Sepsis-induced regulation of lipoprotein lipase expression in rat adipose tissue and soleus muscle

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Abstract Hypertriglyceridemia of sepsis is associated with suppressed tissue lipoprotein lipase (LPL) activities. We investigated the effect of sepsis on lipoprotein lipase gene expression in epididymal fat and soleus muscle from control and septic rats in the fasted and fed state. After 24 h of sepsis, LPL activity decreased significantly in epididymal fat from fasted rats by 45% along with a 57% reduction in LPL mRNA levels and LPL mass. Transcription rate, measured by nuclear run-on assay, decreased by 70% in epididymal fat from fasted septic rats compared to fasted control rats. The synthesis rate of LPL in epididymal fat decreased by 31% while the LPL relative synthesis declined by 50% during sepsis. The turnover rate was not altered. Sepsis in fed rats did not lead to a significant decrease in LPL mRNA in epididymal fat but did lower LPL activity and LPL mass by 45%. The sepsis-induced suppression in soleus LPL activity, LPL mRNA levels, and LPL mass in the fasted state also was observed when septic rats were fed. The results indicate that during fasting sepsis, LPL expression in epididymal fat and possibly soleus muscle involves transcriptional regulation. During sepsis in the fed state, the regulation of LPL in adipose tissue may involve posttranslational mechanisms.—**Lanza-Jacoby, S., N. Sedkova, H. Phetteplace, and D. Perrotti.** Sepsis-induced regulation of lipoprotein lipase expression in rat adipose tissue and soleus muscle. *J. Lipid Res.* 1997. **38:** 701–710.

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Sepsis is associated with complex disturbances in lipid metabolism with the redistribution of lipid substrates resulting in hypertriglyceridemia and lipid accumulation in the liver. Previously, we have reported that the hypertriglyceridemia of sepsis in the rat resulted from a 67% decrease in the clearance rate of very low density lipoprotein triglycerides (TG) which was associated with reduced activities of lipoprotein lipase (LPL) in muscle, adipose tissue, and heart (1, 2). The hypertriglyceridemia in endotoxin-treated rats was attributed to a reduction in TG clearance rate and depressed tissue activities of LPL (3). LPL hydrolyzes the circulating TG from chylomicrons and very low density lipoproteins thus releasing free fatty acids (FFA) to the peripheral tissues which subsequently facilitates TG removal from the blood. In addition to contributing to the impaired clearance rate of TG, the reduction in LPL activity contributes to the shift of lipid fuels from the peripheral tissues to the liver where lipids accumulate leading to impaired liver function. Gouni et al. (4) reported that adipose tissue LPL from fed endotoxin-treated rats was regulated posttranscriptionally as LPL synthesis and LPLmRNA levels were not altered.

There are no studies investigating the mechanisms of how sepsis alters LPL expression. In addition to adipose tissue, altered regulation of LPL in skeletal muscle during sepsis could have a significant impact on the clearance of TG as most of the LPL found in the body is in the skeletal muscle. We have examined the effects of *E. coli* sepsis on the regulation of LPL by measuring the activity, the mRNA levels, transcription rate, synthetic/degradation rates, and protein mass of LPL in adipose tissue and skeletal muscle of rats. Additionally, the effect of nutritional status on the regulation of LPL during sepsis was investigated by conducting experiments with fasted and fed control and septic rats.

METHODS

Materials

[^{35}S]methionine (1000 Ci/mM), [^{35}S]cysteine (600 Ci/mM), [^{32}P]dCTP (3000 Ci/mM), and [^{3}H]triolein

Abbreviations: LPL, lipoprotein lipase; TG, triglyceride; FFA, free fatty acids; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; S-14, spot-14; PBS, phosphate-buffered saline; SSC, sodium chloride citrate buffer; MEM, modified Eagle's medium; TCA, trichloroacetic acid; SEM, standard error of the mean; TNF, tumor necrosis factor; INF, interferon; IL, interleukin.

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(10 Ci/mM) were purchased from DuPont-New England Nuclear, Boston, MA. DNA labeling kit was purchased from Amersham Corp. (Arlington Heights, IL). Rabbit antichicken IgG was purchased from Cappel (Durham, NC). Affinity-purified chicken anti-bovine LPL antibody and biotinylated anti-LPL were obtained from Dr. John W. F. Goers (Cal Poly, San Luis Obispo, CA). The guinea pig LPL cDNA and chicken β actin cDNA were gifts from Dr. Phillip H. Pekala (East Carolina University, Greenville, NC). The human ribosomal S-14 cDNA was a gift from Dr. Jaime Caro, Department of Medicine, Jefferson Medical College. The monoclonal antibody to bovine milk LPL 5D2#9 was a gift from Dr. John D. Brunzell (University of Washington, Seattle, WA). Restriction enzymes were purchased from Boehringer Mannheim (Indianapolis, IN). Bovine serum albumin (BSA) Fraction V was from Calbiochem (La Jolla, CA). Sodium dodecyl sulfate (SDS)-polyacrylamide gels, 3%–27% gradient were purchased from Jule, Inc. (New Haven, CT). All other chemicals including protein G and protease inhibitors were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals

Male Lewis rats (275–300 g), obtained from Harlen Sprague-Dawley (Walkerville, MD) were used in all experiments. The rats were housed in temperature- and humidity-controlled rooms on a 12-h light (0600–1800 h) dark schedule. All the rats had free access to standard rat diet (Purina Rat Chow #5001, Purina Mills; Inc., St. Louis, MO) ad libitum.

Experimental protocol

After 1 week of acclimation, the rats were assigned to four groups: fasted control, fasted septic, fed control, and fed septic. Three days prior to inducing sepsis, the fed rats underwent placement of a jugular vein catheter for infusion of a nutritionally complete diet consisting of 75% of the nonprotein calories as glucose and 25% as lipid (5). Sepsis was induced by intravenous injection of 4×10^8 live *E. coli* colonies/100 g body weight every 24 h; the control groups of rats were injected with 0.09% NaCl. Both septic and control rats were fasted after *E. coli* injection to equalize the nutritional state as food consumption is reduced during sepsis (2). All rats were killed by decapitation 24 h or 48 h after *E. coli* injection. Epididymal fat pads and soleus muscles were excised, placed in liquid N_2, and stored at −80°C until analyzed. When LPL synthesis and degradation were determined, epididymal fat pads were removed and processed immediately. To measure LPL synthesis in the soleus muscle, the lower left hind limb was perfused.

Lipoprotein lipase activity

LPL activity was assayed as described in previous reports (1, 2). Total LPL activity in epididymal fat was measured in deoxycholate extracts. Soleus muscles were homogenized in NH_4-HCl (pH 8.1) using a Polytron (Brinkman Instruments, Westbury, NY) with a PTA7 probe at maximum speed for 2 min at 4°C. The extracts were diluted 1:7 in detergent-free buffer and 150 μl was mixed with 150 μl of glycerol-stabilized [^{3}H]triolein substrate emulsion of Nilsson-Ehle and Schotz (6) and incubated for 2 h at 37°C. Activity was expressed as μmol FFA/mg protein per h.

Lipoprotein lipase mRNA level

The total RNA in adipose tissue was isolated by the procedure of Chomczynski and Sacchi (7) with modifications described by Louveau, Chaudhuri, and Etherton (8). Soleus muscle RNA was extracted by homogenizing the tissues in a urea/LiCl lysis buffer followed by phenol extraction as described by Tushinski et al. (9). Ten μg RNA was subjected to formaldehyde electrophoresis and Northern blotting onto nylon membranes. All cDNA probes were labeled with [d-^{32}P]dCTP (3000 Ci/mM) by a random priming DNA Labeling Kit (Amersham Corporation) Hybridizations were carried out with ^{32}P-labeled guinea pig LPL cDNA for 24 h at 65°C, washed, and autoradiographed. The filters were stripped and rehybridized with Spot-14 (S-14) and the LPL mRNA signals were normalized to the S-14 signal. S-14 is a cytosolic protein which may have a role in lipid metabolism (10). In a preliminary trial, the filters were stripped and rehybridized with β actin. As sepsis increased the β actin signal, this probe could not be used as an internal control.

LPL transcription rate

Nuclear run-on assay, described by Bentley and Groudine (11) and modified by Raschella et al. (12), was used to measure LPL mRNA transcription rates. Nuclei were isolated from adipocytes that were washed 2× with ice-cold phosphate-buffered saline (PBS), lysed in 10 mM Tris-HCl, pH 7.9, 2 mM $MgCl_2$, 3 mM $CaCl_2$, 0.3 M sucrose, 3 mM dithiothreitol in the presence of 0.2% NP-40, centrifuged (500 *g* for 5 min at 4°C), and layered onto a 0.75 M sucrose cushion. Transcription was initiated by resuspending nuclei from adipocytes in 200 μl transcription buffer (1 mM $MgCl_2$, 70 mM KCl, 15% glycerol, 1.25 mM dithiothreitol) containing 0.25 mM each of GTP, ATP, and CTP, and 0.5 mCi α-[^{32}P]UTP. The transcription reaction was performed at 26°C for 20 min and was stopped by placing on ice and adding 30 U DNase-I. The labeled RNA was extracted by

method of Chomczynski and Sacchi (7) and purified by passage through a G-50 RNase-free Sephadex spin column (Boehringer Mannheim). An equal number of counts of nascent radiolabeled transcripts were hybridized to a nitrocellulose membrane (Schleicher and Schuell, Keene, NH), containing 10 μg each of immobilized LPL cDNA, S-14 cDNA (positive control), and Bluescript (control for nonspecific hybridization), and spotted using a slot-blot apparatus. Hybridizations were performed for 72 h at 42°C in 50% formamide, 5 × sodium chloride citrate buffer (SSC), 1 × Denhardt's solution, 50 mM N_2PO_4 buffer, 100 μg/ml salmon sperm DNA, 0.1% SDS. Membranes were washed in 2× SSC/0.1% SDS (2× at room temperature and 1× at 37°C, 20 min each), followed by a single wash in 0.1 × SSC/0.1% SDS (20 min at 65°C). Autoradiography was performed at −70°C for 5 days with intensifying screens and the bands were quantitated by laser scanning densitometry.

LPL protein mass

LPL was extracted from adipose tissue and soleus as described above for measuring LPL activity except that protease inhibitors were added (1 mM phenyl methyl sulfonyl fluoride, 1 mM benzamidine, 1 mM EDTA, and 0.5 mM aprotinin). Mass was determined by enzyme-linked immunoadsorbent assay as described by Goers et al. (13) using an affinity-purified chicken anti-bovine LPL.

LPL synthesis and degradation

Adipose tissue. Epididymal fat was placed in warm PBS, minced into 5-mg fragments, and washed. Tissue fragments (200 mg) were suspended in 2 ml modified Eagle's medium (MEM) containing 5% BSA, 10 U/ml streptomycin, 10 U/ml penicillin, and incubated for 30 min at 37°C under O_2/CO_2 (19:1). After preincubation, 100 μCi/ml of [^{35}S]methionine was added. For pulse experiments, aliquots were removed at specified times and washed 1× with MEM plus 20 mM unlabeled methionine and 3× with PBS plus 20 mM methionine. The tissue was placed in 2 ml ice-cold lysis buffer (1% Triton X-100, 50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 20 μg/ml leupeptin, 100 μg/ml phenylmethylsulfonyl fluoride, and 0.7 μg/ml pepstatin), sonicated briefly (15 pulses), and centrifuged at 10,000 rpm in a microfuge at 4°C. The infranatant below the fat cake was frozen at −80°C for immunoprecipitation of LPL and total trichloroacetic acid (TCA)-precipitable protein. TCA-precipitable protein was determined by precipitating 5 μl labeled infranatant in 20 μl distilled H_2O plus 10 ml 12% TCA on a GF-C filter and washing 5× with distilled H_2O, 4× with methanol, and 3× with acetone. The filters were dried and counted. First, an aliquot of tissue lysate was TCA-precipitated and counted; then the amount of sample used for LPL immunoprecipitation was adjusted to yield equal TCA counts between samples. Protein G-Sepharose was added to the infranatant for 60 min at 4°C. After microfuging at 12,000 rpm, supernatants were immunoprecipitated overnight with an appropriate volume (5–10 μl) of LPL monoclonal antibody. The immunocomplexes were washed with 1× 0.5 M LiCl in 0.1 M Tris-HCL, pH 8.0, and 2× with PBS with 0.1% N-laurylsarcosine. The complex was dissociated by addition 30 μl of 60 mM Tris-HCL, pH 6.8, 10% glycerol, 5% SDS plus 100 mM dithiothreitol and boiled for 5 min. After separating the immunoprecipitated radioactive proteins by SDS-polyacrylamide gel electrophoresis and fluorography, the bands corresponding to LPL were cut from the gel and counted in a liquid scintillation counter. A second immunoprecipitation did not yield any detectable band, which suggested that all of the labeled LPL had been complexed with the LPL antibody in the initial reaction.

To determine degradation of LPL, pulse-chase experiments were conducted. Aliquots of epididymal fat were pulsed for 15 min with 100 μCi/ml of [^{35}S]methionine at 37°C under O_2/CO_2 (19:1) and chased with 5% BSA in MEM buffer supplemented with 20 mM unlabeled methionine. At the specified time intervals aliquots of tissue and media were removed, washed, and prepared for TCA precipitation and immunoprecipitation of LPL.

Muscle. Incorporation of [^{35}S]methionine into total protein and immunoprecipitable LPL in soleus muscle was determined by perfusing the left hindlimb as described by Gorski, Hood, and Terjung (14) and Jurasinski, Kilpatrick, and Vary (15). Briefly, the ipsilateral abdominal, epigastric, and superior vesicle arteries were cauterized. The femoral artery and vein were catherized; after 15 min of perfusion, the first 25 ml of perfusate was discarded. The hindlimb was perfused for 60 min at 37°C in a recirculating system with 125 ml of Krebs-Ringer bicarbonate buffer containing 4% BSA, 30% washed human erythrocytes, 11 mM glucose, 5 U heparin, 1.1 mCi [^{35}S]methionine plus 0.2 mCi [^{35}S]cysteine, and all amino acids at the concentration observed in rat plasma (14). The perfusate was maintained at pH 7.4 and gased with O_2/CO_2 (19:1). Oxygen uptake was monitored by measuring arteriovenous PO_2 difference with blood gas analyzer and was 30 to 35 Torr.

After 60 min of perfusion the soleus muscle was removed and homogenized in 0.025 M ammonium buffer, pH 8.2, containing 5 U/ml, 10 μg/ml leupeptin, 1 μg/ml pepstatin, 25 KIU/ml, 5 nM EDTA, 0.8% Triton X-

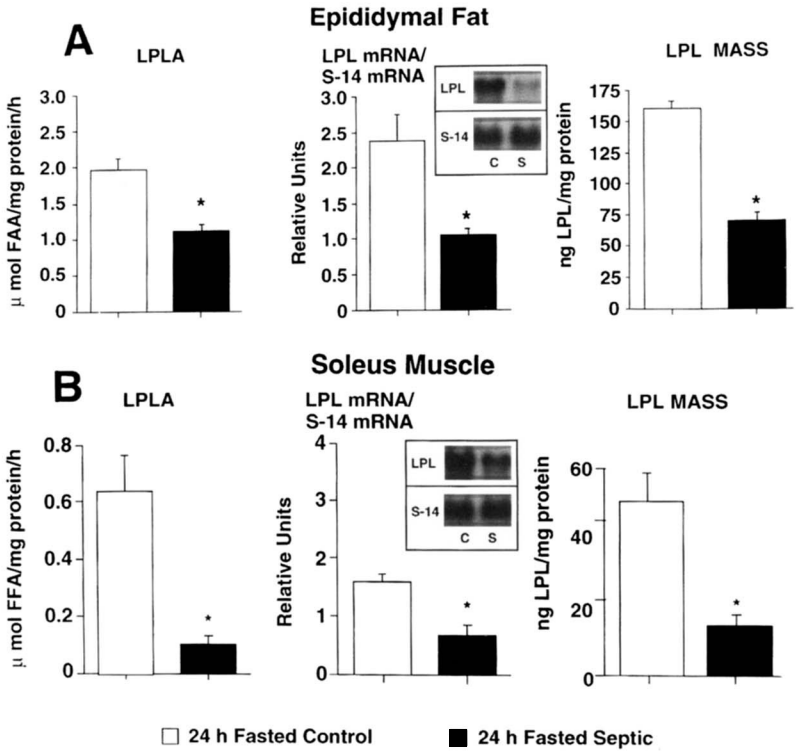

Fig. 1. LPL activity, lipoprotein lipase mRNA/S-14 and LPL mass: A, epididymal fat and B, soleus muscle from control (c) and septic (s) rats after 24 h of fasting. Total LPL activity was measured on tissue homogenates; Northern blot hydridization was used to measure LPL relative message and LPL mass was determined by enzyme-linked immunoadsorbent assay. Bars are means ± SEM (n = 7–10 for epididymal fat and n = 5–7 for soleus muscle); * means significantly different from control values ($P < 0.05$).

120, and 0.04% SDS, and centrifuged in a microfuge at 10,000 rpm. The supernatant was processed as described above for the epididymal fat tissue.

Statistical analysis

All data were expressed as the mean ± standard error of the mean (SEM). The means of control and septic groups were compared by a two-tailed Student's *t* test. *P* values less than 0.05 were considered significant.

RESULTS

Sepsis alters LPL activity, LPL mRNA, and LPL mass

To determine whether the reduction in LPL activity could be attributed to decreased LPL mRNA or LPL mass, we compared LPL mRNA levels and LPL protein in adipose tissue and soleus muscle from control and septic rats fasted for 24 h. **Figure 1A** shows, as we have observed in previous reports (1, 2), that LPL activity declined significantly by 45% ($P \leq 0.05$) in epididymal fat from 24-h fasted septic rats. The autoradiographic images obtained from the Northern blot analysis of the isolated RNA using the LPL cDNA were quantitated by scanning densitometry. The expression of β-actin, which is frequently used as a standard mRNA for Northern analysis, was increased in epididymal fat and soleus muscle from septic rats. To be certain of equal loading of the gel, the blot was hybridized with cDNA for S-14. The signal for the S-14 was not regulated by sepsis in epididymal fat (Fig. 1A) or in soleus muscle (Fig. 1B) from control and septic rats. Based on these findings the values obtained by the densitometer for LPL mRNA were normalized to those obtained for S-14 mRNA. Figure 1A shows that the relative levels of LPL mRNA and LPL mass decreased by 57% ($P \leq 0.05$) in epididymal fat from 24-h fasted septic rats compared with their control rats.

Figure 1B shows the effect of sepsis for 24 h in fasted rats on LPL activity, LPL mRNA levels, and LPL mass in soleus muscle. The activity of LPL in the soleus muscle from 24-h fasted septic rats was reduced by 76% ($P \leq 0.05$) compared with the fasted control rats. Analysis of the Northern blot indicated that the decreases in LPL activity in the soleus muscle were similar to the reductions in LPL mRNA levels and LPL mass which suggests that sepsis also regulates LPL in soleus muscle at the mRNA level. LPL mRNA normalized to S-14 mRNA in soleus muscle from septic rats was reduced by 65% ($P \leq 0.05$); the mass decreased by 70% ($P \leq 0.05$) in comparison to the fasted control rats.

Effect of sepsis on transcription

To elucidate the possible mechanism causing the decrease in LPL mRNA levels in adipose tissue from septic

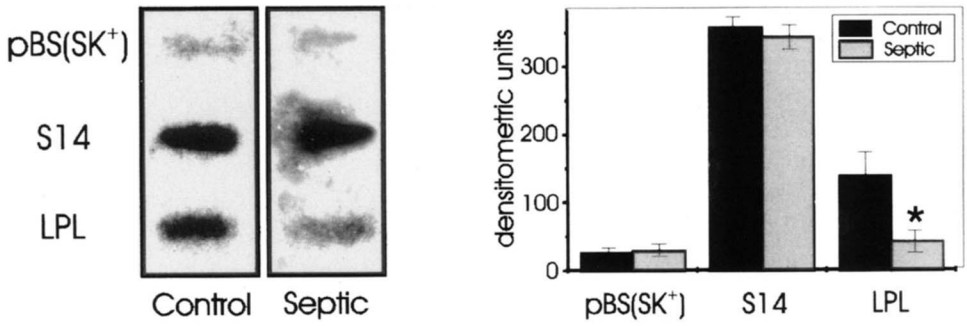

Fig. 2. Transcription of the LPL gene in adipose tissue from 24 h fasted control and septic rats. Epididymal adipose tissue from two rats were pooled and nuclei were isolated as described in the Methods section. The radiolabeled mRNA was hybridized to slot blots prepared with linearized plasmids for LPL, S-14, and pBluescript. Autoradiographs, as shown in the inset, were quantitated by scanning densitometry and LPL was normalized to S-14. Bars are means ± SEM for 3 separate experiments; * means significantly different from control value ($P < 0.05$).

rats, in vitro transcription of the LPL gene was measured. Nuclear run-on assays were performed on adipose nuclei from control and septic rats in the presence of [^{32}P]UTP and the rate of LPL transcription was normalized to S-14. As **Fig. 2** illustrates, sepsis reduced LPL transcription rate by 70% ($P \leq 0.05$), as determined by densiometric scanning, in comparison to the control rats. Transcription rate of the control, S-14, was not altered; hybridization to the negative control, p Bluescript, was at low levels. This decrease in LPL gene transcription rate was sufficient to account for the observed reductions in adipose tissue LPL activity, LPL mRNA levels, and LPL mass.

Effect of sepsis on LPL synthesis in epididymal fat

To determine whether the decrease in LPL activity and LPL mass were due to an effect on translation, the synthesis of LPL was measured by pulse-labeling pieces of adipose tissue from 24-h fasted control and fasted septic rats with [^{35}S]methionine for selected time periods. **Figure 3** shows that total protein synthesis, as determined by TCA precipitation, was linear in epididymal tissue from both groups; incorporation of radioactivity into total protein was greater in septic rats. Rates of LPL synthesis at the 15-min pulse, measured by immunoprecipitation, were significantly ($P \leq 0.05$) lower in the epididymal fat from the septic rats (19.8 ± 2.1 cpm/mg × 10^3) than the control rats (28.9 ± 4.5 cpm/mg × 10^3). In contrast, total protein synthesis in the epididymal tissue from the septic rats (48.0 ± 0.9 cpm/mg × 10^6) increased 49% ($P \leq 0.01$) above the control rats (32.2 ± 2.9 cpm/mg × 10^6). Thus, the decrease in LPL synthesis cannot be accounted for by a reduction in total protein synthesis which indicates that the effect of sepsis is specific for LPL.

The relative rate of LPL synthesis (incorporation of [^{35}S]methionine into LPL as a percentage of radioactiv-

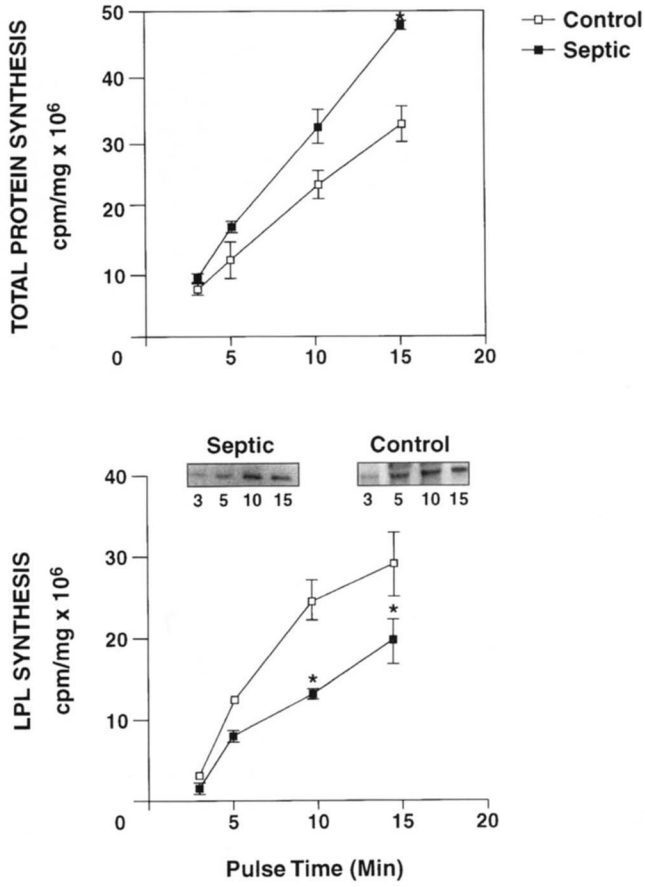

Fig. 3. Effect of sepsis on synthesis of total protein and LPL in epididymal fat from 24 h fasted control and fasted septic rats. Pieces of tissue were pulse-labeled with [^{35}S]methionine for the indicated times and the incorporation of radioactivity into TCA-precipitable protein (top panel) and into immunoprecipitable LPL (bottom panel) was measured. The inset shows a fluorogram of one of five representative experiments; * means significantly different from control values ($P < 0.05$).

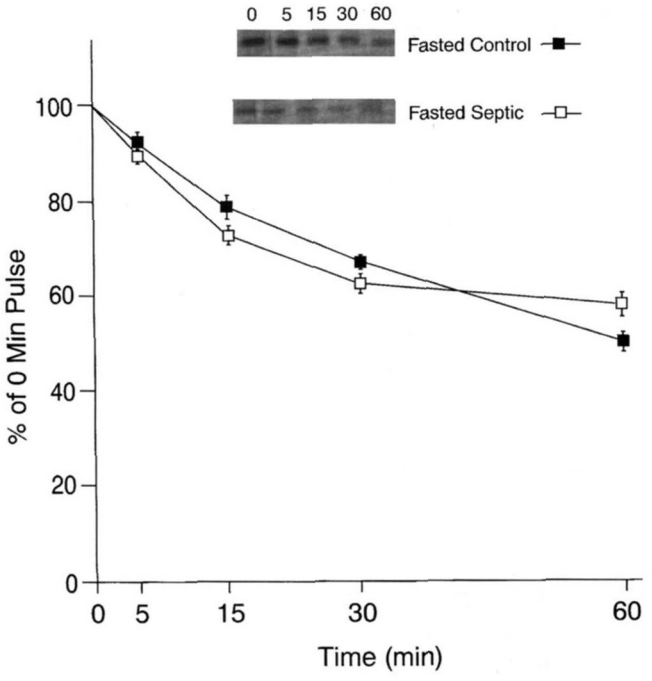

Fig. 4. Effect of sepsis on the degradation of LPL in epididymal fat from 24 h fasted control and fasted septic rats. Pieces of tissue were pulse-labeled with [^{35}S]methionine for 15 min and then chased for 60 min with unlabeled methionine. The inset shows the fluorogram of the immunoprecipitated LPL of the pulse (0 time) and each time point of the chase. One of four representative experiments is shown.

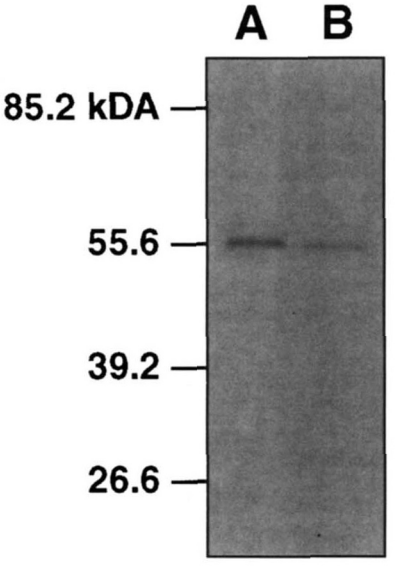

Fig. 5. Effect of sepsis on the incorporation of [^{35}S]methionine plus [^{35}S]cysteine into immunoprecipitable LPL in the soleus muscle from 24 h fasted control and fasted septic rats. The left lower limb was perfused continuously for 60 min with [^{35}S]methionine plus [^{35}S]cysteine; the soleus muscle was removed, minced, and homogenized for immunoprecipitation. A representative fluorogram is shown after 1 week exposure. Lane A: soleus muscle LPL from fasted control rat; lane B: soleus muscle LPL from fasted septic rat. One of four typical experiments is shown.

ity in total TCA-precipitable protein) was lower in epididymal fat from septic rats (0.042 ± 9%) than control rats (0.084 ± 6%) which was due to a combination of an increase in total protein synthesis and decrease in LPL synthesis.

It is also possible that an increase in the rate of degradation could account for the decrease in activity and protein mass of LPL. To answer this question, pieces of adipose tissue were pulsed with [^{35}]methionine for 15 min and then chased with medium containing unlabeled methionine for 120 min. As shown in **Fig. 4,** the degradation rate was not altered. The apparent half-life of the 15 min pulse-labeled LPL was 62 min and 66 min for adipose tissue from the fasted control rats and fasted septic rats, respectively.

Effect of sepsis on LPL synthesis in soleus muscle

To determine whether the reduced levels of LPL mRNA in the soleus muscles from 24-h fasted septic rats were associated with reduced synthesis of LPL, we measured the synthetic rate of immunoprecipitable LPL and TCA-precipitable proteins in soleus muscle by perfusing the lower left hindlimb for 60 min with [^{35}S]methionine plus [^{35}S]cysteine. The incorporation of labeled substrates into total proteins was not altered in the soleus muscles from the fasted septic rats (3.57 ± 0.84 cpm/g × 10^6) compared to the fasted control rats (3.94 ± 0.77 cpm/g × 10^6), which was similar to the observations of Cooney et al. (16). **Figure 5** shows that the incorporation of labeled substrates into immunoprecipitable LPL was less in the soleus muscle from the fasted septic rats (3856 ± 82 cpm/g) than the control rats (6802 ± 167 cpm/g). LPL relative synthesis in soleus muscle from septic rats (0.111 ± 0.01%) was lower than controls (0.193 ± 0.03%). Thus, the decrease in LPL synthetic rate observed in the soleus muscles from the septic rats was not due to a decrease in total protein synthesis but the effect of sepsis was specific for LPL synthesis.

Effect of feeding during sepsis on LPL activity, LPL mRNA, and LPL mass

Feeding is known to increase LPL activity in adipose tissue (17–19) while in skeletal muscle LPL activity may increase or remain the same (18, 20–22). Rats were fed by continuous intravenous feeding for 3 days prior to inducing sepsis and feeding was continued during the 24 or 48 h of sepsis. Feeding during sepsis resulted in decreased adipose LPL activity and LPL mass by 45% ($P \leq 0.05$), but LPL mRNA levels were not altered (**Fig. 6A**) LPL synthetic rate was measured after incubating the adipose tissue for 15 min with [^{35}S]methionine. In-

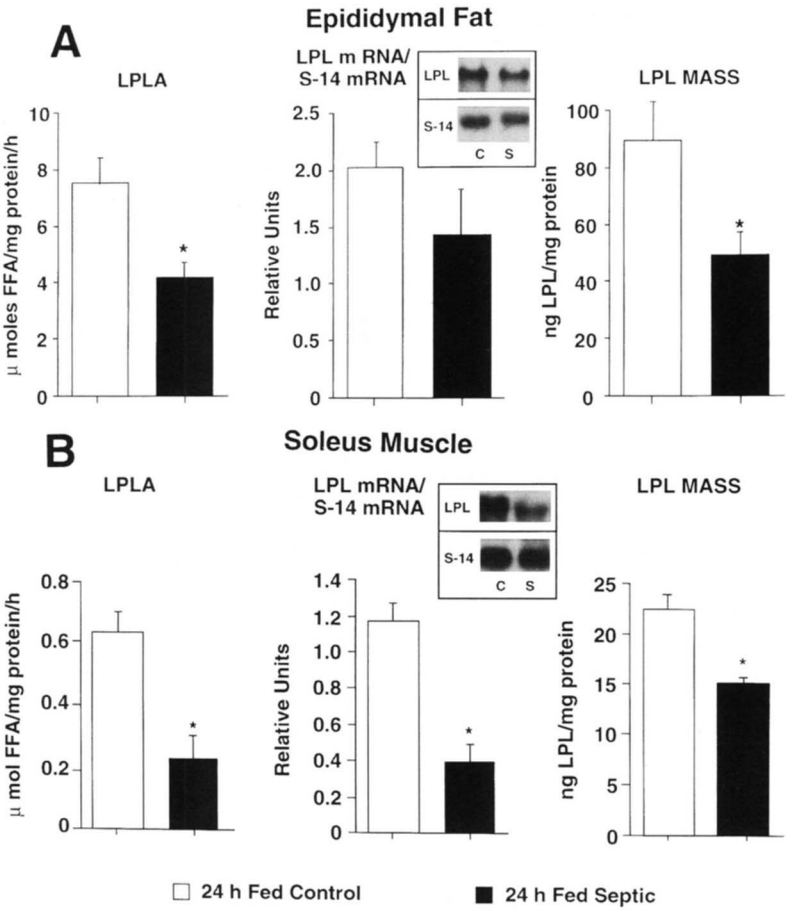

Fig. 6. LPL activity, LPL mRNA/S-14 mRNA, and LPL mass, A, in epididymal fat and B, in soleus muscle from control and septic rats after 24 h of feeding. The inset shows a Northern blot from which the figure was derived. See Fig. 1 for details. Bars are means ± SEM (n = 8–10 for epididymal fat and n = 5–6 for soleus muscle); * means significantly different from control values ($P < 0.05$).

corporation of label into total protein (C = 73 ± 12 cpm/mg × 10^6, S = 88 ± 6 cpm/mg × 10^6) and immunoprecipitable LPL (C = 25 ± 5 cpm/mg × 10^3, S = 23 ± 2 cpm/mg × 10^3) was not altered, which confirmed that the decrease in LPL activity and mass were not due to a reduction in synthesis. The effect of sepsis on LPL regulation in soleus muscle also was evident during feeding; LPL activity, LPL mRNA, and LPL mass were reduced by 64%, 66%, and 28% ($P \leq 0.05$), respectively (Fig. 6B).

We wanted to observe whether a longer duration of sepsis would have an effect on the gene expression of LPL. Rats were made septic for 48 h and fed during this period as described above. After 48 h of sepsis, adipose tissue LPL activity and LPL mass decreased by 56% and 41%, respectively; there was no significant change LPL mRNA (**Table 1**). The longer duration of sepsis produced similar changes in soleus muscle; LPL activity decreased by 56%, LPL mRNA levels by 45%, and LPL mass by 29%. These changes in soleus muscle LPL were similar to those observed after 24 h of fed sepsis, which indicated that the duration of sepsis did not alter the initial regulatory effect.

DISCUSSION

The sepsis-induced suppression of LPL activities in adipose tissue and skeletal muscle is associated with impaired clearance of TG which eventually leads to hypertriglyceridemia. We examined the effect of sepsis on the gene expression of LPL in adipose tissue and soleus muscle from rats during the fasting and fed state. This is the first study to demonstrate that sepsis decreases adipose tissue LPL transcriptionally, leading to the suppression of LPL mRNA levels and LPL synthesis. Reductions in skeletal muscle LPL activity have been reported in septic patients (22), endotoxin-treated rats (23) and mice (24), and septic rats (1, 2) with elevations in plasma TG. This study shows that the sepsis-induced suppression of LPL activity in soleus muscle was accompanied by reductions in LPL mRNA levels that led to decreases in LPL synthesis and LPL mass. These observations were consistent in both fasted and fed septic rats. Although the transcription rate of LPL was not measured in soleus muscle, these findings strongly suggest transcriptional regulation.

Feeding appeared to modify the sepsis-induced regu-

TABLE 1. Effect of sepsis for 48 h on LPL activity, LPL relative message, and LPL mass in epididymal fat and soleus muscle from fed control and fed septic rats

Tissue	LPL Activity	LPL mRNA/S-14 mRNA	LPL Mass
	μmol FFA/mg pro/h	*relative units*	*ng LPL/mg pro*
Epididymal fat			
Control	7.25 ± 0.97	1.15 ± 0.046	65.6 ± 8.2
Septic	3.15 ± 0.27[a]	1.10 ± 0.016	38.5 ± 3.2[a]
Soleus muscle			
Control	0.57 ± 0.03	1.27 ± 0.062	16.6 ± 1.12
Septic	0.32 ± 0.01[b]	0.70 ± 0.020[b]	11.8 ± 0.47[a]

Animals were injected intravenously with either normal saline (controls) or 4×10^8 live *E. coli* colonies/100 g body weight, and maintained on total parenteral nutrition. Values are means ± SEM for n = 8–10 rats. Total lipoprotein lipase (LPL) activity was analyzed on tissue homogenates; relative LPL mRNA, measured by Northern blot hybridization, and LPL mass were determined by ELISA.

[a] $P < 0.01$.

[b] $P < 0.001$.

lation of LPL in adipose tissue. The decrease in LPL mRNA levels after 24 h of fed sepsis was associated with a high variability and could not be duplicated at 48 h of sepsis. Moreover, LPL synthesis was not altered at 24 or 48 h after sepsis. Gouni et al. (4) also found that the decreased LPL activities in adipose tissue from fed endotoxin-treated rats were not accompanied by a reduction in LPL mRNA or by any change in LPL synthesis in adipose tissue. The decrease in adipose LPL mass observed in our study was likely the result of a decrease in LPL intracellular transport with an increase in degradation rate which may have resulted from alterations in oligosaccharide processing. Together these findings suggest that transcriptional mechanisms may have a limited role in the regulation of adipose LPL when sepsis was induced in the fed state as compared with the fasting state. Additionally, posttranslational factors are likely to be already in place in fed rats when sepsis is induced.

Feeding affects the regulation of LPL in adipose tissue, skeletal muscle, and heart. Several studies have found that feeding has a reciprocal effect on LPL in muscle and adipose tissue by increasing activity in adipose tissue and decreasing activity in muscle (17–21). In this study, feeding parenterally also increased LPL activity in adipose tissue from control and septic rats. The values of LPL activity were comparable in adipose tissue from rats fed the liquid diet parenterally (7.4 ± 0.9 μmol FFA/mg protein/h) and the rats fed a standard pellet diet (8.6 ± 0.9 μmol FFA/mg protein/h). In this study LPL activity in soleus muscle from control rats was not altered by feeding. Yet, LPL mass was lower in the soleus muscle from fed than fasted rats. Ong et al. (21) have also shown that LPL activity in the skeletal muscle was not responsive to feeding. In exercised rats, these investigators (25) observed that feeding led to a decrease in LPL mass in soleus muscle with no change in LPL activity or LPL mRNA levels. As LPL activity was not altered in the soleus muscle with feeding, the difference we observe in LPL mass may indicate that the distribution between the active and inactive forms of LPL is altered with a greater proportion of the inactive LPL in the soleus muscle from the fasted rats. The mechanism for the decrease in LPL mass in soleus muscle in fed compared with the fasted control rats is not known but may be related to changes in posttranslational events such as glycosylation, intracellular transport, binding to heparan sulfate proteoglycans, and degradation (26, 27).

It is well known that sepsis elevates the plasma concentrations of several hormones such as insulin, the catecholamines, and the glucocorticoids which have been shown to regulate LPL. Previously, we reported that insulin concentrations were higher in septic versus control rats (1). Lang, Dobrescu, and Meszaros (28) have reported that the peripheral insulin resistance of sepsis was attributed to impaired glucose uptake by skeletal muscle. The presence of insulin resistance in the soleus muscle may account for the down-regulation of LPL during sepsis. The glucocorticoid, dexamethasone, when incubated with rat adipocytes or injected into rats, decreased LPL activity by reducing LPL mRNA levels and LPL synthesis rates (29). In our septic model, plasma concentrations of corticosterone are elevated at 3 h after sepsis (C = 115 ± 9 ng/ml, S = 460 ± 17 ng/ml), and could mediate the changes in LPL found in this study. The increased plasma concentrations of the catecholamines during sepsis (30) may also be responsible for the down-regulation of LPL. Epinephrine has been shown to lower LPL activity by decreasing LPL synthesis (31).

The suppression of LPL that occurs in sepsis and endotoxemia may be mediated, in part, by several cytokines, in particular tumor necrosis factor (TNF), interferon (INF), leukemia inhibitory factor, interleukin (IL)-1, IL-6, and IL-11 (32). In vivo administration of human TNFα to rats decreased adipose tissue but not skeletal muscle LPL activity (33) and the decline in adipose LPL activity did not occur until 16 h after TNF

administration. In our septic model, we have observed that LPL activity began to decline after 3 h of sepsis in adipose tissue (C = 4.4 ± 0.31 μmol/mg protein/h, S = 2.5 ± 0.27 μmol/mg protein/h, $P \leq 0.05$). In addition to TNF, IL-1, leukemia inhibitory factor, INF-α, and INF-γ decrease LPL activity in adipose tissue but only IL-1 and INF-γ decreased LPL activity in the skeletal muscle (24). Inhibition of TNF with TNF antibodies or IL-1 with IL-1 receptor antagonist in endotoxin-treated mice did not prevent the decrease in LPL activities in adipose tissue or skeletal muscle (24) which indicated that TNF or IL-1 were not mediators in the endotoxin-induced inhibition of LPL activity in these tissues. TNF antibodies also were not effective in preventing endotoxin-induced suppression of human macrophage LPL activity (34). Thus, the effects of sepsis on LPL may not be mediated directly by TNF or IL-1 but by other cytokines and/or neuroendocrine hormones.

The down-regulation of LPL contributes to the hypertriglyceridemia of sepsis by reducing the peripheral uptake of triglyceride-rich lipoproteins. The physiological significance of the hypertriglyceridemia during sepsis has not been elucidated but may be part of the host's defense against infection. Recent reports have provided evidence that chylomicrons and lipoproteins improve survival by binding to endotoxin, increasing their clearance, and facilitating their excretion in bile (35).

In conclusion, these findings indicate that sepsis regulates LPL in adipose tissue and soleus muscle transcriptionally. When sepsis is induced in the fed state, posttranslational factors appear to have a role in regulating adipose LPL in that LPL mRNA and LPL synthesis were not altered.

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