Effect of Endotoxin and Cytokines on Lipoprotein Lipase Activity in Mice


Abstract Endotoxin (lipopolysaccharide [LPS]) stimulates the production of cytokines, which mediate many of the metabolic effects associated with infection. In LPS-sensitive C57Bl/6 mice, LPS doses as low as 0.01 μg per mouse decreased adipose tissue lipoprotein lipase (LPL) activity by greater than 50%. In LPS-resistant C3H/HeJ mice, which do not produce cytokines in response to LPS, doses of LPS as high as 10 μg per mouse did not affect LPL activity in adipose tissue. In muscle of C57Bl/6 mice, LPL activity was decreased by 27% after 10 μg of LPS, whereas in C3H/HeJ mice there was no effect. These results indicate that the LPS-induced decrease in both adipose and muscle LPL activity is mediated by cytokines. Tumor necrosis factor (TNF), interleukin (IL)-1, leukemia-inhibiting factor (LIF), interferon alfa, and interferon gamma all decreased adipose tissue LPL activity in intact mice. In skeletal and cardiac muscle, only IL-1 and interferon gamma decreased muscle LPL activity, whereas TNF, LIF, and interferon alfa had no effect. Inhibition of TNF activity blocked the increase in serum triglycerides that is characteristically observed after LPS but did not affect the ability of LPS to decrease adipose tissue LPL activity. Inhibition of IL-1 activity with IL-1 receptor antagonist partially inhibited the increase in serum triglycerides; however, the ability of LPS to decrease LPL activity in either adipose or muscle tissue was not affected. These data indicate that although TNF and IL-1 play a role in mediating the increase in serum triglyceride levels, these cytokines do not play a crucial role in the inhibition of either adipose or muscle LPL activity. These results also indicate that the decrease in LPL activity is not a key event responsible for the increase in serum triglyceride levels. In conclusion, although the inhibition of LPL activity produced by LPS is mediated by cytokines, these effects are not dependent on either TNF or IL-1. Changes in LPL are not essential for the induction of hypertriglyceridemia but may influence the distribution of nutrients.

Key Words • tumor necrosis factor • interleukins • interferons • serum triglycerides • leukemia inhibitory factor

Bacterial, viral, and parasitic infections are frequently associated with hypertriglyceridemia secondary to elevations in very-low-density lipoprotein (VLDL) levels. Endotoxin (LPS) administration, which mimics Gram-negative bacterial infections, has been shown to produce hypertriglyceridemia by stimulating the hepatic production of VLDL and/or by inhibiting the clearance of triglyceride-rich lipoproteins. The activity of lipoprotein lipase (LPL), a key regulatory enzyme in the catabolism and clearance of triglyceride-rich lipoproteins, is decreased after LPS treatment in both adipose tissue and muscle. Recent studies have suggested that the decrease in adipose tissue LPL activity is due to a posttranslational effect. Infection and LPS administration stimulate the production of a large number of cytokines, the hormones of the immune system, and these cytokines are thought to mediate many of the metabolic effects associated with infection and LPS treatment. In adipocytes in culture, studies by our and other laboratories have demonstrated that many cytokines, including tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, IL-11, leukemia-inhibiting factor (LIF), interferon alfa (IFN-α), and interferon gamma (IFN-γ) inhibit LPL activity. However, to date, the administration only of TNF and IL-6 has been shown to decrease adipose tissue LPL activity in intact animals. TNF decreased epididymal fat LPL activity without affecting activity in several other sites. Moreover, the effect of cytokines on muscle LPL activity in vivo and in vitro has not been extensively explored. Our studies have demonstrated that in rats, TNF does not decrease muscle LPL activity.

The purpose of the present study was to determine the following: (1) whether the LPS-induced decrease in adipose and muscle LPL activity requires cytokine production; to determine this we used HeJ mice, which do not produce cytokines in response to LPS; (2) the effect of TNF, IL-1, LIF, IFN-α, and IFN-γ on adipose and muscle LPL activity in intact mice; and (3) whether either TNF or IL-1, the major cytokines produced in response to LPS administration, mediate the LPS-induced changes in LPL activity.

Materials

Tritiated triolein was purchased from New England Nuclear. Triolein, lecithin, and fatty acid-free bovine serum albumin were purchased from Sigma Chemical Co. CytoScent scintillation fluid was purchased from ICN Biochemical, Inc. Endotoxin (Escherichia coli, strain 055:B5) was purchased from Difco Laboratories. Murine TNF with a specific activity of 2.9×10^7 U/mg, murine IFN-γ with a specific activity of 5×10^4 U/mg, and human LIF at a concentration of 2.05 mg/mL were kindly provided by Genentech Inc. Recombinant human IL-1β (amino acids 112 to 185) with a specific activity of 2.0×10^5 U/mg was purchased from Genzyme.
269) with a specific activity of $5 \times 10^{11}$ U/mg was produced as described previously and kindly provided by Dr Charles A. Dinarello of Tufts University, Boston, Mass.\textsuperscript{24} Recombinant human IFN-α (A/D) (specific activity 7.9 $\times 10^{10}$ U/mg) was kindly provided by Drs M. Brunda and P. Sorter of Hoffmann-La Roche. Human IFN-α (A/D) hybrid has been shown to regulate mouse tissues in a manner similar to that of murine IFN-α.\textsuperscript{25} Antibodies against TNF were generated by immunization of New Zealand White rabbits by standard techniques at Caltag Laboratories. Immunoglobulins were purified from serum by ammonium sulfate precipitation by means of previously described procedures to avoid LPS contamination. IL-1 receptor antagonist (IL-1ra) was kindly provided by Dr Robert C. Thompson of Syngen, Inc.

Animal Procedures

C57BI/6 male mice (weight, 18 to 20 g) were purchased from Simonens Laboratories (Gilroy, Calif), and C3H/HeJ male mice (weight, 18 to 20 g) were purchased from Jackson Laboratories (Bar Harbor, Me). The animals were maintained in a normal 12-hour light/dark cycle and were fed Purina mouse chow (Ralston Purina) and water ad libitum. On the evening before the study (16 hours before study), animals were injected with the indicated doses of LPS (10 μg per mouse), TNF (1 μg per mouse), IL-1 (80 ng per mouse), LIF (5 μg per mouse), IFN-α (30 μg per mouse), or IFN-γ (50 μg per mouse), or with the appropriate vehicle alone (controls). The doses of TNF, IL-1, IFN-α, and IFN-γ have previously been shown by our laboratory to stimulate hepatic lipid synthesis.\textsuperscript{26} The dose of LIF used has been shown by other investigators to be effective in vivo.\textsuperscript{27} LPS was administered intraperitoneally in 0.9% saline solution. TNF, IL-1, LIF, IFN-α, and IFN-γ were administered intraperitoneally in 0.1% human serum albumin solution. Food was withdrawn from the animals after the injection because LPS and cytokines have been shown to induce anorexia. When indicated, animals were injected intraperitoneally with saline or anti-TNF antibodies (quantity of antibodies sufficient to neutralize 34 μg of TNF) 4 hours before LPS administration. When indicated, animals were injected subcutaneously with IL-1ra (1 mg per mouse in 0.1% human serum albumin) at 0, 2, 4, and 6 hours after LPS administration. It has been shown by several investigators that the effect of LPS on IL-1 secretion and mRNA induction is maximal within 60 to 90 minutes\textsuperscript{28,29}; hence, the IL-1ra dose schedule used here should be able to block the effect of IL-1 induced by a single bolus of LPS.

LPL Activity

Mice were killed 16 hours after LPS or cytokine administration, and then the epididymal fat pad, quadriceps muscle, a combination of biceps and triceps muscle, and heart 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 mol/L NaCl, 0.005 mol/L KCl, 0.0012 mol/L KH₂PO₄, 0.0012 mol/L MgSO₄, 0.55 mol/L CaCl₂, and heparin (4 U/mL) for 60 minutes at 37°C. Lipolytic activity was determined as described previously.\textsuperscript{21} Briefly, the substrate (unlabeled triolein) and 18.75 μCi of triitated triolein were homogenized with 3.0 mg of lecithin, 1.2 mL of 20% fatty acid-free bovine serum albumin, 0.5 mL normal human plasma (LPL cofactor), 0.5 mL 1% Triton X-100, 15 U heparin, and 6.8 mL of 1 mol/L tris(hydroxymethyl)aminomethane buffer, pH 8.6. An aliquot of the resultant emulsion (0.1 mL) and 0.4 mL of the extracted medium of the tissue were incubated in a metabolic shaker at 37°C for 60 minutes. The reaction was stopped by addition of 4.0 mL of isopropanol–sulfuric acid reagent (10 mL 3N sulfuric acid, 400 mL isopropanol). Subsequently, the triitated oleic acid was separated from triolein by sequential hexane extraction and alkalinization; an aliquot of the alkaline medium was counted. One

![Graph](https://example.com/graph.png)

Fig 1. Line graph shows dose-response curves for the effect of endotoxin (lipopolysaccharide [LPS]) on adipose tissue lipoprotein lipase (LPL) activity in C57BI/6 and C3H/HeJ mice. Animals were injected with the LPS dose indicated on the abscissa. Sixteen hours later the animals were killed, and adipose tissue LPL activity was determined as described in "Methods." Values are mean±SEM; n=10 for controls and n=5 for each dose in C57BI/6 and C3H/HeJ mice. *P<0.01 compared with controls. Control LPL activity in C57BI/6 mice was 138 and in C3H/HeJ mice 87 nmol/h per 100 mg tissue.

Serum Triglyceride Levels

We measured serum triglyceride levels using Sigma Diagnostic Kit No. 337 (Sigma Chemical Co). Serum glycerol levels were subtracted from total serum triglyceride levels.

Statistical Analysis

Data are presented as mean±SEM. Statistical significance between groups was calculated by Student's t test. Because baseline LPL activity varied between experimental groups, only animals that were studied simultaneously under identical conditions were compared.

Results

Effects of LPS on Serum Triglyceride and LPL Activity

The effects of LPS on serum triglyceride levels in C57BI/6 and C3H/HeJ mice have been previously reported.\textsuperscript{30} In C57BI/6 mice serum triglyceride levels were increased 62% and 76% by 16 hours after the administration of 1 and 10 μg of LPS, respectively.\textsuperscript{30} In contrast, in LPS-resistant C3H/HeJ mice, serum triglyceride levels were not significantly increased by LPS doses as high as 100 μg per mouse.\textsuperscript{30} These results indicate that the increase in serum triglyceride levels induced by LPS is mediated by cytokines secreted by macrophages.

The effect of LPS on adipose tissue LPL activity is shown in Fig 1. In LPS-sensitive C57BI/6 mice, LPS doses as low as 0.01 μg per mouse decreased LPL activity by greater than 50%. Higher doses of LPS did not result in a further reduction in LPS activity. In LPS-resistant C3H/HeJ mice, doses of LPS as high as 10 μg per mouse did not affect LPL activity in adipose tissue. In quadriceps muscle of C57BI/6 mice, LPL activity was decreased by 27% 16 hours after administration of 10 μg LPS (Fig 2). In cardiac muscle we observed only a 16% decrease in LPL activity after LPS administration, and this decrease was not statistically significant (mean of four separate experiments; n=5 for
control and LPS treatment for each experiment). In C3H/HeJ mice, 10 μg LPS had no effect on quadriceps muscle LPL activity (Fig 2). The lack of response of C3H/HeJ mice indicates that the effect of LPS on both adipose and quadriceps muscle LPL activity is mediated by cytokines.

**Effect of Cytokines on Serum Triglyceride and LPL Activity**

The effect of cytokines on serum triglyceride levels is shown in Fig 3. As reported previously, both TNF and IL-1 increased serum triglyceride levels (TNF, 72%; IL-1, 110%). LIF administration also produced an increase in serum triglyceride levels (41%). With IFN-γ there was a trend toward an increase (35%), but this did not reach statistical significance. IFN-α administration did not increase serum triglyceride levels.

The effect of cytokines on adipose tissue LPL activity is shown in Fig 4. TNF, IL-1, LIF, IFN-α, and IFN-γ all decreased epididymal adipose tissue LPL activity. In contrast, in two sites of skeletal muscle (quadriceps [Fig 5, top panel] and triceps/biceps [Fig 5, bottom panel]),
Previous studies by this laboratory and other laboratories have shown that LPS decreasing LPL activity in both adipose tissue and muscle. Furthermore, these results indicate that although TNF is an important mediator of the increase in serum triglyceride levels, TNF is not crucial for the LPS-induced inhibition of adipose tissue LPL activity. However, these results suggest that a decrease in adipose tissue LPL activity is not the key event responsible for the increase in serum triglyceride levels.

To inhibit IL-1 activity, we used an antibody that we have previously shown neutralizes IL-1 activity in vivo. Pretreatment with TNF antibody blocked the increase in serum triglyceride levels that is characteristically observed after LPS administration (Fig 7, top panel). However, as shown in the bottom panel of Fig 7, the ability of LPS to decrease adipose tissue LPL activity is not affected by pretreatment with TNF antibodies. These results indicate that although TNF is an important mediator of the increase in serum triglyceride levels, TNF is not crucial for the LPS-induced inhibition of adipose tissue LPL activity. Moreover, these results suggest that a decrease in adipose tissue LPL activity is not the key event responsible for the increase in serum triglyceride levels.

To inhibit IL-1 activity, we used IL-1ra, which we have previously shown neutralizes IL-1 activity in vivo. As shown in the top panel of Fig 8, administration of IL-1ra partially inhibits the increase in serum triglyceride levels induced by LPS. However, the ability of LPS to decrease LPL activity in either adipose tissue (Fig 8, middle panel) or muscle (Fig 8, bottom panel) is not affected by inhibition of IL-1 activity. These data indicate that although IL-1 plays a role in mediating the increase in serum triglyceride levels, it does not play a crucial role in the inhibition of either adipose or muscle LPL activity induced by LPS.

**Discussion**

Low-dose LPS administration has been shown to increase serum triglyceride levels in rats by stimulating the secretion of VLDL by the liver, whereas high-dose LPS inhibits the clearance of triglyceride-rich lipoproteins. The delay in lipoprotein clearance has been attributed to LPS decreasing LPL activity in both muscle and adipose tissue. Previous studies by this laboratory and other laboratories have shown that cytokine production is required for the LPS-induced increase in serum triglyceride levels. LPS administration to C3H/HeJ mice, which are incapable of producing cytokines in response to LPS, fails to result in an increase in serum triglyceride levels. In the present study we demonstrate that LPS treatment of C3H/HeJ mice also does not decrease LPL activity in adipose tissue. In contrast, in C57Bl/6 mice, which produce cytokines in response to LPS, treatment with LPS decreases adipose tissue LPL activity. These results confirm the studies of Kawakami and Cerami, who previously demonstrated the failure of LPS to decrease adipose tissue LPL activity in C3H/HeJ mice. Moreover, we now demonstrate that the LPS-induced inhibition of muscle LPL activity also requires cytokine production. In C3H/HeJ mice, LPS treatment had no effect on muscle LPL activity, whereas in C57Bl/6 mice, LPS treatment decreased muscle LPL activity. Thus, the decrease in LPL activity in both adipose tissue and muscle that occurs after LPS treatment is mediated by cytokine production.

Previous studies have shown that a variety of different cytokines, including TNF, IL-1, IL-6, IL-11, LIF, IFN-α, and IFN-γ, inhibit the activity of LPL in cultured adipocytes. However, in intact animals only TNF and IL-6 have previously been shown to decrease adipose tissue LPL activity. Interferons decrease
postheparin LPL activity in plasma, but whether this is
due to a decrease in adipose tissue LPL activity is
unknown. IL-1 did not affect LPL activity in post-
heparin plasma of primates and caused only a small
nonsignificant decrease in LPL activity in adipose tissue
of rats. In the present study we demonstrate that
the administration of TNF, IL-1, LIF, IFN-α, and IFN-γ
to intact mice decreases LPL activity in adipose tissue.
Thus, as observed in tissue culture studies, a large
number of different cytokines are capable of decreasing
adipose tissue LPL activity.

Studies in rodents have suggested that muscle LPL
activity accounts for a substantial portion of the clear-
ance of triglyceride-rich lipoproteins from the circula-
tion. Moreover, during fasting, the activity of LPL in
adipose tissues decreases, and the importance of muscle
in the clearance of triglyceride-rich lipoproteins in-
creases. Therefore, the status of muscle LPL activity
is of great importance in determining the rate of clear-
ance and the distribution of uptake of triglyceride-rich
lipoproteins. The effect of cytokines on muscle LPL
activity in vivo and in vitro has not been extensively
explored. Studies by our laboratory and by Semb et
al have shown that in intact rats, TNF does not
decrease LPL activity in muscle. In the present study we
demonstrate that in intact mice IL-1 and IFN-γ de-
crease skeletal muscle and heart LPL activity, whereas
TNF, LIF, and IFN-α have no effect. Thus, while many
cytokines inhibit adipose tissue LPL activity, only IL-1
and IFN-γ have been shown to decrease LPL activity in
muscle. One can speculate that this selective effect on
LPL could influence the distribution of nutrients be-
tween tissues.

In the present study we measured LPL activity in
adipose and muscle tissue, which includes both intracel-
lar and extracellular enzymes. The enzyme activity
that is important in lipoprotein metabolism is localized
to the endothelial surface. Unfortunately, at the present
time assays to specifically determine the activity of LPL
on the endothelial cell surface are not available. Nev-
ertheless, studies have shown that LPL activity mea-
sured in whole tissue reflects the uptake by these tissues
of labeled lipid in triglyceride-rich lipoproteins.

We next determined whether either TNF or IL-1,
the primary cytokines secreted in response to LPS, is
responsible for the decrease in LPL activity in either
adipose tissue or muscle. Neutralization of TNF with
TNF antibodies markedly diminished the ability of LPS
to increase serum triglyceride levels, indicating that
TNF is an important mediator of hypertriglyceridemia.
However, the inhibition of adipose tissue LPL activity
was not affected by the inhibition of TNF action,
indicating that TNF is not crucial for the LPS-induced
decrease in adipose tissue LPL activity. When IL-1
activity was inhibited by administration of IL-1ra, there
was a partial inhibition of the LPS-induced increase in
serum triglycerides, indicating that IL-1 contributes to
hypertriglyceridemia. However, similar to our observa-
tions with TNF, the inhibition of IL-1 activity did not
affect the ability of LPS to decrease either adipose or
muscle LPL activity, indicating that IL-1 does not play
a critical role in mediating the LPS inhibition of LPL
activity. From experiments in HeJ mice it is apparent
that cytokines play a key role in mediating changes in
LPL activity. Which cytokine or combinations of cyto-
kines are responsible for the LPS effect on LPL activity
is not clear. Based on previous studies and the experi-
ments reported here, a large number of cytokines could
be responsible for the inhibition in adipose tissue,
whereas in muscle tissue, the number of potential
candidates uncovered thus far is small. Unfortunately,
reagents to inhibit the activity of these varied cytokines
in intact animals are not currently available to us.

It should also be recognized that while the inhibitors
of either TNF or IL-1 action had no effect on LPL
activity, these inhibitors were able to blunt the increase
in serum triglyceride levels induced by LPS. This dis-
cordant effect on LPL activity and triglyceride levels
suggests that the increase in serum triglycerides is not related to the inhibition of LPL activity. This is in agreement with previous studies that have demonstrated that LPS administration under certain circumstances increases serum triglyceride levels by stimulating hepatic VLDL secretion. Furthermore, we and other laboratories have also shown that TNF and IL-1 increase serum triglyceride levels by increasing hepatic VLDL secretion without affecting the clearance of triglyceride-rich lipoproteins. Here we also report that IFN-α and IFN-γ can decrease LPL activity without increasing serum triglyceride levels at 16 hours. Thus, the present studies provide further evidence that changes in serum triglyceride levels are not necessarily mediated by changes in LPL activity. The changes in LPL activity may not regulate serum triglyceride levels, but rather they could alter the distribution of nutrients between various tissues. It is well recognized that cytokines play a predominant role in the development of cachexia during chronic infections and cancer, and it is possible that cytokine-induced alterations in LPL activity could contribute to these changes.

In summary, the present study demonstrates that the inhibition of LPL activity in adipose tissue may be mediated by a wide variety of cytokines, whereas the inhibition of LPL activity in muscle tissue is produced by a more limited number of cytokines. However, the effects of LPS on LPL activity are not mediated by TNF or IL-1. The question of which cytokine or combinations of cytokines mediate LPS-induced changes in LPL activity awaits the development of additional blocking reagents.

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