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Recombinant Human Cachectin/Tumor Necrosis Factor but Not Interleukin-1α Downregulates Lipoprotein Lipase Gene Expression at the Transcriptional Level in Mouse 3T3-L1 Adipocytes

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Lipoprotein lipase (LPL) is synthesized primarily in muscle and adipose tissue and by hydrolyzing triglycerides in chylomicrons and very low density lipoprotein allows uptake of the resultant free fatty acids by these tissues. This report describes the cloning of the mouse LPL gene from which probes were derived to study the regulation of LPL synthesis in the 3T3-L1 adipocyte cell culture system. Preconfluent 3T3-L1 preadipocytes had very small amounts of LPL mRNA (<1 pg/μg of RNA). At confluency, LPL mRNA levels increased to 5 to 15 pg/μg of RNA. After insulin and dexamethasone were added, LPL activity and mRNA levels rose in parallel. Peak mRNA levels were reached within 4 to 10 days, achieving LPL mRNA concentrations of 150 to 500 pg/μg of RNA. This represents a 15- to 50-fold increase over confluent cells. Two cytokines known to diminish adipose tissue LPL activity were studied to see how their effects were regulated. Recombinant human cachectin/tumor necrosis factor diminished both LPL activity and LPL mRNA levels. The effect on LPL activity compared with mRNA levels was quicker, at a lower dose, and more complete (95 versus 75% maximum effect). The effect of recombinant human cachectin tumor necrosis factor on LPL mRNA levels was shown by nuclear run-on experiments to be exerted transcriptionally. It was also independent of new protein synthesis. Recombinant human interleukin-1α diminished only LPL activity but not mRNA levels. This study suggests that during times of stress, cytokines secreted by activated macrophages can alter energy balance by affecting transcriptional and posttranscriptional processes in adipocytes.

Lipoprotein lipase (LPL) plays a central role in the metabolism of lipids and lipoproteins. Its main purpose is to hydrolyze triglycerides in triglyceride-rich lipoproteins, which allows uptake of free fatty acids by extrahepatic tissues. The protein has an apparent molecular weight of 60,000, is bound to the luminal surface of capillary endothelial cells, and is activated by apolipoprotein CII (28, 37). A genetic deficiency of LPL exists which is a rare inherited disease that causes type I hyperlipoproteinemia (27). Affected individuals have very high plasma triglyceride levels and can have clinical symptoms including abdominal pain, pancreatitis, and hepatosplenomegaly (15).

The tissue-specific regulation of LPL activity is poorly understood. LPL is synthesized principally in adipose and muscle cells. It is then secreted and found in active form on the capillary endothelia of these tissues. During fasting, enzymatic activity is high in muscle and low in adipose tissue, whereas during the fed state, activity is high in adipose and low in muscle tissue (10). This suggests a possible gatekeeping function for this enzyme in the trafficking of nutrients (13).

Suppression of LPL is thought, at least in part, to be responsible for the cachexia associated with diseases caused by infections or malignancies (3). This phenomenon is due to the selective inhibition of adipose tissue LPL by various cytokines (1, 16) produced by stimulated macrophages (6). One substance that inhibits adipose tissue LPL activity, called cachectin, has been purified and characterized (5). N-terminal sequencing and cDNA cloning revealed the identity of cachectin with tumor necrosis factor (TNF), a protein previously described that causes lysis of certain tumor cells (4). A second cytokine which inhibits LPL activity is interleukin-1α (IL-1α). In differentiated 3T3-L1 adipocytes, recombinant human (rh) cachectin/TNF inhibits LPL activity essentially by 100%, whereas rhIL-1α only has a 60 to 90% suppression. rh cachectin/TNF and rhIL-1α do not inhibit enzyme activity directly, but decrease enzyme mass in adipocytes (32; S. R. Price, S. B. Mizel, and P. H. Pekala, Biochim. Biophys. Acta, in press). In vivo, rh cachectin/TNF was found to decrease adipose tissue LPL activity but to have no effect on LPL in muscle, macrophages, or total plasma (36).

This report describes the cloning of the mouse LPL gene and the use of molecular probes to study its transcriptional regulation in mouse 3T3-L1 adipocytes after treatment with rh cachectin/TNF and rhIL-1α.

MATERIALS AND METHODS

Materials. rh TNF was obtained from Chiron Corp. (Emeryville, Calif.); recombinant material had a specific activity of 3.0×10^7 units per mg. rhIL-1α was the kind gift of Peter Lomedico (Department of Molecular Genetics, Hoffmann-La Roche Inc., Nutley, N.J.). The recombinant material had a specific activity of 2.1×10^7 units per mg. Dulbecco modified Eagle medium (DME) and fetal bovine serum were purchased from GIBCO Laboratories (Grand Island, N.Y.). Dexamethasone was obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.), and bovine insulin was from Calbiochem-Behring (La Jolla, Calif.). Glycerol tri[9,10(*n*)-^{3}H]oleate was purchased from Amersham Corp. (Arlington Heights, Ill.), and unlabeled glycerol trioleate was from Nu Check Prep (Elysian, Minn.). Guanidinium isothiocyanate, formaldehyde (37% acid free), and chloroform were obtained from Fluka Chemie A.G. (Bucha, Switzerland).

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For Southern and Northern (RNA) blotting, Biotrace RP-nylon was purchased from Gelman Sciences, Inc. (Ann Arbor, Mich.). Radioactive nucleotides came from Amersham. Cold nucleotides were obtained from Pharmacia, Inc. (Piscataway, N.J.). Restriction enzymes were purchased from New England BioLabs, Inc. (Beverly, Mass.). All other chemicals used in this study were obtained from Fisher Scientific Co. (Fair Lawn, N.J.) if not otherwise stated. Oligonucleotides were prepared on a nucleotide synthesizer (model 381A; Applied Biosystems, Forest City, Calif.) and purified by the procedure recommended by the company.

Mouse genomic library screening. Two oligonucleotides corresponding to mouse LPL cDNA sequence (bases 511 to 556 and 557 to 602 [18]) were used to screen a mouse genomic library. This library was prepared from total DNA from mice (strain 129), partially digested with *Mbo*I, and cloned into the lambda EMBL 3 *Bam*HI site (gift from R. Costa) (8). A total of 600,000 recombinants were plated, and filters were lifted in duplicate. After the filters were processed and baked (23), they were prehybridized for 12 h at 60°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7.0)–0.1 M Na_2HPO_4-NaH_2PO_4 (pH 7.0)–5× Denhardt solution (0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% Ficoll 400)–0.1% sodium dodecyl sulfate (SDS)–20 mg of denatured salmon sperm DNA (Sigma Chemical Co., St. Louis, Mo.) per ml. Filters were then probed with ^{32}P-end-labeled oligonucleotides (23) in hybridization buffer (same as above containing 10% dextran sulfate) for at least 20 h at 60°C. The filters were washed three times in 2× SSC–0.1% SDS at 55°C for 30 min each and autoradiographed. Duplicate positives were plaque purified (23). To confirm the identity of these clones, genomic fragments were subcloned into M13mp19 and sequenced by the dideoxy method (34). Six overlapping clones spanning 30 kilobases (kb) of genomic DNA were isolated which contain coding sequences identical to the sequences published by Kirchgessner et al. (18) for the mouse LPL cDNA.

Cell culture. 3T3-L1 mouse embryo fibroblasts were obtained from American Type Culture Collection (Rockville, Md.) and cultured as described previously (22, 39). For the studies described herein, cells were plated in 100-mm Falcon petri dishes (Becton Dickinson Labware, Oxnard, Calif.) at a seeding density of 5.0×10^5 cells per dish in DME supplemented with 10% fetal bovine serum, 8.0 μg of biotin per ml, and 4.0 μg of pantothenic acid per ml (this medium is subsequently referred to as DME complete). Cultures were maintained at 37°C in an atmosphere of 5% CO_2, and the medium was changed every 2 days. 3T3-L1 cells were differentiated to form adipocytes by a modification of the method of Rubin et al. (33). Two days after reaching confluence, monolayers of 3T3-L1 cells were exposed to DME complete containing 5.0 μg of bovine insulin per ml and 0.39 μg of dexamethasone per ml. This medium was removed after 48 h, and monolayers were incubated with DME complete containing 50 ng of bovine insulin per ml for an additional 48 h. Thereafter, cultures were maintained in DME complete and used for cytokine stimulation, LPL activity measurement, and RNA isolation at the times indicated for each experiment.

LPL assay. After treatment of the 3T3-L1 cultures by protocols described in the text, cells were assayed for heparin-releasable LPL activity by the method of Nilsson-Ehle and Schotz (29) with the modifications described by Kawakami et al. (17). Briefly, medium was aspirated from cell monolayers and replaced with 4 ml of DME complete containing 10 units of heparin per ml. Cultures were incubated for 60 min at 37°C in an atmosphere of 5% CO_2, at which time duplicate 75-μl samples of the medium were removed from each dish. Each 75-μl sample was mixed with 25 μl of a substrate solution containing 22.6 mM [^{3}H]triolein (1.4 μCi/μmol), 2.5 mg of lecithin per ml, 40 mg of bovine serum albumin per ml, 33% (vol/vol) human serum, and 33% (vol/vol) glycerol in 0.27 M Tris hydrochloride (pH 8.1) and incubated at 37°C for 90 min. One milliunit of enzyme activity is defined as the release of 1 nmol of fatty acid per min.

RNA isolation and quantitation. Total RNA from the 3T3-L1 cells was extracted by a modification of the method of Chirgwin et al. (7). Cell cultures in 100-mm petri dishes were dissolved with 7 ml of cold GTC buffer (4 M guanidinium isothiocyanate, 0.05 M Tris hydrochloride [pH 7.5], 0.01 M EDTA, 2% *N*-lauroylsarcosine, 1% β-mercaptoethanol) for 2 min. Subsequently, 6 ml of 100% ethanol was added and total RNA was precipitated overnight at −20°C. The RNA was pelleted by centrifugation at 10,000 × *g* for 20 min at 4°C, resuspended in 4 ml of GTC buffer, and again precipitated with 3.5 ml of 100% ethanol. After centrifugation, the RNA pellet was dissolved in 0.8 ml of SDSEB buffer (50 mM Tris hydrochloride [pH 9.0], 100 mM NaCl, 10 mM EDTA, 0.5% SDS), extracted twice with 0.6 ml of phenol, and reextracted with 0.4 ml of chloroform. The top layer was removed, 1 ml of ethanol and 50 μl of 3 M sodium acetate were added, and RNA was precipitated for 12 h at −20°C. Finally, the RNA was pelleted by centrifugation in a microcentrifuge, washed with 100% ethanol, air dried, and suspended in 100 μl of sterile water. Total RNA was quantitated by measuring the A_{260} and the A_{280}. Poly(A)$^+$ RNA was determined by a slot-blot assay in which total RNA was bound to nitrocellulose paper and probed with T4 polynucleotide kinase-labeled oligo(dT)$_{12-18}$ (Schleicher & Schuell, Inc., Keene, N.H.) (14).

LPL mRNA analysis. (i) Northern blotting. Total RNA was electrophoresed on 1% agarose (International Biotechnologies, Inc., New Haven, Conn.) gels containing 6% formaldehyde (23) and blotted onto nylon paper. Prehybridization and hybridization were performed in the same buffer mixtures as described for the library-screening procedure except that all buffers contained 50% formamide. Blots were prehybridized for 6 h at 42°C and then hybridized for 20 h at 42°C in the presence of a probe derived by nick translating an LPL genomic fragment (23). Blots were washed for 30 min in 3× SSC–0.5% SDS at 65°C and after that two times for 30 min each in 0.3× SSC–0.5% SDS at the same temperature. In some experiments, nick-translated mouse actin cDNA was used as a second probe (gift from Bruce Spiegelman) (38) to serve as a control.

(ii) Solution hybridization. The solution hybridization assay for LPL mRNA quantitation was done by the method of Newman et al. (26) with an M13mp19-derived clone (pLPL3171) containing a 119-base-pair (bp) *Bam*HI fragment which spans the region 522 to 641 of the mouse cDNA sequence of Kirchgessner et al. (18). The template was primed with the universal primer (NEB 1211). The probe was synthesized with Klenow fragment to a specific activity of 400 Ci/mmol, using [α-^{32}P]dATP as the labeled deoxynucleotide. Single-stranded probe was isolated by a combination of gel isolation and hydroxyapatite fractionation such that S1 resistance of the probe was less than 1%. The solution hybridization mix used 80 pg of probe in a 100-μl reaction mix which resulted in >90% hybridization when incubated for 60 h at 68°C with total RNA. The hybrid

material was digested with 26 U of S1 nuclease (P-L Biochemicals, Inc., Milwaukee, Wis.) for 2 h at 45°C. Double-stranded material was precipitated by the addition of carrier DNA and 7.5% trichloroacetic acid. The precipitate was collected on glass fiber filters and washed with cold 7.5% trichloroacetic acid. The dried filters were counted in Hydrofluor in a Beckman LS 8100 scintillation counter for 2 min. The amount of RNA in each sample was determined by comparing the counts in the experimental samples with a standard curve derived from the hybridization of the probe to known amounts of the M13 template. Assays were done in duplicate at three different concentrations to ensure that the assay would be in the linear range of the standard curve (42).

Nuclear run-on transcription. The protocol for nuclear run-on transcription experiments was modified from the procedures described by Einat et al. (12) and Leff et al. (21). Nuclei were isolated from 5×10^7 3T3-L1 cells by placing the culture dishes on liquid ice immediately after incubation, removing the medium, washing the cells twice with phosphate-buffered saline, and adding 5 ml of precooled lysis buffer (10 mM Tris hydrochloride [pH 7.5], 5 mM $MgCl_2$, 25 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Nonidet P-40) to each dish. This was pipetted up and down five to ten times, and lysates from equally treated dishes were combined. A nuclear pellet was derived by spinning at $4,000 \times g$ for 5 min, and the supernatant discarded. The pelleted nuclei were resuspended in 5 ml of lysis buffer and carefully layered over 10 ml of nuclear storage buffer (50 mM Tris hydrochloride [pH 7.8], 5 mM $MgCl_2$, 0.1 mM EDTA). After recentrifugation at $2,000 \times g$ for 10 min, the nuclei were resuspended in 0.4 ml of nuclear storage buffer and immediately frozen at −80°C.

For the transcription reaction, 40 μl of the nuclear suspension was incubated with 20 μl of 5× transcription buffer (120 mM Tris hydrochloride [pH 7.8], 50 mM NaCl, 350 mM $(NH_4)_2SO_4$, 4 mM $MnCl_2$, 0.24 mM EDTA)–5μl of heparin (20 μg/μl) (Sigma)–5 μl of NTP-Mix (20 mM ATP, 20 mM UTP, 20 mM GTP)–100 μCi of [α-^{32}P]CTP in a total volume of 100 μl for 45 min at 32°C. After the 45 min, the reaction was stopped by adding 0.4 ml of termination mix. This was prepared by incubating 25 μl of DNase I (1 μg/ml) and 25 μl of proteinase K (1 μg/ml) for 30 min at 37°C, after which 0.35 ml of buffer containing 20 mM Tris hydrochloride (pH 7.8), 10 mM $CaCl_2$, and 10 μg of yeast tRNA was added. After incubation of the transcription reaction with the termination mix for 30 min at 37°C, 50 μl of 0.2 M EDTA, 25 μl of 20% SDS, and 25 μl of H_2O were added. The whole mixture was incubated for 15 min at 37°C, extracted with 0.5 ml of phenol, and precipitated with 600 μl of ice-cold (20%) trichloroacetic acid for 15 min on ice. After centrifugation, the pellet was rinsed twice with ice-cold trichloroacetic acid, dissolved in TE buffer (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA), and ethanol precipitated. The labeled nuclear RNA was then used as a probe in slot-blot assays to assess the LPL transcription rate.

In these assays, unlabeled DNA fragments representing mouse LPL genomic sequences, mouse actin cDNA, M13 single-stranded DNA, and M13 double-stranded DNA were immobilized on nitrocellulose. Baked filters were prehybridized in RNA prehybridization buffer for 4 h and then hybridized at 42°C in the same buffer containing equal counts of radiolabeled RNA from each run-on transcription reaction (5×10^6 cpm) for 24 to 48 h. After hybridization, blots were washed in 2× SSC twice at 37°C, RNase treated for 30 min at room temperature (20 μg of RNase A per ml), washed for another hour in 2× SSC–0.5% SDS at 42°C, and then washed three times for 30 min each at 50°C in 0.5× SSC–0.5% SDS and exposed for 12 to 48 h. Specific signals were quantitated by laser densitometry (LKB Ultrascan XL laser scanner).

RESULTS

Cloning the mouse LPL gene. To study the transcriptional regulation of the LPL gene, we isolated mouse genomic clones. A mouse genomic library in the EMBL 3 lambda bacteriophage vector was screened with two labeled oligonucleotide probes simultaneously. The oligonucleotides were each 45 bases in length and corresponded to adjacent segments of the mouse LPL cDNA sequence reported by Kirchgessner et al. (18). This resulted in the isolation of 10 independent positive clones which actually represented six different overlapping genomic fragments spanning a region of 30 kb. In one of the overlapping regions, a 119-bp *Bam*HI fragment was identified that hybridized to both oligonucleotides and which, on DNA sequencing (34), corresponded to the mouse LPL cDNA (Fig. 1).

Mapping of the cloned genomic DNA was also done with several restriction enzymes. To verify that the mouse LPL gene was single copy and that the genomic clones were from this region and not rearranged, one of the genomic clones was nick translated and, after preassociation (35) with total mouse genomic DNA, used as a probe in Southern blotting analysis with *Eco*RI-digested mouse DNA blotted onto nylon. The banding pattern corresponded to the expected *Eco*RI fragments found by mapping the genomic clones (data not shown).

Figure 1 also indicates which probes were used in this study to analyze and quantitate LPL mRNA from mouse 3T3-L1 adipocytes. The indicated 1.3-kb *Xba*I fragment was shown by sequencing to contain 349 bp of exon (data not shown) and was used to detect LPL mRNA in Northern blotting analysis (Fig. 2). The 119-bp *Bam*HI fragment indicated in Fig. 1 was subcloned into M13 and used to obtain a uniformly labeled single-stranded probe for the solution hybridization assays (see Materials and Methods). This 119-bp fragment is derived entirely from an exon portion of the 1.3-kb *Xba*I fragment.

LPL gene regulation during differentiation of 3T3-L1 cells. 3T3-L1 preadipocytes can be differentiated into adipocytes by growing the cells to confluency and then treating them with insulin and dexamethasone. These cells then express large amounts of heparin-releasable LPL activity (33). In the preconfluent state, small amounts of LPL mRNA were detectable by Northern blotting analysis (<1 pg/μg of RNA). LPL mRNA levels increased significantly when the cells reached confluency (5 to 15 pg/μg of RNA). After the addition of insulin and dexamethasone to confluent cells, both mRNA and LPL activity increased dramatically, reaching a maximum 4 to 10 days after treatment. Figure 3 shows LPL mRNA levels compared with heparin-releasable LPL activity in the culture medium in a typical experiment in which 3T3-L1 cells were differentiated and cultivated for 6 days. During this period, LPL activity and LPL mRNA (150 to 500 pg/μg of RNA) levels were induced 15- to 50-fold as compared with the level in non-hormone-treated confluent cells. LPL mRNA concentrations correlated very well with heparin-releasable LPL activity over the entire time course. At the time of highest LPL activity and mRNA levels, over 90% of the cells contained lipid droplets, indicating efficient differentiation.

rh cachectin/TNF effect on LPL mRNA levels. The addition of rh cachectin/TNF to 3T3-L1 adipocytes at any point

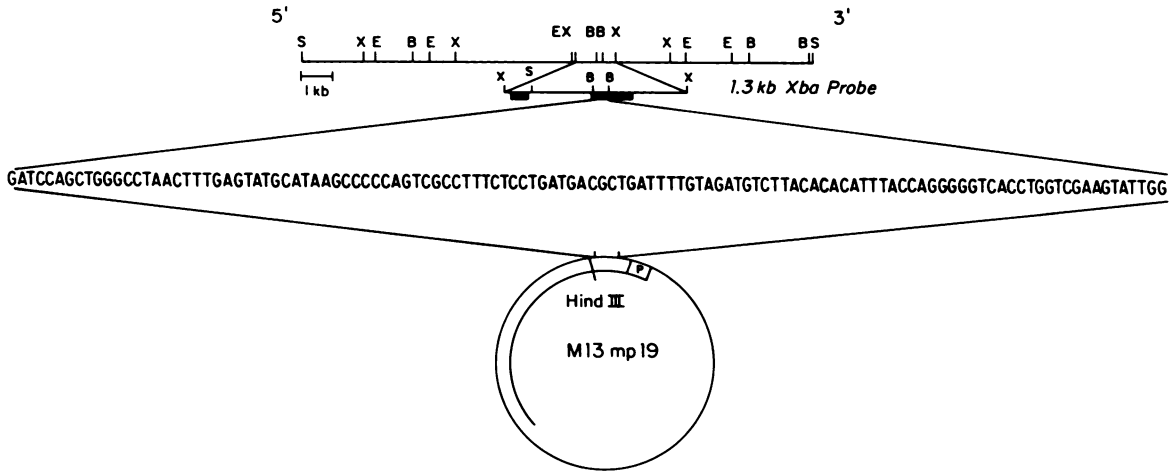

FIG. 1. Map of the mouse genomic LPL clone MG9. The whole clone was cut at the EMBL 3 *Sal*I (S) sites to liberate the insert, which was then digested with *Eco*RI (E), *Bam*HI (B), and *Xba*I (X) either singly or in combination to map these restriction enzyme cutting sites. A 1.3-kb *Xba*I fragment containing two exons of the LPL gene (solid bars) was used as a probe in all Northern blotting experiments. The figure also displays the sequence of a 119-bp *Bam*HI fragment that was subcloned into M13mp19 to produce a uniformly labeled single-stranded probe used in solution hybridization assays (for details see Materials and Methods).

during the entire time course of differentiation decreased LPL activity and mRNA levels (Table 1). The time course of the rh cachectin/TNF effect was studied in maximally differentiated cells (Fig. 3). LPL activity was reduced 50% in approximately 2.5 h and 95% in 12 h. The LPL mRNA level was reduced 50% in approximately 5 h and was reduced a maximum of 75% in 12 h. The more rapid and complete reduction of LPL activity compared with mRNA levels was a consistent finding in all experiments.

The dose response of the rh cachectin/TNF effect on LPL activity and mRNA levels in maximally differentiated 3T3-L1 adipocytes was also studied (Fig. 4). Lower doses of rh cachectin/TNF were required to reduce enzyme activity than to reduce mRNA levels. To achieve a 50% decrease of mRNA levels required more than 10 times as much rh cachectin/TNF. Even at large doses of rh cachectin/TNF (150 ng/ml), LPL mRNA levels were reduced no more than 75%, whereas LPL activity was virtually abolished. LPL mRNA levels measured by solution hybridization were due to full-length LPL transcripts as determined by Northern blotting analysis (Fig. 2). In addition, rh cachectin/TNF had no effect on total RNA, poly(A)$^+$ RNA, or actin mRNA

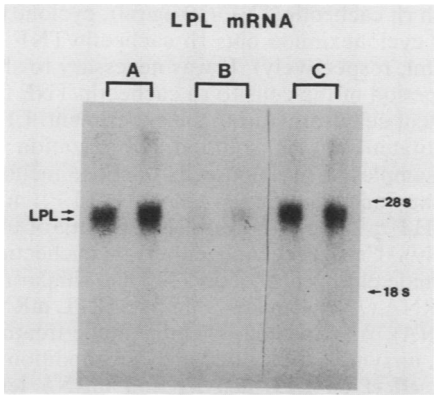

FIG. 2. Northern blot analysis of 3T3-L1 adipocytes 8 days after the induction of differentiation and then treated with or without cytokines for 6 h. Duplicate 10-μg samples of total RNA were electrophoresed on a formaldehyde gel and blotted against the mouse LPL 1.3-kb *Xba*I fragment as indicated in Materials and Methods. The two major LPL mRNA species are indicated by the arrows and are approximately 3.6 and 3.4 kb. The positions of the 28S and 18S RNAs are indicated by the arrows on the right of the figure. Lanes A, RNA from cells receiving no cytokine treatment. Lanes B, RNA from cells treated for 6 h with rh cachectin/TNF at 10 ng/ml. Lanes C, RNA from cells treated for 6 h with rhIL-1α at 10 IU/ml.

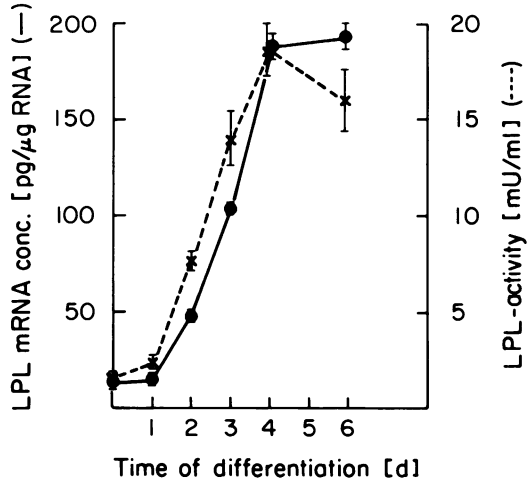

FIG. 3. LPL activity and LPL mRNA concentrations during differentiation of mouse 3T3-L1 cells. Confluent cells were differentiated on day 0 by the addition of insulin and dexamethasone. On the indicated days, heparin-releasable LPL activity was measured and RNA was isolated immediately thereafter from the same cells. LPL mRNA concentration was measured by solution hybridization assay. At each point, two separate dishes were studied. For each dish, duplicate activity assays and triplicate solution hybridization mRNA assays were performed. The indicated result represents the mean ± standard deviation (bars) of the four activity and six mRNA determinations.

TABLE 1. Effect of rh cachectin/TNF on LPL activity and LPL mRNA levels during the time course of 3T3-L1 cell differentiation

Day	LPL activity (mU/ml)[a]		LPL mRNA (pg/μg of RNA)[a]	
	−	+	−	+
0	1.7 ± 0.2	0.8 ± 0.1	13.3 ± 1.9	10.3 ± 3.8
1	2.4 ± 0.3	0.2 ± 0.1	15.0 ± 1.2	10.3 ± 2.1
2	18.4 ± 0.3	4.3 ± 0.6	48.2 ± 3.0	24.4 ± 5.8
3	14.6 ± 1.4	5.5 ± 0.2	103.6 ± 6.6	44.8 ± 5.2
4	20.0 ± 1.3	4.2 ± 0.2	188.1 ± 18.0	81.5 ± 7.5
6	16.0 ± 1.5	2.0 ± 0.4	195.0 ± 19.2	98.6 ± 13.5

[a] LPL activity and solution hybridization assays were performed on cells 16 h after the addition of rh cachectin/TNF (+; −, no cachectin/TNF). Control cultures were maintained during that time in DME complete medium.

concentration. It has previously been shown that TNF treatment of 3T3-L1 cultures causes no general effect on total protein synthesis (31, 32), and polyacrylamide gel electrophoresis reveals that only a few proteins have visibly different amounts (32).

rh cachectin/TNF effect on LPL transcription rate. To test whether the rh cachectin/TNF effect on LPL mRNA concentration was at the level of transcription, we performed nuclear run-on experiments. In these experiments, nuclear RNA was radiolabeled by in vitro RNA elongation while reinitiation of RNA synthesis was prevented. LPL primary transcripts were quantitated by hybridization to an unlabeled LPL genomic fragment immobilized on nitrocellulose in a slot-blot assay. The transcription rate of the LPL gene was normalized to the transcription rate of the actin gene, which is not responsive to rh cachectin/TNF treatment. When cells were treated with rh cachectin/TNF at a concentration of 10 ng/ml for 12 h, LPL-specific nuclear RNA decreased by 68 and 71%, respectively, in two independent experiments (Fig. 5). rh cachectin/TNF did not appear to change the transcription of the mouse actin gene. These results are in excellent agreement with the quantitative data obtained from the solution hybridization assay for total LPL mRNA in similarly treated cells.

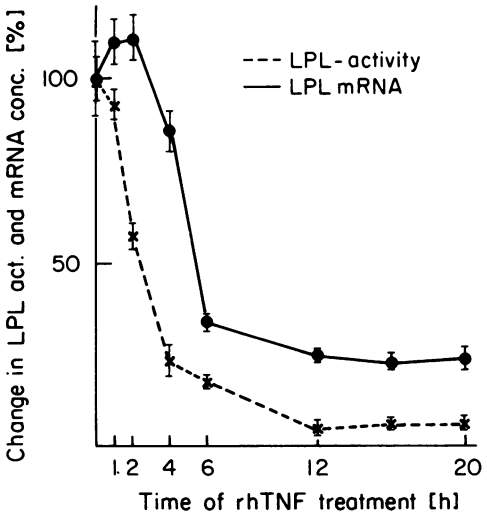

FIG. 4. Time course of LPL activity and LPL mRNA levels in response to rh cachectin/TNF. Thirteen days after the induction of differentiation, 3T3-L1 adipocytes were treated with 10 ng of rh cachectin/TNF per ml at time zero. At the indicated time points, LPL activity measurements and solution hybridization assays were performed as described in the legend to Fig. 3. The indicated percent change was derived from the values of treated cells compared with untreated cells. Bars, standard deviation.

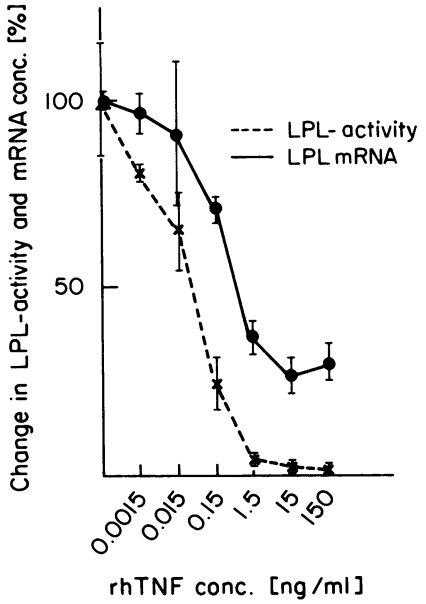

FIG. 5. Dose response of LPL activity and LPL mRNA levels to increasing amounts of rh cachectin/TNF. Nine days after the induction of differentiation, 3T3-L1 cells were treated with the indicated concentrations of rh cachectin/TNF. The cells were analyzed for LPL activity and mRNA concentrations 18 h after the addition of the cytokine as described in the legend to Fig. 3. The indicated percent change was derived from values of treated cells compared with untreated cells. Bars, Standard deviation.

To study whether the rh cachectin/TNF effect on LPL mRNA levels was dependent on active protein synthesis, we exposed 3T3-L1 adipocytes to rh cachectin/TNF in the presence of cycloheximide. Differentiated cells were treated for 6 h with rh cachectin/TNF (10 ng/ml), cycloheximide (10 μg/ml), or cycloheximide plus rh cachectin/TNF (10 μg/ml and 10 ng/ml, respectively). It was necessary to shorten the exposure period of the cells to rh cachectin/TNF from 12 to 6 h to prevent cell death during the experiment. LPL mRNA was quantitated by the solution hybridization assay on duplicate samples. Untreated cells or those incubated with only cycloheximide had similar amounts of LPL mRNA (118 ± 14 and 114 ± 22 pg of LPL mRNA per μg of total RNA, respectively). Cells receiving either rh cachectin/TNF or cycloheximide plus rh cachectin/TNF had similar reductions of LPL mRNA (77 ± 9 and 69 ± 15 pg of LPL mRNA per μg of total RNA). As expected, cycloheximide treatment abolished LPL enzyme activity under these conditions.

rhIL-1α effect on LPL activity and mRNA level. Since rhIL-1α had also been shown to suppress LPL activity (1), we studied its mechanism of action. The time course of the rhIL-1α effect in maximally differentiated 3T3-L1 adipocytes is shown in Fig. 6. LPL activity was reduced 50% in approximately 3 h and to a maximum of 75% in 8 h. There was no significant effect of rhIL-1α on LPL mRNA levels (Fig. 2). The rhIL-1α dose-response curve is shown in Fig. 7. LPL activity decreased more than 50% at an rhIL-1α concentration of 0.01 IU/ml, whereas no significant reduction of LPL mRNA levels was seen even at 1,000 IU/ml. The maximum reduction of LPL activity was 85%. Even at the

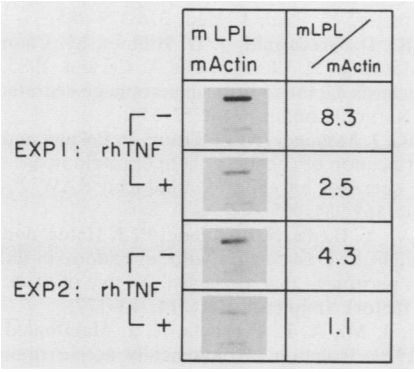

FIG. 6. Nuclear run-on transcription of untreated and rh cachectin/TNF (10 ng/ml)-treated differentiated 3T3-L1 adipocytes. Cells differentiated for 9 days were combined from 10 plates, and nuclei were isolated (for details, see Materials and Methods). The in vitro-^{32}P-labeled nuclear RNA was hybridized to the 6-kb *Bam*HI mouse genomic LPL fragment located 5' to the 119-bp *Bam*HI region (Fig. 1), and a 0.3-kb mouse actin cDNA fragment was immobilized on nitrocellulose. To demonstrate specificity of the observed signals, the labeled nuclear RNA was also hybridized to M13mp19 double-stranded and single-stranded DNA and pBR322. None of these controls gave detectable signals (data not shown). Hybridization to other LPL genomic fragments gave results identical to the one displayed. The numbers were obtained from scanning specific signals and dividing the LPL signal by the actin signal.

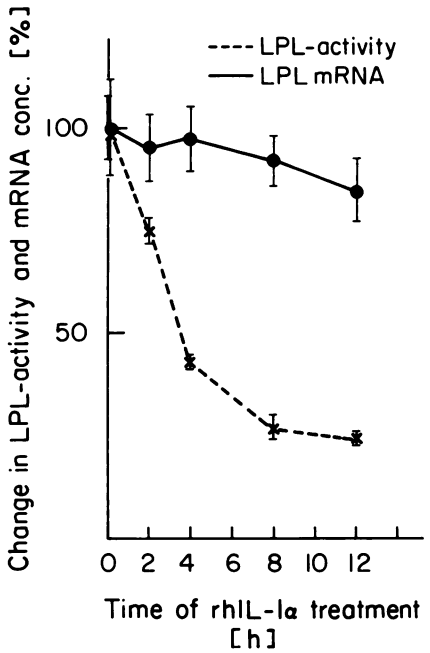

FIG. 7. Time course of LPL activity and LPL mRNA levels in response to rhIL-1α. 3T3-L1 adipocytes differentiated for 9 days were treated with rhIL-1α at a concentration of 19 IU/ml at time zero. At the indicated time points, LPL activity assays were performed and RNA was isolated. LPL mRNA levels were deduced from Northern blotting analysis. Total 3T3-L1 RNA (10 μg) was separated by electrophoresis on formaldehyde agarose gels and blotted onto nylon. These blots were probed with a nick-translated genomic LPL probe and an actin cDNA probe. LPL mRNA levels were then determined densitometrically and normalized to the actin signal. Each point represents the mean ± standard deviation (bars) of duplicate determinations from two separate dishes.

highest dose, rhIL-1α had no effect on total RNA, poly(A)$^+$ RNA, or actin mRNA concentration. Therefore, the observed suppression of LPL activity of rhIL-1α does not appear to be regulated at the level of mRNA.

DISCUSSION

The LPL gene was cloned from a mouse genomic library, and probes to detect LPL mRNA were isolated. The LPL probes were used to investigate LPL gene regulation in mouse 3T3-L1 adipocytes. LPL mRNA increased steadily upon the induction of differentiation in 3T3-L1 cells and reached maximal levels in 10 days. The 15- to 50-fold induction of mRNA reflected the same pattern seen for the induction of enzyme activity. Experiments were performed on the 3T3-L1 adipocytes with rh cachectin/TNF and rhIL-1α, two cytokines previously shown to decrease LPL activity in these cells (2). In agreement with previous results, we found that both cytokines decreased LPL activity, but we showed here for the first time that they act, at least in part, by different mechanisms. rh cachectin/TNF decreased both LPL activity and mRNA levels, whereas rhIL-1α decreased only LPL activity. rh cachectin/TNF decreased LPL activity by 95% and mRNA by only 75%. The LPL mRNA response required more cachectin/TNF and a longer time to decrease levels to 50% compared with what was necessary for the same reduction of LPL activity. In nuclear run-on experiments, we showed that the rh cachectin/TNF effect in LPL mRNA level was exerted by regulating transcription but not the stability of the mRNA. Thus, cachectin/TNF appears to affect LPL activity both by decreasing LPL gene transcription and by another posttranscriptional mechanism. The effect of rhIL-1α on LPL activity appears to be completely posttranscriptional. The current study did not allow us to determine whether the posttranscriptional mechanisms for the two cytokines are the same or different, but it is apparent that full-length transcripts are present, thus indicating control at the level of translation, protein modification, and/or translocation, all of which are necessary for LPL activity as measured in our experiments. Price et al. (32) have shown that cachectin/TNF treatment of 3T3-L1 cells does not affect the size of LPL after 17 h of treatment (indicative of altered glycosylation) and that there is no apparent change in the half-life of the protein activity.

Changes in transcriptional activity after cachectin/TNF treatment as described in this paper for the LPL gene have also been shown for several other genes. In other murine adipocyte systems, glycerophosphate dehydrogenase, adipsin, and the aP2 mRNA levels are reduced by cachetin/TNF treatment of the differentiated cells (25, 40). For c-*myc* (20, 43), cachectin/TNF mediated a decrease in mRNA and the transcription rate was found to be independent of active protein synthesis, as shown here for LPL. This suggests a direct effect of cachectin/TNF or the cachectin/TNF-cachectin/TNF receptor complex either on the transcription complex itself or in activating preexisting cellular factors. In contrast, the class I HLA gene expression is increased by cachectin/TNF, and this was shown to depend on the synthesis of β_2-interferon (19, 24).

Interestingly, in vivo cachectin/TNF downregulation of LPL activity and protein mass occurs only in adipose tissue but not in heart muscle, skeletal muscle, brain tissue, or macrophages (36). Since all these cells express cachectin/TNF receptors (5, 9, 41), adipocytes must have a postreceptor mechanism to regulate LPL activity not shared by these other cell types. This could be due, in part, to tissue

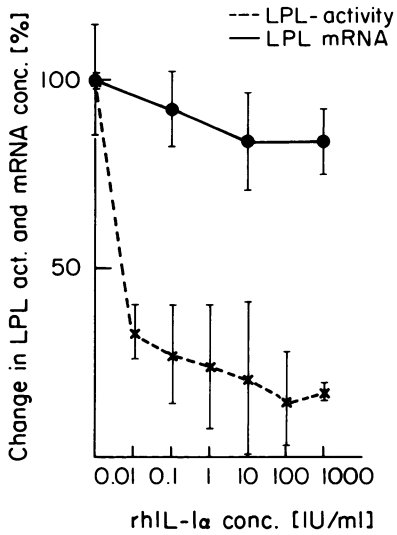

FIG. 8. Dose response of LPL activity and LPL mRNA levels to increasing amounts of rhIL-1α. 3T3-L1 cells differentiated for 9 days were treated with the indicated concentrations of rhIL-1α and analyzed for LPL activity and mRNA concentrations 18 h after the addition of the cytokine as described in the legend to Fig. 3. The indicated percent change was derived from values of treated cells compared with untreated cells. Bars, Standard deviation.

differences in *trans*-acting factors that regulate LPL gene transcription.

Finally, the possible physiological significance of the downregulation of adipose tissue LPL by cachectin/TNF and IL-1 should be discussed. It has been speculated that in endotoxic shock the organism reacts by liberating cytokines to diminish triglyceride storage and make lipid, an excellent energy-producing substrate, available. This may be part of a cytokine-coordinated strategy to fight this stress situation. In agreement with this are the previous demonstrations that cachectin/TNF both increases intracellular lipolysis (30), enhancing triglyceride mobilization, and inhibits adipose tissue glycerophosphate dehydrogenase, promoting triglyceride storage (11). It has also been reported that cachectin/TNF inhibits acetate uptake into fat tissue (30). While these effects of cachectin/TNF may be beneficial during the acute response to endotoxin, prolonged action may lead to cachexia as observed after prolonged exposure.

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