Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance

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Abstract Hyperlipidemia frequently accompanies infectious diseases and may be due to increases in lipoprotein production or decreases in lipoprotein clearance. The administration of endotoxin (LPS) has been used to mimic infection and prior studies demonstrate that LPS produces hypertriglyceridemia. In the present study in rodents, the dose of LPS necessary to induce hyperlipidemia was orders of magnitude less than that necessary to induce shock and death. As little as 10 ng/100 g body weight induced hypertriglyceridemia and this increase in serum triglyceride levels occurred rapidly (78% increase at 2 h). At high doses of LPS (50 μg/100 g body weight), the clearance of triglyceride-rich lipoproteins was decreased. At low doses of LPS (100 ng/100 g body weight), triglyceride clearance was not altered but the hepatic secretion of triglyceride was increased. Low dose LPS stimulated hepatic de novo fatty acid synthesis and lipolysis, both of which provided a source of fatty acids for the increase in hepatic triglyceride production. High dose LPS did not increase hepatic fatty acid synthesis or peripheral lipolysis, and hepatic triglyceride secretion was not stimulated. Thus, low dose LPS produces hypertriglyceridemia by increasing hepatic lipoprotein production, while high dose LPS produces hypertriglyceridemia by decreasing lipoprotein catabolism. Administration of anti-tumor necrosis factor (TNF) antibodies or interleukin 1 (IL-1) receptor antagonist did not prevent the increase in serum triglyceride levels induced by LPS. However, anti-TNF antibodies and interleukin 1 receptor antagonist (IL-1ra) blocked the increase in serum triglycerides induced by TNF or IL-1, respectively. These data suggest that neither of these cytokines is absolutely required for the increase in serum triglycerides induced by LPS, raising the possibility that other cytokines, small molecular mediators, or LPS itself may play a crucial role.—**Feingold, K. R., I. Staprans, R. A. Memon, A. H. Moser, J. K. Shigenaga, W. Doerrler, C. A. Dinarello, and C. Grunfeld.** Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J. Lipid Res.* 1992. **33:** 1765–1776.

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Hyperlipidemia frequently accompanies infectious and inflammatory diseases (1–5). The hypertriglyceridemia associated with infection has been attributed to both increases in lipoprotein production and decreases in lipoprotein clearance. For example, Kaufman et al. (6) have shown that experimental infections impair the clearance of triglyceride-rich lipoproteins from the circulation. Furthermore, infection has been shown to produce a decrease in the activity of adipose tissue lipoprotein lipase (LPL), a key enzyme in triglyceride catabolism, that could account for this clearance defect (6–11). In contrast, Wolfe and co-workers (12) have demonstrated that sepsis increases hepatic VLDL production, which could contribute to hyperlipidemia. The increase in hepatic VLDL production secondary to infection may be due to an increase in de novo fatty acid synthesis in the liver (11, 13, 14) and/or to an increase in reesterification of plasma fatty acids derived from a stimulation of lipolysis (1, 7, 11, 12, 15).

The administration of endotoxin (LPS) has been used to mimic infections and studies have demonstrated that a single dose of LPS is sufficient to produce hypertriglyceridemia (16–18). Similar to experimental infections, LPS administration has been shown to decrease LPL activity and to delay the clearance of triglyceride-

Abbreviations: LPL, lipoprotein lipase; LPS, endotoxin; PIA, R-2-phenylisopropyl adenosine; TNF, tumor necrosis factor; IL, interleukin; IL-1ra, interleukin 1 receptor antagonist; BW, body weight; VLDL, very low density lipoprotein.

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rich lipoproteins from the circulation (19–23). Additionally, LPS treatment in some (17, 19, 24, 25), but not all studies (18, 20, 23), stimulated lipolysis. However, the effect of LPS administration on hepatic lipid synthesis and VLDL production has not been adequately addressed.

Infections, inflammation, and LPS administration stimulate the production of a wide array of cytokines including TNF and IL-1 (26–28). Many of the lipid changes that accompany infection can be induced by the administration of these cytokines (29, 30). We reported that the administration of TNF or IL-1 increases serum triglyceride levels within 1–2 h (29, 30). Our studies and those of others have demonstrated that the TNF- and IL-1-induced increase in serum triglyceride levels is primarily due to cytokine-induced stimulation of hepatic lipoprotein production (30–33). In the case of TNF, both an increase in de novo hepatic fatty acid synthesis and an increase in lipolysis with accelerated re-esterification contribute to the elevated production of lipoproteins by the liver (34). In contrast, the increase in hepatic lipoprotein production induced by IL-1 is primarily due to increases in de novo fatty acid synthesis in the liver (30). In acute studies (90–120 min after cytokine administration), neither TNF nor IL-1 affects the clearance of triglyceride-rich lipoproteins from the circulation (30, 32, 35).

The previous studies examining the effects of LPS on lipid metabolism were carried out many hours after LPS administration. As described above our recent studies have demonstrated that cytokines are capable of rapidly altering lipid metabolism. Thus, the aims of the present study were threefold. First, to determine whether LPS acutely raises serum triglyceride levels. Second, to define the mechanism(s) by which LPS acutely induces hyperlipidemia. Third, to determine if this hyperlipidemia could be accounted for by an LPS-induced stimulation of TNF or IL-1 secretion.

METHODS

Materials

^3H_2O (5 Ci/g), [26-^{14}C]cholesterol, [^{14}C]oleic acid, and [^{3}H]triolein were purchased from New England Nuclear (Boston, MA); thin-layer chromatography polygram sil-G plates were from Brinkmann Instruments (Westbury, NY); Triton WR-1339 was from Ruger Chemical Company (Irvington, NJ); BetaMax ES scintillation fluid was purchased from ICN Biomedicals Inc. (Irvine, CA); R-2-phenylisopropyl adenosine (PIA) was from Boehringer Mannheim (Mannheim, Germany); rat chow was from Simonsen Laboratories (Gilroy, CA). Murine TNF alpha (2.9×10^7 units/mg) was kindly provided by Genentech, Inc. (South San Francisco, CA). The cDNA for TNF was kindly provided by Dr. Bruce Beutler of the University of Texas, Southwestern Medical Center. Recombinant human IL-beta (112–269) with a specific activity of 5×10^7 units/mg was produced as described previously (36). The cDNA for murine IL-1β was kindly provided by Dr. Arjun Singh of Genentech, Inc. Antibodies were generated against mTNF in rabbits by immunization of New Zealand White Rabbits by standard techniques at Caltag Laboratories (South San Francisco, CA). Serum was treated by ammonium sulfate precipitation using previously described precautions to avoid LPS contamination (37). IL-1ra (37a, 37b) was kindly provided by Dr. Robert C. Thompson of Synergen (Boulder, CO). Endotoxin (*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). WEHI 164 clone 13 cells were kindly provided by Dr. M. Palladino of Genentech, Inc.

Animal procedures

Male Sprague-Dawley rats (approximately 200 g) were purchased from Bantin and Kingman (Fremont, CA). The animals were maintained in a reverse light cycle room (3:00 AM–3:00 PM dark, 3:00 PM–3:00 AM light), and were provided with rat chow and water ad lib. Experiments were initiated at 8:30 AM, with interruption of the light cycle. In previous studies the stimulation of lipid synthesis and the increase in serum triglyceride levels induced by cytokines were seen during both the dark and light phase of the light cycle (38). Anesthesia with isoflurane was induced, then animals were injected via the tail vein with LPS or cytokines at the indicated doses in 0.5 ml 0.9% saline or with saline alone (control). Subsequently food was withdrawn because LPS, TNF, and IL-1 induce anorexia. Where indicated, animals were injected subcutaneously with 0.15 μmol/kg PIA in 0.3 ml saline or an equal volume of saline alone (control) 30 min before LPS administration. Where indicated, animals were injected intraperitoneally with anti-murine TNF antibodies (quantity of antibody sufficient to neutralize 170 μg of mTNF) 17 h prior to LPS administration. Where indicated, animals were injected intraperitoneally with 3 mg/kg IL-1ra just prior to and 1 h after LPS administration. In some experiments animals were fed a fat-free high sucrose diet consisting of 20% vitamin-free casein, 4% salt (Hegstadt ICN), 76% sucrose, and 22 g/kg diet vitamin mixture (ICN) for 3 days prior to study.

Lipogenesis

One hour after LPS administration the animals were injected intraperitoneally with 50 mCi ^3H_2O. After a 1-h period of in vivo labeling, the animals were anesthetized and weighed, and a blood specimen was obtained. The incorporation of ^3H_2O into fatty acids in the liver and the quantity of labeled lipids in the serum were determined as described previously (29, 39, 40). Briefly, samples were

weighed and the lipids were saponified in 45% KOH–water–70% ethyl alcohol 2:1:5 (v/v) which cleaves fatty acids from triglyceride, phospholipid, and cholesteryl esters. After adding an internal standard of [^{14}C]oleic acid, the total nonsaponifiable lipids were extracted with petroleum ether. The saponified material was brought to pH < 2 and the fatty acids were then extracted with petroleum ether and counted by liquid scintillation with correction for recovery of the internal standard, as described previously (29, 39, 40). Values are expressed as ^3H_2O incorporated into fatty acid per hour of in vivo labeling. Because lipid is secreted from liver and cleared from plasma during this time period, these values represent relative rates.

Chylomicron clearance studies

Chylomicron clearance was determined as described previously (32). Briefly, after mesenteric lymph duct cannulation, rats were administered 100 μCi [^{3}H]triolein and [^{14}C]cholesterol in 2 ml corn oil/milk emulsion by gastric lavage. The lymphatic drainage was collected in iced tubes for 18 h. Chylomicrons were isolated by centrifugation at 5×10^6 $g \cdot$ min at 10°C. Labeled chylomicrons (10 mg triglyceride) were injected via the tail vein into rats that had been administered either saline or LPS 90 min prior to study. Blood samples (0.075 ml) were obtained from the tail vein every 2 min for 20 min, extracted with Dole's reagent, added to scintillation fluid, and counted. The $t_{1/2}$ of disappearance from the circulation of chylomicron [^{3}H]triglyceride and [^{14}C]cholesterol was calculated by linear regression analysis of a semi-log clearance curve.

Hepatic triglyceride secretion

Hepatic triglyceride secretion was determined as previously described (31) after administration of Triton WR-1339, a nonionic detergent that traps lipoprotein in the plasma and thereby allows for the determination of the rate of secretion of lipoproteins (41–43). For 3 days prior to study the animals were fed a fat-free diet consisting of 20% vitamin-free casein, 4% salt (Hegstadt ICN), 76% sucrose, and 22 g/kg diet vitamin mixture (ICN) to minimize the small intestine's contribution. Rats were administered LPS and then 120 mg Triton WR-1339 in 0.9 saline intravenously at time zero. One and 2 h later a blood sample was obtained for determination of serum triglyceride levels. The difference between these values is equivalent to the rate of secretion of triglyceride-containing lipoproteins.

Lipoprotein lipase activity

Postheparin lipase activity was determined 15 min after the intravenous administration of 50 units of heparin. Postheparin plasma lipase activity was measured by a minor modification of the method of Krauss et al. (44). Briefly 50 μl of plasma was pre-incubated for 15 min at 27°C in the presence or absence of protamine sulfate (3 mg/ml) to inhibit LPL. In this assay equivalent inhibition is achieved with protamine or 1 M NaCl (not shown). Subsequently, the plasma was incubated for 30 min at 27°C with 0.9 ml of a labeled triglyceride emulsion consisting of 19 μCi [^{3}H]triolein and 75 mg unlabeled triolein that had been sonicated in 9 ml of 0.194 M Trizma–0.15 M NaCl–3.3% BSA–0.05% Triton X-100, pH 8.6, buffer. After the incubation, the reaction was terminated with KOH and free fatty acids were extracted by the procedure of Schotz et al. (45). Total lipase activity is measured in the absence of protamine and hepatic lipase in the presence of protamine. LPL is the difference between total and hepatic lipase. Results are expressed as μmole free fatty acid released/ml plasma per h.

Serum TNF assay

TNF activity in serum was measured using cytotoxicity in the TNF-sensitive cell line, WEHI 164 clone 13. Cytotoxicity was assessed using the MTT tetrazolium method (46). Concentrations were calculated by comparison with a recombinant murine TNF alpha standard. The minimal detectable concentration of TNF in this assay is 10 pg/ml.

TNF and IL-1 mRNA levels

Total RNA was isolated from liver and spleen using the guanidine isothiocyanate phenol–chloroform extraction method (47) and batch-absorbed to oligo (dT)-cellulose; polyadenylated mRNA was then batch-eluted (48). Identical amounts of RNA were fractionated in 1% agarose gels containing 2.2 M formaldehyde, and then were transferred to nylon membranes electrophoretically. Membranes were prehybridized at 65°C for 1 h in 5 × SSC, 2 × Denhardt's, 2% SDS, and 10% dextran sulfate containing 100 μg/ml sheared salmon sperm DNA. cDNA probes were labeled by the random priming technique (48) using the Multiprime DNA labeling system. Hybridization with [^{32}P]cDNA probes was conducted at 65°C overnight in the same buffer. Blots were washed with 0.2 × SSC, 0.1% SDS at room temperature for 30 min, then at 65°C for 1 h. Blots were exposed to Kodak XAR-5 film at −70°C using Cronex intensifying screens. Autoradiograph intensity was quantified by densitometry.

Serum lipid levels

Serum triglyceride levels were measured using Sigma Diagnostic Kit #405 (Sigma Chemical Company, St. Louis, MO) after extraction with Dole's reagent. Serum cholesterol levels were measured using Sigma Diagnostic Kit #351. Serum free fatty acids were measured using the Wako NEFA-c Kit (Dallas, TX). Serum glucose levels were measured using a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH).

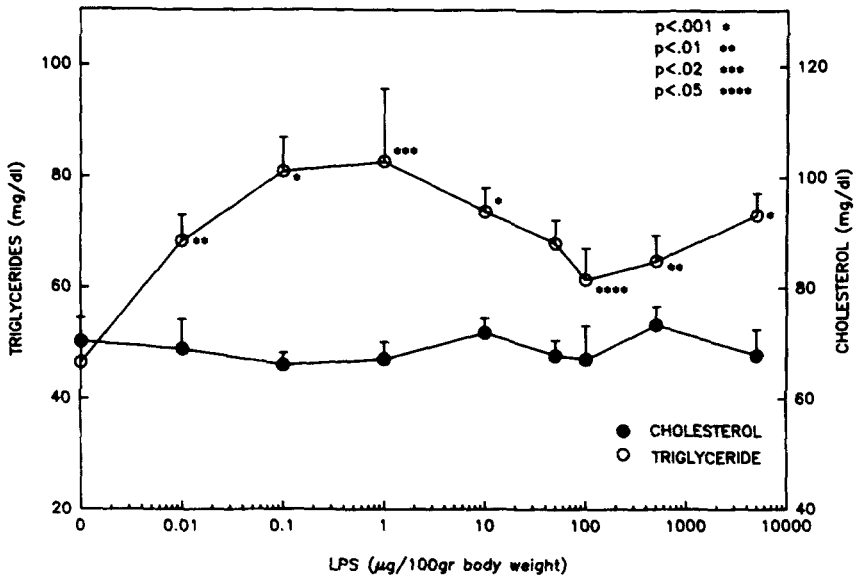

Fig. 1. Animals were injected intravenously with the indicated doses of LPS. Two hours later the animals were killed and serum triglyceride and cholesterol levels were measured; n = 5 for each group.

Statistics

Statistical differences were determined using a two-tailed Student's *t*-test. Because of variations in the rate of lipid synthesis and secretion from month to month, only animals that were studied under identical conditions were compared.

RESULTS

Effect of LPS on serum lipid and TNF levels

The effect of various doses of LPS on serum triglyceride and cholesterol levels is shown in **Fig. 1.** By 2 h after LPS administration, doses as low as 10 ng/100 g body weight (BW) caused a significant increase in serum triglyceride concentrations. The maximal increase in serum triglyceride levels was seen at 0.1 μg/100 g BW (78% increase). A marked increase in the dose of LPS to levels as high as 5 mg/100 g BW, which is approximately the LD50 dose, did not produce a further increase in serum triglyceride levels. At 1 h after LPS administration, an increase in serum triglyceride levels was not observed. No significant effect on serum cholesterol levels was observed in these acute experiments at any LPS dose.

For comparison, the dose response for the LPS-induced increase in serum TNF levels is shown in **Fig. 2.** Serum TNF concentrations were first detectable after 1 μg/100 g BW of LPS and reached a maximum increase at 1 mg/100 g BW. Thus, the maximal increase in serum triglyceride levels is induced by a lower LPS dose than needed for maximal serum TNF levels. However, doses of LPS similar to those that increase serum triglyceride concentrations were able to increase TNF and IL-1 mRNA levels by over 5-fold in the liver (**Fig. 3**). Similarly, low doses of LPS also increased TNF and IL-1 mRNA levels in the spleen (3 ng/100 g: TNF 5.7-fold, IL-1 8.9-fold; 10 ng/100 g: TNF 7.5-fold, IL-1 10.8-fold).

Effect of endotoxin on hepatic lipid synthesis and secretion

To determine the mechanism by which LPS acutely increases serum triglyceride levels, we first measured hepatic lipid secretion using the Triton WR-1339 technique. For optimal quantification of hepatic lipid secretion using this technique, animals must be fed a sucrose diet to eliminate the contribution of intestinal chylomicrons (49). The sucrose diet results in an increase in baseline serum triglyceride levels but nevertheless both low and high dose LPS increases serum triglyceride levels (sucrose diet control 123 ± 12.8 mg/dl, sucrose diet + low dose LPS (100 ng/100 g BW) 186 ± 8.4 mg/dl; sucrose diet + high dose LPS (50 μg/100 g BW) 243 ± 21.2 mg/dl, n = 5 for each group, $P < 0.01$ compared to control for both low and high dose LPS). With the Triton WR-1339 technique (**Fig. 4A**), a low dose of LPS (100 ng/100 g BW) induced a 35% increase in hepatic lipid secretion ($P < 0.05$). In contrast, a higher dose of LPS (50 μg/100 g BW) did not significantly increase hepatic lipid secretion (Fig. 4B). These results suggest that at low doses, LPS increases serum triglyceride levels by increasing hepatic lipid secretion, whereas at higher doses, other mechanisms must be involved.

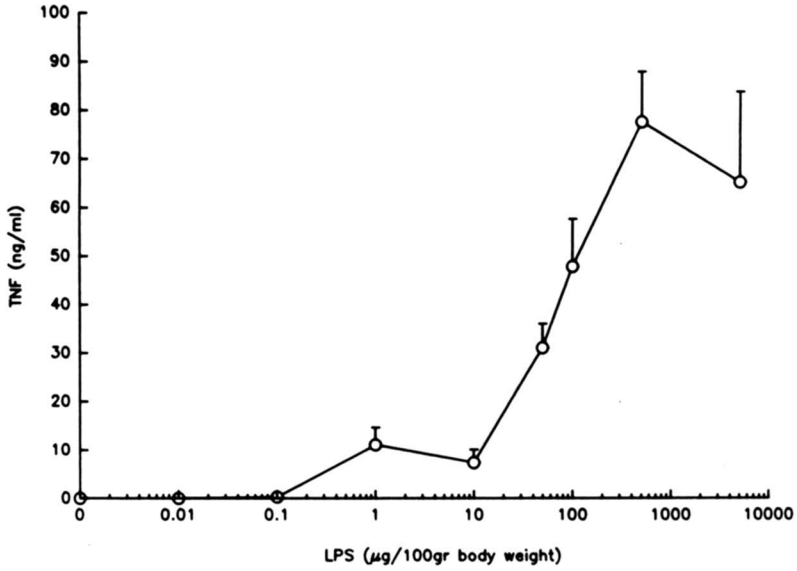

Fig. 2. Animals were injected intravenously with the indicated doses of LPS. Two hours later the animals were killed and serum TNF levels were determined using the cell line, WEHI 164 clone 13; n = 5 for each group.

A low dose (100 ng/100 g BW) of LPS caused a 73% increase in de novo hepatic fatty acid synthesis measured by the in vivo incorporation of ^3H_2O (**Table 1**). However, high doses of LPS (50 μg/100 g BW) did not increase fatty acid synthesis in the liver. Moreover, the quantity of labeled fatty acids that appears in the serum of animals administered a low dose of LPS was increased 2.2-fold, whereas the administration of higher doses had no significant effect on labeled fatty acid concentrations in serum (Table 1). Thus, similar to the effects on hepatic lipid secretion, low doses of LPS stimulate hepatic lipid synthesis while high doses of LPS have no significant effect.

Role of lipolysis in the endotoxin-induced hypertriglyceridemia

The administration of low dose LPS (100 ng/100 g BW) resulted in a 39% increase in serum free fatty levels whereas a higher dose of LPS (50 μg/100 g BW) had no significant effect (**Fig. 5**). To determine the importance of lipolysis and the increased delivery of free fatty acids to the liver in the LPS-induced hyperlipidemia, we used the drug PIA to inhibit LPS-induced adipose tissue lipolysis (34). PIA induced a small (21 μg/dl) decrease in plasma triglycerides in control animals. PIA pretreatment blunted the increase in serum free fatty acids induced by low dose LPS (LPS 444 ± 20 vs. LPS + PIA 283 ± 37 nmol/ml, $P < 0.01$). As shown in **Fig. 6,** pretreatment with PIA markedly inhibited the increase in serum triglyceride levels induced by low doses of LPS. In contrast, PIA pretreatment had no effect on the ability of high dose LPS to increase serum triglycerides (Fig. 6).

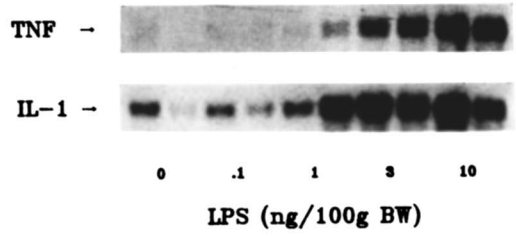

Fig. 3. Effect of LPS on hepatic cytokine mRNA. Rats were injected with normal saline or LPS (ng/100 g BW). Ninety min later the livers were removed, RNA was extracted, and polyadenylated RNA was isolated as described in Methods. mRNA was subjected to gel electrophoresis, electrophoretically transferred, and probed with random-primed [^{32}P] cDNA probes for TNF or IL-1 as indicated.

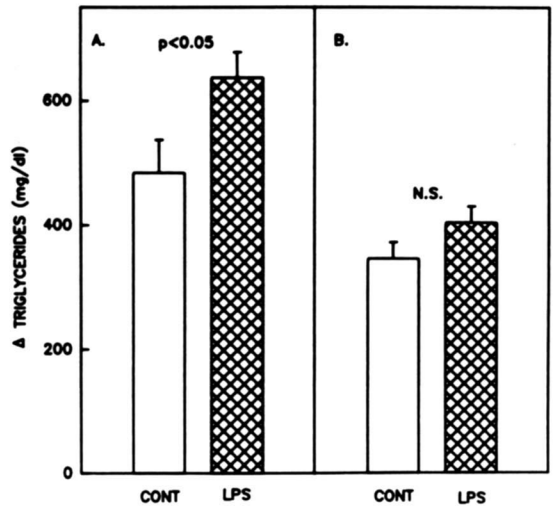

Fig. 4. Animals were administered 120 mg Triton WR-1339 and LPS (A, 100 ng/100 g BW; B, 50 μg/100 g BW) or saline (control) intravenously at time zero. Blood samples were obtained 1 and 2 h later for determination of serum triglyceride levels. The difference in serum triglyceride levels between 1 and 2 h is equivalent to the rate of secretion of triglyceride-containing lipoproteins; n = 10 for each group.

TABLE 1. Effect of endotoxin on serum triglycerides and de novo fatty acid synthesis

Treatment	Serum Triglycerides	Liver Fatty Acid	Serum Fatty Acid
	mg/dl	*μmol ^3H_2O inc/h/organ*	*μmol ^3H_2O inc/h/ml*
Low dose endotoxin			
Control (n = 5)	53 ± 1.8	32.7 ± 2.3	0.258 ± 0.022
100 ng/100 g body wt (n = 5)	81 ± 8.2	56.5 ± 7.33	0.576 ± 0.045
	$P < 0.02$	$P < 0.02$	$P < 0.001$
High dose endotoxin			
Control (n = 5)	67 ± 8.4	53.1 ± 4.6	0.229 ± 0.031
50 μg/100 g body wt (n = 5)	113 ± 11.1	38.5 ± 5.9	0.263 ± 0.020
	$P < 0.02$	NS	NS

Animals were injected intravenously with LPS or saline. One hour later, the animals were injected intraperitoneally with 50 mCi ^3H_2O and after a 1-h in vivo labeling period, the animals were killed. The quantity of labeled lipids in the liver and serum was determined as described in Methods.

These observations suggest that low dose LPS stimulates lipolysis and that the increased free fatty acid delivery to the liver contributes to the hypertriglyceridemia. That serum triglyceride levels are still somewhat increased in the low dose LPS-PIA group compared to time-matched controls suggests that, while lipolysis is a major contributor, it is likely that the LPS-induced increase in hepatic de novo fatty acid synthesis also plays a role in providing fatty acids for triglyceride synthesis and secretion.

Effect of endotoxin on triglyceride-rich lipoprotein clearance

Low dose LPS (100 ng/100 g BW) had no significant effect on the clearance of either chylomicron triglyceride or cholesterol (**Fig. 7**). In contrast, higher doses of LPS (50 μg/100 g BW) significantly slowed the clearance of labeled chylomicrons from the circulation ($T_{1/2}$ for triglyceride increased 2.3-fold; $T_{1/2}$ for cholesterol increased 1.85-fold). These results suggest that the increase in serum triglyceride levels induced by high doses of LPS is due to a delay in the clearance of triglyceride-rich lipoproteins from the circulation.

The effect of high doses of LPS on hepatic lipase and LPL activity in postheparin plasma is shown in **Table 2.** Postheparin plasma LPL activity decreased by 41% after high dose LPS. In contrast, hepatic lipase activity was slightly (24%) increased in the high dose LPS group compared to controls.

Role of TNF and IL-1 in endotoxin-induced hyperlipidemia

The next series of experiments were designed to determine whether the LPS-induced increase in serum triglyceride levels could be inhibited by blockade of TNF

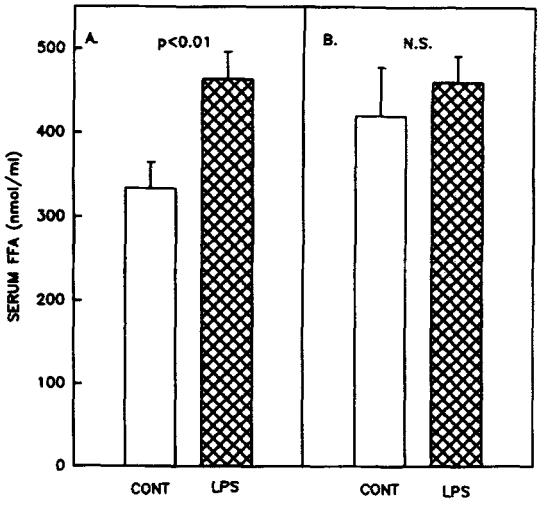

Fig. 5. Animals were administered LPS (A, 100 ng/100 g BW; B, 50 μg/100 g BW) or saline (control) intravenously. Two hours later the animals were killed and serum free fatty acid levels were measured; n = 10 for each group.

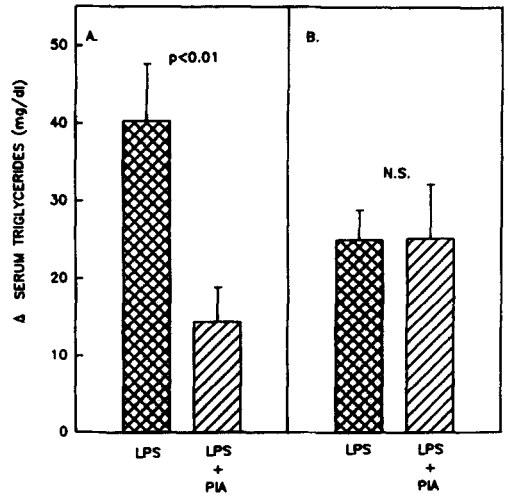

Fig. 6. Animals were injected subcutaneously with 0.15 μmol/kg PIA or saline 30 min before LPS administration (A, 100 ng LPS/100 g BW; B, 50 μg LPS/100 g BW, intravenously). Two hours after LPS administration, animals were killed and serum triglyceride levels were measured. Data are expressed as the increase in triglycerides induced by LPS over control animals treated with or without PIA, respectively; n = 10 for each group.

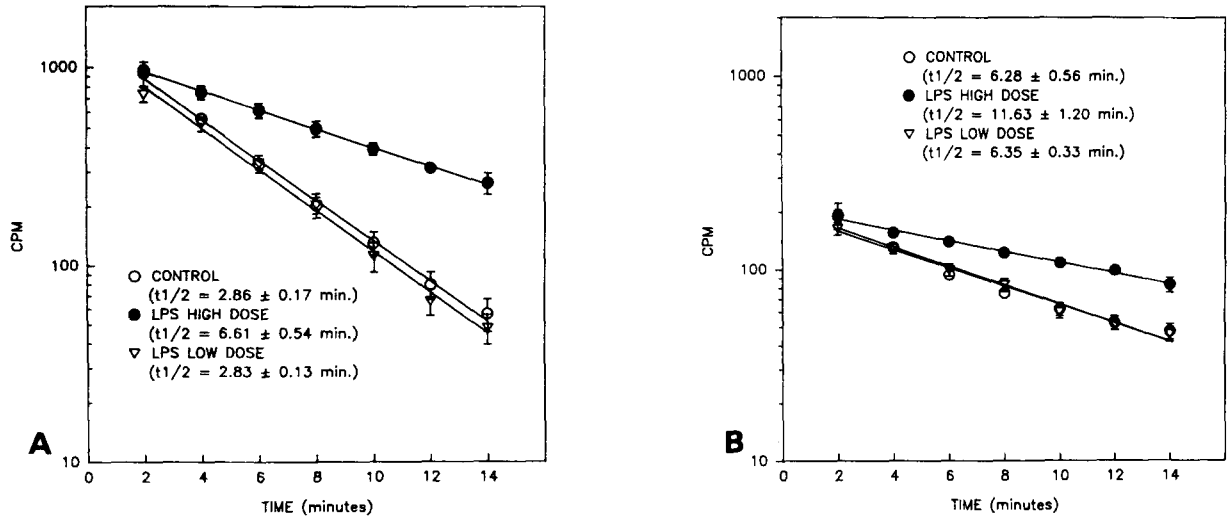

Fig. 7. Animals were injected intravenously with saline (control) or LPS (low dose, 100 ng/100 g BW; high dose, 50 μg/100 g BW). Ninety min later the animals were injected with [^{3}H]triglyceride- and [^{14}C]cholesterol-labeled chylomicrons and the clearance was determined. A: triglyceride clearance; B:: cholesterol clearance; n = 5 for each group. $P < 0.001$, triglyceride $T_{1/2}$ high dose LPS versus control or low dose LPS. $P < 0.01$, cholesterol $T_{1/2}$ high dose LPS versus control or low dose LPS.

and IL-1. As shown in Fig. 2, and by the studies of other investigators (50), LPS administration stimulates TNF secretion. Antibodies that neutralize TNF activity have been shown to prevent the toxicity and death caused by LPS (51). Similarly, an antibody developed in our laboratory that neutralizes TNF cytotoxicity in vitro in WEHI cells also prevented LPS-induced death (data not shown). Furthermore, pretreatment with this antibody can prevent the increase in serum triglyceride levels that is characteristically observed after TNF administration (**Table 3**). Additionally, pre-treatment with this antibody can neutralize the increase in serum TNF activity after LPS administration by greater than 90% (serum TNF levels – low dose LPS 8.57 ± 1.25 ng/ml vs. LPS + TNF antibody 0.84 ± 0.57, $P < 0.001$; high dose LPS 226 ± 34.9 ng/ml vs. LPS + TNF antibody 13.5 ± 11.5, $P < 0.001$). However, pretreatment with this TNF-neutralizing antibody did not prevent the increase in serum triglyceride levels induced by either low or high doses of LPS (Table 3). These results suggest that TNF alone is not the crucial mediator of either low or high dose LPS-induced hypertriglyceridemia.

TABLE 2. Effect of endotoxin on postheparin plasma lipase activity

	Control	Endotoxin	*P*
	μmol free fatty acid/ml/h		
Postheparin lipoprotein lipase	40.6 ± 1.39	24.1 ± 0.09	<0.001
Postheparin hepatic lipase	26.0 ± 1.97	32.3 ± 1.59	<0.05

Animals were injected intravenously with LPS (50 μg/g BW) or saline. Ninety min later the animals were injected intravenously with 50 U heparin, and postheparin plasma lipase activity was determined as described in Methods; n = 5 for controls and for LPS groups.

IL-1ra is an antagonist to the IL-1 receptor and recent studies have shown that this protein can block many of the effects of IL-1 (28, 52). Furthermore, pretreatment of animals with IL-1ra can prevent the septic shock and death that occurs with LPS administration (52). Treatment of animals with IL-1ra can prevent the characteristic increase in serum triglyceride levels that is induced by IL-1 (**Table 4**). However, IL-1ra treatment with doses

TABLE 3. Effect of TNF neutralizing antibodies on serum triglyceride levels

Experiment	Triglycerides
	mg/dl
Exp. 1	
Control	61 ± 4.0
TNF (5 μg)	108 ± 12.1[a]
Antibody	69 ± 8.9
Antibody + TNF	72 ± 12.1
Exp. 2	
Control	51 ± 7.9
Low dose endotoxin (100 ng/100 g BW)	84 ± 5.7[a]
Antibody + low dose endotoxin	92 ± 16.2[b]
Exp. 3	
Control	55 ± 5.9
High dose endotoxin (50 μg/100 g BW)	114 ± 9.4[a]
Antibody + endotoxin	104 ± 5.8[a]

Animals were injected intraperitoneally with antibodies against mTNF (quantity of antibody sufficient to neutralize 170 μg of mTNF) 17 h prior to the study. On the morning of the study, the animals were injected intravenously with 5 μg TNF (Exp. 1), 100 ng/100 g BW or 50 μg/100 g BW LPS (Exp. 2 and Exp. 3, respectively), or saline (control). Two hours later the animals were killed and serum triglyceride levels were measured; n = 5–10 for each group.

[a] $P < 0.01$ versus control.

[b] $P < 0.1$ versus control.

TABLE 4. Effect of IL-1ra on serum triglyceride levels

Experiment	Serum Triglycerides
	mg/dl
Exp. 1	
Control	42 ± 5.3
IL-1 (1 μg)	74 ± 10.5[a]
IL-1 + IL-1ra	36 ± 2.2
Exp. 2	
Control	27 ± 4.6
Low dose endotoxin (100 ng/100 g BW)	82 ± 7.5[b]
Endotoxin + IL-1ra	82 ± 11.5[b]
Exp. 3	
Control	34 ± 3.5
High dose endotoxin (50 μg/100 g BW)	71 ± 8.0[b]
Endotoxin + IL-1ra	83 ± 3.7[b]

Animals were injected intraperitoneally with 3 mg/kg IL-1ra just prior to and 1 h after LPS or IL-1 administration. The animals were injected intravenously with 1 μg IL-1 (Exp. 1), 100 ng/100 g BW or 50 μg/100 g BW LPS (Exp. 2 and Exp. 3, respectively), or saline (control). Two hours after IL-1 or endotoxin administration, the animals were killed and serum triglyceride levels were measured; n = 5 for each group.

[a] $P < 0.05$ versus control.

[b] $P < 0.01$ versus control.

similar to those that prevent shock (52) did not prevent the increase in serum triglyceride levels caused by either low or high doses of LPS (Table 4).

Because these two cytokines frequently have overlapping and synergistic effects, we next determined the effect of simultaneously treating animals with both TNF-neutralizing antibody and IL-1ra. The combination of TNF-neutralizing antibodies and IL-1ra did not prevent the increase in serum triglyceride levels induced by either low or high doses of LPS (**Table 5**). These results suggest that the increase in serum triglyceride levels that occurs after LPS is not mediated solely by increases in TNF and IL-1.

A high dose of LPS (50 μg/100 g BW) produced a decrease in serum glucose levels 120 min later. Animals in our experiments were allowed to eat ad lib up to the time of LPS treatment; it is likely that decreases in serum glucose levels may have been due to decreased absorption secondary to decreased intestinal motility (38). Hourly treatment with IL-1ra (3 mg/kg) inhibited the decrease in serum glucose levels induced by LPS (control, 177 ± 6.6 mg/dl, LPS, 50 μg/100 g BW 141 ± 3.3 mg/dl, $P < 0.01$; LPS + IL-1ra, 175 ± 8.3 mg/dl, NS vs. control, $P < 0.01$ vs. LPS alone). In contrast, administration of TNF-neutralizing antibodies did not alter the glucose-lowering effect of LPS (control, 176 ± 9 md/dl, LPS, 50 μg/100 g BW 140 ± 4.4 mg/dl, $P < 0.01$; LPS + TNF antibody, 152 ± 4.9 mg/dl, $P < 0.05$ vs. control; NS vs. TNF alone). Thus, while inhibition of TNF or IL-1 activity does not affect the LPS-induced changes in lipid metabolism, blocking IL-1 activity ameliorates the decrease in serum glucose levels produced by LPS.

DISCUSSION

LPS administration leads to hypertriglyceridemia (16–18) which has been attributed to a delay in the catabolism of circulating lipids secondary to decreases in the activity of LPL (19–23). LPL activity and triglyceride clearance are also decreased during infections (6–11). However, the overproduction of lipid by the liver may also play a role in the hypertriglyceridemia associated with infection (11–14). Studies have demonstrated that during infection hepatic lipoprotein secretion is increased (12), that the de novo synthesis of lipid in the liver is enhanced (11, 13, 14), and that an increased delivery of free fatty acids to the liver could lead to increased triglyceride formation (1, 7, 11, 12, 15). In some studies LPS stimulates lipolysis (17, 19, 24, 25) but LPS has not been previously shown to stimulate de novo hepatic lipid synthesis or hepatic lipoprotein production in intact animals. In fact, at high LPS doses in fasted animals a decrease in triglyceride production has been noted (23). Thus, sepsis or LPS administration is capable of inducing an array of alterations in lipid metabolism that can lead to hypertriglyceridemia. Which of these alterations is dominant may depend on a variety of factors including the animal species studied, the nutritional status of the animal, and the dose and type of LPS administered. Lipoprotein metabolism differs markedly in humans compared to rodents and whether the changes observed in lipid metabolism after infections or LPS in experimental animals also occur in humans is unknown. In addition, the species of microorganism causing the infection and the time after LPS or sepsis in which experiments are carried out may play a role.

In the present study we have made a number of new observations relating to the effect of LPS on lipid metabolism. First, the dose of LPS necessary to induce

TABLE 5. Effect of TNF neutralizing antibodies and IL-1ra on serum triglyceride levels

Experiment	Serum Triglycerides
	mg/dl
Exp. 1	
Control	48 ± 3.6
Low dose endotoxin (100 ng/100 g BW)	84 ± 15.2[a]
Endotoxin + IL-1ra + TNF antibody	75 ± 6.0[b]
Exp. 2	
Control	47 ± 2.0
High dose endotoxin (50 μg/100 g BW)	81 ± 5.4[b]
Endotoxin + IL-1ra + TNF antibody	78 ± 8.1[b]

Animals were treated with antibodies against TNF and IL-1ra as described in Tables 3 and 4. Saline (control) or 100 ng/100 g BW (Exp. 1) or 50 μg/100 g BW (Exp. 2) of LPS was injected intravenously. Two hours later the animals were killed and serum triglyceride levels were measured; n = 5 for each group.

[a] $P < 0.05$ versus control.

[b] $P < 0.01$ versus control.

hypertriglyceridemia is surprisingly very small. Previous studies have used much higher doses of LPS. In contrast, we administered very low doses of LPS and showed that as little as 10 ng/100 g BW produces a significant increase in serum triglyceride levels and that 100 ng/100 g BW produces close to a maximal increase. These doses of LPS are far below the doses required to cause death in our laboratory (LD_{50} approximately 5 mg/100 g BW) indicating that hypertriglyceridemia is among the most sensitive host responses to LPS occurring at 1/500,000th of the LD_{50}.

Second, LPS administration rapidly increases serum triglyceride levels. In previous studies, the effect of LPS on lipid metabolism was usually examined many hours after treatment. In contrast, we have demonstrated that lipid metabolism is altered and serum triglyceride levels are increased as early as 2 h after LPS. This observation is similar to our previous studies where TNF and IL-1 produce rapid changes in lipid metabolism, increasing serum triglyceride levels within 45–90 min (29, 30).

The third observation is that the pathophysiology of the hypertriglyceridemia induced by LPS is dependent on the dose of LPS studied. At high doses of LPS, the increase in serum triglycerides is due to the decreased clearance of triglyceride-rich lipoproteins from the circulation (Fig. 7), with no effect seen on hepatic lipoprotein production. Other investigators, at later time periods after high dose LPS administration, have also demonstrated a delay in triglyceride-rich lipoprotein clearance from the circulation (19–23) with no increase seen in VLDL production (23); this decrease in clearance is associated with a marked decrease in LPL activity (19–23). In our studies, at an earlier time point, high dose LPS caused a 41% reduction in postheparin plasma LPL activity. It is possible that this decrease in postheparin plasma LPL activity reflects an even more marked decrease in LPL activity on the endothelial wall, the key site of enzyme activity involved in lipoprotein catabolism.

In contrast, low doses of LPS (100 ng/100 g BW) did not alter triglyceride-rich lipoprotein clearance (Fig. 7), but increased the hepatic secretion of triglyceride due to increases in both hepatic de novo fatty acid synthesis (Table 1) and peripheral lipolysis (Fig. 5), both of which could provide a source of fatty acids for the increase in hepatic triglyceride production. In the studies using PIA, a drug that inhibits lipolysis (34), we have shown that both de novo synthesis and lipolysis contribute to the hypertriglyceridemia (Fig. 6). These results demonstrate that low dose LPS can induce changes in lipid metabolism in adipose and hepatic tissue that lead to the overproduction and secretion of lipid by the liver resulting in hypertriglyceridemia, observations similar to those reported to occur in experimental infections (1, 11–15) and after cytokine administration (29–35).

It is not clear why high doses of LPS do not cause alterations in hepatic lipid secretion. High doses of LPS might decrease blood flow to the liver or induce other toxic effects that override the stimulation of hepatic lipid secretion. It is also possible that high doses of LPS induce the secretion of different cytokines or other compounds than low dose LPS, and that these compounds inhibit hepatic lipid secretion. Recent studies by our laboratory have demonstrated that at least one cytokine, IL-4, can prevent the stimulation of hepatic lipogenesis that is induced by TNF, IL-1, and IL-6 (53). Lastly, in primary cultures of rabbit hepatocytes, low concentrations of LPS increased hepatic lipid synthesis and the secretion of lipid into the media, whereas high doses of LPS did not affect lipid homeostasis (54). Thus it is possible that LPS has a biphasic effect on the liver that could account for our findings.

The last major observation is that neither TNF nor IL-1 alone appears to mediate the acute changes in lipid metabolism induced by LPS. High dose LPS produces hypertriglyceridemia by delaying the clearance of triglyceride-rich lipoproteins, whereas previous studies have shown that both TNF and IL-1 do not affect triglyceride clearance (30, 32, 35). Additionally, inhibition of the action of TNF and/or IL-1 with TNF-neutralizing antibodies and/or IL-1ra does not prevent the increase in serum triglyceride levels induced by either low or high doses of LPS. Our TNF antibody was used at doses that can prevent LPS-induced death and which neutralize over 90% of the serum TNF activity induced by LPS administration. IL-1ra was used at doses that prevent shock (52). It is of course possible that the systemic administration of TNF antibodies or IL-1ra is not capable of blocking the local (paracrine) effects of cytokines on lipid metabolism. The ability of low doses of LPS that increase serum triglyceride levels to increase hepatic mRNA levels of TNF and IL-1 suggests that these cytokines are produced in the liver under conditions where LPS affects hepatic lipid metabolism. However, in contrast to the lack of an effect of inhibiting TNF or IL-1 activity on LPS-induced changes in lipid metabolism, blocking IL-1 activity with IL-1ra ameliorated the decrease in serum glucose levels produced by LPS. Administration of TNF-neutralizing antibodies did not alter the effect of LPS on glucose metabolism. These effects on glucose metabolism confirm recent reports by Vogel et al. (55, 56) demonstrating that IL-1ra reverses LPS-induced hypoglycemia while TNF antibodies do not. These observations suggest that separate metabolic effects induced by LPS may be mediated by different cytokine pathways.

Recent studies by our laboratory have shown that the ability of LPS to stimulate hepatic fatty acid synthesis is markedly reduced in LPS-resistant mice (C3H/HeJ), whose macrophages do not produce cytokines, such as TNF or IL-1, in response to LPS (57). Thus, LPS activation of macrophages is important in stimulating hepatic

lipogenesis in mice. However, cytokines other than TNF or IL-1 that are induced by LPS or infection, including lymphotoxin, mCSF, IL-6 and the interferons, can affect various aspects of lipid metabolism in a manner that could contribute to hypertriglyceridemia (58–60). Moreover, other compounds induced by LPS and sepsis, such as platelet-activating factor, have also been reported to affect lipid metabolism (61). Lastly, it is possible that LPS has direct effects on lipid metabolism that elevate serum triglyceride levels. In vitro studies have demonstrated that LPS can increase lipolysis in primate adipocytes (25) and increase lipid synthesis in rabbit hepatocytes (54). Thus, there are multiple potential agents, including LPS itself, that could produce changes in lipid metabolism that lead to hyperlipidemia.

Given the very low amounts of LPS that induce hypertriglyceridemia, one can speculate that the increase in serum triglycerides could be a protective response by the host. Studies have demonstrated that lipoprotein particles, including triglyceride-rich lipoproteins, bind LPS and protect animals from the toxic effects of LPS (37, 62–66). Additionally, viruses have been shown to complex with lipoproteins, thereby resulting in a decrease in the pathogenicity of these viruses (67–69). It is possible that the increase in serum triglyceride levels induced by LPS and cytokines represents a beneficial host response to infection.

In summary, both low and high doses of LPS induce hypertriglyceridemia but the pathophysiological mechanisms for these increases differ. Low doses of LPS increase circulating triglyceride levels by stimulating hepatic lipid secretion while high doses of LPS decrease the clearance of triglyceride-rich lipoproteins. Additionally, our data raise the possibility that these effects may not be due to TNF or IL-1 secretion but rather may be secondary to other as yet uncharacterized cytokines or small molecule mediators or to direct effects of LPS.

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