Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in Syrian hamsters

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Abstract Endotoxin (LPS) administration, which mimics infection, stimulates the production of many cytokines, including TNF, that are thought to mediate the alterations in lipid metabolism that occur during infection. The aims of this study were to determine the effect of LPS or TNF administration on plasma LCAT activity and hepatic LCAT mRNA levels in Syrian hamsters. Plasma LCAT activity was decreased 8 h after LPS administration, reached a maximum level of inhibition at 16 h which persisted for at least 24 h, at which time the activity was 53% of control values. The decrease in plasma LCAT activity was first seen at an LPS dose of 0.01 μg/100 g body weight and reached a maximum at 50–100 μg/100 g body weight. The ratio of free to esterified cholesterol in the plasma increased in the LPS-treated animals. Moreover, LPS administration decreased LCAT mRNA levels in the liver. The decrease in hepatic LCAT mRNA levels preceded the decrease in plasma LCAT activity. Additionally, TNF treatment (16.7 μg/100 g body weight) decreased plasma LCAT activity by 35% and LCAT mRNA levels in the liver by 60% 16 h after administration. Lastly, in cultured rat H35 hepatocytes, TNF decreased LCAT mRNA levels by 50% with a 1/2 maximal dose of approximately 1 ng/ml. Thus, plasma LCAT activity and hepatic mRNA levels are decreased by LPS or TNF treatment. LCAT is a member of a group of proteins that affect lipid and lipoprotein metabolism whose levels are altered during the host's acute phase response.—**Ly, H., O. L. Francone, C. J. Fielding, J. K. Shigenaga, A. H. Moser, C. Grunfeld, and K. R. Feingold.** Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in Syrian hamsters. *J. Lipid Res.* 1995. **36:** 1254–1263.

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Infection and inflammatory diseases are frequently associated with multiple disturbances in lipid and lipoprotein metabolism (1–4). LPS administration, which mimics infection, stimulates the production of many cytokines, including tumor necrosis factor (TNF), that are thought to mediate many of the host's metabolic responses that occur during infection (5, 6). Administration of LPS or TNF to rodents increases serum triglyceride and cholesterol levels (see ref. 4 for review). The hypertriglyceridemia is secondary to increases in hepatic VLDL secretion and/or decreases in clearance of triglyceride (TG)-rich lipoproteins due to inhibition of lipoprotein lipase activity (7–12). The increase in serum cholesterol levels may be due to an increase in de novo hepatic cholesterol synthesis, secondary to an increase the activity of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol synthesis (10, 13–15). As LPS and TNF have minimal effects on LDL receptor protein and mRNA levels in the liver, decreased cholesterol clearance is not likely to be the cause of the hypercholesterolemia (14). Additionally, serum HDL cholesterol levels are decreased after LPS or TNF administration (3, 14, 16).

In contrast to the increase in serum cholesterol levels in rodents and rabbits, LPS or TNF administration produces a decrease in serum cholesterol levels in humans and non-human primates (see ref. 4 for review). The mechanism accounting for the different responses in serum cholesterol levels after LPS or TNF administration in different species is not understood. Recently, studies have shown that LPS or TNF administration results in a decline in plasma activity of lecithin:cholesterol acyltransferase (LCAT) in monkeys secondary to a decrease in LCAT mass (17, 18). This finding led Auerbach and Parks (17) and Ettinger et al. (18) to hypothesize that the

Abbreviations: LPS, endotoxin (lipopolysaccharide); TNF, tumor necrosis factor; LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; i.p., intraperitoneal.

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hypocholesterolemia that occurs in primates during the acute inflammatory state might be due to a decrease in plasma LCAT activity. LCAT is an important enzyme in reverse cholesterol transport, playing a central role in the transport of excess cholesterol from peripheral tissues to HDL (19, 20). It hydrolyzes the *sn*-2 fatty acid from lecithin and transfers it to unesterified cholesterol in HDL to form cholesteryl esters.

The purpose of the present study was threefold: first, to determine whether LPS or TNF administration decreases plasma LCAT activity in Syrian hamsters; second, to elucidate the mechanism(s) for this decrease; and third, to evaluate the hypothesis that the decrease in plasma LCAT activity is responsible for hypocholesterolemia that follows LPS/TNF administration. We have used Syrian hamsters in our experiments because, in comparison to other rodents, cholesterol and lipoprotein metabolism more closely resembles that of humans (21–23).

METHODS

Materials

[1,2-^{3}H-(n)]cholesterol (53.3 Ci/mmol, 1 mCi/ml) and α-[^{32}P]dCTP (3000 Ci/mmol, 10 mCi/ml) were purchased from New England Nuclear (Boston, MA). LPS (*E. coli* 055:B5) was purchased from Difco Laboratories (Detroit, MI) and was freshly diluted to desired concentrations in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Inc., Irvine, CA). Human TNF-α with a specific activity of 5.6×10^7 units/mg was kindly provided by Genentech, Inc. (South San Francisco, CA). Multiprime DNA labeling system was purchased from Amersham International (Amersham, United Kingdom). Mini spin columns (G50) were purchased from Worthington Biochemical Corporation (Freehold, NJ). Oligo (dT)-cellulose, type 77F was purchased from Pharmacia LKB Biotechnology AB (Upsala, Sweden). Nitrocellulose was purchased from Schleicher and Schuell (Keene, NJ). Kodak XAR5 film was used for autoradiography. A full-length human LCAT cDNA was obtained by reverse transcription polymerase chain reaction of HepG2 poly A+ mRNA as previously described (24). All chemicals used to measure LCAT activity, except apoA-I, were purchased from Sigma (St. Louis, MO). ApoA-I was isolated from fresh human plasma by a method described previously (25). Thin-layer chromatography polygram Sil G plates (TLC, 25 mm silica gel) were purchased from Brinkmann Instruments (Westbury, NY). Betamax ES scintillation fluid was purchased from ICN Biomedical, Inc. (Irvine, CA).

Animal procedures and experimental protocol

Male Syrian hamsters (100–150 g) were obtained from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a standard light cycle room (6 AM to 6 PM light, 6 PM to 6 AM dark) and were fed rodent chow and water ad libitum. Animals were anesthetized using isofluorane and were injected intraperitoneally (i.p.) with LPS or TNF at the indicated doses in 0.5 ml of either 0.9% saline (for LPS) or 0.1% human serum albumin (HSA) for TNF. Control animals were injected with either 0.9% saline or 0.1% HSA alone. Food was withdrawn from both control and treated animals immediately after injection because LPS and cytokines can induce anorexia. Animals were studied between 4 and 24 h after LPS and cytokine administration as indicated in the text.

Cell culture

Rat hepatoma cell line H35 was obtained from American Tissue Type Culture Collection (Rockville, MD). The cells were maintained in DMEM containing 10% fetal calf serum (FCS, Hyclone Lab, UT) and subcultured at a ratio of 1:10 weekly. For the experiments, cells were subcultured into 100-mm culture dishes (Lux, Nunc, Naperville, IL) after reaching confluency. The cells were washed two times with PBS and incubated for 24 h with fresh medium containing the appropriate concentration of TNF. Cells were then harvested and poly A+ mRNA was isolated for Northern blot analysis.

Measurements of total cholesterol and triglycerides

Plasma cholesterol levels were measured using Sigma Diagnostic Kit No. 351 (Sigma Chemical Co., St. Louis, MO). Free and esterified cholesterol were determined by a variation of the method of Gamble et al. (26). Briefly, plasma isolated from control and LPS-treated animals was diluted in potassium phosphate buffer, pH 7.4, and sonicated for 1 min. Sonicated samples (0.1 ml) were mixed with 20 μl of a solution of 20 mM sodium cholate and 1% Triton X-100 in assay buffer followed by 25 μl of 95% ethanol. The assay solution for free cholesterol consisted of potassium phosphate buffer, pH 7.4, cholesterol oxidase, horseradish peroxidase, and *p*-hydroxyphenyl acetic acid. Samples were incubated for 1 h at 37°C and fluorescence was determined (excitation, 325 nm; emission, 415 nm). To determine total plasma cholesterol a second set of samples was assayed identically to those above except that cholesteryl ester hydrolase was added. Esterified cholesterol was calculated by subtracting free from total cholesterol. Plasma triglyceride levels corrected for glycerol concentrations were measured using Sigma Diagnostic Kit No. 337.

Measurement of LCAT activity (exogenous assay)

LCAT activity was measured in freshly collected hamster plasma by the addition of a radiolabeled artificial substrate to plasma using a modification of the method of Chen and Albers (27). Briefly, artificial substrate liposomes consisting of human apoA-I, egg yolk lecithin, and cholesterol at a molar ratio of 0.8:250:12.5 were prepared

by the cholate dialysis method (28). Each assay mixture containing 250 nmol lecithin, 7.5 nmol unlabeled cholesterol, 50 pmol, 2.5 μCi [^{3}H]cholesterol, 0.8 nmol (or 22 μg) apoA-I, and 0.5% (w/v) HSA was preincubated at 37°C for 15 min; then 5 mM β-mercaptoethanol and 15 μl hamster plasma were added to a final volume of 0.5 ml. The assay mixture was vortexed immediately and incubated in a shaking water bath at 37°C for 30 min. The reaction was stopped by an addition of 2 ml ethanol. The lipids were extracted twice with 4 ml hexane containing 50 mg unlabeled cholesterol and cholesteryl oleate as carriers. The extract was dried under nitrogen and redissolved in chloroform. The lipids were then separated by TLC using a hexane–ethyl acetate 9:1 (v/v) solvent system. The cholesteryl ester and unesterified cholesterol bands were identified, scraped off, and the radioactivities were measured by liquid scintillation counting. LCAT activity was determined from conversion of [^{3}H]cholesterol to labeled cholesteryl ester. Control reactions containing no enzyme were run simultaneously to correct for nonenzymatic reaction. The rate of LCAT reaction is expressed as molar activity (nmol cholesterol esterified/h per ml plasma) and reported as percentage of controls. LCAT activity after LPS or TNF administration was expressed as percent control to allow for comparison between experiments carried out at different times. We found that LCAT activity in controls varied from experiment to experiment.

Measurement of LCAT activity (endogenous assay)

LCAT activity was also measured as the rate of utilization of free cholesterol in native plasma. This assay measures activity in the presence of the endogenous plasma lipoproteins (29). The endogenous rate is always lower, because the enzyme is reacting with the natural mixture of HDL species present in plasma instead of the optimal, discoidal HDL substrate. The free cholesterol content of plasma was measured colorimetrically in pentuplicate by autoanalyser (29) at zero time and after 1 and 2 h at 37°C. LCAT activity (which was linear) was expressed as nmoles free cholesterol consumed/h per ml plasma.

Isolation of RNA and Northern blotting

Poly A+ mRNA was isolated from hamster liver by a variation of the guanadinium thiocyanate method (30) and Northern blots were prepared as described previously (14, 16, 31). Blots were exposed to X-ray film for various durations to ensure that measurements were on the linear portion of the curve, and bands were quantified by densitometry.

Statistics

The results are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using a two-tailed Student's *t*-test.

RESULTS

Effect of endotoxin (LPS) on plasma lipid levels and LCAT activity

The effect of high dose LPS (50 μg/100 g body weight) on plasma lipid levels is shown in **Table 1.** Plasma triglyceride levels were increased by 62%, 33%, and 50% at 4 h, 8 h, and 16 h after LPS administration, respectively. The increase was most significant at 24 h administration, where the triglyceride levels were increased 3.5-fold. LPS administration also significantly increased plasma cholesterol levels by 22% and 72% at 16 h and 24 h, respectively. A small decrease in total cholesterol was seen at 4 h after LPS treatment. In a separate experiment, total, free, and esterified plasma cholesterol were measured 16 h after LPS treatment. In this experiment, total

TABLE 1. Effect of endotoxin on plasma lipid levels

	4 h (n = 5)	8 h (n = 6)	16 h (n = 15)	24 h (n = 6)
		mg/dl		
Cholesterol				
Control	80 ± 1.6	76 ± 3.8	111 ± 4.6	58 ± 5.0
LPS	71 ± 2.6[a]	68 ± 3.8	135 ± 2.6[b]	100 ± 5.8[b]
HDL-Cholesterol				
Control	46 ± 1.5	39 ± 3.0	45 ± 2.8	42 ± 5.0
LPS	27 ± 3.4[b]	25 ± 3.2[b]	39 ± 1.0[a]	35 ± 1.5
Triglycerides				
Control	112 ± 13.0	102 ± 10.8	149 ± 10.5	122 ± 27.0
LPS	181 ± 32.3[a]	136 ± 22.0	224 ± 16.8[b]	423 ± 63.8[b]

Animals were injected i.p. with either saline (controls) or LPS (LPS, 50 μg/100 g body weight). Food was withdrawn from both control and treated animals immediately after injection. At the indicated time the animals were killed and plasma cholesterol, HDL-cholesterol, and triglyceride levels were determined. Data are mean ± SEM. [a] $P < 0.05$ and [b] $P < 0.005$.

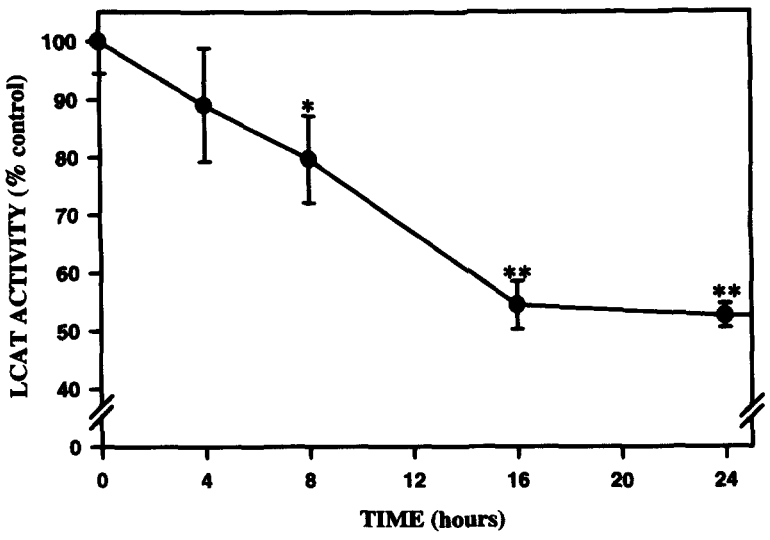

Fig. 1. Time course of the effect of LPS on plasma LCAT activity. Animals were injected i.p. with either saline (controls) or LPS (50 μg/100 g body weight). At the indicated times, the animals were killed and plasma LCAT activity was determined as described in Methods. Data are means of 5, 6, 15, and 6 hamsters for 4, 8, 16, and 24 h, respectively. Data are presented as mean ± SEM; $^{*}P < 0.05$ and $^{**}P < 0.005$ vs. control.

plasma cholesterol was increased 38.8% (control 148 ± 7 vs. LPS 205 ± 6 mg/dl, $P < 0.001$, n = 8 for both groups). Moreover, the increase in total cholesterol primarily consisted of an increase in free cholesterol (93% increase) (control 42 ± 3 vs. LPS 83 ± 2 mg/dl, $P < 0.001$, n = 8 for both groups). In contrast, esterified plasma cholesterol was increased only 16% in the LPS-treated animals (control 105 ± 5 vs. LPS 122 ± 5, $P < 0.05$, n = 8 for both groups). As a consequence, the free to esterified cholesterol ratio was 0.40 in controls but 0.68 in LPS-treated animals.

HDL-cholesterol levels were decreased after LPS administration with a maximal decrease (41%) at 4 h. By 8 h and 16 h after LPS administration, HDL-cholesterol levels were decreased by 35% and 13%, respectively (Table 1). Previous studies from our laboratory have shown that the composition of HDL 16 h after LPS treatment is very similar to control HDL with only a small decrease in the percent cholesterol (control 15.43 ± 0.33% vs. LPS 13.61 ± .28%, $P < 0.01$) and a small increase in the percent phospholipid (control 33.0 ± 0.59 vs. LPS 36.2 ± 0.29%, $P < 0.01$) (14). Protein and triglyceride were not altered by LPS administration (14). In the present study we found that LPS treatment resulted in an increase in the percent of cholesterol that was free in HDL (free cholesterol was 13.8 ± 0.38% of total HDL cholesterol in controls and 21.3 ± 0.51% of total HDL cholesterol in LPS-treated $P < 0.001$, n = 8 for both groups).

The disproportionate increase in plasma free cholesterol versus cholesteryl ester in LPS-treated animals suggested that LPS may decrease plasma LCAT activity in hamsters. As shown in **Fig. 1,** high dose LPS (50 μg/100 g body weight) decreased plasma LCAT activity as measured by the exogenous assay (see Methods). A significant decrease was first observed at 8 h after LPS administration, reaching a maximum decrease at 16 h that persisted for at least 24 h, at which time the activity was only 53% of control (Fig. 1).

We next determined the effect of different doses of LPS on plasma lipid levels (**Table 2**) and LCAT activity (Fig. 2) at 16 h, the time point at which inhibition of LCAT was maximal. An LPS dose as low as 0.1 μg/100 g body weight

TABLE 2. Effect of endotoxin on plasma lipid levels

		Cholesterol		Triglyceride		HDL-Cholesterol	
LPS Conc.		Control	LPS	Control	LPS	Control	LPS
μg/100 g BW		*mg/dl*		*mg/dl*		*mg/dl*	
0.01	n = 12	103 ± 4.0	110 ± 4.2	124 ± 18.3	134 ± 15.7	43 ± 1.6	42 ± 1.6
0.10	n = 14	104 ± 2.6	117 ± 5.1[a]	141 ± 13.9	173 ± 23.4	42 ± 1.8	42 ± 2.0
1.00	n = 7	103 ± 4.1	123 ± 4.8[a]	112 ± 11.7	134 ± 19.2	43 ± 2.7	42 ± 3.0
10	n = 8	107 ± 3.7	137 ± 4.3[b]	133 ± 16.6	218 ± 24.9[b]	44 ± 2.6	39 ± 1.8[a]
50	n = 15	110 ± 4.6	135 ± 2.6[b]	149 ± 10.5	224 ± 16.8[b]	45 ± 2.8	39 ± 1.0[a]
100	n = 9	100 ± 2.8	136 ± 3.9[b]	150 ± 19.3	230 ± 21.5[b]	40 ± 1.6	41 ± 1.8

* Animals were injected i.p. with either saline (controls) or LPS at the indicated doses. Sixteen hours after injection the animals were killed and plasma cholesterol, HDL-cholesterol, and triglyceride levels were determined. Data are mean ± SEM.
[a] $P < 0.05$ and [b] $P < 0.005$.

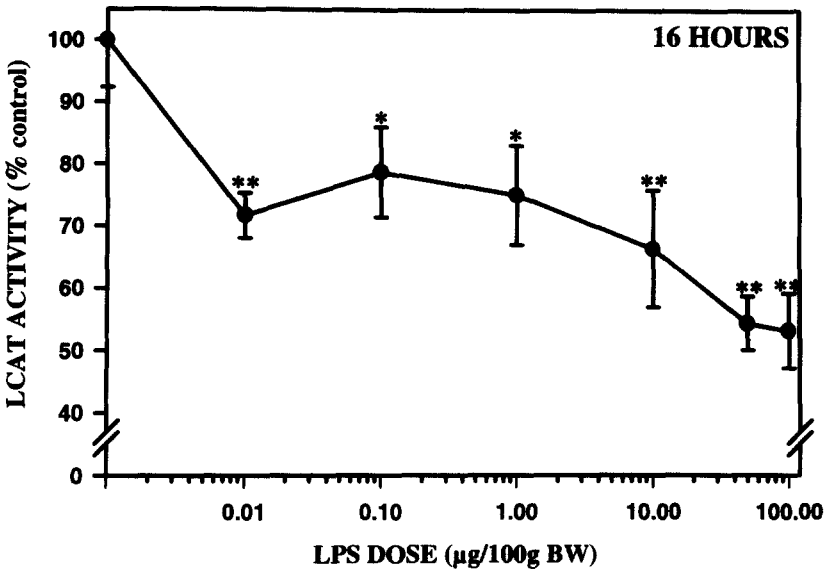

Fig. 2. Dose-response curve of the effect of LPS on plasma LCAT activity. Animals were injected i.p. with either saline (controls) or LPS at the indicated doses. Sixteen hours after the injections, the animals were killed and plasma LCAT activity was determined as described in Methods. Data are presented as mean ± SEM with n = 12, 13, 9, 8, 14, and 9 for 0.01, 0.1, 1, 10, 50, and 100 µg/100 g body weight, respectively; $^{*}P < 0.05$ and $^{**}P < 0.005$ vs. control.

increased plasma cholesterol levels, while higher doses of LPS (10 µg/100 g body weight or more) were required to increase plasma triglyceride levels at 16 h (Table 2). Plasma HDL levels were marginally affected at 16 h (Tables 1 and 2). **Figure 2** shows the effect of different doses of LPS on plasma LCAT activity at 16 h. LPS inhibited LCAT, an effect seen with as little as 10 ng/100 g body weight, and the maximal effect occurred at an LPS dose of 50–100 µg/100 g body weight with plasma LCAT activity decreased to only 50% of control (Fig. 2).

Table 3 shows LCAT activity from the same plasma samples measured with either the optimized (exogenous substrate) assay, or as the rate of consumption of native plasma free cholesterol (endogenous substrate). LPS caused a similar reduction in LCAT activity (approximately 40%) in each case. These data indicate that the level of LCAT was rate-limiting for cholesteryl ester synthesis in plasma, and that it was considerably decreased after LPS injection.

TABLE 3. Comparison of plasma LCAT activity measured by either artifical liposomal substrates (exogenous method) or pooled substrates (endogenous method)

	LCAT Activity	
	Exogenous Method	Endogenous Method
	nmol/ml/hr	
Control	271 ± 2.1	85 ± 2.3
LPS injected	162 ± 9.0[a]	48 ± 14.2[a]
% Control	60 ± 3.3[a]	57 ± 15.1[a]

Animals were injected i.p. with either saline (controls) or LPS (LPS, 50 µg/100 g body weight). Sixteen hours later the animals were killed and plasma LCAT activity was determined either by a method using artificial liposomal substrates (exogenous method) or pooled substrates (endogenous method) as described in the Methods. Data are mean ± SEM of five animals in each group.
[a] $P < 0.005$.

Effect of LPS on hepatic LCAT mRNA levels

To elucidate the mechanism involved in the LPS-induced decrease in plasma LCAT activity, we next examined LCAT mRNA levels in the liver. LPS induced a dose-dependent decrease in hepatic LCAT mRNA levels (**Fig. 3A**). The time course of the effect of LPS on LCAT mRNA levels is shown in Fig. 3B and demonstrates that the decrease in LCAT mRNA occurs as early as 4 h, which precedes the decrease in plasma LCAT activity. By 8 h, high dose LPS decreased hepatic mRNA levels by 90%, while low dose LPS decreased mRNA levels by 60% of the control (Fig. 3B). Thus, LPS administration not only inhibits plasma LCAT activity but also decreases the levels of LCAT mRNA in the liver.

Effect of TNF on plasma LCAT activity and hepatic mRNA

As TNF mediates many of the actions of LPS on lipid metabolism, we next studied the effect of TNF on plasma LCAT activity (**Fig. 4**) and hepatic mRNA levels (**Fig. 5**). TNF (16.7 µg/100 g body weight) decreased LCAT activity in the plasma by 35% and LCAT mRNA in the liver by 60% 16 h after administration. It has previously been reported by our laboratory (16) that 16 h after TNF administration, serum cholesterol and triglyceride levels are increased and HDL-cholesterol levels are decreased in Syrian hamsters.

We next investigated the effect of TNF on LCAT mRNA levels in rat H35 hepatocytes in culture (**Fig. 6**). Exposure of H35 hepatocytes for 24 h led to a dose-dependent decrease in LCAT mRNA levels. Maximal inhibition occurred at a TNF dose of 10 ng/ml, which

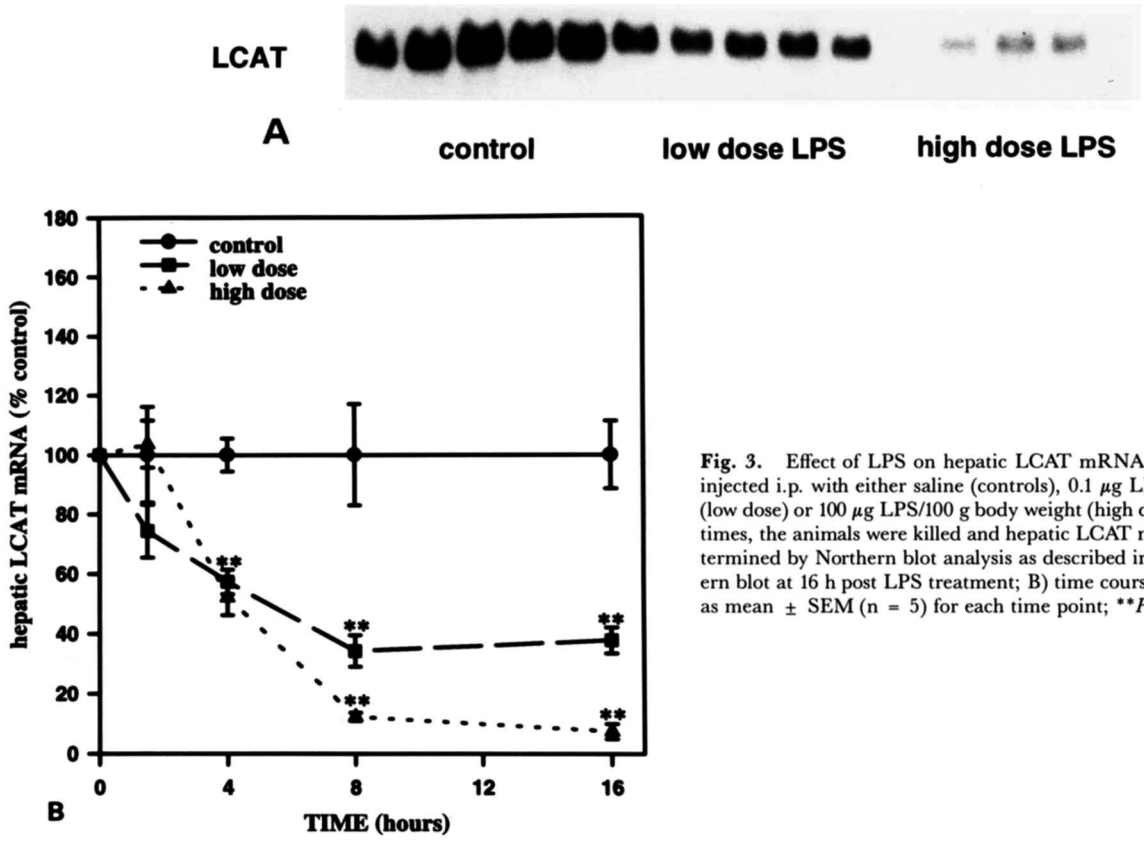

Fig. 3. Effect of LPS on hepatic LCAT mRNA levels. Animals were injected i.p. with either saline (controls), 0.1 μg LPS/100 g body weight (low dose) or 100 μg LPS/100 g body weight (high dose). At the indicated times, the animals were killed and hepatic LCAT mRNA levels were determined by Northern blot analysis as described in Methods. A) Northern blot at 16 h post LPS treatment; B) time course. Data are presented as mean ± SEM (n = 5) for each time point; $^{**}P < 0.005$ vs. control.

decreased LCAT mRNA levels to 50% of control values (Fig. 6). The 1/2 maximal dose was approximately 1 ng/ml. Thus, the effect of TNF on LCAT mRNA levels in rat H35 hepatocytes in vitro was similar to its effect in the hamster liver in vivo.

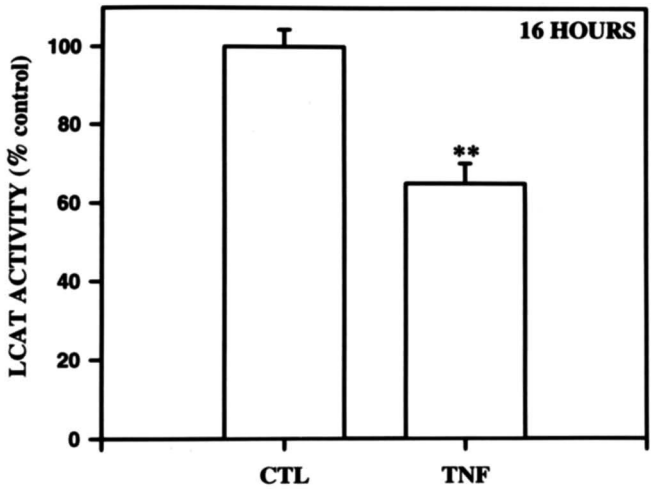

Fig. 4. Effect of TNF on plasma LCAT activity. Animals were injected i.p. with either 0.1% HSA (controls) or TNF (16.7 μg/100 g body weight). Sixteen hours after injection the animals were killed and plasma LCAT activity was determined as described in Methods. Data are presented as mean ± SEM with n = 11; $^{*}P < 0.005$ vs. control.

DISCUSSION

In the present study, we evaluated the hypothesis, proposed by Parks and colleagues (17, 18), that the decrease in serum cholesterol levels that occurs during the acute inflammatory state is due to inhibition of plasma LCAT activity. They observed that LPS or TNF administration in monkeys decreases total serum cholesterol levels as well as decreasing plasma LCAT activity (17, 18). In the present study, we report that LPS or TNF treatment also decreases plasma LCAT activity in Syrian hamsters. In contrast to what is observed in primates, LPS or TNF administration increases total serum cholesterol levels in Syrian hamsters (14, 16). In Syrian hamsters, this increase in plasma cholesterol levels is accounted for by an increase (93%) in free cholesterol with only a modest increase occurring in the esterified cholesterol fraction. Thus, the decrease in plasma LCAT activity induced by LPS or TNF cannot by itself be responsible for the changes in serum cholesterol levels that occur during the acute inflammatory state. LPS also increases serum cholesterol levels in mice, but contradictory findings have been described with regard to plasma LCAT activity with Kitagawa et al. (32) reporting that LPS decreases and Sakaguchi (33) reporting that LPS increases plasma LCAT activity.

The explanation for the difference in response of serum cholesterol levels to LPS or TNF in primates and rodents

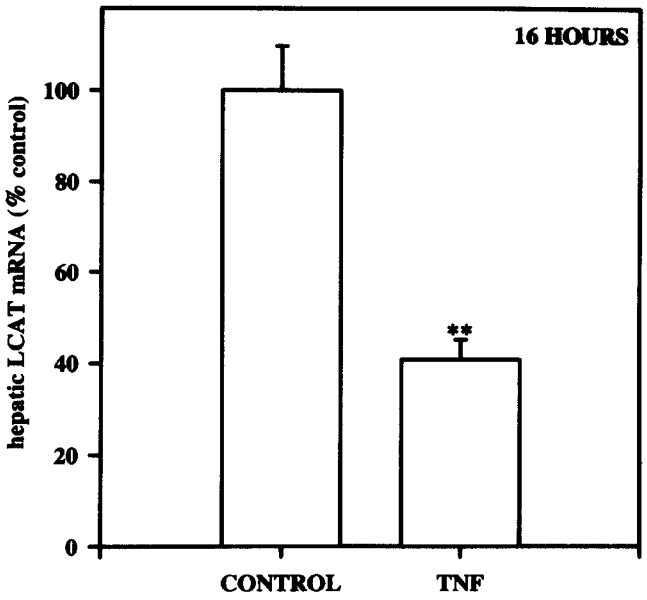

Fig. 5. Effect TNF on hepatic LCAT mRNA levels. Syrian hamsters were injected i.p. with either 0.1% HSA or TNF (16.7 μg/100 g body weight). Sixteen hours after injection the animals were killed and hepatic LCAT mRNA levels were determined as described in Methods. Data are presented as mean ± SEM with n = 5 for each point; $*P < 0.005$ vs. control.

is unclear. In vivo studies have indicated that LPS and TNF enhance hepatic VLDL secretion and cholesterol synthesis in rodents (12, 34, 35). In contrast, Ettinger et al. (36) have demonstrated in HepG2 cells in vitro that TNF, IL-1 beta, and IL-6 decrease cholesterol and apolipoprotein B secretion. It is possible that the response of the liver to cytokines differs between rodents and primates. Such species differences have been noted with regard to the ability of cytokines to stimulate the production of other acute phase proteins (37). For example, alpha2 macroglobulin synthesis is increased many-fold by cytokines in rat liver but is unaffected by cytokines in human liver.

HDL-cholesterol levels decrease after LPS or TNF administration in both primates and Syrian hamsters (3, 14, 16–18). The decrease in plasma LCAT activity could contribute to this decrease in HDL-cholesterol levels. In support of this, we found that the proportion of cholesterol that was unesterified was increased in HDL obtained from LPS-treated animals. Additionally, a decrease in HDL-cholesterol levels is also observed in humans with familial LCAT deficiency providing support for this possibility (38, 39). However, we found that the decrease in HDL-cholesterol precedes the decrease in plasma LCAT activity in hamsters, suggesting that LPS does not lower HDL cholesterol levels by decreasing LCAT activity. The mechanism by which LPS administration decreases HDL cholesterol levels is unknown.

Recently, interest in plasma LCAT activity has increased because of its key role in HDL metabolism and reverse cholesterol transport. In this process cell-derived cholesterol is transported from cell membranes to HDL particles, the free cholesterol in HDL is esterified by LCAT and packaged into the HDL core (19). Whether the decrease in plasma LCAT activity that occurs during inflammation impedes reverse cholesterol transport is unknown. Epidemiological studies have suggested that chronic infections might be associated with an increased risk of coronary heart disease (40–42).

Only the liver, brain, testes, and skin have been shown to have significant quantities of LCAT mRNA, with the liver containing the vast majority (43, 44). The liver is considered to be the major source of plasma LCAT activity because destruction of the liver by either galactosamine or praseodymium nitrate results in the virtual absence of LCAT activity in plasma (45, 46). Additionally,

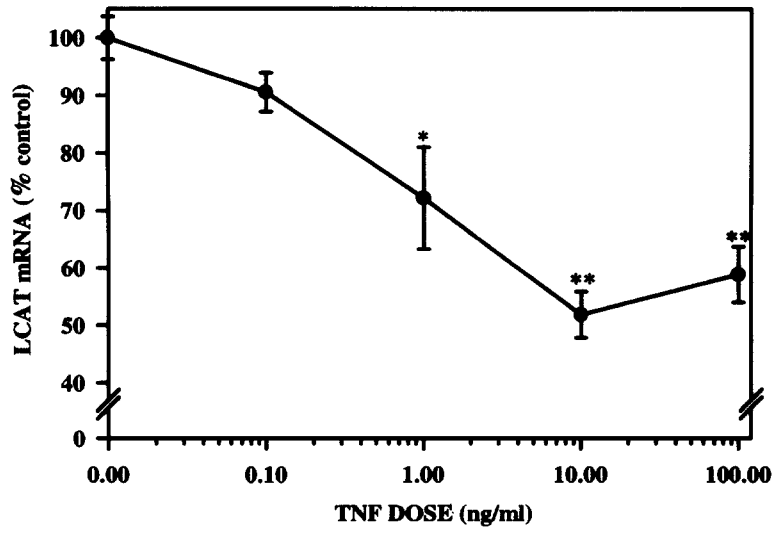

Fig. 6. Dose–response curve effect of TNF on LCAT mRNA levels in rat hepatoma H35 cells. Rat H35 hepatoma cells were grown to confluence as described in Methods. At the start of the experiment, fresh medium containing the indicated doses of TNF was added to the culture dishes and the cells were incubated for 24 h. TNF treatment did not cause toxicity; cell density and protein levels were similar in control and TNF-treated dishes. Cells were then collected, poly A+ mRNAs were isolated, and Northern blots were prepared as described in Methods. Data are presented as mean ± SEM with n = 4; $*P < 0.05$ and $**P < 0.005$ vs. control.

in diseases that impair hepatic function, such as alcoholic hepatitis, plasma LCAT activity is decreased (47). In the present study, we demonstrated that the administration of LPS or TNF decreases LCAT mRNA levels in the liver of intact animals. This decrease is not due to a general inhibition of mRNA synthesis, as our previous studies demonstrated that LPS administration increases HMG-CoA reductase and β-actin mRNA levels in the liver (14). This decrease in LCAT mRNA occurred more rapidly than the decrease in plasma LCAT activity, first being observed 4 h after LPS treatment. Of note, is that injury to the liver results in a decrease in plasma LCAT activity that exhibits a time course similar to that reported here (45), suggesting that the LPS-induced decrease in hepatic LCAT mRNA levels accounts for the decrease in plasma LCAT activity. Moreover, in hepatic cells in culture, TNF decreased LCAT mRNA levels. The half maximal inhibitory dose of TNF was 1 ng/ml which is very similar to the TNF doses required to regulate acute phase proteins, a well-known biological function of TNF. Thus, our studies coupled with those of Ettinger et al. (36) which demonstrated that cytokines inhibit LCAT secretion into the media of HepG2 cells in culture, suggest that LPS and TNF decreased plasma LCAT activity, at least in part, by inhibiting the synthesis of LCAT in the liver secondary to a decrease in LCAT mRNA levels. A variety of other manipulations that affect lipoprotein metabolism (such as a high fat diet, most cholesterol-lowering drugs, and hormones including estrogens, L-thyroxine, and hydrocortisone) do not to alter hepatic LCAT mRNA levels (43, 48). In fact, the only other treatment shown to affect hepatic LCAT mRNA levels is the administration of fibrates, such as fenofibrate (48). Thus, TNF is the only endogenous regulator of hepatic LCAT mRNA levels that has been reported.

Infection, inflammation, or trauma stimulate cytokine production which results in marked changes in the serum concentrations of specific plasma proteins (acute phase proteins) (37, 49). The hepatic synthesis of certain proteins such as C-reactive protein and fibrinogen increases (positive acute phase proteins) while the synthesis of other proteins such as albumin and transferin is inhibited (negative acute phase proteins). The results of previous studies and the present study indicate that LCAT is a negative acute phase protein. It is believed that the changes in acute phase proteins induced by cytokines play an important homeostatic role in host defense, as the beneficial properties have been elucidated for some of these proteins (49). For example, complement 3 and C-reactive protein help in the opsonization of bacteria, immune complexes, and foreign particles (49). The potential beneficial role of a decrease in plasma LCAT activity is unknown but one can speculate that a decrease in the removal of cholesterol from cells could be beneficial to the function of the cells that are activated during the immune response or are involved in tissue repair.

In addition to the decrease in plasma LCAT activity, other factors that affect HDL metabolism are altered during the acute phase response. The levels of two HDL-associated apolipoproteins, serum amyloid A (SAA) and apolipoprotein J, increase after LPS or TNF administration while apoA-I levels decrease (4, 49–51). Moreover, hepatic mRNA levels of SAA and apoJ are increased after LPS or TNF treatment (51, 52). SAA-rich HDL have a reduced affinity for hepatocytes and an increased affinity for macrophages, suggesting that HDL-cholesterol may be preferentially directed to macrophages (53). Furthermore, changes in HDL structure may alter its ability to serve as a substrate for LCAT. For example, Steinmetz et al. (54) have shown that SAA associated with HDL-like particles inhibits LCAT activity. Thus, alterations in HDL structure coupled with changes in plasma LCAT activity may result in a redirection of HDL metabolism in a manner that may be beneficial to the host during the acute phase response.

In summary, the present study demonstrates that in Syrian hamsters plasma LCAT activity and hepatic mRNA levels are decreased by LPS or TNF administration. Thus, LCAT is a member of a group of proteins that affect lipid and lipoprotein metabolism whose levels are altered during the host's acute phase response.

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