

# Lipoteichoic acid stimulates lipolysis and hepatic triglyceride secretion in rats in vivo

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**Abstract** The host response to infection is frequently accompanied by changes in lipid metabolism. Previous studies have shown that endotoxin (LPS), a component of the cell wall of gram-negative bacteria, increases serum lipid levels. In this study, we demonstrate that lipoteichoic acid (LTA), a component of the cell membrane of gram-positive bacteria, also increases serum lipid levels in rats in a dose-dependent manner (0.1–300  $\mu\text{g}/200$  g body weight). Serum triglyceride levels increased within 2 h after LTA administration with peak values at 4 h (2-fold increase). Serum cholesterol levels also increased but the effect was delayed occurring at 16 h and was relatively small (1.2-fold increase). LTA (10  $\mu\text{g}/200$  g BW) did not decrease adipose tissue lipoprotein lipase activity or the clearance of triglyceride-rich lipoproteins. Rather, the LTA-induced hypertriglyceridemia is due to an increase in hepatic triglyceride secretion. LTA stimulates both hepatic de novo fatty acid synthesis and lipolysis. The increased delivery of free fatty acids to the liver plays a major role in the LTA-induced hypertriglyceridemia. Pretreatment with phentolamine, an  $\alpha$ -adrenergic receptor antagonist, and alprenolol, a  $\beta$ -adrenergic receptor antagonist, or phentolamine alone significantly suppressed the hypertriglyceridemia induced by LTA. These adrenergic inhibitors had no significant effect on the increase in lipolysis. ■ These results indicate that catecholamines are involved in mediating the LTA-induced increase in hepatic triglyceride secretion via  $\alpha$ -adrenergic receptors. These changes in lipid metabolism may play an important role in the organism's response to gram-positive infection.—Nonogaki, K., A. H. Moser, X.-M. Pan, I. Staprans, C. Grunfeld, and K. R. Feingold. Lipoteichoic acid stimulates lipolysis and hepatic triglyceride secretion in rats in vivo. *J. Lipid Res.* 1995. **36**: 1987–1995.

**Supplementary key words** lipoteichoic acid • gram positive • triglyceride • cholesterol • lipolysis • catecholamines

Gram-negative bacterial infection is often accompanied by profound disturbances in intermediary metabolism (1). A frequent occurrence is hypertriglyceridemia which has been attributed to both increases in lipoprotein production and decreases in lipoprotein clearance (for review see reference 1). The administration of

endotoxin (LPS), a major component of the cell wall of gram-negative bacteria, has been used to mimic gram-negative infections and studies have demonstrated that a single dose of LPS is sufficient to produce hypertriglyceridemia (1). We recently demonstrated that LPS is capable of rapidly altering lipid metabolism (90–120 min after LPS administration), and that the mechanism of the hypertriglyceridemia is dependent on the dose of LPS (2). Low dose LPS (100 ng/100 g body weight) stimulated hepatic de novo fatty acid synthesis and lipolysis, both of which provided a source of fatty acids for an increase in hepatic triglyceride production. In contrast, high dose LPS (50  $\mu\text{g}/100$  g body weight) did not increase hepatic triglyceride secretion or lipolysis, but rather produced hypertriglyceridemia by decreasing post-heparin lipoprotein lipase activity and clearance of triglyceride-rich lipoproteins. Further studies have shown that LPS-induced hypertriglyceridemia is mediated by endogenous catecholamines via  $\alpha$ -adrenergic receptors in rats (3).

In contrast to gram-negative bacteria infections, the effect of gram-positive bacteria on lipid metabolism has been less extensively investigated. Gallin, Kaye, and O'Leary (4) and Alvarez and Ramos (5) have reported that patients with severe infection caused by gram-positive cocci have increased serum triglyceride levels. Moreover, several investigators have shown that serum triglyceride levels are also increased in experimental gram-positive infections in monkeys (6, 7), rabbits (8), and rats (9). In rats, the administration of live *Staphylococcus aureus* (*S. aureus*) results in a decrease in lipopro-

Abbreviations: LTA, lipoteichoic acid; LPS, endotoxin; LPL, lipoprotein lipase; PIA, R-2-phenylisopropyl adenosine; TNF, tumor necrosis factor; IL, interleukin; FFA, free fatty acid; HDL, high density lipoprotein; TNS, total nonsaponifiable lipids.

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tein lipase (LPL) activity in diaphragm, heart, and adipose tissue (9).

Lipoteichoic acid (LTA) is a component of the cell membrane of gram-positive bacteria that is thought to be analogous to LPS, and can mediate many of the toxic and metabolic effects of gram-positive infections (10). Recently, several studies have demonstrated the ability of LTA to activate phagocytes, including release of cytokines and oxygen radicals (11–18) as well as induction of tumoricidal activity (17). However, the effect of LTA on lipid metabolism has not yet been evaluated. The purpose of the present study was to characterize the effect of LTA administration on serum lipid levels and determine the mechanisms by which LTA induces hypertriglyceridemia in rats.

## MATERIALS AND METHODS

### Materials

*S. aureus*-derived LTA, (R)-N<sup>6</sup>-2-phenylisopropyl adenosine (PIA), phentolamine, alprenolol, triolein, lecithin, and fatty acid-free bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO). Triton WR-1339 was purchased from Ruger Chemical Co. (Irvington, NJ). [<sup>3</sup>H]triolein, 1-[<sup>14</sup>C]sodium acetate (56.2 mCi/mmol), 1,2-[<sup>3</sup>H]cholesterol (31 Ci/mmol), and [<sup>3</sup>H]oleic acid (2–10 Ci/mmol) were purchased from New England Nuclear, Boston, MA. Ready Safe scintillation fluid was purchased from Beckman (Fullerton, CA).

The LTA contained 580 pg endotoxin/μg of LTA measured with a Limulus assay (obtained from Sigma Chemical Co. and Chromogenix). However, studies by other investigators have shown that LTA alone has activity in the Limulus assay (19) and therefore the positive Limulus assay does not necessarily indicate LPS contamination. Moreover, the administration of 5–6 ng/200 g BW LPS to rats (the quantity of LPS in 10 μg/200 g BW of LTA) does not increase serum triglyceride levels, indicating that LPS contamination could not account for the LTA-induced alterations in lipid metabolism.

### Animal procedures

Male Sprague-Dawley rats (approximately 200 g) were purchased from Simonsen Animal Vendors. Animals were maintained on a reverse 12-h light cycle (0300–1500 h dark, 1500–0300 h light) and fed Simonsen rat chow (Simonsen, Gilroy, CA) and water ad libitum unless otherwise indicated. Simonsen rat chow contains 24% protein, 6% fat, 6% ash, 3.5% fiber, and 60% complex carbohydrate, all derived from ground wheat, soybean meal, or rice bran.

The animals were injected via the tail vein with LTA in 0.5 ml 0.9% saline or saline alone under halothane anesthesia. At 1, 2, 4, 8, and 16 h, blood samples were taken to measure serum triglyceride and cholesterol levels. Where indicated, animals were injected subcutaneously (s.c.) with 0.15 μmol/kg phenylisopropyl adenosine (PIA) in 0.3 ml saline or an equal volume of saline alone (control) 30 min before LTA administration. Where indicated, animals were injected intraperitoneally (i.p.) with the α-adrenergic receptor antagonist, phentolamine (5 mg/kg), the β-adrenergic receptor antagonist, alprenolol (6 mg/kg), or saline alone. Phentolamine was dissolved in 0.5 ml pyrogen-free water. Alprenolol was dissolved in 0.5 ml 0.9% saline. After the injection of LTA or saline, food was removed to control for the effect of anorexia that can be induced by LTA.

### Chylomicron clearance studies

Chylomicron clearance was determined as described previously (20). The major mesenteric lymph duct was cannulated in rats. After establishment of lymph flow, the animals were administered 100 μCi of [<sup>3</sup>H]triolein in 2 ml of a corn oil–milk emulsion intragastrically. The lymphatic drainage was collected in iced tubes for 18 h. Chylomicrons were isolated by layering 5 ml of lymph under 0.15 mol NaCl and centrifuging at  $5 \times 10^6 g \cdot \text{min}$  at 10°C. Labeled chylomicrons (10 mg of triglyceride) were injected via the tail vein into rats to whom either 10 μg LTA or saline had been administered intravenously 2 h before study. Blood samples (0.075 ml) from the tail vein were obtained at 1.5, 3, 4.5, 6, 7.5, and 9 min after chylomicron administration. The blood samples were extracted with Dole's reagent. The lipid phase was counted by liquid scintillation counting. The  $t_{1/2}$  of disappearance from the circulation of the [<sup>3</sup>H]triglyceride associated with the chylomicrons was calculated by linear regression analysis of a semi-log clearance curve.

### Adipose tissue lipoprotein lipase analysis

Two hours after LTA administration, animals were killed and the epididymal fat pads were removed and frozen in liquid nitrogen. The frozen tissue was weighed, chopped into fine pieces, and transferred to a 15-ml centrifuge tube. LPL was extracted with a phosphate buffer (pH 7.5) containing 0.118 M NaCl, 0.005 M KCl, 0.0012 M KH<sub>2</sub>PO<sub>4</sub>, 0.0012 M MgSO<sub>4</sub>, 0.55 M CaCl<sub>2</sub>, and heparin (4 U/ml) for 60 min at 37°C. Lipolytic activity was determined as described previously (21). Briefly, the substrate, unlabeled triolein (75 mg), 18.75 μCi of [<sup>3</sup>H]triolein, and 3.0 mg of lecithin, was homogenized with 1.2 ml of 20% fatty acid-free bovine albumin, 0.5 ml of normal human plasma (LPL cofactor), 0.5 ml 1% Triton X-100, 15 U heparin, and 6.8 ml of 1.0 M tris

(hydroxymethyl) aminomethane buffer, pH 8.6. An aliquot of the resultant emulsion (0.1 ml) and 0.4 ml of extracted medium of epididymal adipose tissue were incubated in a metabolic shaker at 37°C for 60 min. The reaction was stopped by addition of 4 ml of isopropanol-sulfuric acid reagent (10 ml 3 N sulfuric acid, 400 ml isopropanol). Subsequently, the released [<sup>3</sup>H]oleic acid was separated from triolein by sequential hexane extraction and alkalization; an aliquot of the alkaline medium was counted.

### Hepatic triglyceride secretion

Hepatic triglyceride secretion was determined using the Triton WR-1339 technique. Triton WR-1339 is a nonionic detergent that traps lipoproteins in the plasma (22, 23) and thereby allows determination of the rate of secretion of hepatic triglyceride lipoproteins (24). Rats were administered LTA or saline i.v., followed 30 min later by the i.v. administration of 120 mg Triton WR-1339 in 0.9% saline. One and 2 h later, blood was obtained for the determination of serum triglyceride levels. The increase in serum triglyceride levels from 1 to 2 h is equivalent to the rate of secretion of triglyceride-containing lipoproteins.

### Lipogenesis in vitro

Two hours after LTA or saline administration, the animals were killed and the liver was rapidly removed. Slices (0.5-mm thick) were prepared with a McIlwain tissue slicer and 200 mg of tissue was placed in the outer well of a 25-ml center-well flask containing 2 ml of Krebs-Ringer phosphate buffer and 1 [<sup>14</sup>C]acetate (1 μCi/1019 nmol/100 μl). The flasks were incubated for 2 h at 37°C in a Dubnoff metabolic shaker at 100 oscillations/min. The tissues were then saponified by refluxing overnight in a solution of 45% KOH, water, and 70% ethyl alcohol (2:1:5) and then analyzed for <sup>14</sup>C-labeled lipids as described previously (25). In these experiments <sup>3</sup>H internal standards were employed.

### Serum chemistries

Serum triglyceride levels were measured using Sigma Diagnostic Kit 405 (Sigma Chemical Co., St. Louis, MO). Serum cholesterol levels and serum high density lipoprotein (HDL) cholesterol levels after the separation of the HDL fraction using HDL cholesterol reagent (PTA/MgCl<sub>2</sub>) (Sigma Chemical Co.) were measured using Diagnostic kit No. 351 (Sigma Chemical Co.). Serum FFA levels were measured using the Wako NEFA-c Kit (Dallas, TX).

### Statistics

Data are presented as mean ± SEM. Statistical significance between two groups was determined using Stu-

dent's *t* test. Comparisons among more than two groups were done by analysis of variance using Duncan's test.

## RESULTS

### Effect of LTA on serum lipid levels

Figure 1 shows the effect of LTA on serum triglyceride (Fig. 1A) and FFA (Fig. 1B) levels 2 h after administration. LTA resulted in an increase in serum triglyceride levels in a dose-dependent manner with 0.1, 1, 10, 100, and 300 μg causing a 69%, 81%, 100%, 110%, and 108% increase, respectively (Fig. 1A). The administration of LTA (10, 100, 300 μg) caused serum FFA levels to increase 42%, 37%, and 70%, respectively (Fig.

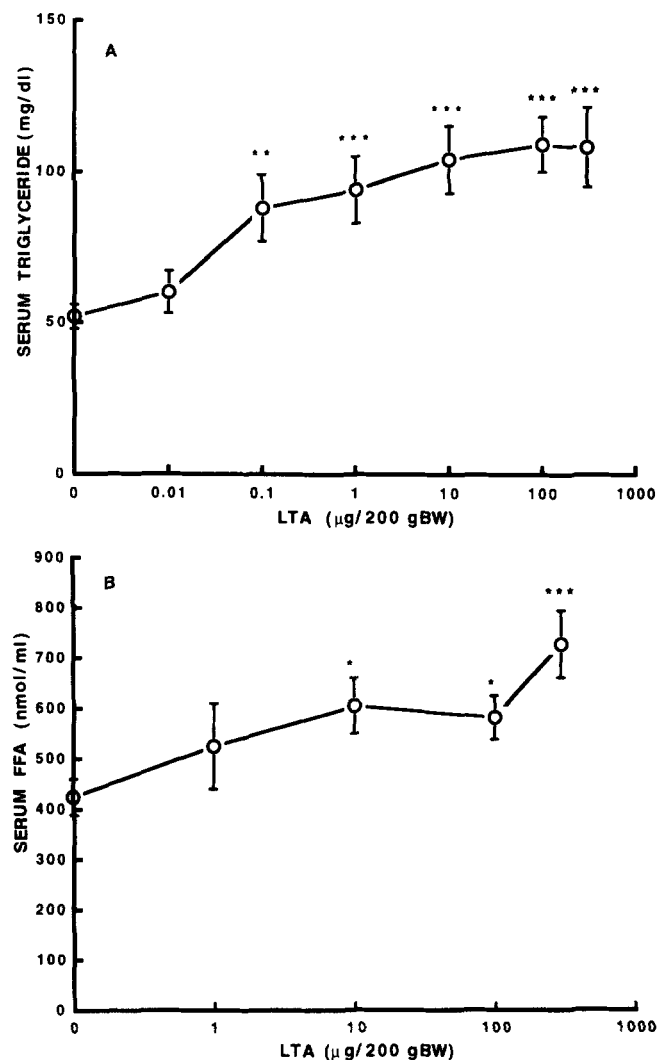
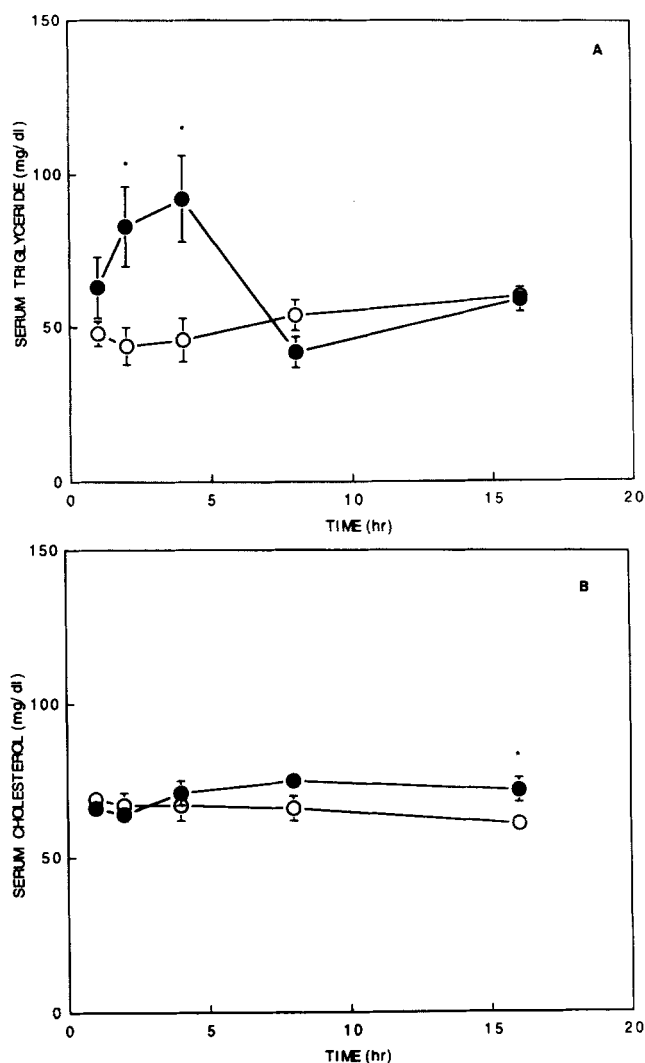


Fig. 1. Dose-response curve of the effect of LTA on serum triglyceride (A) and FFA levels (B). Animals were administered LTA (0.1–300 μg/200 g BW) or saline i.v. as indicated. Blood samples were obtained 2 h later for the determination of serum triglyceride or FFA levels. Values are the mean ± SE (n = 5 for each group); \*, *P* < 0.05; \*\*, *P* < 0.01; and \*\*\*, *P* < 0.005 vs. controls.



**Fig. 2.** Time course of the effect of LTA on serum triglyceride (A) or cholesterol (B) levels. Animals were administered LTA (10  $\mu$ g/200 g BW) or saline i.v. as indicated. Blood samples were obtained 1, 2, 4, 8, 16 h later for the determination of serum triglyceride and cholesterol levels; (○) saline controls; (●) LTA-treated animals ( $n=5$ ). Values are the mean  $\pm$  SE ( $n=5$  for each group); \*,  $P < 0.05$  vs. controls.

1B). To determine the time course for the increase in serum triglyceride and cholesterol levels induced by LTA (10  $\mu$ g), we next examined serum triglyceride (Fig. 2A) and cholesterol levels (Fig. 2B) 1, 2, 4, 8, and 16 h after LTA administration. Serum triglyceride levels were significantly increased 2 h after LTA administration and reached a peak value at 4 h (200%). By 8 h serum triglyceride levels had returned to baseline (Fig. 2A). Serum cholesterol levels were slightly but significantly increased (20%) 16 h after LTA administration (Fig. 2B). Serum HDL-cholesterol levels were also significantly increased (25%) 16 h after LTA administration (controls;  $24 \pm 1$  mg/dl vs. LTA;  $30 \pm 0.3$  mg/dl,  $P < 0.005$ ). These findings demonstrate that LTA induces hyperlipidemia in rats.

### Adipose tissue lipoprotein lipase activity

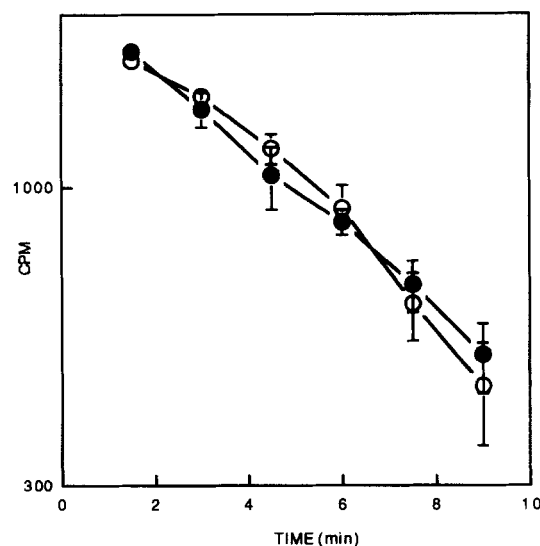
To determine the mechanism by which LTA acutely increases serum triglyceride levels, we first measured adipose tissue lipoprotein lipase activity 2 h after LTA administration. LTA administration did not affect adipose tissue lipoprotein lipase activity at this time point (control;  $175 \pm 55$  nmol FFA/ml per h vs. LTA;  $182 \pm 42$  nmol FFA/ml per h) indicating that a decrease in adipose tissue lipoprotein lipase activity does not contribute to the LTA-induced increase in serum triglyceride levels.

### Chylomicron clearance

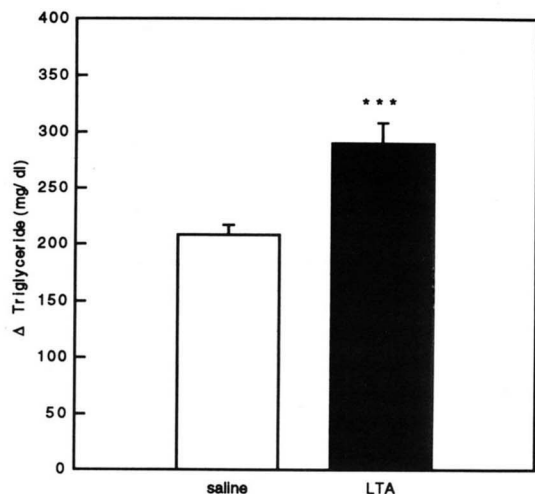
We next measured the clearance rate of triglyceride-rich lipoproteins (Fig. 3). The  $t_{1/2}$  of disappearance of the triglyceride from the circulation was not delayed in LTA-treated animals (saline control vs. LTA-administered animals,  $4.8 \pm 0.2$  min vs.  $4.8 \pm 0.1$  min). This data indicates that a decrease in the clearance of triglyceride-rich lipoproteins is not the mechanism by which LTA acutely increases serum triglyceride levels.

### Hepatic triglyceride secretion

We next determined whether LTA stimulates the hepatic secretion of lipoproteins (Fig. 4). The secretion of triglyceride was increased 40% in animals administered LTA as compared to controls (control vs. LTA



**Fig. 3.** Effect of LTA on chylomicron clearance; (○) saline controls; (●) LTA-treated animals ( $n=5$ ). Mesenteric lymph from rats administered [ $^3$ H]triolein was collected, and chylomicrons were isolated by centrifugation. Labeled chylomicrons (10 mg triglyceride) were injected intravenously into rats that had been administered 10  $\mu$ g LTA or saline 120 min before study. Blood samples were obtained every 90 s for 9 min, extracted with Dole's reagent, and lipid phase was counted by liquid scintillation. Values are the mean  $\pm$  SE ( $n=5$  for each group);  $t_{1/2}$  of disappearance from circulation was calculated as described in Methods; saline control vs. LTA-administered animals,  $4.8 \pm 0.2$  vs.  $4.8 \pm 0.1$  min.



**Fig. 4.** Effect of LTA on hepatic triglyceride secretion. Animals received saline or LTA (10  $\mu\text{g}/200$  g BW) and 30 min later 120 mg Triton WR-1339 i.v. as indicated. Blood samples were obtained 1 h 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. Values are the mean  $\pm$  SE (n = 5 for each group); \*\*\*,  $P < 0.005$  vs. controls.

treatment;  $208 \pm 9$  mg/dl vs.  $290 \pm 18$  mg/dl;  $P < 0.005$ ). This finding suggests that the increase in serum triglyceride levels in LTA-treated animals is due to an increase in hepatic lipoprotein secretion.

### Lipogenesis in vitro

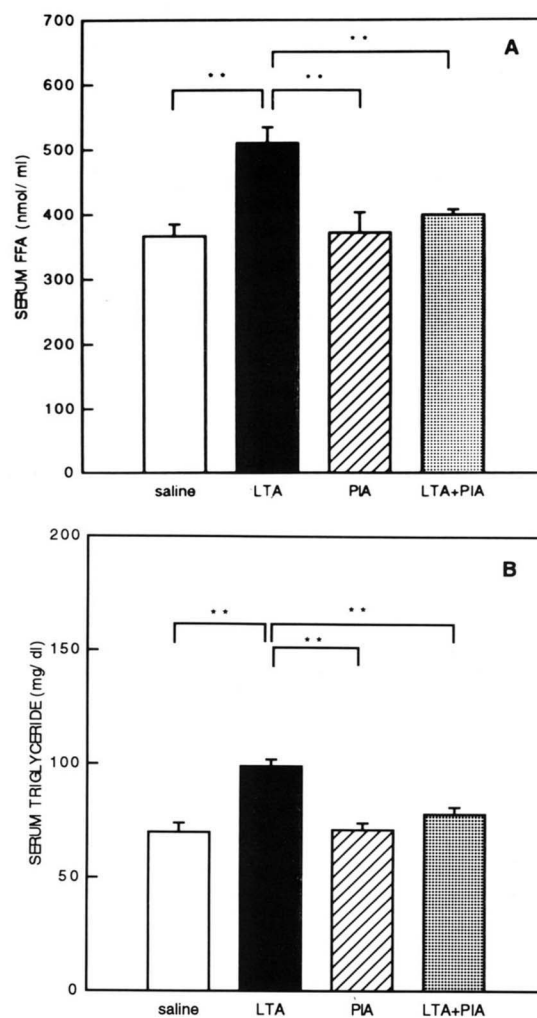
The incorporation of acetate into fatty acid, cholesterol, and total nonsaponifiable lipids was measured in liver slices from control and LTA-treated animals 2 h after LTA administration (Table 1). Fatty acid synthesis was significantly increased in liver slices from animals given LTA as compared to controls. In contrast, cholesterol synthesis and total nonsaponifiable lipid synthesis were not altered by LTA treatment animals indicating that at this time point LTA specifically increases fatty acid synthesis.

TABLE 1. Effect of LTA on lipogenesis in the liver in vitro

	Cholesterol	TNS	Fatty Acids
		<i>nmol<sup>a</sup></i>	
Control	18.4 $\pm$ 5	28.2 $\pm$ 11	11.4 $\pm$ 1
LTA	19.7 $\pm$ 4	37.4 $\pm$ 12	22.5 $\pm$ 3
	NS	NS	$P < 0.05$

Two h after the intravenous administration of LTA or saline (controls) the animals were killed, and 200 mg of liver slices was placed in center-well flasks containing 2 ml of Krebs-Ringer phosphate buffer and [ $1\text{-}^{14}\text{C}$ ]acetate (1  $\mu\text{Ci}/1019$  nmol per 100  $\mu\text{l}$ ). The flasks were incubated at 37°C for 2 h, after which the incorporation of label into cholesterol and fatty acids was determined. Values are mean  $\pm$  SE (n = 4 for each animal). TNS, total nonsaponifiable lipids.

<sup>a</sup>nmol incorporated/200 mg tissue per h.



**Fig. 5.** Effect of PIA on changes in serum FFA (A) and triglyceride (B) levels. PIA (0.15  $\mu\text{mol}/\text{kg}$ ) were administered s.c. as indicated. Thirty min later, animals received saline or LTA (10  $\mu\text{g}/200$  g BW) i.v. as indicated. Two h later, blood samples were obtained for determination of serum triglyceride and FFA levels. Values are the mean  $\pm$  SE (n = 5 for each group); \*\*,  $P < 0.01$ .

### Role of lipolysis in LTA-induced hypertriglyceridemia

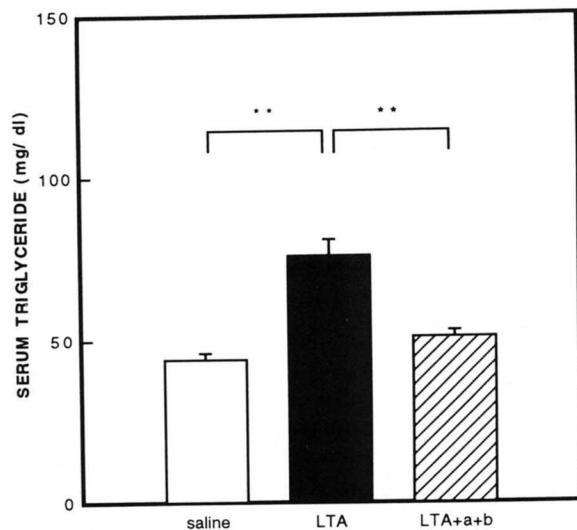
To determine the relative importance of hepatic lipogenesis versus increased lipolysis with increased delivery of FFA to the liver in mediating the LTA-induced hypertriglyceridemia, we next determined the effect of prior treatment with PIA, a drug that inhibits adipose tissue lipolysis (Fig. 5). Two hours after LTA administration, pretreatment with PIA completely inhibited the increase in serum FFA (Fig. 5A) levels induced by LTA. Moreover, pretreatment with PIA markedly diminished the increase in serum triglyceride levels induced by LTA

(Fig. 5B). PIA alone did not affect either serum FFA or triglyceride levels. These findings indicate that LTA stimulates lipolysis and the increased delivery of free fatty acids to the liver plays a major role in the LTA-induced hypertriglyceridemia.

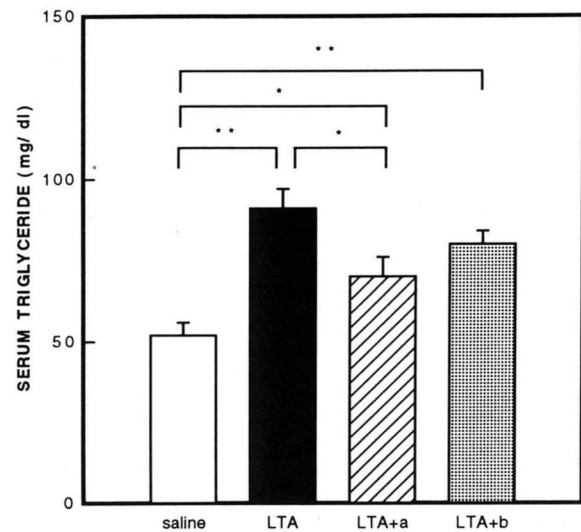
### Effects of adrenergic receptor antagonists on LTA-induced hypertriglyceridemia

We previously reported that endogenous catecholamines via  $\alpha$ -adrenergic receptors are involved in mediating the hypertriglyceridemia induced by LPS in rats (3). We therefore examined the effects of adrenergic receptor antagonists on the hypertriglyceridemia induced by LTA. Figure 6 shows the effect of i.p. injection of phentolamine (5 mg/kg) and alprenolol (6 mg/kg) on the increase in serum triglyceride levels induced by LTA. The pretreatment with phentolamine and alprenolol significantly suppressed the increase in serum triglyceride levels induced by LTA. However, this pretreatment did not suppress the increase in serum FFA levels (saline group,  $212 \pm 18$  nmol/ml; LTA alone group,  $367 \pm 28$  nmol/ml; phentolamine and alprenolol treatment group,  $387 \pm 38$  nmol/ml). These findings indicate that catecholamines are involved in the hypertriglyceridemia induced by LTA, but they do not mediate the increase in lipolysis.

To determine the role of  $\alpha$ - or  $\beta$ -adrenergic receptors, we next examined the effect of phentolamine alone or alprenolol alone on the increase in serum triglyceride



**Fig. 6.** Effect of i.p. injection of phentolamine and alprenolol on the increase in serum triglyceride levels induced by LTA. Phentolamine (5 mg/kg) and alprenolol (6 mg/kg), or saline were administered i.p. as indicated. Thirty min later, animals received saline or LTA ( $10 \mu\text{g}/200 \text{ g BW}$ ) i.v. as indicated. Blood samples were obtained 2 h later for the determination of serum triglyceride levels. Values are the mean  $\pm$  SE ( $n = 10$  for each group); a: phentolamine; b: alprenolol; \*\*,  $P < 0.01$ .



**Fig. 7.** Effect of i.p. injection of phentolamine alone or alprenolol alone on the increase in serum triglyceride levels induced by LTA. Phentolamine (5 mg/kg), alprenolol (6 mg/kg), or saline were administered i.p. as indicated. Thirty min later, animals received saline or LTA ( $10 \mu\text{g}/200 \text{ g BW}$ ) i.v. as indicated. Blood samples were obtained 2 h later for the determination of serum triglyceride levels. Values are the mean  $\pm$  SE ( $n = 15$  for each group); a: phentolamine; b: alprenolol; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

and FFA levels induced by LTA. Pretreatment with phentolamine alone significantly suppressed the increase in serum triglyceride levels, whereas alprenolol had no significant effect (Fig. 7). Neither phentolamine nor alprenolol had a significant effect on the increase in serum FFA levels induced by LTA (saline group,  $208 \pm 19$  nmol/ml; LTA alone group,  $358 \pm 26$  nmol/ml; phentolamine treatment group,  $360 \pm 20$  nmol/ml; alprenolol treatment group,  $306 \pm 14$  nmol/ml). These adrenergic receptor antagonists by themselves did not affect serum triglyceride levels in control animals (data not shown). These findings indicate that catecholamines are involved in mediating the hypertriglyceridemia induced by LTA predominantly via  $\alpha$ -adrenergic, rather than  $\beta$ -adrenergic receptors. However, some synergistic effect of combined blockade cannot be ruled out.

### DISCUSSION

Infection leads to an increase in serum triglyceride levels and, in some species, serum cholesterol levels. The increase in serum lipid levels associated with infection may be beneficial to the host. The increase in serum lipids may result in the enhanced delivery of lipids to cells that are activated during the immune response and to cells involved in tissue repair. Additionally, lipoproteins bind LPS and can protect the animal from the toxic effects of endotoxin (26–31). Lipoproteins also bind a

variety of viruses blocking their cytopathic effects (32–34) and can induce the lysis of the parasite, *Trypanosoma brucei* (35). Considered in this light, the increase in lipids during infection may be postulated to be a part of the acute phase response with lipoproteins serving not only as a potential source of metabolic fuel (36) but also as direct participants in the protective process (37).

Although gram-positive bacterial infection increases serum lipids, the mechanisms of these effects have not been well studied. LTA is a component of the cell membrane of gram-positive bacteria (10). Analogous to LPS, LTA has been shown to produce nitric oxide synthase (15), activate complement activation (16), and stimulate the secretion of cytokines such as TNF, IL-1, IL-6, and IL-8 (12, 14, 16, 18). The purpose of the present study was to determine the effect of LTA administration on serum lipid levels and the mechanisms by which LTA induces hypertriglyceridemia in rats.

The present study demonstrates that a single dose of as little as 0.1  $\mu\text{g}$  of LTA is sufficient to rapidly (within 2 h) produce hypertriglyceridemia in rats. Serum triglyceride levels reached peak values at 4 h (2-fold increase) after LTA administration. LTA also increased serum cholesterol levels but the effect was delayed occurring at 16 h, relatively modest (1.2-fold increase), and primarily due to an increased serum HDL-cholesterol levels.

The present study further demonstrates that the hypertriglyceridemia induced by LTA treatment is due to an increase in hepatic triglyceride secretion (1.4-fold) as demonstrated using the Triton WR-1339 technique. In contrast, the clearance of triglyceride-rich lipoproteins was not delayed by LTA treatment, nor was the activity of adipose tissue lipoprotein lipase, an important enzyme involved in the clearance of triglyceride-rich lipoproteins, decreased. These findings indicate that decreased clearance is not the mechanism for the hypertriglyceridemia but rather enhanced secretion of lipoproteins by the liver accounts for the increase.

The fatty acids required for the increase in hepatic triglyceride secretion induced by LTA treatment could be derived from at least two sources. First, the present study demonstrates that LTA induces lipolysis, resulting in an increase in circulating FFA levels and the increased delivery of FFA to the liver. Second, LTA administration also stimulates hepatic de novo fatty acid synthesis, which could also provide a source of fatty acids for the increase in hepatic triglyceride secretion. That the increase in lipolysis is of major importance is shown by the effect of PIA, an adenosine agonist, that blocks lipolysis by interacting with specific cell surface receptors that inhibit adenylate cyclase activity (38, 39). Pretreatment with PIA prevented the LTA-induced increase in serum

FFA levels and attenuated the increase in serum triglyceride levels, indicating the importance of lipolysis in providing FFA to the liver. In recent studies, we found that LTA did not stimulate lipolysis in 3T3-L1 adipocytes in culture (data not shown), suggesting that LTA may stimulate lipolysis via second messengers in vivo. LTA has been shown to induce macrophage secretion of TNF, IL-1, and IL-6 (12, 14, 16). Our laboratory has shown that TNF, IL-1, and IL-6 stimulate lipolysis (40, 41, 42), and it is therefore possible that these cytokines stimulate lipolysis in vivo. Of course it is also possible that LTA could have direct effects on adipocytes in vivo or could stimulate the production of second messengers other than cytokines that effect adipose tissues.

Previous studies by our laboratory and others have extensively characterized the effects of LPS on lipid metabolism (2, 37). The alterations in triglyceride metabolism induced by LTA have many similarities to that observed after the administration of low doses of LPS. First, neither low dose LPS nor LTA decreased the clearance of triglyceride-rich lipoproteins. Second, both LPS and LTA increased hepatic triglyceride secretion. Third, both LPS and LTA stimulated lipolysis which provided a major source of FFA for hepatic triglyceride secretion. Fourth, both LPS and LTA acutely increased hepatic fatty acid synthesis without altering cholesterol synthesis. Lastly,  $\alpha$ -adrenergic receptor antagonists inhibit the hypertriglyceridemia induced by LPS as well as LTA.

In the present study, we demonstrate that catecholamines play an important role in mediating the hypertriglyceridemia induced by LTA in rats. Generally, catecholamines have been recognized to be primarily lipolytic hormones, and these effects are mediated by  $\beta$ -adrenergic receptors in adipose tissues. However, chronic treatment with prazosin, an  $\alpha$ 1-adrenergic receptor antagonist, reduced triglyceride levels and secretion rates in rats and human (43, 44), a finding that is difficult to reconcile with an action of catecholamines on FFA and hepatic triglyceride secretion through  $\beta$ -adrenergic receptors. We previously reported that, in rats, none of the changes in lipid metabolism induced by low-dose LPS, including lipolysis, are mediated by  $\beta$ -adrenergic receptors. Rather,  $\alpha$ -adrenergic receptors mediate the hypertriglyceridemia induced by low-dose LPS via increasing hepatic triglyceride secretion (3). The present study also indicates that catecholamines are involved in mediating the increased hepatic triglyceride secretion induced by LTA as well as LPS primarily via  $\alpha$ -adrenergic receptors.

In summary, the present study demonstrates that LTA induces hyperlipidemia in rats. LTA-induced hypertriglyceridemia is caused by increased hepatic triglyceride secretion with the fatty acids provided by lipolysis. These

changes in lipid metabolism may play an important role in the organism's response to gram-positive infection. ■■

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