Lipopolysaccharide and tumor necrosis factor cause a fall in plasma concentration of lecithin: cholesterol acyltransferase in cynomolgus monkeys[1]

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Abstract The effects of intravenous injection of lipopolysaccharide (LPS) and tumor necrosis factor alpha (TNF) were investigated in cynomolgus monkeys (*Macaca fascicularis*). Injection of 20 μg/kg of LPS from *E. coli* (serotype 055:B5) into cynomolgus monkeys fed a monkey chow diet caused a twofold increase in plasma triglyceride and a 25% reduction in plasma cholesterol 48 h after injection. Similar results were found with injection of recombinant human TNF at a dose of 20 μg/kg into chow-fed animals. However, injection of the same dose of LPS or TNF into animals fed an atherogenic diet containing saturated fat and cholesterol resulted in a 2.4- to 5-fold increase in plasma triglyceride concentrations and no significant change in plasma cholesterol levels. The fall in plasma cholesterol levels observed in chow-fed animals was associated with a 57% decrease in the cholesteryl ester (CE) content in low density lipoprotein (LDL) and 35% decrease in CE in high density lipoprotein (HDL) in LPS-injected animals, and a decrease of 33% in CE concentration of LDL and 41% in CE of HDL in animals injected with TNF. In animals fed the atherogenic diet containing saturated fat and cholesterol, the injection of both LPS and TNF also resulted in a significant decrease in the CE content of LDL and HDL. However, the plasma total cholesterol levels did not change in the animals fed saturated fat and cholesterol because the decrease in CE content of LDL and HDL was offset by an increase in very low density lipoprotein (VLDL)-CE. Plasma lecithin:cholesterol acyltransferase (LCAT) activity declined to 22% and 54% of control values 24 h after LPS and TNF injection, respectively. Similarly, plasma concentration of LCAT mass determined by radioimmunoassay declined 35% in animals injected with LPS and 27% in animals injected with TNF within 24 h. Direct addition of LPS or TNF to an in vitro assay of LCAT activity in control plasma had no effect on LCAT activity suggesting that LPS and TNF did not directly inhibit LCAT activity. A part of the lipoprotein changes observed after LPS injection may be due to a decline in LCAT mass and a decrease in generation of cholesteryl ester in plasma. The cytokine, TNF, is an important mediator of these phenomena. **—Ettinger, W. H., L. D. Miller, J. J. Albers, T. K. Smith, and J. S. Parks.** Lipoplysaccharide and tumor necrosis factor cause a fall in plasma concentration of lecithin:cholesterol acyltransferase in cynomolgus monkeys. *J. Lipid Res.* 1990. **31:** 1099–1107.

Supplementary key words VLDL • LDL • HDL • hypocholesterolemia • cholesteryl esters

Acute inflammation is associated with changes in lipid and lipoprotein metabolism (1). Hypertriglyceridemia is a consistent finding during the acute states of inflammatory process and cytokines such as tumor necrosis factor alpha (TNF) and interleukin-1 beta (IL-1), among others, are thought to mediate the effects of inflammation on triglyceride metabolism (2, 3). Tumor necrosis factor inhibits synthesis of lipoprotein lipase in cell culture systems and several animal species, and also increases hepatic synthesis of triglyceride in rats, both of which may result in hypertriglyceridemia (2, 4–8). Administration of interferon gamma to humans inhibits postheparin lipase activity and causes hypertriglyceridemia (9). Interleukin-1 also has been reported to cause an increase in plasma triglyceride concentrations in rodents (10).

Much less is known about the effects of acute inflammation and cytokines on cholesterol metabolism. The reported changes in plasma cholesterol concentrations are variable and appear to be species-specific. In rodents and rabbits, cholesterol levels increase during the acute phase response (4, 11) while in nonhuman primates and humans, infection and other inflammatory conditions almost always lead to hypocholesterolemia (12–21). The mechanisms by which cholesterol levels decrease in primates are not well studied. Recently, we showed that subcutaneous injection of LPS resulted in the development of hypocholesterolemia, as well as a decline in plasma activity of lecithin: cholesterol acyltransferase (LCAT) in African green monkeys (20). Thus, we have hypothesized that one

Abbreviations: LCAT, lecithin:choleterol acyltransferase; LPS, lipopolysaccharide; TNF, tumor necrosis factor; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; PLA, postheparin plasma lipase activity; LDL, lipoprotein lipase.

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mechanism for the development of hypocholesterolemia during the acute inflammatory state is a diminished generation of cholesteryl esters in plasma. To further investigate this hypothesis we undertook this study to answer the following questions. *1)* Is the decrease in LCAT activity after injection with LPS due to inhibition of the enzyme or to a change in mass of the enzyme? *2)* Does TNF mediate the changes in cholesterol concentrations and LCAT activity?

METHODS

Animals

The animals used in this study were adult male cynomolgus monkeys (*Macaca fascicularis*) weighing 4.6–6.1 kg. Animals were fed either monkey chow (Purina monkey chow-25) or a semi-synthetic diet containing 0.38 mg of cholesterol/kcal and 40% of calories as fat (polyunsaturated to saturated fat ratio = 0.3), 15% as protein, and 45% as carbohydrate (22). The animals were fed 150 kcal/kg per day in two feedings at 8 AM and 2 PM. The animals are part of the National Heart, Lung, and Blood Institute-supported Primate Resource Colony at the Bowman Gray School of Medicine of Wake Forest University.

Experimental protocol

The animals were fasted overnight (18 h) prior to the experimental procedures. A baseline blood sample was obtained from the femoral vein after administration of intramuscular ketamine (15 mg) and the animals then were injected intravenously with either lipopolysaccharide from *Escherichia coli*, serotype 055:B5 (Sigma Chemical Company), 20 μg/kg in 0.5 ml of a 0.9% NaCl solution, recombinant human tumor necrosis factor alpha, 0.5–20 μg/kg in 0.5 ml of saline, or 0.5 ml of saline alone. Additional blood samples were obtained at 3 h, 24 h, and 48 h after injection. For experiments designed to measure the TNF concentrations in plasma, blood samples were obtained at 30, 60, 90, and 180 min after injection. Animals were given their regular feedings after the 3-h sample and throughout the remainder of the study.

Measurement of lipoproteins

Blood was collected in tubes containing a final concentration of 0.1% EDTA. Blood cells were separated from plasma by centrifugation at 39,000 *g*-min at 4°C. Lipoproteins were then isolated from plasma by adjusting the solvent density to 1.225 g/ml by the addition of solid KBr, followed by centrifugation of plasma for 20 h at 50,000 rpm at 4°C, in a Ti-70 rotor (Beckman Instruments, Inc., Palo Alto, CA). The lipoproteins were removed by slicing the top 1 cm of the tube and aspirating the supernatant and were fractionated by size into VLDL, LDL, and HDL using a Superose 6B column (Pharmacia, Piscataway, NJ) with a Rainin high performance liquid chromatography system (23). A solution of 0.15 M NaCl, 0.01% EDTA, and 0.01% sodium azide was used to elute the lipoproteins at a flow rate of 1 ml/min.

Total plasma and individual lipoprotein cholesterol and triglyceride were measured by enzymatic methods (Boehringer Mannheim, Single Vial-Reagent System, Indianapolis, IN). Free cholesterol was measured using an enzymatic method (Waco Biochemical Diagnostics, Inc., Usaka, Japan). Esterified cholesterol was estimated by subtracting the free cholesterol from total cholesterol. Protein was measured by the method of Lowry et al. (24), after extraction of the developed color with hexane. Phospholipid phosphorus was measured by the method of Fiske and SubbaRow (25).

Measurement of LCAT activity and mass

Plasma LCAT activity was measured by the addition of a radiolabeled artificial substrate to plasma using a modification of the method of Chen and Albers (26). Briefly, recombinant particles consisting of egg yolk lecithin, [^{14}C]cholesterol, and human apoA-I (250:8:0.8 molar ratio) were made by the cholate dialysis method. Each assay contained 10 μl of plasma, 4 nmol of substrate cholesterol, and 2% human serum albumin and 10 nmol β-mercaptoethanol in a final volume of 495 μl. 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 of cholesteryl oleate and free cholesterol as carriers. The extract was dried under nitrogen and redissolved in chloroform. The lipids were then separated by thin-layer chromatography using a solvent system of hexane–ethylether–acetic acid 80:20:2 (v/v/v). The cholesteryl ester band was scraped off and the radioactivity was measured in a liquid scintillation counter. LCAT activity was reported as nmol cholesteryl ester formed per ml of plasma per h.

LCAT mass was measured using a competitive displacement double antibody radioimmunoassay (RIA) for human LCAT (27). One hundred microliters of a 1:50 dilution of monkey plasma was added to 100 μl of goat anti-human LCAT sera (diluted 1:450). Samples were diluted with RIA buffer containing 1% (v/v) albumin and 0.1% (vol/vol) Tween-20 (Sigma Chemical Co.). The samples were incubated for 48 h, then 100 μl of (1:100) normal goat serum and 300 μl of rabbit anti-goat IgG were added. The radioactivity was measured and tubes were incubated overnight at 4°C. The tubes were centrifuged and resulting precipitates were washed with RIA buffer containing 1% albumin and then measured for radioactivity.

Measurement of postheparin plasma triglyceride lipase activity

Postheparin plasma lipase activity was measured using Intralipid (Cutter Laboratories, Berkeley, CA) containing tri[9,10-^{3}H]oleoylglycerol as the substrate as previously described (20). Postheparin plasma was obtained 10 min after intravenous injection of heparin, 100 units/kg body weight. Ten μl of postheparin plasma was added to 90 μl of substrate solution. The mixture was incubated at 37°C for 60 min and the reaction was stopped with a mixture of benzene-chloroform-methanol followed by 0.3 N NaOH. Radioactivity in the upper phase was determined by liquid scintillation counting. Results are reported as μmol of oleic acid hydrolyzed per h per ml.

Measurement of tumor necrosis factor

TNF concentrations in plasma were measured using a sandwich ELISA assay kit for human TNF (Biokine TNF test kit, T Cell Sciences, Inc., Cambridge, MA).

Statistical analysis

Values are given as mean ± standard error of the mean. Statistical comparisons were done using analysis of variance for repeated measures and analysis of covariance. Associations between continuous variables were measured by Pearson's product moment correlations.

RESULTS

Clinical response to LPS and TNF

Lipopolysaccharide, 20 μg/kg, caused a significant increase in core temperature of the monkeys 1 h after injection (1.0 ± 0.3°C, $P < 0.05$) as did 20 μg/kg of TNF (0.6 ± 0.2°C, $P < 0.05$). There was no significant change in core temperature 1 h after injection with saline (−0.2 ± 0.1°C). The plasma concentration of TNF increased more than 1,000-fold 90 min after LPS injection but rapidly decreased towards preinjection levels by 180 min (**Fig. 1**). Most of the animals were observed to be less physically active than normal for several hours after LPS and to a lesser extent after TNF injection. The animals injected with either LPS or TNF would usually not eat their first feeding (given 3 h after injection) and occasionally would not eat until the next day.

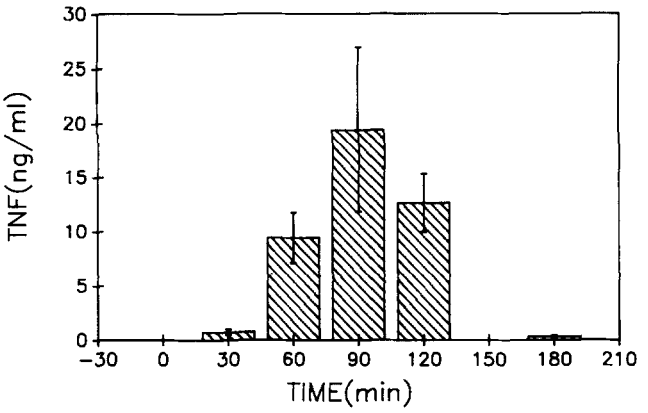

Fig. 1. Mean (± SE) plasma concentration of TNF in serum of four cynomolgus monkeys injected with LPS (20 μg/kg). The assay for TNF was done using a sandwich ELISA assay for human TNFα.

Effects on lipoprotein concentrations

Injection of saline had no significant effect on plasma lipid concentrations; however, there were marked changes in plasma lipid concentrations after LPS injection, and the effects were modified by diet (**Table 1**). Plasma TG concentration increased 2-fold 48 h after LPS injection in chow-fed animals, but TG concentration increased more than 6-fold within 24 h in saturated fat-cholesterol-fed animals. In contrast, there was a 25% reduction in plasma total cholesterol at 24 h which persisted at least 48 h after LPS injection in chow-fed animals, but there was no significant change in plasma cholesterol in the animals fed saturated fat-cholesterol. Similar results were seen in animals injected with TNF. Chow-fed animals had a modest but significant 1.4-fold increase in TG concentration after TNF (20 μg/kg) whereas the animals fed saturated fat-cholesterol had a 3-fold increase. Plasma cholesterol decreased 25% in chow-fed animals, but there was no significant difference in the animals fed saturated fat and cholesterol.

TABLE 1. Effect of LPS and TNF on plasma lipid concentrations in cynomolgus monkeys fed a diet containing monkey chow or saturated fat and cholesterol

Diet	Lipid	Lipopolysaccharide			Tumor Necrosis Factor		
		0 h	24 h	48 h	0 h	24 h	48 h
		mg/dl ± SE			*mg/dl ± SE*		
Monkey chow (n = 4)	TG	45 ± 7	48 ± 6	95 ± 20*	42 ± 3	57 ± 2**	58 ± 4**
	Chol	171 ± 6	116 ± 8*	126 ± 20*	125 ± 4	95 ± 5*	87 ± 6*
Saturated fat and cholesterol (n = 6)	TG	37 ± 4	234 ± 20*	189 ± 16*	45 ± 4	136 ± 8*	107 ± 6*
	Chol	395 ± 18	410 ± 20	401 ± 30	314 ± 20	325 ± 27	292 ± 18

TG, triglyceride; Chol, cholesterol. Statistical comparisons were done using analysis of variance with repeated measures; *, $P < 0.01$; **, $P < 0.05$.

Injection of LPS caused changes in lipoprotein composition within 24 h (**Table 2**). In chow-fed animals, all of the constituents of VLDL increased. In contrast, the concentration of cholesteryl ester decreased by 57% in LDL while TG concentration increased by 50%. There was also a significant decrease in the phospholipid content of LDL. Similarly, the HDL in chow-fed animals showed a 34% decrease in CE concentration and an increase in TG concentration. In animals fed saturated fat and cholesterol, LPS injection was associated with at least a 3-fold increase in concentration of all VLDL constituents. There was a significant 43% decrease in LDL-CE concentration with a concomitant increase in LDL-TG concentration. There was also a 58% decrease in HDL-CE concentration and 54% decrease in HDL protein after LPS injection in saturated fat-cholesterol-fed animals.

Injection of TNF into monkeys resulted in changes in lipoprotein composition that were similar to those seen after LPS injection (**Table 3**). The concentration of VLDL increased 3-fold after TNF injection into chow-fed animals. In contrast, the cholesteryl ester concentration of LDL and HDL decreased by 33% and 41%, respectively, while the concentration of triglyceride increased. Similar effects were noted in saturated fat-cholesterol-fed animals: TNF injection increased VLDL mass by 3-fold whereas the CE concentrations of LDL and HDL were both decreased significantly. The TG concentration of LDL also increased significantly but there was no change in the protein or phospholipid content of LDL or HDL.

Postheparin lipase activity

Total postheparin plasma lipase activity (PLA) was measured in animals injected with LPS or TNF. There were five animals in each group, three were fed the saturated fat-cholesterol diet and two the monkey chow diet. Total PLA was significantly lower 24 h after injection of LPS (41 ± 6 vs 28 ± 4 μmol/h per ml, $P<0.05$) and TNF (39 ± 3 vs 27 ± 5 μmol/h per ml, $P<0.05$). There was no statistically significant interaction between diet and total PLA or change in PLA by analysis of covariance.

TABLE 2. Composition of lipoprotein particles at baseline and 24 h after intravenous injection of LPS in cynomolgus monkeys fed a diet containing monkey chow or saturated fat and cholesterol

Fraction	Time	Phospholipid	Protein	Triglyceride	Free Cholesterol	Cholesteryl Ester
		Monkey Chow (n = 3)				
	h			*mg/dl ± SE*		
VLDL	0	2.5 ± 0.7 (19%)	1.4 ± 2 (10%)	7 ± 2 (52%)	0.2 ± 0.01 (1%)	2.4 ± 2 (18%)
VLDL	24	10 ± 2* (21%)	7 ± 3* (15%)	19 ± 3* (40%)	1 ± 0.5 (2%)	10 ± 2* (21%)
LDL	0	72 ± 7 (23%)	73 ± 4 (24%)	23 ± 1 (7%)	27 ± 1 (9%)	113 ± 4 (37%)
LDL	24	55 ± 4** (26%)	60 ± 7 (28%)	31 ± 1* (14%)	19 ± 2 (9%)	49 ± 4* (23%)
HDL	0	183 ± 10 (33%)	253 ± 7 (45%)	11 ± 1 (2%)	19 ± 1 (3%)	92 ± 5 (16%)
HDL	24	227 ± 23 (37%)	270 ± 31 (44%)	34 ± 3** (5%)	21 ± 2 (3%)	61 ± 7* (10%)
		Saturated Fat-Cholesterol (n = 4)				
				mg/dl ± SE		
VLDL	0	19 ± 2 (21%)	16 ± 4 (18%)	16 ± 9 (18%)	8 ± 2 (9%)	32 ± 3 (34%)
VLDL	24	93 ± 5* (16%)	49 ± 2* (8%)	217 ± 40* (36%)	46 ± 9* (8%)	190 ± 42* (32%)
LDL	0	162 ± 9 (21%)	159 ± 9 (21%)	14 ± 9 (2%)	76 ± 4 (10%)	360 ± 31 (47%)
LDL	24	158 ± 5 (24%)	138 ± 12 (21%)	79 ± 13* (12%)	71 ± 6 (11%)	205 ± 15* (31%)
HDL	0	94 ± 25 (26%)	187 ± 40 (52%)	12 ± 4 (3%)	14 ± 4 (4%)	53 ± 6 (15%)
HDL	24	65 ± 20 (34%)	86 ± 20* (45%)	10 ± 1 (5%)	8 ± 4 (4%)	22 ± 7* (12%)

Values in parentheses are percentage mass distribution. Statistical comparisons were made using analysis of variance of repeated measures; *, $P < 0.01$; **, $P < 0.05$.

TABLE 3. Composition of lipoprotein particles at baseline and 24 h after intravenous injection of TNF in cynomolgus monkeys fed a diet containing monkey chow or saturated fat and cholesterol

Fraction	Time	Phospholipid	Protein	Triglyceride	Free Cholesterol	Cholesteryl Ester
		Monkey Chow (n = 3)				
	h			*mg/dl ± SE*		
VLDL	0	3 ± 0.5 (26%)	2 ± 1 (17%)	4 ± 9 (35%)	0.5 ± 0.1 (4%)	2 ± 1 (17%)
VLDL	24	4 ± 1 (13%)	4 ± 1 (13%)	19 ± 6** (60%)	0.5 ± 1 (2%)	4 ± 0.5 (13%)
LDL	0	47 ± 1 (23%)	50 ± 2 (25%)	15 ± 2 (7%)	21 ± 4 (10%)	71 ± 8 (35%)
LDL	24	48 ± 8 (25%)	47 ± 6 (25%)	26 ± 1** (14%)	22 ± 3 (11%)	48 ± 5* (25%)
HDL	0	147 ± 6 (34%)	175 ± 10 (41%)	13 ± 3 (3%)	10 ± 1 (2%)	83 ± 6 (19%)
HDL	24	139 ± 4 (36%)	163 ± 10 (43%)	15 ± 1 (4%)	16 ± 2** (4%)	49 ± 3* (13%)
		Saturated Fat-Cholesterol (n = 4)				
				mg/dl ± SE		
VLDL	0	7 ± 3 (15%)	8 ± 2 (17%)	16 ± 9 (34%)	4 ± 2 (9%)	12 ± 2 (26%)
VLDL	24	19 ± 8** (10%)	15 ± 3** (8%)	57 ± 12* (30%)	9 ± 3** (5%)	40 ± 6** (21%)
LDL	0	180 ± 16 (26%)	163 ± 8 (23%)	15 ± 6 (2%)	74 ± 7 (11%)	264 ± 7 (38%)
LDL	24	161 ± 13 (28%)	142 ± 12 (25%)	25 ± 2** (4%)	71 ± 8 (12%)	180 ± 4* (31%)
HDL	0	100 ± 5 (34%)	135 ± 25 (46%)	6 ± 3 (2%)	12 ± 4 (4%)	39 ± 6 (14%)
HDL	24	95 ± 10 (39%)	105 ± 15 (43%)	11 ± 2 (4%)	11 ± 5 (4%)	23 ± 4** (9%)

Values in parentheses are percentage mass distribution. Statistical comparisons were done using analysis of variance of repeated measures; *, $P < 0.01$; **, $P < 0.05$.

LCAT activity and mass

Plasma LCAT activity was measured, using an exogenous substrate, in animals after injection with LPS, TNF, and saline. There was no significant interaction between diet and change in LCAT activity after injection with LPS or TNF so the data from animals fed both diets were pooled for statistical analysis. Plasma LCAT activity decreased within 3 h in the LPS- and TNF-injected animals and remained depressed by 50% ($P<0.01$) and 30% ($P<0.01$) of control, respectively, at 48 h (**Fig. 2**). There was no significant change in LCAT activity in animals injected with saline. Plasma LCAT mass concentration decreased by 35% ($P<0.01$) in animals injected with LPS at 24 h and the concentration of LCAT protein remained 24% ($P<0.05$) below baseline at 48 h (**Fig. 3**). In the animals injected with TNF, the LCAT mass was significantly reduced by 27% ($P<0.01$) at 24 h and 15% ($P<0.05$) at 48 h. There was no statistically signficant change in LCAT mass in the animals injected with saline. The change in LCAT activity between 0 and 24 h after injection with LPS and TNF was correlated with the change in LCAT mass ($r = 0.68$, $P<0.01$).

To determine whether LPS or TNF directly inhibited LCAT in vitro, these substances were added to the reaction mixture containing plasma from control animals in

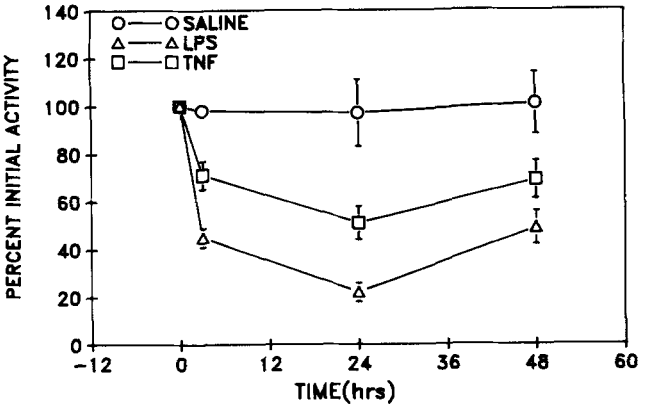

Fig. 2. Percent change in the activity of plasma LCAT after injection of cynomolgus monkeys with LPS (20 μg/kg), TNF (20 μg/kg), or saline. Results are expressed as the percent of initial enzyme activity and are the mean values (± SE) of six animals in each group. The mean (± SE) initial enzyme activities (nmol cholesteryl ester formed/ml per h) for each group were: saline (29 ± 6), LPS (34 ± 5), TNF (38 ± 7).

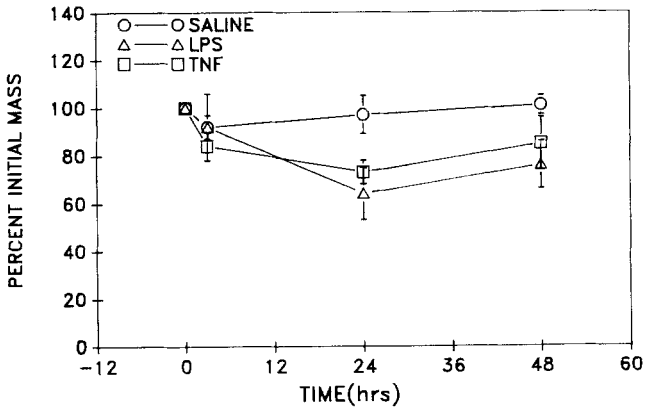

Fig. 3. Percent change in plasma concentration of LCAT after injection of LPS (20 μg/kg), TNF (20 μg/kg), or saline. The concentrations of LCAT were measured using a radioimmunoassay for human LCAT and are expressed as percent of initial enzyme mass, mean ± SE, of six animals in each group. The mean (± SE) initial mass (μg/ml) of LCAT was: saline (2.27 ± 0.11), LPS (2.34 ± 0.80), TNF (2.01 ± 0.08).

concentrations similar to those in vivo. Neither LPS nor TNF inhibited the generation of cholesteryl esters using an exogenous substrate in vitro.

LCAT activity and mass were measured at baseline and 24 h after various doses of TNF (0.20 μg/kg–20 μg/kg) were injected into the monkeys (**Fig. 4**). These data indicate that the decrease in LCAT activity and mass was directly related to the amount of TNF injected. Two hundred ng per kg caused no change in LCAT activity, whereas 20 μg/kg resulted in a 60% decrease in activity and 21% decrease in LCAT mass.

DISCUSSION

We have shown previously that injection of LPS into African green monkeys resulted in a decrease in plasma LCAT activity and a decrease in plasma lipoprotein cholesteryl ester content (20). The present study confirms and extends our initial observations in another nonhuman primate species, *Macaca fascicularis*, and specifically addresses two experimental questions: *1)* Is the decrease in LCAT activity due to an inhibition of the plasma enzyme or to a decrease in plasma LCAT mass? *2)* Does TNF mediate the decrease in plasma cholesterol esters and LCAT activity? The results showed that injection of LPS into monkeys, at a dose that caused fever and prostration, resulted in a decrease in both the cholesteryl ester content of LDL and HDL and in the activity and concentration of LCAT in plasma. Plasma levels of the cytokine tumor necrosis factor alpha increased markedly after LPS injection. Intravenous injection of recombinant human TNF caused low-grade fever and prostration and resulted in changes in lipoprotein composition and in LCAT concentration and activity that were similar to those observed after LPS injection. These data suggest that LPS causes a relative plasma LCAT deficiency, which may account for the low cholesteryl ester concentration in plasma. TNF is an important mediator of this response.

The injection of either LPS or TNF resulted in consistent changes in lipoprotein concentration and composition. In all animals, regardless of diet, there was an increase in the total plasma triglyceride, as well as the triglyceride content of all the lipoprotein particles, and a decrease in the concentration of cholesteryl esters in LDL and HDL. However, the net effect of LPS and TNF on total plasma triglyceride and cholesterol concentrations differed depending on dietary intake. Animals fed saturated fat and cholesterol had a greater absolute increase in VLDL concentration after LPS or TNF injection compared to the animals fed monkey chow, and as a result had a 3-fold greater increase in total plasma triglyceride. Additionally, because the VLDL in animals fed the the saturated fat cholesterol diet were more enriched in cholesteryl ester, the reduction in cholesteryl ester content of HDL and LDL was offset by the increase in VLDL cholesteryl ester concentration; the net result being that there was no change in total plasma cholesterol concentration. The most likely explanation for the latter observa-

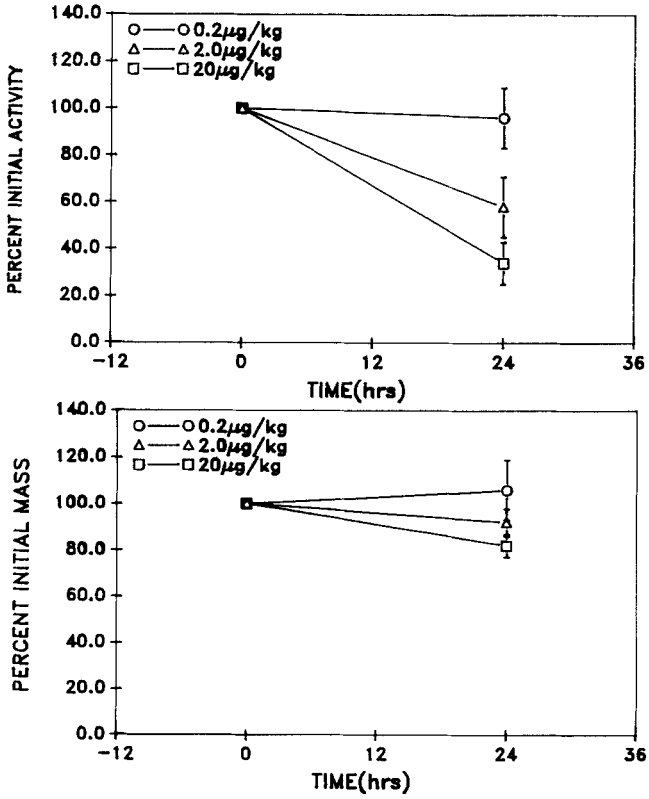

Fig. 4. The effect of TNF on plasma LCAT activity (upper panel) and mass (lower panel) 24 h after injections of different amounts of TNF. Results are shown as the mean (± SE) percentage of inital activity or mass for four animals.

tion is that cynomolgus monkeys fed saturated fat and cholesterol derived a large proportion of plasma cholesteryl ester from hepatic secretion, whereas in chow-fed animals most plasma cholesteryl ester comes from the LCAT reaction. Liver perfusion studies of African green monkeys fed high fat–cholesterol-containing diets similar to the one used in the present study have shown a strong correlation between hepatic cholesteryl ester content and secretion rate of hepatic VLDL cholesteryl ester, as well as the cholesterol ester content of plasma LDL (28). In addition, cynomolgus monkeys fed a diet enriched in saturated fat and cholesterol have increased hepatic stores of cholesteryl esters compared to those animals fed lesser amounts of dietary cholesterol (unpublished observations, F. Johnson and L. Rudel).

Thus, the net effect of the inflammatory process on plasma cholesteryl ester may depend on the relative contribution of hepatic- versus plasma-generated cholesteryl esters as well as the species-specific response to the cholesterol-enriched diet. In the African green monkey fed saturated fat and cholesterol, relatively less cholesteryl ester comes from hepatic secretion than in cynomolgus monkeys and therefore these animals develop hypocholesterolemia during acute inflammation. The situation in humans is more like that of the African green monkey than that of the cynomolgus monkey in that most of the cholesteryl esters in humans are thought to derive from the LCAT reaction (29). However, it should be noted that other factors may be important in interpreting these data. Cholesterol esterification in vivo is affected by dietary intake of cholesterol and postprandial lipemia (30) and therefore experiments designed to test the interactions of the latter factors and the response to inflammation are needed to fully understand our data.

We were unable to determine, from steady state measurements, whether the larger increase in VLDL in saturated fat-fed animals after LPS injection was due to a relatively greater increase in the hepatic synthesis and/or secretion rate of VLDL and/or a greater impairment in VLDL metabolism in the saturated fat–cholesterol-fed animals compared to those fed monkey chow. However, there was no difference between the two diet groups in the initial total postheparin lipase activity, nor was there any significant difference in the decline in activity at 24 h. These data are consistent with the hypothesis of increased VLDL secretion in the saturated fat- and cholesterol-fed animals after LPS injection.

The changes in lipoprotein composition of LDL and HDL after injection of TNF and LPS are similar, though not identical, to particles seen in LCAT-deficient patients and in nonhuman primate liver perfusions in which LCAT activity is low (29, 31, 32). The low cholesteryl ester content of LDL and HDL particles may reflect decreased generation of cholesteryl ester in plasma by LCAT. The plasma activity of LCAT was significantly inhibited in all animals after injection of both LPS and TNF and changes in LCAT activity were detectable within 3 h of injection. There also was a significant reduction in LCAT concentration after LPS and TNF injection which was highly correlated with the change in enzyme activity, but the magnitude of change was less than that in enzyme activity. It is not known why there was a discrepancy between changes in LCAT mass and activity. The assay used for LCAT mass uses human LCAT and anti-human LCAT antibodies and may underestimate the mass of nonhuman primate LCAT. Other possibilities include an increased amount of endogenous substrate (discoidal HDL particles) in the plasma of animals injected with LPS or TNF which may compete with the exogenous labeled substrate, giving a falsely low measurement of activity (20), secretion of LCAT protein that has lost enzyme activity but not antigenic determinants, or the presence of an inhibitor of LCAT during the inflammatory process. It did not appear that TNF or LPS directly inhibited the LCAT reaction since direct addition of these substances to the in vitro reaction did not change measured activity. Nonetheless, this does not rule out the possibility that other cytokines or substances that are released during the inflammatory process may inhibit the LCAT enzyme.

Taken together, these data support the hypothesis that the decreased activity of LCAT during the inflammatory process is at least partially the result of a decreased mass of enzyme in plasma. A possible explanation for the fall in LCAT concentration is that hepatic synthesis and/or secretion of LCAT is inhibited by TNF or other cytokines. Tumor necrosis factor has been shown to inhibit gene transcription and secretion of several hepatic proteins (33). Consistent with this hypothesis, we have found that secretion of LCAT was decreased in HepG2 cells incubated with TNF (V. Varma, J. Parks, and W. Ettinger, unpublished observations). Nonetheless, increased catabolism of LCAT protein or a shift of LCAT mass out of the plasma compartment cannot be excluded and has been reported for other plasma proteins during the acute phase reaction (34).

Some of the lipoprotein changes during inflammation may be due to a decrease in the numbers of LDL and HDL particles as several clinical studies have shown low concentrations of apolipoprotein B and A-I in patients with inflammatory diseases (12, 17, 19). In our previous study (20), as well as this one, we did not find consistent evidence of a change in the number of lipoprotein particles in plasma, although in the animals fed saturated fat and cholesterol in this study, HDL protein was reduced by more than half suggesting a decreased number of particles. A longer time course may be necessary to observe alterations in production rate and/or catabolism of LDL or HDL particles. There are theoretical reasons why the

inflammatory process would decrease the concentration of LDL and HDL particles. VLDL-TG catabolism is impaired in nonhuman primates during infection and this is thought to be due to a decrease in lipoprotein lipase (LPL) synthesis, which results in impaired hydrolysis of triglyceride (13, 20). Our data also show a decrease in plasma LPL activity. Acute inhibition of LPL, by anti-LPL antibodies, results in decreased production of LDL (35). Thus, LDL production may be lowered during acute inflammatory states in which LPL activity is inhibited. Similarly, since the LPL reaction is thought to be an important mediator of HDL concentrations, inhibition of this enzyme may also result in lower plasma concentrations of HDL (35, 36). Hepatic synthesis and secretion of apoproteins also may be suppressed in inflammatory states (33).

Tumor necrosis factor caused changes in lipoprotein metabolism and enzyme activity similar to those seen with LPS. This suggests that TNF is an important, if not the sole, mediator of the observed changes in lipoprotein metabolism after LPS injection in the nonhuman primate. These experiments confirm several previous reports that have shown that TNF causes hypertriglyceridemia in a variety of experimental animals by increasing hepatic synthesis of triglyceride, inhibiting hydrolysis of triglyceride, or both (2, 4, 7, 8). However, this is the first report to show that recombinant TNF injected into nonhuman primates results in hypocholesterolemia and decreased generation of cholesteryl esters in plasma. Nontheless, other cytokines have been shown to affect lipoprotein metabolism (3, 9, 10) and we cannot exclude the possibility that these mediators, either alone or in synergy with TNF, did not affect the observed changes in cholesterol metabolism. Experiments in which animals receive passive immunization against TNF and other cytokines may address this issue.

In conclusion, the metabolic response to acute inflammation is complex and the resultant changes in lipoprotein metabolism depend on the species studied, the specific inflammatory or traumatic stimulus, and the nutritional state of the animal. Available data suggest that the inflammatory response affects hepatic synthesis and secretion of lipoproteins, intravascular metabolism of lipoprotein particles by the triglyceride lipases and LCAT, and perhaps lipoprotein particle catabolism. Further work is needed to understand the mechanisms by which the inflammatory process alters lipoprotein metabolism, as well as the clinical significance of these observations.

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