Endotoxin, tumor necrosis factor, and interleukin-1 decrease hepatic squalene synthase activity, protein, and mRNA levels in Syrian hamsters

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Abstract Recent studies have shown that endotoxin (LPS) administration to Syrian hamsters markedly increased hepatic HMG-CoA reductase activity, protein mass, and mRNA levels, but only produced a modest increase in hepatic cholesterol synthesis, suggesting that LPS may also influence other key enzymes involved in the regulation of cholesterol metabolism. In the present study, we have examined the effect of LPS and cytokines on the activity, protein mass, and mRNA level of squalene synthase, which is the first committed enzyme in cholesterol biosynthesis and is located at a branch point in the mevalonate pathway. Our results demonstrate that LPS administration produces a marked decrease in the mRNA levels of squalene synthase. This decrease in squalene synthase mRNA occurred very rapidly (90 min after LPS) and required relatively small doses of LPS (1 μg/100 gm body weight). LPS also significantly decreased squalene synthase activity and protein mass. Finally, LPS produced a marked decrease in squalene synthase mRNA, activity, and protein levels when the basal levels of squalene synthase expression were increased 4-fold by prior treatment with bile acid binding resin, colestipol. Tumor necrosis factor and interleukin-1, which mediate many of the metabolic effects of LPS, also decreased hepatic squalene synthase activity and mRNA levels. Taken together, our results suggest that the discordant regulation of HMG-CoA reductase and squalene synthase during the host response to infection and inflammation may have substantial effects on the regulation of substrate flux into the non-sterol pathways of mevalonate metabolism.—**Memon, R. A., I. Shechter, A. H. Moser, J. K. Shigenaga, C. Grunfeld, and K. R. Feingold.** Endotoxin, tumor necrosis factor, and interleukin-1 decrease hepatic squalene synthase activity, protein, and mRNA levels in Syrian hamsters. *J. Lipid Res.* 1997. **38:** 1620–1629.

Supplementary key words cytokines • acute phase response • cholesterol metabolism • HMG-CoA reductase • cholesterol feeding • colestipol feeding

The host response to infection and inflammation is associated with multiple changes in triglyceride and cholesterol metabolism (1–5). These metabolic changes can also be induced by the administration of endotoxin (LPS) which mimics gram-negative infections and by pro-inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) that mediate many of the pathophysiologic and metabolic responses that occur during infection. For example, LPS and cytokines increase serum triglyceride levels and VLDL production by stimulating hepatic lipogenesis, suppressing fatty acid oxidation, and increasing re-esterification of peripherally derived fatty acids (6, 7).

Several studies have demonstrated that LPS also exerts profound effects on cholesterol metabolism which may support increased VLDL production. LPS administration increases serum cholesterol levels and stimulates de novo hepatic cholesterol synthesis in mice, rats, and hamsters (8–10). Recent studies from our laboratory have focused on the mechanisms of LPS-induced changes in cholesterol metabolism in Syrian hamsters (10–13), because in contrast to other rodents, lipid and lipoprotein metabolism in hamsters more closely resembles that in humans. We have shown that LPS produces an increase in the activity, protein mass, and mRNA levels of hepatic hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in the cholesterol biosynthesis pathway (10). The effect of LPS on HMG-CoA reductase mRNA is mediated through an increase in its transcription rate (12). However, while LPS produces a dramatic 8- to 10-fold increase in the activity, protein mass, and mRNA levels of HMG-CoA reductase, it produces only a modest 2-fold increase in

Abbreviations: LPS, endotoxin; TNF, tumor necrosis factor; IL-1, interleukin-1; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein; VLDL, very low density lipoprotein; FPP, farnesyl pyrophosphate; BW, body weight.

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de novo hepatic cholesterol synthesis (10), suggesting that LPS may also affect other key enzymes or proteins that are involved in the regulation of cholesterol metabolism. LPS did not increase the mRNAs for HMG-CoA synthase or farnesyl pyrophosphate (FPP) synthase (12), enzymes that are usually coordinately regulated by sterols. LPS also has no effect on the protein or mRNA levels of the hepatic LDL receptor (10).

Squalene synthase is the first committed enzyme in the biosynthesis of cholesterol and is located at a branch point in the mevalonate pathway (14, 15). It catalyzes the condensation of two FPP molecules to produce presqualene diphosphate which is subsequently reduced to squalene in the presence of reduced nucleotides. In addition to the synthesis of sterols, molecules in the pathway of mevalonate metabolism prior to presqualene diphosphate are utilized to synthesize several other compounds such as dolichol, ubiquinone, and prenylated proteins (14, 15). Because of its unique location in the mevalonate pathway, squalene synthase is a logical site for metabolic regulation. A decrease in squalene synthase coupled with a large increase in HMG-CoA reductase could support some increase in cholesterol synthesis while redirecting mevalonate metabolites into other non-sterol pathways.

Squalene synthase has been recently purified and cloned from rat liver (16, 17) and these advances have now made it possible to study the in vivo regulation of squalene synthase. In this study we have examined the effects of endotoxin, TNF, and IL-1 administration on the activity, protein, and mRNA levels of hepatic squalene synthase in Syrian hamsters. Furthermore, we have determined the effect of dietary manipulations that are known to regulate squalene synthase activity on the ability of LPS to alter squalene synthase activity and mRNA levels.

MATERIALS AND METHODS

Materials

[α-^{32}P]dCTP (3,000 Ci/mmol, 10 mCi/ml) and [1-^{3}H]farnesyl pyrophosphate (triammonium salt, sp.act. 22.5 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Endotoxin (*E. coli* 55: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 5×10^7 U/mg was kindly provided by Gennentech, Inc. (South San Francisco). Recombinant human IL-1β with a specific activity of 1×10^9 U/mg was generously provided by Immunex (Seattle, WA). The cytokines were freshly diluted to desired concentrations in pyrogen-free 0.9% saline containing 0.1% human serum albumin. Multiprime DNA labeling system was purchased from Amersham International (Amersham, United Kingdom); minispin G-50 columns were from Worthington Biochemical Corporation (Freehold, NJ); oligo (DT) cellulose type 77F was from Pharmacia LKB Biotechnology AB (Upsala, Sweden); nitrocellulose and Nytran were from Schleicher & Schuell (Keene, NH). Kodak XAR5 film was used for autoradiography. The cDNA for squalene synthase was prepared as described previously (16, 17). Purified rat liver squalene synthase was used for the generation of monoclonal rabbit anti-rat squalene synthase antibodies (16, 17). Goat anti-rabbit IgG conjugated with alkaline phosphatase and alkaline phosphatase substrate reaction kit were purchased from Bio-Rad Laboratories (Hercules, CA).

Animal procedures

Male Syrian hamsters (approximately 100–150 g) were purchased from Simonsen Laboratories (Gilroy, CA). The animals were maintained on a normal light cycle (6 AM to 6 PM light, 6 PM to 6 AM dark) and were provided with rodent chow (Simonsen Laboratories) and water ad libitum. Where indicated, cholesterol or colestipol (UpJohn, Kalamazoo, MI) was added to the chow diet (2% by weight) and the animals were fed for 7 days prior to study. Animals were injected intraperitoneally (i.p.) with LPS, TNF, IL-1, or TNF+IL-1 at the indicated doses in 0.5 ml 0.9% saline or with saline alone. Subsequently, food was withdrawn from both control and treated animals because LPS and cytokines can induce anorexia. Animals were studied between 1.5 and 16 h after LPS or cytokine administration as indicated in the text. The doses of LPS used (0.1 to 100 μg/100 g body weight (BW)) are far below the doses required to cause death in rodents in our laboratory (LD_{50} ~5 mg/100 g BW) but have significant effects on triglyceride and cholesterol metabolism in Syrian hamsters (10–13). Similarly, the doses of TNF and IL-1 used (17 μg/100 g BW and 1 μg/100 g BW, respectively) have marked effects on lipid metabolism and reproduce many of the effects of LPS on lipid metabolism in Syrian hamsters (11, 13, 18).

Squalene synthase activity assay

Squalene synthase activity was measured in freshly isolated microsomes by the method of Shechter et al. (16). Briefly, the livers were homogenized in a 0.3 M sucrose buffer containing 10 mM HEPES, 1.0 mM DTT, and 1.0 mM EDTA and the microsomes were isolated. The assay mixture contained 100 mM potassium phosphate buffer, pH 7.4, 5 mM $MgCl_2$, 5 mM CHAPS, 10 mM dithiothreitol, 2 mM NADPH, and 25 μM [^{3}H]farnesyl pyrophosphate in a final volume of 100 ul. The assays were

carried out in 96-well microtitration plates and the reaction was initiated by the addition of 10 μg microsomal protein. The plates were incubated for 30 min at 37°C. The reaction was terminated by the addition of 20 μl of 0.5 M EDTA (pH 8.0) and 10 μl of unlabeled 0.5% squalene in ethanol was added as carrier. The labeled squalene was isolated by thin-layer chromatography and the radioactivity in squalene was measured by liquid scintillation counting. Control reactions containing no microsomal protein were run in parallel to correct for the background. Squalene synthase activity is expressed as pmoles squalene synthesized per mg protein per minute. Protein was assayed by the method of Bradford (protein assay, Bio-Rad Laboratories).

SDS-gel electrophoresis and immunoblotting

Microsomes were isolated and the proteins were separated on a 10% polyacrylamide minigel. The separated proteins were electrophoretically transferred to nitrocellulose membrane. The blocking and washing of the membrane was performed as described by Shechter et al. (16). The membrane was incubated with rabbit anti-rat squalene synthase anti-sera (1:5000) overnight. Alkaline phosphatase conjugated goat anti-rabbit (1:5000) was used as a second antibody. Finally, the membrane was incubated with alkaline phosphatase substrates (5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium) according to the Bio-Rad protocol. After the development of sufficient color (2–5 min), the reaction was stopped by extensively rinsing the membrane with deionized water. The membranes were air-dried and the squalene synthase band density was measured using a Bio-Rad GS-670 imaging densitometer (Bio-Rad Laboratories).

Isolation of RNA and Northern blotting

Total RNA was isolated by a variation of the guanidinium thiocyanate method (10). Poly A^+ RNA was isolated using oligo dT cellulose and quantified by measuring absorption at 260 nm. Equal amounts of poly A^+ RNA (10 μg) were loaded on 1% agarose-formaldehyde gels and electrophoresed. The uniformity of sample applications was checked by ultraviolet visualization of the acridine orange-stained gel before transfer to Nytran membranes. In order to avoid any potential problems with RNA transfer from the gels, the exposure to acridine orange was kept to a minimum by staining the gels for only 1 min followed by 1 h of destaining. We and others have found that LPS increases mRNA levels of actin in liver (10, 19). LPS also increases hepatic mRNA levels for glyceraldehyde 3-phosphate dehydrogenase (G-3PD) and cyclophilin (unpublished observations by this laboratory). Therefore, the mRNA levels of actin, G-3PD and cyclophilin, which are widely used for normalizing data, cannot be used to study LPS induced regulation of proteins in liver. However, the differing direction of the changes in mRNA levels for specific proteins after LPS, cytokines, colestipol administration or cholesterol feeding, the magnitude and consistency of the alterations, and the relatively small standard error of the mean make it unlikely that the changes observed are due to unequal loading of mRNA. cDNA probe hybridization was performed in 0.75 M sodium chloride, 0.075 M sodium citrate, 2% SDS, 10% dextran sulfate, 2 × Denhardt's solution and 100 μg/ml sheared salmon sperm DNA at 65°C overnight. Blots were washed in 0.2× SSC and 0.1% SDS at room temperature for 30 min and at 65°C for 1 h. The blots were exposed to X-ray films for various durations to ensure that measurements were done on the linear portion of the curve, and the bands were quantified by densitometry.

Statistics

The results are presented as means ± SEM. Statistical significance between two groups was determined by using the Student's *t*-test. Comparison among several groups were done by analysis of variance and statistical significance was calculated using Bonferroni multiple comparison test.

RESULTS

Effect of LPS on hepatic squalene synthase activity, protein, and mRNA levels

The data presented in **Fig. 1** show the time course of LPS (100 μg/100 g BW) effect on squalene synthase mRNA and activity. Ninety minutes after LPS administration squalene synthase mRNA levels were decreased by 53% ($P < 0.01$) whereas by 4 h after LPS squalene synthase mRNA levels had decreased by 88% ($P < 0.001$). At 8 and 16 h after LPS administration, squalene synthase mRNA levels were less than 5% of the controls (Fig. 1A). LPS also produced a significant decrease in hepatic squalene synthase activity at all time points studied (Fig. 1B). The maximal decrease in activity was 65% and was observed at 16 h after LPS administration.

We next determined the dose response for LPS-induced decrease in squalene synthase mRNA levels. As shown in **Fig. 2A,** a dose of 1 μg/100 g BW produced a 71% decrease in squalene synthase mRNA levels 16 h after administration. On the other hand, a dose of 0.1 μg/100 g BW was ineffective, suggesting that the half-maximal dose of LPS effect on squalene synthase mRNA levels is between 0.1 and 1 μg/100 g BW. Doses of 10 and 100 μg/100 g BW produced a greater than 95%

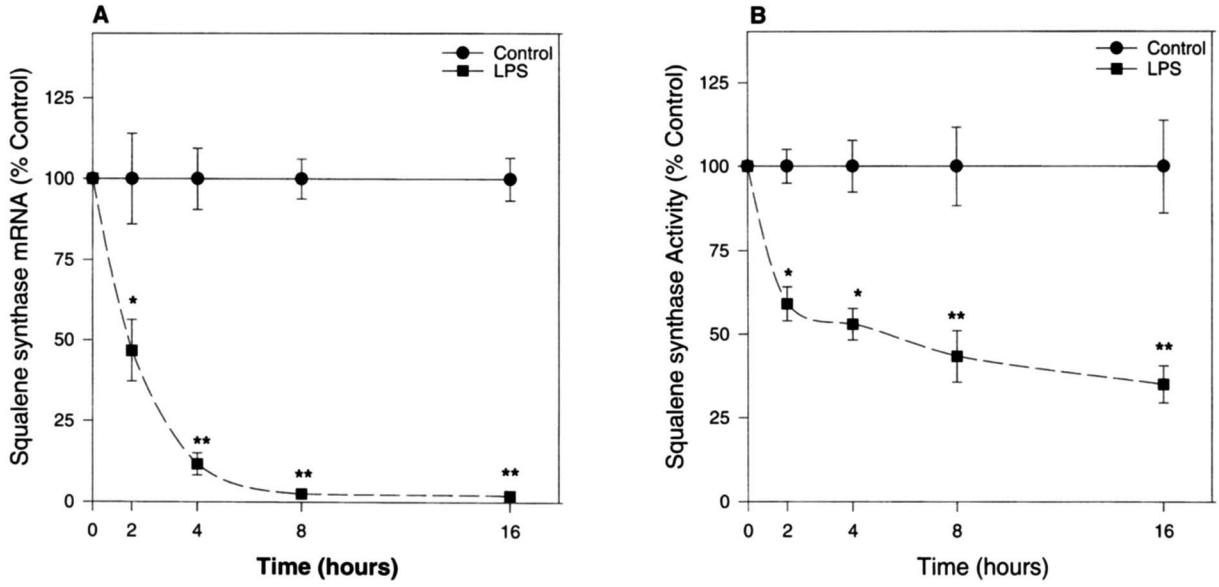


Fig. 1. Time course of LPS effect on hepatic squalene synthase mRNA (panel A) and activity (panel B). Syrian hamsters were injected i.p. with saline or LPS (100 μg/100 g BW). At the indicated times animals were killed and livers were removed. Squalene synthase mRNA and activity were determined as described in Methods. Data are presented as mean ± SEM; n = 5 for each time point; A: $*P < 0.01$; $**P < 0.001$; B: $*P < 0.01$; $**P < 0.001$.

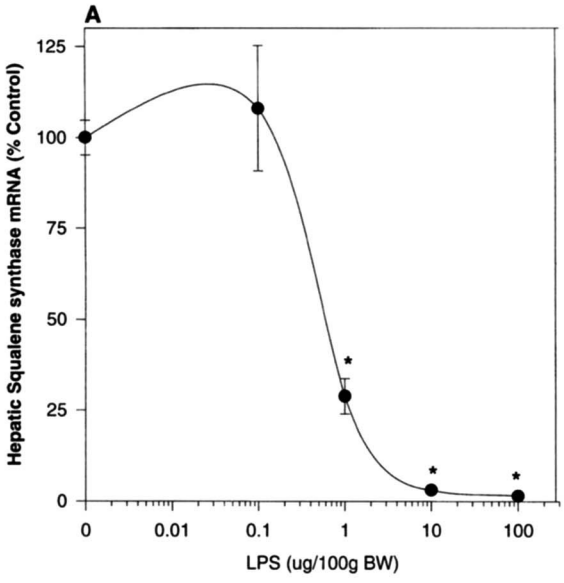
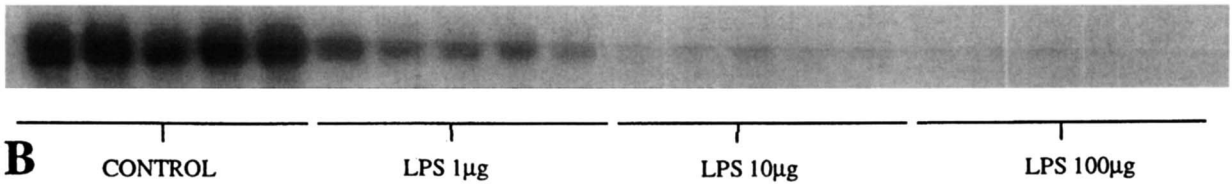


Fig. 2. Dose-response of LPS effect on hepatic squalene synthase mRNA. Syrian hamsters were injected with various doses of LPS and 16 h later animals were killed and livers were obtained for RNA isolation. Squalene synthase mRNA levels were determined by Northern blotting as described in Methods. A: Data presented as % control as quantified by densitometry. Data are presented as mean ± SEM; n = 5 for each dose; $*P < 0.001$. B: Representative Northern blot probed for squalene synthase mRNA.



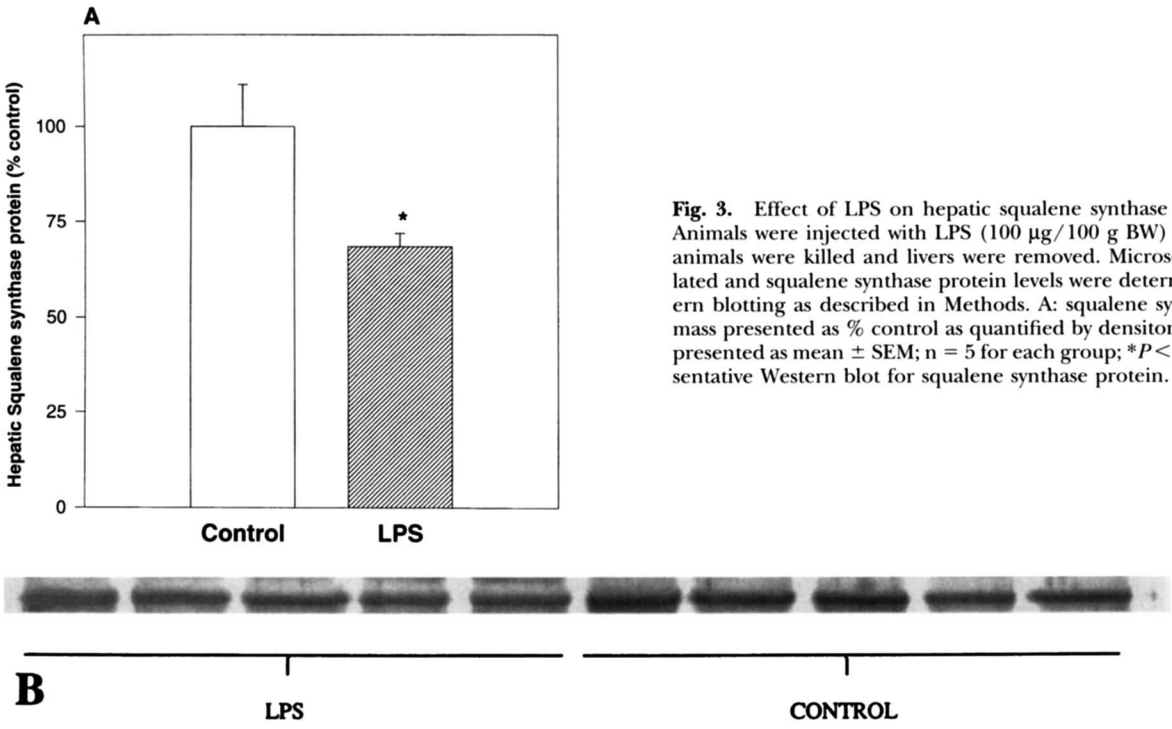


Fig. 3. Effect of LPS on hepatic squalene synthase protein levels. Animals were injected with LPS (100 μg/100 g BW) and 16 h later animals were killed and livers were removed. Microsomes were isolated and squalene synthase protein levels were determined by Western blotting as described in Methods. A: squalene synthase protein mass presented as % control as quantified by densitometry. Data are presented as mean ± SEM; n = 5 for each group; $*P < 0.02$. B: Representative Western blot for squalene synthase protein.

decrease in squalene synthase mRNA levels. Figure 2B shows a representative Northern blot of LPS effect on squalene synthase mRNA in liver.

The effect of LPS administration on squalene synthase protein level was determined by Western blotting and quantified by densitometry. The data presented in **Fig. 3A** demonstrate that 16 h after LPS (100 μg/100 g BW), squalene synthase protein mass was significantly decreased (32% decrease, $P < 0.02$), although the decrease in protein mass was smaller in magnitude than that observed for activity or mRNA levels at the same dose and time-point. Figure 3B shows a representative Western blot for LPS effect on squalene synthase protein.

Effect of cytokines on hepatic squalene synthase mRNA levels and activity

Our previous studies have shown that pro-inflammatory cytokines such as TNF and IL-1 mediate many of the metabolic effects of LPS, hence we next examined the ability of TNF (17 μg/100 g BW) and IL-1 (1 μg/100 g BW) to mimic the effect of LPS on squalene synthase mRNA and activity. These doses of TNF and IL-1 have previously been shown to have significant effects on lipid and lipoprotein metabolism in Syrian hamsters (11, 13, 18). Eight hours after administration, TNF decreases squalene synthase mRNA by 46% whereas IL-1 either alone or in combination with TNF produced a greater than 95% decrease in squalene synthase mRNA levels (**Fig. 4A**). Figure 4B shows a representative Northern blot for the TNF and IL-1 effects on squalene synthase mRNA in liver. TNF and IL-1 produced a 32% and 43% decrease in squalene synthase activity, respectively, while the combination of both cytokines decreased squalene synthase activity by 60% (Fig. 4C).

Effect of dietary manipulations on the ability of LPS to decrease squalene synthase mRNA, activity, and protein levels

Earlier studies have shown that feeding bile acid binding resins to rats up-regulates squalene synthase activity and mRNA levels (20, 21) whereas feeding a diet containing cholesterol down-regulates squalene synthase activity, protein mass, and mRNA in rat liver (16, 21, 22). We first compared the effects of cholesterol and colestipol (bile acid binding resin) feeding with that of LPS treatment on squalene synthase activity and mRNA levels in hamsters. Hamsters were fed a 2% cholesterol or colestipol diet for 7 days or treated with LPS for 16 h. The data presented in **Fig. 5A** demonstrate that, similar to what has been previously reported for rats, cholesterol feeding to hamsters decreased squalene synthase mRNA levels by 75% while colestipol feeding produced a 4-fold increase in squalene synthase mRNA levels. On the other hand, in the same experiment LPS (100 μg/100 g BW) produced a 80% decrease in squalene synthase mRNA levels (Fig. 5A). Cholesterol feeding decreased squalene synthase activity by 82%, whereas co-

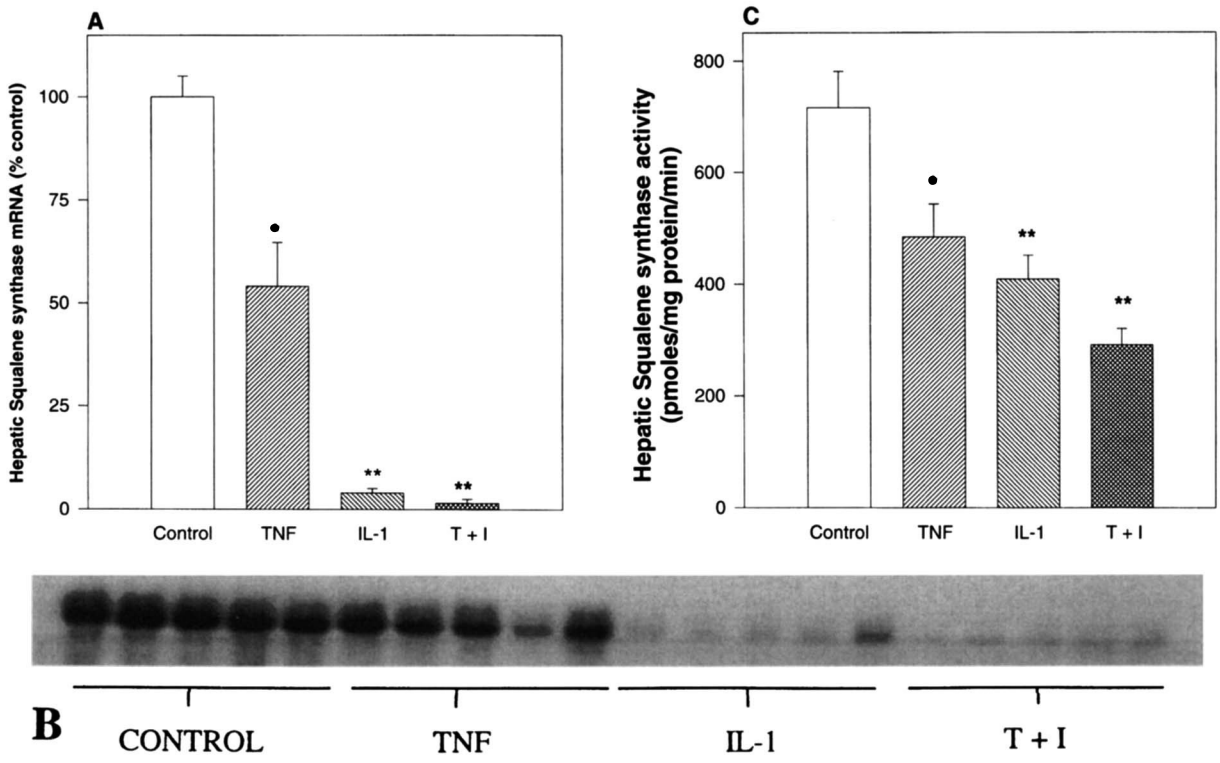


Fig. 4. Effect of TNF, IL-1, and the combination of TNF and IL-1 on squalene synthase mRNA (panel A and B) and activity (panel C) levels. Syrian hamsters were injected TNF and IL-1 at the doses indicated in text and 8 h later animals were killed. Livers were obtained and squalene synthase mRNA and activity were determined as described in Methods. Data are presented as mean ± SEM; n = 5 in each group for mRNA determinations and 10 for each group for squalene synthase activity measurements; A: $*P < 0.01$, $**P < 0.001$; C: $*P < 0.05$, $**P < 0.001$.

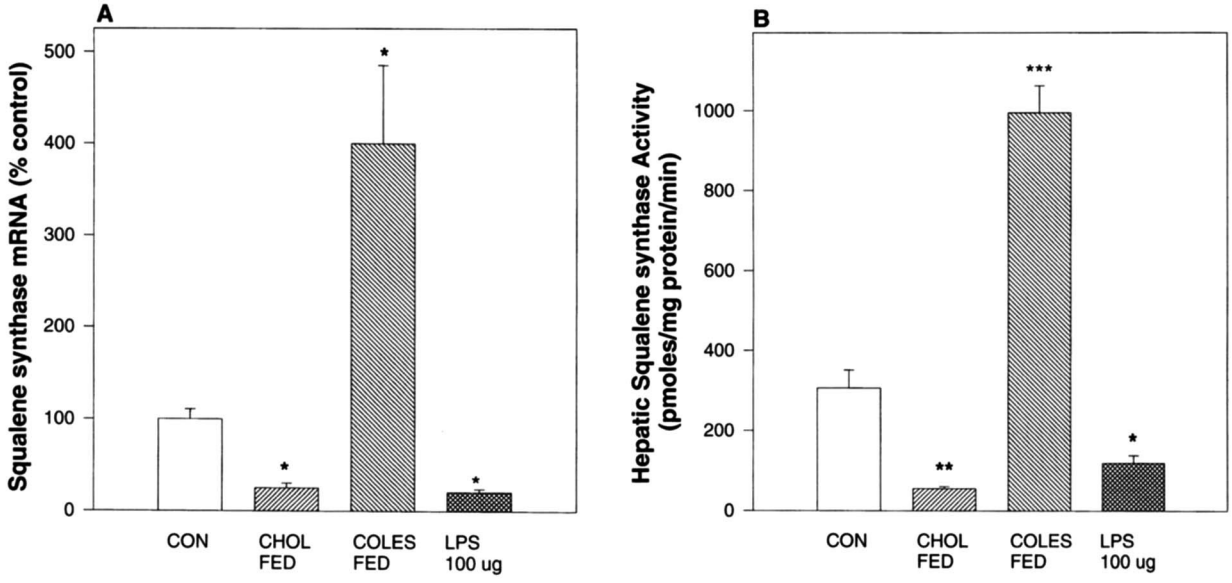


Fig. 5. Effect of diet and LPS on hepatic squalene synthase mRNA (panel A) and activity (panel B) levels. Animals were fed either rodent chow alone or rodent chow containing 2% by weight cholesterol or colestipol for 7 days. Another group of animals fed rodent chow alone was also injected with LPS (100 μg/100 g BW) 16 h prior to the study. The animals were killed and livers were obtained for squalene synthase mRNA and activity determinations. Data are presented as mean ± SEM; n = 5 for each group; A: $*P < 0.001$; B: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

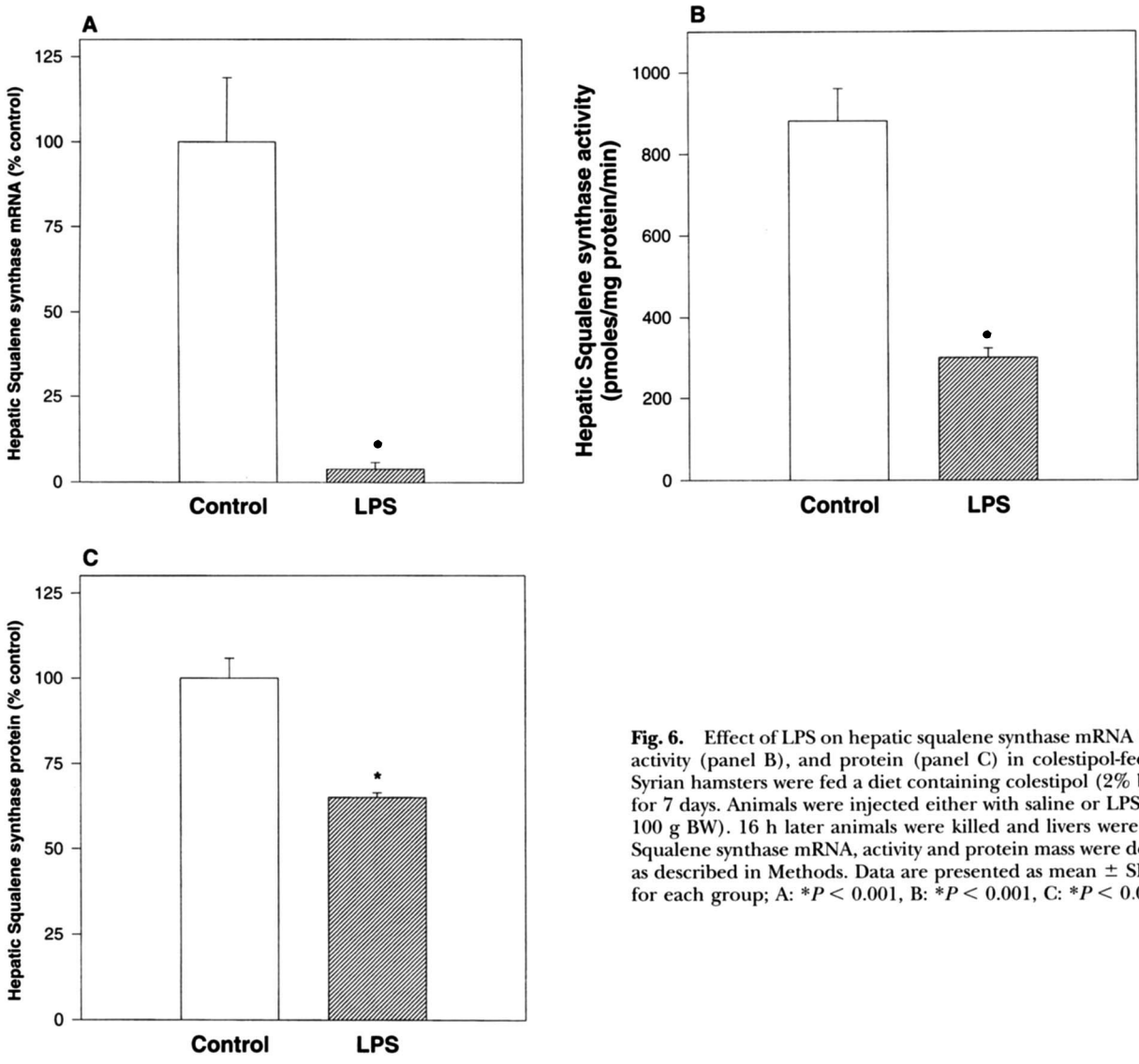


Fig. 6. Effect of LPS on hepatic squalene synthase mRNA (panel A), activity (panel B), and protein (panel C) in colestipol-fed animals. Syrian hamsters were fed a diet containing colestipol (2% by weight) for 7 days. Animals were injected either with saline or LPS (100 μg/100 g BW). 16 h later animals were killed and livers were obtained. Squalene synthase mRNA, activity and protein mass were determined as described in Methods. Data are presented as mean ± SEM; n = 5 for each group; A: $*P < 0.001$, B: $*P < 0.001$, C: $*P < 0.004$.

lestipol feeding produced a 3.2-fold increase in squalene synthase activity (Fig. 5B). In the same experiment LPS treatment produced a 60% decrease in squalene synthase activity.

We next examined the effect of LPS administration in colestipol-fed hamsters to determine whether LPS can lower squalene synthase mRNA, activity, and protein levels when it has been up-regulated by prior treatment with colestipol feeding. The data presented in **Fig. 6** shows that LPS decreased squalene synthase mRNA levels by 96% (Fig. 6A), activity by 66% (Fig. 6B), and protein mass, as determined by immuno-reactivity, by 35% (Fig. 6C). These results are almost identical to those seen in chow-fed animals indicating that LPS exerts a pronounced effect on squalene synthase, even in the up-regulated state. The differences between the magnitude of LPS effect on mRNA and protein levels suggest that LPS may regulate squalene synthase by multiple mechanisms.

DISCUSSION

Squalene synthase occupies a central position in the metabolism of cholesterol because it catalyzes the conversion of FPP into squalene, the first committed step in the biosynthesis of sterols. It has been suggested that the regulation of squalene synthase plays a major role in the diversion of flux of intermediates to sterol or nonsterol pathways (23). Our previous studies have shown that LPS induces a marked increase in hepatic HMG-CoA reductase activity, protein and mRNA levels but only a modest increase in cholesterol synthesis, sug-

gesting that LPS may down-regulate some distal enzyme in the mevalonate pathway.

The results of our present study demonstrate that LPS down-regulates hepatic squalene synthase. LPS administration produced a pronounced decrease in hepatic squalene synthase mRNA levels. The LPS induced decrease in squalene synthase mRNA is rapid, occurring as early as 90 min after administration, is maximal by 16 h, and requires a relatively small dose of LPS. The LPS-induced decrease in squalene synthase mRNA is also accompanied by a decrease in squalene synthase activity and protein mass, although the decrease in protein mass is comparatively smaller in magnitude. The rapid and profound effects of LPS on squalene synthase mRNA indicate that the primary site of LPS-induced regulation is at the mRNA level. The reason for the difference in the magnitude of inhibition for squalene synthase protein mass and mRNA levels is not clear, however, there are several potential explanations for this observation. First, squalene synthase protein may have a long half-life and therefore a marked decrease in mRNA may not be acutely reflected in a comparable decrease in protein mass or activity. The half-life of squalene synthase protein in vivo is not known. Second, LPS may exert additional regulatory effects by either increasing the rate of translation or by decreasing the rate of degradation for squalene synthase protein. Third, LPS may regulate squalene synthase activity independent of its regulatory effect on mRNA and protein levels. Further studies are needed to address these issues.

Infection or LPS administration stimulate the production of several cytokines including TNF and IL-1 which mediate many of the host responses that occur during infection. Both TNF and IL-1 raise serum triglyceride and cholesterol levels, stimulate hepatic fatty acid and cholesterol synthesis (8), and increase hepatic HMG-CoA reductase activity (8) and mRNA levels (18). In the present study, we demonstrate that like LPS, both TNF and IL-1 decrease hepatic squalene synthase activity and mRNA levels. The effect of IL-1 alone on squalene synthase mRNA levels was more pronounced than that of TNF and was comparable to that of the high dose of LPS. As seen with LPS, the effect of TNF and IL-1 on squalene synthase activity was smaller in magnitude. A combination of both cytokines produced a decrease in squalene synthase activity that was comparable to that produced by high dose of LPS. We have earlier shown that the combination of TNF and IL-1 is usually more effective than either cytokine alone and reproduces many of the effects of LPS on lipid and lipoprotein metabolism (18).

Previous studies have shown that several proteins involved in hepatic cholesterol homeostasis including HMG-CoA synthase, FPP synthase, squalene synthase, and the LDL receptor are usually coordinately regulated with HMG-CoA reductase (24–26). Likewise, the activities of HMG-CoA reductase and squalene synthase change in parallel in response to cholesterol feeding or upon treatment with bile acid binding resins or HMG-CoA reductase inhibitors (16, 20, 27, 28). Our previous studies have shown that LPS produces a dramatic increase in HMG-CoA reductase mRNA levels without significantly altering HMG-CoA synthase, FPP synthase, or LDL receptor mRNA levels (12). The results of the present study indicate that under similar conditions when LPS up-regulates HMG-CoA reductase activity, protein, and mRNA levels, there is, in contrast, a dramatic decrease in squalene synthase activity, protein, and mRNA levels. Additionally, our data demonstrate that LPS markedly decreases squalene synthase mRNA, protein mass, and activity when it has been up-regulated by prior treatment with colestipol, indicating that the regulation of squalene synthase by LPS is independent of dietary status. We have previously also shown that LPS increases HMG-CoA reductase mRNA when it has been up-regulated by treatment with colestipol or down-regulated by cholesterol feeding (12). Taken together, these results confirm the discordant regulation of HMG-CoA reductase and squalene synthase by LPS. Furthermore, these data suggest that LPS-induced regulation of HMG-CoA reductase and squalene synthase is different from sterol regulation and may not be dependent upon hepatic sterol levels.

Our results concerning LPS- and cytokine-induced regulation of squalene synthase (present study) and HMG-CoA reductase (10, 12) point towards a different facet of regulation of isoprenoid metabolism during infections and inflammation. Under normal conditions the rate of hepatic cholesterol synthesis is far greater than the rates of dolichol and ubiquionone synthesis (29, 30). Earlier studies have indicated that the key enzymes of isoprenoid metabolism are coordinately regulated and therefore dietary and pharmacological manipulations that produce large changes in the rates of cholesterol synthesis have negligible effects on the synthesis of non-sterol products (31). It has also been suggested that the enzymes of non-sterol pathways are generally saturated at lower substrate concentrations due to their higher affinities for mevalonate-derived products (23). However, as a number of enzymes involved in different branch point reactions have not been purified, information about their substrate affinities can only be considered approximate. For example, studies in isolated hepatocytes indicate that both the cholesterol and dolichol pathways are saturated at the same substrate concentration (29). Our present results demonstrate that LPS and cytokines down-regulate squalene synthase while up-regulating HMG-CoA reductase (10, 12).

This discordance can explain the modest increase in hepatic cholesterol synthesis despite a much more pronounced increase in HMG-CoA reductase activity, protein and mRNA levels reported earlier (10, 12). Moreover, it is likely to result in an accumulation of intermediates between mevalonate and squalene which may have a substantial effect on the flux of substrate into the non-sterol pathways particularly towards the synthesis of dolichol, ubiquinone and/or isoprenylation of proteins during the host response to infectious and inflammatory stimuli. Dolichol is involved in the glycosylation of proteins (32) and the synthesis of several acute phase proteins that are glycosylated is markedly elevated in liver in response to LPS and cytokines (33, 34). In fact, it has been shown that the synthesis of dolichol phosphate is increased in liver during turpentine-induced inflammation (35). LPS also induces the isoprenylation of several proteins in macrophages (36, 37). Further studies are needed to determine whether LPS and/or cytokines alter the flux of substrate in one or more of these many alternate non-sterol pathways of mevalonate metabolism in liver and to define the significance of these metabolic alterations to the host acute phase response.

In summary, the results of the present study demonstrate that LPS, TNF, and IL-1 produce a marked decrease in squalene synthase activity, protein mass, and mRNA levels. LPS also suppresses squalene synthase when it has been up-regulated by prior treatment with colestipol. The down-regulation of squalene synthase by LPS and cytokines may have substantial effect on the regulation of substrate flux into the non-sterol pathways of mevalonate metabolism.

This work was supported by grants from the Research Service of the Department of Veterans Affairs and the National Institutes of Health (DK 40990, DK49448, and HL 48540).

Manuscript received 14 February 1997 and in revised form 1 May 1997.

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