Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters

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Abstract Infection and inflammation increase serum triglyceride and cholesterol levels in rodents and rabbits. Endotoxin (LPS) has been used as a model of infection and its effects on triglyceride metabolism have been previously characterized. In the present study we demonstrate that both low (100 ng/100 g body weight) and high dose (100 μg/100 g body weight) LPS increase serum cholesterol levels in hamsters. The increase in serum cholesterol is first observed 16 h after LPS and persists for at least 24 h. This increase is primarily due to an increase in low density lipoprotein (LDL) cholesterol. High density lipoprotein (HDL) cholesterol levels decrease after LPS treatment. Both low and high dose LPS increase hepatic cholesterol synthesis (low dose 85%, high dose 205%) and total HMG-CoA reductase activity (low dose 2.97-fold, high dose 9.96-fold). However, the proportion of HMG-CoA reductase in the active form is reduced by LPS treatment. Additionally, the mass of HMG-CoA reductase protein in the liver, measured by Western blotting, is increased after LPS. Moreover, LPS increases hepatic HMG-CoA reductase mRNA levels (low dose 3.1-fold, high dose 14.2-fold). The increase in hepatic HMG-CoA reductase mRNA levels is first seen 4 h after LPS and persists for at least 24 h. In contrast, LPS had only minimal effects on hepatic LDL receptor protein and mRNA levels. These results suggest that LPS increases serum cholesterol levels by increasing hepatic cholesterol synthesis. LPS administration decreases apoE mRNA levels in the liver while having no effect on apoA-I mRNA levels. These results suggest that HMG-CoA reductase is a member of a group of hepatic proteins that are positively regulated by inflammatory stimuli (acute phase proteins) while apoE can be considered a negative acute phase protein in hamsters. It is possible that increases in hepatic HMG-CoA reductase provide cholesterol that allows for the increased production of lipoproteins and elevations in serum lipid levels that may be beneficial to the body's host defense.—**Feingold, K. R., I. Hardardottir, R. Memon, E. J. T. Krul, A. H. Moser, J. M. Taylor, and C. Grunfeld.** Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. *J. Lipid Res.* 1993. **34:** 2147–2158.

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Changes in triglyceride and cholesterol metabolism frequently accompany infections and inflammatory diseases (1–5). The administration of endotoxin (LPS) has been used to mimic infections. Both infections and LPS administration produce hypertriglyceridemia by increasing hepatic lipoprotein secretion and reducing lipoprotein clearance, presumably by decreasing lipoprotein lipase activity (6–12). Recent studies by our laboratory have shown that low doses of LPS acutely increase serum triglyceride levels by rapidly stimulating the hepatic secretion of lipids without altering triglyceride clearance (13). Within 1–2 h, low dose LPS stimulates hepatic de novo fatty acid synthesis and adipose tissue lipolysis, both of which could provide a source of fatty acids for the increase in hepatic triglyceride production (13). In contrast, higher doses of LPS do not acutely increase hepatic fatty acid synthesis or peripheral lipolysis, and hepatic triglyceride secretion is not stimulated (13). Rather, high dose LPS decreases the activity of lipoprotein lipase and slows the clearance of triglyceride-rich lipoproteins (13).

Infections, inflammatory diseases, and LPS administration, in addition to altering triglyceride metabolism, also produce increases in serum cholesterol levels in rodents and rabbits (1, 5, 11, 14–16). Moreover, increased hepatic de novo cholesterol synthesis and/or HMG-CoA reductase activity has been observed in several different animal infections (11, 17, 18). Specifically, Canonico et al.

Abbreviations: LPS, endotoxin (lipopolysaccharide); VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

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(17) have demonstrated that pneumococcal sepsis in rats increases the incorporation of [^{14}C]acetate into cholesterol in isolated hepatocytes and increases the activity of HMG-CoA reductase in the liver. Lanza-Jacoby and Taberes (11) have reported that *E. coli* gram negative sepsis increases the incorporation of $^{3}H_2O$ into cholesterol in the liver of intact rats. Lastly, de Vasconcelos et al. (18) have demonstrated that, in rats, cecal ligation and puncture, which causes peritonitis, increases both the incorporation of $^{3}H_2O$ into cholesterol in the liver in vivo and the activity of hepatic HMG-CoA reductase.

The purpose of the present study was twofold; first, to determine whether LPS administration increases serum cholesterol levels in Syrian hamsters; and, second, to elucidate the mechanism(s) for this increase. We have used Syrian hamsters in our experiments because, in contrast to other rodents, studies have indicated that cholesterol and lipoprotein metabolism in Syrian hamsters more closely resembles that in humans. For example, the effects of dietary cholesterol and fatty acids on serum cholesterol levels and LDL metabolism are similar in Syrian hamsters and humans (19, 20). Additionally, like humans, hepatic VLDL from Syrian hamsters contains no apoB-48 and hamsters have cholesteryl ester transfer protein activity in the plasma (21, 22).

METHODS

Materials

$^{3}H_2O$ (5 Ci/g), [26-^{14}C]cholesterol, [^{14}C]HMG-CoA (54.2 mCi/mmol), [^{3}H]mevalonic acid (300 Ci/mmol), and alpha ^{32}P-dCTP (3,000 Ci/mmol, 10 mCi/ml) were from New England Nuclear (Boston, MA); thin-layer chromatography polygram Sil G plates were from Brinkmann Instruments (Westbury, NY); Betamax ES scintillation fluid was from ICN Biomedical, Inc. (Irvine, CA); rat chow was from Simonsen Laboratories (Gilroy, CA); LPS (*E. coli* 055:B5) was from Difco Laboratories (Detroit, MI) and was freshly diluted to desired concentrations in pyrogen-free 0.9% saline (Kendall McGraw Laboratories, Inc., Irvine, CA). Multiprime DNA labeling system was from Amersham International (Amersham, United Kingdom); Mini spin columns (G50) were from Worthington Biochemical Corporation (Freehold, NJ); oligo (dt)-cellulose, type 77F was from Pharmacia LKB Biotechnology AB (Upsala, Sweden); Western light chemiluminescent detection system was from Tropix, Inc. (Bedford, MA); and nitrocellulose was from Schleicher and Schuell (Keene, NH). Kodak XAR5 film was used for autoradiography. The complimentary DNA for HMG-CoA reductase and for the LDL receptor was from the American Type Culture Collection (Rockville, MD): clone name pH Red-102 ATTC No. 57042 and clone name P LDLR3 ATTC No. 57004. Human beta actin cDNA was kindly provided by Dr. Peter Gunning of Stanford University. Murine apoE and A cDNA were prepared as described previously (23). HMG-CoA reductase antiserum was kindly provided by Drs. A. Alberts and J. Germershausen of Merck, Sharp and Dohme, Inc. LDL receptor anti-serum was kindly provided by Dr. A. Cooper of Palo Alto Medical Foundation.

Animal procedures

Male Syrian hamsters (approximately 100–120 g) were purchased from Simonsen Laboratories (Gilroy, CA). The animals were maintained in a reverse light cycle room (3 AM to 3 PM dark, 3 PM to 3 AM light) and were provided with rodent chow and water ad libitum. Anesthesia with isofluorane was induced and the animals were injected intraperitoneally with LPS at the indicated dose in 0.5 ml 0.9% saline or with saline alone. Subsequently, food was withdrawn because LPS can induce anorexia. Animals were studied between 1.5 and 24 h after LPS as indicated in the text.

Lipoprotein analysis

Lipoproteins were separated by density gradient ultracentrifugation (24). Serum (1 ml) was brought to a density (d) of 1.21 g/ml with potassium bromide and then floated on a cushion of d 1.353 g/ml solution in centrifuge tubes. Samples were then sequentially layered with 3 ml of sodium chloride solutions d 1.05, d 1.025, and d 1.006 g/ml. The gradients were then centrifuged at 40,000 rpm for 22 h at 12°C in a Beckman SW 41 TI rotor. Fractions (0.5 ml) were collected and cholesterol and triglyceride levels were determined. The fractions were then combined into three fractions (VLDL d < 1.006, LDL d 1.006–1.063, and HDL d 1.063–1.21 g/ml), diluted with solutions of d 1.006, d 1.063, and d 1.21 g/ml, respectively, and centrifuged at 44,000 rpm for 22 h. The top 0.5 ml was collected, the fractions were weighed, and cholesterol, triglyceride, phospholipid, and protein concentrations were determined.

Lipogenesis

The animals were injected intraperitoneally with 50 mCi $^{3}H_2O$. After 1 h, the animals were anesthetized and weighed; a blood specimen was obtained and the liver and small intestine were removed. The incorporation of $^{3}H_2O$ into cholesterol and fatty acids in the liver and small intestine was determined as described in previous publications (25, 26).

HMG-CoA reductase activity

The animals were killed and liver microsomes were isolated as described previously in the presence of NaCl or NaF (25, 27). The activity of HMG-CoA reductase in liver microsomes was determined as described in previous publications (25, 27).

Western blotting

For the determination of HMG-CoA reductase protein, SDS-PAGE was performed in the presence of urea, as described in previous publications (28). For measurement of LDL receptor protein, SDS-PAGE was carried out under nonreducing conditions (28, 29). Protein was transferred to nitrocellulose (0.2 μm pore size). The level of HMG-CoA reductase protein was determined using HMG-CoA reductase anti-serum and the level of LDL receptor protein was determined using LDL receptor anti-serum (28, 29). A Tropix Western Light chemiluminescent detection system was used and the duration of film exposure was varied to allow measurements on the linear portion of the curve. After X-ray film development, band density was measured using an EC Apparatus Corporation Densitometer.

Isolation of RNA and Northern blotting

Total RNA was isolated by a variation of the guanadinium thiocyanate method (30) and Northern blots were prepared as described previously (28). 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.

To ensure the accuracy of the Northern blot analysis, several steps were taken. First, for a particular blot the same quantity of poly A RNA (10 μg) was loaded on all lanes. Second, the experiments were repeated several times to ensure a sufficient "N" that would minimize analytical errors. Third, in some instances, the specimens were analyzed using duplicate Northern blots and the same relative changes were observed demonstrating the reproducibility of the method. Lastly, we measured several different mRNA species and did not observe similar changes, indicating that the results were not due to loading artifacts.

Serum lipid levels

Serum cholesterol levels were measured using Sigma Diagnostic Kit No. 351 (Sigma Chemical Company, St. Louis, MO). HDL cholesterol was measured after precipitation of VLDL and LDL with phosphotungstic acid and $MgCl_2$. HDL cholesterol concentrations were measured with Sigma Diagnostic Kit No. 352. Serum triglyceride levels were measured using Sigma Diagnostic Kit No. 337-B. Serum triglyceride levels were corrected for glycerol concentrations. Phospholipid concentrations were measured with Wako Phospholipids B Kit (Wako Pure Chemical Industries, Osaka, Japan). Proteins were determined by the Bio-Rad DC Protein Assay (Bio-Rad Laboratories, Richmond, CA).

Statistics

Statistical significance was determined using a two-tailed Student's t test.

RESULTS

The effect of the administration of low dose (100 ng/100 g body weight) or high dose (100 μg/100 g body weight) LPS on serum lipid levels 16 h after LPS treatment is shown in **Table 1.** As noted in Methods, both the control and LPS-treated animals were fasted beginning immediately after LPS administration. Serum cholesterol levels were increased 29% and 49% and serum triglyceride levels were increased 48% and 152% in the low and high dose LPS groups, respectively. HDL cholesterol levels were significantly decreased in the high dose LPS group (25% decrease).

Density gradient ultracentrifugation demonstrated that the increase in serum cholesterol levels in the LPS-treated animals was accounted for by an increase in cholesterol in the LDL lipoprotein fraction (**Fig. 1A**). Cholesterol in the HDL lipoprotein fraction was slightly decreased in the LPS-treated animals (Fig. 1A) consistent with our observations using precipitation methods to assay HDL cholesterol levels (Table 1). Additionally, the quantity of triglycerides in the LDL lipoprotein fraction was increased in the LPS-treated animals (Fig. 1B). The composition of VLDL and HDL lipoproteins was similar in control and LPS-treated animals (**Table 2**). However, in the LDL lipoprotein fraction of LPS-treated animals, the percent triglyceride was increased while cholesterol was decreased (Table 2). Thus, LPS administration increases cholesterol and triglyceride in the LDL lipoprotein fraction and results in a triglyceride-enriched LDL particle.

At 4 and 8 h after LPS administration, serum cholesterol levels were not altered in either the low or high dose LPS groups (data not shown). However, the increase in serum cholesterol levels persisted for at least 24 h after LPS administration (control 100 ± 5.2; low dose 141 ± 10.7, $P < 0.01$; high dose 161 ± 8.5 mg/dl, $P < 0.001$). Thus, similar to our previous observations in rats (13), LPS administration does not cause a rapid change in serum cholesterol levels but rather results in a

TABLE 1. Effect of endotoxin on serum lipid levels

Group	Cholesterol	Triglycerides	HDL Cholesterol
	mg/dl	*mg/dl*	*mg/dl*
Control	107 ± 2.8	165 ± 25.1	75 ± 4.5
Low dose LPS	137 ± 3.7[a]	244 ± 28.4[b]	68 ± 3.2
High dose LPS	159 ± 3.2[a]	416 ± 41.1[a]	56 ± 2.3[c]

Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose), or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed and serum cholesterol, serum triglyceride, and HDL cholesterol levels were measured. Data are mean ± SEM; n = 23 for cholesterol and triglycerides; n = 5 for HDL cholesterol.

[a] $P < 0.001$ compared to control.
[b] $P < 0.05$ compared to control.
[c] $P < 0.01$ compared to control.

delayed increase. In contrast, as observed in rats (13), serum triglyceride levels increase soon after LPS administration to hamsters (4 h control 237 ± 26.3 vs. high dose LPS 506 ± 53.1 mg/dl $P < 0.01$).

The effect of the administration of LPS on cholesterol and fatty acid synthesis in the liver and small intestine 16 h after treatment is shown in **Table 3.** In the small intestine, the incorporation of ^3H_2O into cholesterol and fatty

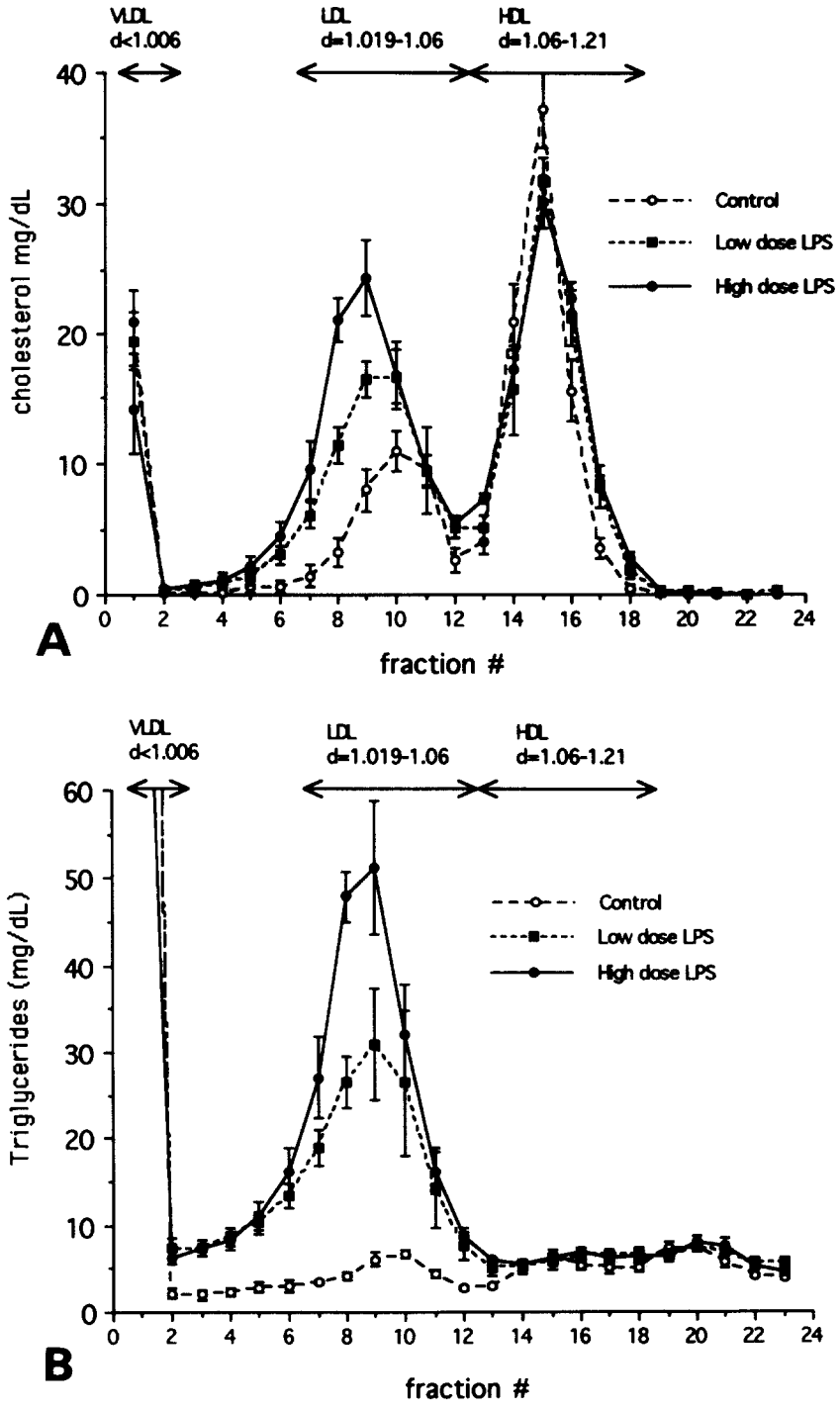

Fig. 1. Density gradient ultracentrifugation. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose), or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed and lipoproteins were separated by density gradient ultracentrifugation. Panels A and B show cholesterol and triglyceride levels, respectively. Data are presented as mean ± SEM; n=5 for each group.

TABLE 2. Composition of lipoprotein fractions in LPS-treated and control animals

Fraction	Cholesterol	Triglycerides	Phospholipids	Protein
	%	%	%	%
VLDL				
Control	7.18 ± 0.43	64.43 ± 0.81	18.17 ± 0.64	10.21 ± 0.31
Low dose LPS	6.67 ± 0.31	63.93 ± 1.07	18.53 ± 0.46	10.86 ± 0.69
High dose LPS	7.82 ± 0.40	60.63 ± 1.62	20.18 ± 0.97	11.37 ± 0.78
LDL				
Control	25.84 ± 2.94	14.46 ± 1.93	22.72 ± 2.02	36.98 ± 6.37
Low dose LPS	18.02 ± 0.89[a]	27.86 ± 1.39[c]	24.57 ± 0.35	29.55 ± 0.53
High dose LPS	14.42 ± 1.37[b]	33.74 ± 2.90[c]	23.77 ± 0.12	28.07 ± 1.48
HDL				
Control	15.43 ± 0.33	1.58 ± 0.25	33.00 ± 0.59	50.00 ± 0.86
Low dose LPS	14.17 ± 0.18[b]	1.79 ± 0.42	36.01 ± 0.31[b]	48.02 ± 0.51
High dose LPS	13.61 ± 0.28[b]	1.86 ± 0.06	36.15 ± 0.29[b]	48.38 ± 0.56

Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose), or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed; lipoproteins were separated by density gradient ultracentrifugation; and cholesterol, triglyceride, phospholipid, and protein levels were determined. The data are presented as mean ± SEM; n = 5 for each group.

[a] $P < 0.05$ compared to control.
[b] $P < 0.01$ compared to control.
[c] $P < 0.001$ compared to control.

acids was decreased in the LPS-treated animals (cholesterol: low dose decreased 26%, high dose decreased 23%; fatty acids: low dose decreased 34%, high dose decreased 41%). In contrast, in the liver, LPS administration had no significant effect on the incorporation of ^3H_2O into fatty acids. However, in the liver, LPS administration markedly increased the incorporation of ^3H_2O into cholesterol (low dose, 85%; high dose, 205%). Thus, LPS stimulates hepatic cholesterol synthesis without enhancing hepatic fatty acid synthesis or small intestinal lipid synthesis.

The effect of LPS on hepatic HMG-CoA reductase activity, mass, and mRNA levels is shown in **Fig. 2.** Maximal activity of HMG-CoA reductase in the liver was increased 2.97-fold and 9.96-fold in the low and high dose LPS treated animals, respectively (Fig. 2A). The mass of HMG-CoA reductase protein, as measured by Western blotting, was also increased in the LPS-treated animals (low dose, 7.15-fold; high dose, 8.58-fold) (Fig. 2B). Moreover, LPS administration markedly increased hepatic mRNA levels (low dose, 3.1-fold; high dose, 14.2-fold) (Fig. 2C). Thus, LPS administration increases HMG-CoA reductase mRNA levels in the liver which could account for the increase in HMG-CoA reductase activity and cholesterol synthesis.

Fig. 3 shows the effect of low and high dose LPS on HMG-CoA reductase mRNA levels in the liver at various times after LPS administration. The increase in HMG-CoA reductase mRNA levels was initially observed 4 h after LPS treatment and persisted for at least 24 h.

TABLE 3. Effect of endotoxin on Lipid Synthesis

	Liver		Small Intestine	
Group	Cholesterol	Fatty Acids	Cholesterol	Fatty Acid
	μmoles ^3H_2O incorporated/organ/h		*μmoles ^3H_2O incorporated/organ/h*	
Control	1.02 ± 0.12	7.34 ± 0.84	1.32 ± 0.04	2.07 ± 0.18
Low dose LPS	1.89 ± 0.20[a]	5.15 ± 0.57	0.97 ± 0.07[a]	1.37 ± 0.20[c]
High dose LPS	3.11 ± 0.50[b]	8.19 ± 0.65	1.01 ± 0.12[c]	1.23 ± 0.30[c]

Animals were injected I.P. with either saline (control), 100 ng/100 g body weight LPS (low dose), or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were injected I.P. with 50 mCi ^3H_2O and after a 1-h in vivo labeling period the animals were killed. The incorporation of ^3H_2O into cholesterol and fatty acids was determined as described in Methods. Data are presented as mean ± SEM; n = 9 for liver samples; n = 5 for intestine samples.

[a] $P < 0.01$ compared to control.
[b] $P < 0.001$ compared to control.
[c] $P < 0.05$ compared to control.

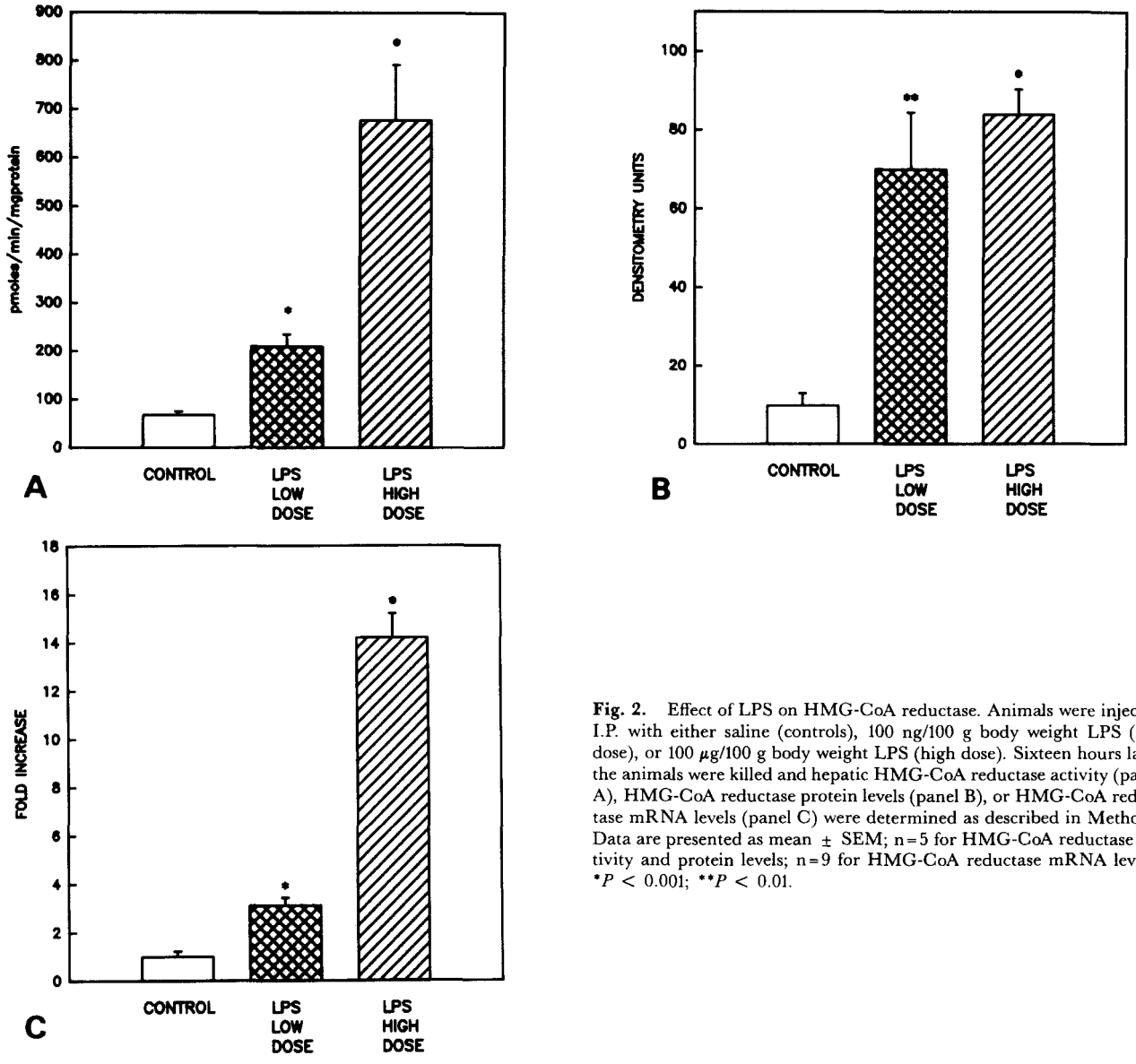

Fig. 2. Effect of LPS on HMG-CoA reductase. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose), or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed and hepatic HMG-CoA reductase activity (panel A), HMG-CoA reductase protein levels (panel B), or HMG-CoA reductase mRNA levels (panel C) were determined as described in Methods. Data are presented as mean ± SEM; n=5 for HMG-CoA reductase activity and protein levels; n=9 for HMG-CoA reductase mRNA levels; $^{*}P < 0.001$; $^{**}P < 0.01$.

The activity of HMG-CoA reductase is dependent on both the total quantity of enzyme and the catalytic activity of the enzyme. It is well recognized that HMG-CoA reductase activity is modulated by a reversible phosphorylation–dephosphorylation with the phosphorylated form of the enzyme being inactive and the dephosphorylated form active (31, 32). During the routine isolation of liver microsomes, dephosphorylation (activation) of HMG-CoA reductase occurs and HMG-CoA reductase assays performed on these samples represents an index of the total quantity of enzyme present in the tissue (27, 31, 32). If, however, microsomes are isolated in the presence of fluoride ion, which blocks dephosphorylation, assays of HMG-CoA reductase activity are indicative of the quantity of dephosphorylated (active) enzyme in situ (27, 31, 32). In most experimental conditions the activity of HMG-CoA reductase increases or decreases to a similar degree regardless of the method of microsome isolation. Because of the modest increase in cholesterol synthesis (Table 3) compared to the marked increase in HMG-CoA reductase activity and mass (Fig. 2), we next determined the quantity of dephosphorylated (active) enzyme. Similar to results described above in Fig. 2A, in microsomes isolated in sodium chloride buffer, high-dose LPS treatment increased HMG-CoA reductase activity 8.8-fold (**Table 4**). However, in microsomes isolated in sodium fluoride buffer, which inhibits dephosphorylation, high-dose LPS treatment increased HMG-CoA reductase activity only 3.8-fold (Table 4). The percentage of reductase in the active dephosphorylated form was decreased in

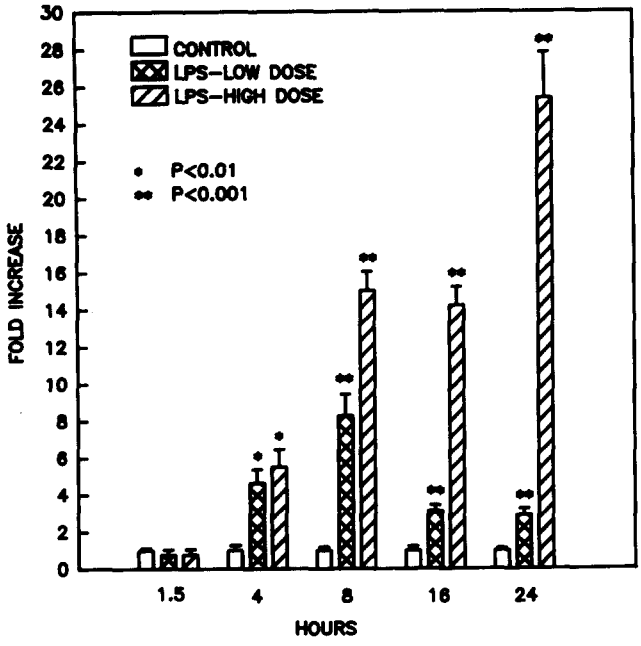

Fig. 3. Time course of effect of LPS on HMG-CoA reductase mRNA levels. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose) or 100 μg/100 g body weight LPS (high dose). At the indicated times animals were killed and hepatic HMG-CoA reductase mRNA levels were determined as described in Methods. Data are presented as mean ± SEM; n = 5 for each time point except n = 9 for 16 h; $^{*}P < 0.01$; $^{**}P < 0.001$.

LPS-treated animals (control 37.4% ± 7.9 vs. LPS 13.5% ± 0.8 in the active form, $P < 0.02$). Thus, while LPS administration increases total HMG-CoA reductase, the proportion of reductase in the active form is reduced.

Total cholesterol levels in the liver were unchanged at 16 h (control, 1.85 ± 0.078; low dose, LPS 1.90 ± 0.104; high dose LPS, 1.94 ± 0.191). Similarly, there were no significant differences in free or esterified cholesterol levels. Therefore, changes in hepatic cholesterol content are unlikely to be responsible for the increase in HMG-CoA reductase.

We next determined whether LPS administration results in an increase in the levels of other mRNA species in the liver. Similar to observations in rats reported by other investigators (33), in Syrian hamsters LPS administration increases beta actin mRNA levels in the liver (low dose, 2.03-fold; high dose, 5.07-fold) (**Fig. 4**). In contrast, low dose LPS treatment had only a minimal stimulatory effect on hepatic LDL receptor mRNA levels and high dose LPS treatment had no significant effect (**Fig. 5A**). The mass of LDL receptor protein, as measured by Western blotting, was slightly increased in the low dose LPS group (increased 1.3-fold) and unchanged in the high dose LPS group (Fig. 5B). These results with the LDL receptor indicate that LPS administration does not cause an increase in the levels of all mRNA species. In fact, while the expression of HMG-CoA reductase and LDL receptor mRNAs is usually coordinately regulated (34), in the present studies LPS markedly elevated hepatic HMG-CoA reductase mRNA levels but had only minimal effects on hepatic LDL receptor mRNA levels.

The effect of LPS on hepatic apoA-I and E mRNA levels is shown in **Fig. 6.** Low dose LPS administration results in a small decrease in apoE mRNA levels (18%) while high dose LPS decreased hepatic apoE mRNA levels 37% (Fig. 6A). In contrast, apoA-I mRNA levels in the liver were not affected by LPS treatment (Fig. 6B). **Fig. 7** shows the effect of LPS on apoE mRNA levels in the liver at various times after LPS administration. After high dose LPS, the decrease in apoE mRNA levels was first observed 8 h after LPS administration and persisted for at least 24 h.

DISCUSSION

The present study demonstrates that in Syrian hamsters LPS treatment results in an increase in serum cholesterol levels after 16 h which is primarily due to an increase in LDL cholesterol. In contrast, HDL cholesterol

TABLE 4. Effect of endotoxin on HMG-CoA reductase activity and activation state

Group	HMG-CoA Reductase Activity		
	Total Enzyme (NaCl Buffer)	Active Enzyme (NaF Buffer)	Activation State (NaF/NaCl)
	pmoles mevalonate formed/min/mg protein		%
Control	102 ± 26.4	31 ± 5.0	37.4 ± 7.9
LPS	900 ± 121.2	118 ± 11.7	13.5 ± 0.8
	$P < 0.001$	$P < 0.001$	$P < 0.02$

Animals were injected I.P. with either saline or 100 μg/100 g body weight LPS. Sixteen hours later the animals were killed, the livers were homogenized in buffers containing either 50 mM NaCl or 50 mM NaF, and microsomes were isolated by ultracentrifugation. HMG-CoA reductase activity was then determined as described in Methods. Data are presented as mean ± SEM; n = 5 for each group.

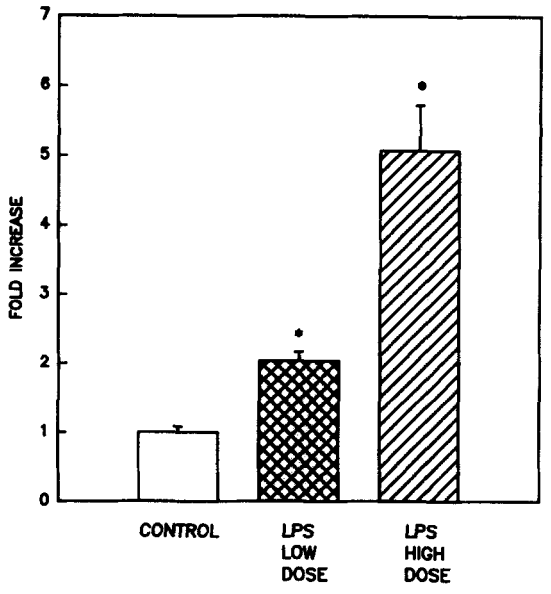

Fig. 4. Effect of LPS on actin mRNA levels. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose) or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed and hepatic actin mRNA levels were determined as described in Methods. Data are presented as mean ± SEM; n=9; $^{*}P < 0.001$.

levels decrease after LPS treatment. The effect of LPS on serum cholesterol and lipoprotein levels in hamsters is similar to observations previously reported by other investigators in rats and mice (14–16). However, the studies in rats and mice used large doses of LPS whereas, in the present study, we observed that a low dose of LPS (100 ng/100 g body weight) also increases serum cholesterol levels.

In several different infections an increase in hepatic de novo cholesterol synthesis and/or HMG-CoA reductase activity has been observed in rats (11, 17, 18). We now demonstrate that LPS administration to Syrian hamsters increases hepatic de novo cholesterol synthesis and HMG-CoA reductase activity, protein mass, and mRNA levels. However, LPS administration decreased the proportion of HMG-CoA reductase in the active dephosphorylated form, which probably accounts for the relatively modest increase in hepatic cholesterol synthesis despite large increases in HMG-CoA reductase protein mass. de Vasconcelos et al. (18) also observed a decrease in HMG-CoA reductase activation state during infection. Thus, infection increases hepatic cholesterol synthesis by increasing the mass of HMG-CoA reductase protein in the liver which can be accounted for by an increase in HMG-CoA reductase mRNA levels.

In contrast to the marked stimulatory effect of LPS on HMG-CoA reductase mRNA levels (increased 14-fold), LPS had only minimal effects on hepatic LDL receptor mRNA levels. HMG-CoA reductase and LDL receptor mRNA levels are usually coordinately regulated with parallel increases or decreases in mRNA levels being observed in response to most stimuli (34). Recently, discordances between HMG-CoA reductase and LDL receptor mRNA levels have been reported in liver and other tissues (35, 36). The marked stimulatory effect of LPS on hepatic HMG-CoA reductase mRNA with only a minimal change in LDL receptor mRNA levels is another example of such discordant regulation.

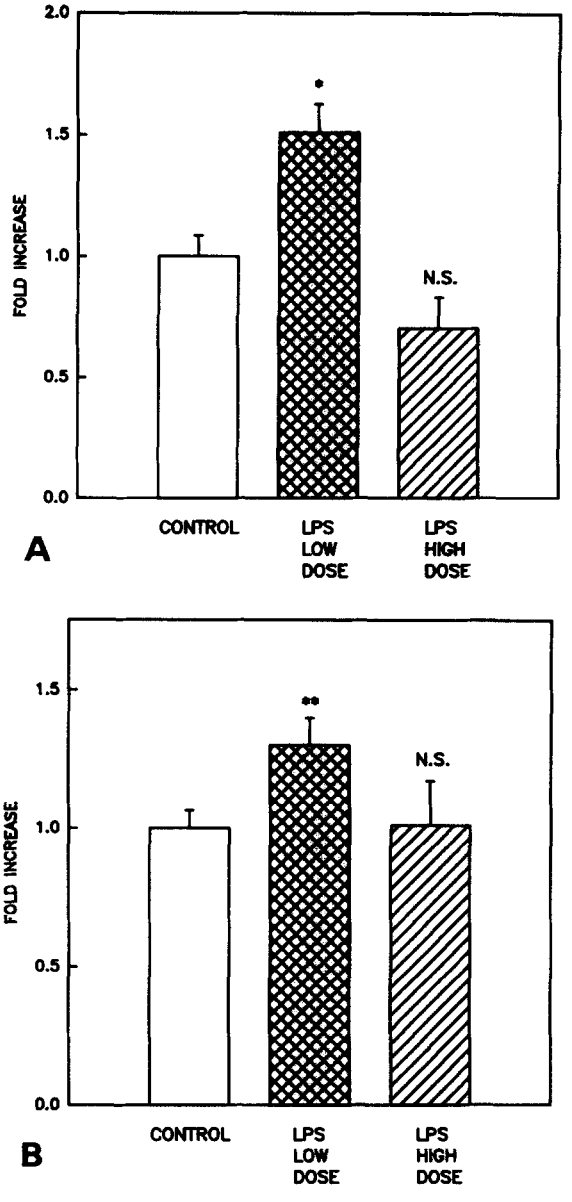

Fig. 5. Effect of LPS on the LDL receptor. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose) or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed and hepatic LDL receptor mRNA (panel A) and hepatic LDL receptor protein (panel B) levels were determined as described in Methods. Data are presented as mean ± SEM; n=9 for LDL receptor protein; n=14 for LDL receptor mRNA; $^{*}P < 0.01$; $^{**}P < 0.05$.

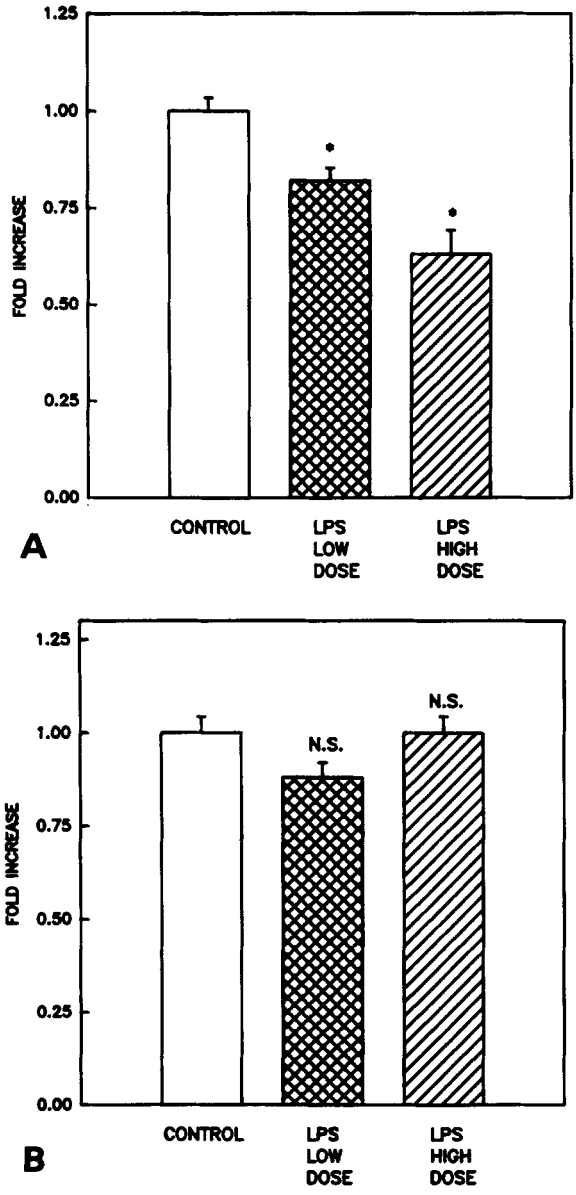

Fig. 6. Effect of LPS on apolipoprotein mRNA levels. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose) or 100 μg/100 g body weight LPS (high dose). Sixteen hours later the animals were killed and hepatic apoE (panel A) and apoA-I (panel B) mRNA levels were determined as described in Methods. Data are presented as mean ± SEM; n=14 for apoE and n=9 for apoA-I; $^*P < 0.001$.

Similar to the effect of LPS on LDL receptor mRNA levels, LPS did not decrease LDL receptor protein levels; therefore, the increase in serum and LDL cholesterol levels cannot be accounted for by changes in the number of hepatic LDL receptors. It is tempting to speculate that the increase in hepatic cholesterol synthesis induced by LPS accounts for the increase in serum cholesterol levels, but data directly proving this hypothesis are not available.

The present study also found that LPS decreased apoE mRNA levels in the liver. Other investigators have not observed a decrease in liver apoE mRNA levels after LPS administration in rats (37). However, turpentine-induced inflammation produced a decrease in hepatic apoE mRNA levels of a magnitude similar to that seen in the present study (38). In macrophages, endotoxin decreases the secretion of apoE (39). Moreover, studies have shown that infection or inflammation leads to a decrease in apoE in VLDL particles (5, 40).

In contrast to the effects on apolipoprotein E mRNA levels, we did not observe any significant change in hepatic apoA-I mRNA levels. No change in hepatic apoA-I mRNA levels in rats was found after turpentine-induced inflammation (38), but a decrease in hepatic apoA-I mRNA levels was found after LPS administration (41). LPS administration has also been shown to decrease apoA-I secretion by hepatocytes in some but not all studies (41–43). A more consistent observation, however, has been that infection and inflammation decrease circulating HDL and apoA-I levels (4, 5, 9). The etiology of the decrease in serum apoA-I needs to be more clearly defined.

Many of the metabolic effects of infection, inflammation, and LPS administration are mediated by cytokines such as TNF, IL-1, and IL-6 (44, 45). These cytokines induce, in the liver, the synthesis of certain proteins (acute phase proteins) while the synthesis of other proteins is in-

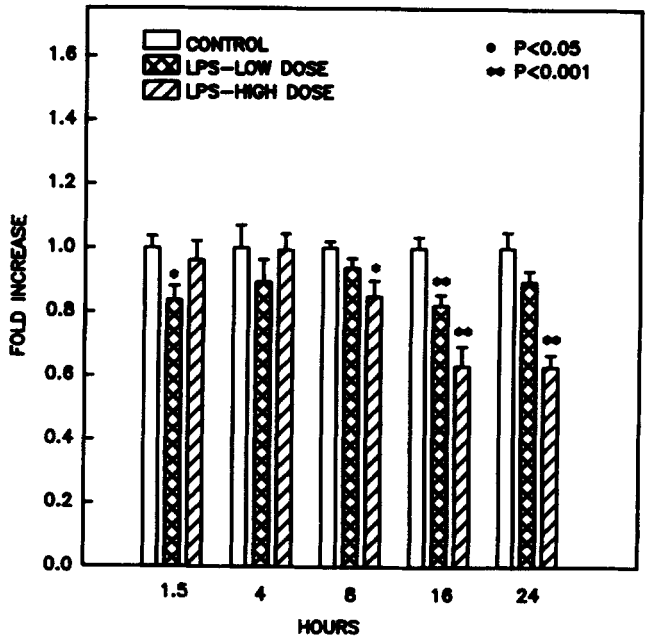

Fig. 7. Time course of effect of LPS on apoE mRNA levels. Animals were injected I.P. with either saline (controls), 100 ng/100 g body weight LPS (low dose) or 100 μg/100 g body weight LPS (high dose). At the indicated times animals were killed and hepatic apoE mRNA levels were determined as described in Methods. Data are presented as mean ± SEM; n=5 for each time point except n=14 for 16 h.

hibited (negative acute phase proteins) (46, 47). The precise mechanisms by which cytokines induce these changes in gene regulation and protein synthesis are not yet resolved. While the sterol-regulating element is similar in HMG-CoA reductase and LDL receptor genes, there is evidence that there are differences in the promoters in the two genes which could allow for the independent regulation of these two proteins (48). The present study demonstrates that HMG-CoA reductase is a member of the group of proteins that is positively regulated by inflammatory stimuli. In contrast, apoE can be considered a negative acute phase protein.

One can speculate on why there is a linkage between inflammation/infection and lipid/lipoprotein metabolism. It is believed that the changes in the pattern of protein synthesis in the liver that comprise the acute phase response have potential beneficial effects (46). A number of studies has suggested that increases in serum lipid and lipoprotein levels may also be beneficial during infection and inflammation. Experiments have demonstrated that lipoproteins bind LPS (49–54). Moreover, this binding can protect animals from the toxic effects of LPS (54). In addition, studies have shown that lipoproteins bind to a variety of viruses blocking their cytopathic effects (55–59) and induce the lysis of the parasite, *Trypanosoma bruceii* (60, 61). Furthermore, lipoproteins bind urate crystals, reducing the inflammatory response to these crystals (62). It is possible that the increase in HMG-CoA reductase provides cholesterol which allows for the production of lipoproteins and elevations in serum lipid levels. A decrease in apoE levels could decrease lipoprotein clearance and thereby also contribute to the hyperlipoproteinemia. Thus, the changes in lipid and lipoprotein metabolism described in this and other studies may serve to facilitate the body's host defense.

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