.05.
FIG. 3. Electron micrographs of VLDL, LDL, and HDL isolated by density gradient centrifugation before (−LPS) and 48 h after (+LPS) injection of 300 μg of LPS/kg body weight.

FIG. 4. Whole plasma LCAT activity measured before and after LPS injection (300 μg/kg body wt) into eight African green monkeys. LCAT activity was measured using an exogenous substrate as described under "Materials and Methods." Each symbol represents duplicate determinations for the time course of an individual animal.

**TABLE V**

| Time after LPS treatment (h) | Protein | Plasma protein (mg/dl) |
|-----------------------------|---------|-----------------------|
|                             | HDL2a   | HDL3b                  |
|                             | (n = 6) | (n = 4) |
| 0                           | 49.5b   | 31.2                  |
|                             | ±11.4   | ±9.8  |
| 24                          | 59.9    | 40.1d                |
|                             | ±4.2    | ±4.2  |
| 48                          | 39.5    | 26.3                 |
|                             | ±10.8   | ±7.1  |

aHDL2a, RP = 0.445–0.711; HDL3b, RP = 0.711–0.781; HDL3b, RP = 0.781–0.962.
bMean ± S.E.
cNo detectable peaks.
dp < 0.05, 0 h versus 24 h.

**DISCUSSION**

This study was initiated to determine the effects of a relatively low dose of LPS on the plasma lipoproteins of African green monkeys. Three-hundred μg of LPS/kg body weight resulted in a decrease in LCAT and post-heparin lipase activities and affected lipoprotein concentrations, compositions, and distribution of the animals. Concentrations of plasma LDL and HDL decreased while those of VLDL increased. All lipoprotein fractions became enriched in triglyceride and depleted of cholesteryl ester and, in the LDL and HDL fractions, the total amount of core material was reduced relative to surface, so that many particles were discoidal in shape or showed evidence of having excess surface material. Many of the characteristics of these plasma particles obtained after LPS injection were similar to those of particles derived from recirculating perfusion of African green monkey livers in which LCAT activity was very low (27, 30, 31) and from the plasma of LCAT-deficient human beings (32, 33). Thus, these findings suggest that the lipoprotein particles found in plasma within 2 days of LPS injection represent predominantly liver-derived particles that are incompletely converted into mature particles. If this is the case the LPS-treated African green monkey may serve as source of relatively unmodified hepatic precursor particles for use for structural and metabolic studies.

Of the lipoprotein compositional changes induced by LPS, the most striking was the decrease in cholesteryl ester content. LCAT is considered the source of most plasma cholesteryl ester in man but in cholesterol-fed monkeys the liver is likely to make a significant contribution to the plasma cholesteryl ester pool (30). Although we have no data concerning the hepatic contribution to the plasma cholesteryl ester pool in this study, we found a 55–64% reduction in LCAT activity, measured with an exogenous substrate, concomitant with a
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reduction of plasma LDL and HDL cholesteryl ester concentrations (Tables III and IV). Sakaguchi (5) has reported an increase in LCAT activity in mice treated with LPS (7.7 mg/kg), but an endogenous assay was utilized and it is unclear whether this outcome was due to effects on enzyme activity or on substrate or a combination of both. Thus, it appears likely that the drop in plasma cholesteryl ester content associated with LPS treatment in this study was caused by a decreased plasma LCAT activity. Although several studies have demonstrated that lipase activity is decreased by LPS in vivo, to our knowledge this is the first report to demonstrate a decrease in plasma LCAT activity with a concomitant depletion of plasma cholesteryl ester. To explain our observations, we hypothesize that one or more monokines (i.e. interleukin-1, cachectin) released by macrophages after interaction with LPS inhibits the synthesis of LCAT analogous to the situation with lipoprotein lipase (12-14). In preliminary studies using the cholesteryl-fed cynomolgus monkey, we found that both plasma LCAT mass and activity were decreased after intravenous injection of 100 µg of LPS. The decrease in plasma LCAT mass and activity also occurred with injection of human recombinant tumor necrosis factor (i.e. cachectin) but not with human recombinant interleukin-1. Direct addition of LPS, tumor necrosis factor, or interleukin-1 to the LCAT assay of control plasma samples did not inhibit the activity of LCAT. These data suggest that LPS, tumor necrosis factor, and perhaps other monokines decrease LCAT activity by inhibition of LCAT synthesis. Whether these same compounds affect LCAT degradation as well must await further studies.

The concentration of plasma VLDL was significantly greater at 48 h after LPS injection compared with baseline concentrations and this increase resulted from a 7-fold or greater concentration of all VLDL chemical constituents except cholesteryl ester, which was 3-fold higher. An increase in plasma VLDL after LPS injection has been documented in other studies and is thought to be secondary to the inhibition of LPL activity (3, 34-36). Available data support the concept that macrophages stimulated by an encounter with LPS secrete several monokines including interleukin-1 and cachectin. These monokines have been shown to inhibit LPL activity in vitro and in vivo (12-14). Thus, an inhibition of LPL would lead to an accumulation of VLDL in plasma. Active LPL also appears to be important in the formation of LDL particles. Recently, Goldberg et al. (37) demonstrated that inhibition of LPL activity with anti-LPL antisera decreased the appearance of endogenously labeled apoB into LDL of cynomolgus monkeys. Based on these data the increase in plasma VLDL and decrease in LDL may have resulted from the inhibition of LPL when LPS was given. Whether LPS also stimulates hepatic secretion of VLDL as a result of increased plasma free fatty acid concentrations is unknown and could possibly contribute to the elevated concentrations of plasma VLDL.

LPS treatment was associated with a redistribution of HDL apoproteins. Before LPS treatment HDL particles contained predominantly apoA-I with smaller amounts of apoA-II and C peptides (Fig. 2, lane e). After LPS treatment apoA-I remained the major HDL apoprotein but the apoA-I, A-II, and C peptides were replaced by a protein similar in size to that of monkey apoSAA (Fig. 2, lanes i and m), an acute phase reactant protein. This protein has a sequence identical to that of human apoSAA in all but 8 of 103 amino acids. In a previous study using short-term chair restraint of monkeys to induce apoSAA, we concluded that apoSAA displaced apoA-I and apoA-II from HDL particles (15). Since total HDL protein did not change with LPS treatment (Table IV) and the relative amount of apoA-I, A-II, and Cs decreased after LPS treatment (Fig. 2; lanes i and m) compared with the 0 h (lane e), it appears that apoSAA displaced HDL apoproteins in this study also without a net change in total HDL protein. In our previous study there was no evidence of a change in the lipid composition or the total protein content of HDL (15), while in the present study there was a profound depletion of HDL cholesteryl ester after LPS treatment. Thus, even though the effects of LPS and chair restraint on HDL composition are distinct from one another, it appears in both situations that apoSAA can displace apoA-I and other apoproteins from the surface of HDL.

HDL particle size distribution, as monitored by protein distribution on gradient gels, was modified by LPS treatment even though total HDL protein concentrations were constant (Table V). Since HDL protein concentration was unchanged with LPS treatment, this outcome may be the result of a redistribution of HDL lipid mass. HDL_b2,4 sized particles are the predominant species that accumulate during recirculating perfusion of African green monkey livers (38). These HDL particles are predominantly discoidal in shape and are deficient in core cholesteryl esters because of the low levels of LCAT in the perfusate (27). Upon incubation of the liver perfusate with exogenous LCAT, HDL cholesteryl ester content increased, the particles became round in appearance by electron microscopy and the size of the particles shifted to that of HDL_b2 (38). Thus, a relative deficiency in LCAT activity caused by LPS injection may slow the conversion of HDL_b2,4 particles to HDL_b2,4 particles and result in the HDL subfraction protein distribution seen in Table V. However, at 48 h after LPS injection the distribution of HDL protein had returned to base-line values even though HDL particles remained discoidal in shape (Fig. 3), LCAT activity remained depressed (Fig. 4), and HDL particles were still deficient in cholesteryl ester (Table IV). It is possible that by 48 h after LPS injection when plasma triglycerides concentrations are elevated (Fig. 1), the HDL_b2,4 particles become relatively enriched from the transfer of triglycerides from VLDL to HDL (Table IV). This triglyceride enrichment could result in a size increase of HDL_b2,4 particle so that they migrate in the HDL_b2,4 size range. Because our chemical compositions were measured on the entire spectrum of HDL particles, it is not possible to know whether the HDL_b2,4 particles were selectively enriched in triglycerides.

It is clear from this and other studies that experimental animals have varying degrees of responsiveness to LPS. In our study monkeys given 300 µg of LPS/kg body weight showed dramatic changes in lipoprotein concentration, distribution, and composition while mice given an equivalent of 1.7 mg/kg dose (assuming 30 g of body weight) showed no such change in HDL composition (39). Even correcting for differences in basal metabolic rate between the mouse (140 kcal/kg/day; Ref. 40) and monkey (48 kcal/kg/day), the animals in our study received approximately half the amount of LPS given to mice. In recent years several studies have used LPS or trauma to study acute phase reactant proteins with regard to HDL metabolism (39, 41-45). The results from this study suggest that in some experimental animals and at some doses there may be marked perturbations of plasma lipoproteins that accompany the appearance of acute phase reactant proteins. These perturbations, if not recognized, may complicate the interpretation of the study of acute phase reactant proteins and their effect on lipoprotein metabolism.

The results of this study as well as others establish an
interrelationship between acute inflammation events and lipoprotein metabolism. Indeed, many pathological states have been associated with one or more alterations in lipoprotein concentrations including increased plasma triglyceride concentrations and decreased total plasma and HDL cholesterol (1-9). The myriad of biological effects that result from LPS are thought to be mediated by monokines, but with regard to the lipid changes only tumor necrosis factor has been shown to inhibit LPL \textit{in vivo} (12). To better understand the role of monokines on lipid metabolism during inflammatory events, more \textit{in vivo} studies using the pure monokines will be necessary. Such studies are currently underway in our laboratory using the nonhuman primate model.

\textbf{Acknowledgments}—We gratefully acknowledge the excellent assistance of Linda Odham in manuscript preparation.

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