Cachectin/tumor necrosis factor decreases human adipose tissue lipoprotein lipase mRNA levels, synthesis, and activity

Susan K. Fried and Rudolf Zechner

Laboratory of Human Behavior and Metabolism and Laboratory of Biochemical Genetics and Metabolism, Rockefeller University, New York, NY 10021

Abstract The effects of the cytokine cachectin/tumor necrosis factor (TNF) on human adipose tissue lipoprotein lipase (LPL) were studied. TNF is produced by activated macrophages and is thought to play a role in mediating hypertriglyceridemia and wasting of adipose tissue triglyceride stores (cachexia) that often accompany infection and malignancy. TNF effects were studied in human adipose tissue fragments maintained in organ culture in the presence of insulin and dexamethasone to induce high LPL activity. Addition of TNF to the culture medium for 20 h caused a dose-dependent inhibition of LPL activity to an average of 37% of controls at 50 U/ml TNF. This inhibition of LPL activity was explained by specific decreases in levels of LPL mRNA (to 40% of controls) and rates of LPL synthesis determined by biosynthetic labeling and immunoprecipitation (to 32% of controls). The decline in LPL synthesis was specific, as it occurred despite a small increase in overall protein synthesis in the presence of TNF. Comparable decreases in LPL activity were observed when TNF was added to adipose tissue cultured solely in the presence of insulin. Thus, similar to results in rodent models, TNF is a potent inhibitor of LPL gene expression in human adipose tissue. TNF may therefore play a role in the disorders of triglyceride catabolism and the pathogenesis of cachexia that occur with stimulation of the immune system in humans. —**Fried, S. K., and R. Zechner.** Cachectin/tumor necrosis factor decreases human adipose tissue lipoprotein lipase mRNA levels, synthesis, and activity. *J. Lipid Res.* 1989. **30:** 1917–1923.

Supplementary key words fat cell • adipocyte (human) • cytokine • cachexia

Infection and malignancy are often accompanied by cachexia, with depletion of adipose tissue stores of triglyceride (1, 2). This catabolic state is characterized by increased circulating triglycerides, which has been attributed, at least in part, to a marked inhibition of adipose tissue lipoprotein lipase (LPL) activity (1). LPL catalyzes the hydrolysis of circulating triglycerides to free fatty acids that can be taken up, esterified, and stored in adipose cells (3). Thus, this enzyme plays a key role in the regulation of lipoprotein catabolism and of adipose cell size. Cytokines, including the macrophage secretory product cachectin/TNF, are thought to play an important role in mediating the inhibition of LPL activity in cachexia since they have been shown to have a suppressive effect on lipoprotein lipase activity in cultured 3T3-L1 mouse adipocytes (4, 5) and upon administration in vivo to animals (6, 7). TNF suppresses LPL activity in 3T3-L1 adipocytes by specifically decreasing LPL gene transcription (8) and therefore levels of LPL mRNA and rates of LPL synthesis (4, 5, 9). However, a recent report could demonstrate no effect of TNF on LPL activity in isolated human adipose cells in primary culture, questioning the role of this cytokine in mediating changes in triglyceride catabolism that result from infection and malignancy in humans (10).

We report here that cachectin/TNF produces a dose-dependent marked suppression of LPL activity in human adipose tissue maintained in organ culture in the presence of insulin or insulin plus dexamethasone. Similar to results in 3T3-L1 adipocytes, TNF decreases LPL activity by acting to suppress its synthesis, at least in part by decreasing levels of LPL mRNA.

MATERIALS AND METHODS

Source of adipose tissues

Human adipose tissue was obtained at surgery (vertical-banded gastroplasty or cholecystectomy) from the epigastric abdominal subcutaneous adipose tissue or by needle aspiration from the abdominal or gluteal region of lean or obese subjects (n = 15). Surgical samples were placed in Medium 199 containing 25 mM HEPES (M199, Gibco, Grand Island, NY) and immediately transported to the laboratory, arriving within 1 h. Needle biopsy samples (typically 2–5 g) were placed in 1 liter of

Abbreviations: TNF, tumor necrosis factor; LPL, lipoprotein lipase; SDS, sodium dodecyl sulfate; mRNA, messenger RNA; cDNA, complementary DNA; rh, recombinant human; TCA, trichloroacetic acid.

sterile 0.9% saline and brought to the laboratory within 15 min. Since results did not vary by source of tissue, all results have been pooled. This study was approved by the Institutional Review Board of the Rockefeller University Hospital.

Organ culture

All subsequent procedures were carried out using sterile precautions under a laminar flow hood. In the case of surgical specimens, tissue was minced into small pieces (< 10 mg each). All samples were washed free of lipid and blood clots with 0.9% saline at 37°C on a 250 μm nylon mesh and placed in organ culture essentially as described by Smith (11). In brief, human adipose tissue fragments were placed in serum-free Medium 199 with Earle's salts, in plastic 100-mm Petri dishes (~0.5 g/15 ml of medium). Tissue pieces floated freely in the medium instead of being "sandwiched" under cover slips as described by Smith (11). Cultures were placed in a humidified incubator and maintained at 37°C under an atmosphere of 5% CO_2. The medium was supplemented with 1 mU/ml insulin (Humulin R, Eli Lilly and Co., Indianapolis, IN) and/or 30 nM dexamethasone (Stetis Laboratories, Phoenix, AZ) and was changed every 2 to 3 days. At day 6 or 7, the medium was changed and samples of adipose tissue were incubated for 20 h with varying concentrations of recombinant human cachectin/tumor necrosis factor (rh TNF) (Chiron Corp., Emeryville, CA; 3×10^7 U/mg) added in the presence of the aforementioned concentrations of insulin and/or dexamethasone.

Measurement of LPL activity

For measurement of heparin-releasable LPL activity, tissue fragments were washed in saline and incubated for 30 min at 37°C in the presence of 5 U/ml heparin in M199. The glycerol-stabilized triolein substrate emulsion of Nilsson-Ehle and Schotz (12) was used as previously described (13). Total tissue LPL activity was measured after detergent extraction basically as described by Iverius and Brunzell (14). After homogenization of tissue fragments (20–50 mg) in 0.2–0.3 ml of extraction buffer (0.5% deoxycholate, 0.2 M Tris, 0.25 M sucrose, 1% bovine serum albumin, 10 U/ml heparin, and 0.02% Nonidet P40, pH 8.3) samples were briefly sonicated and centrifuged for 15 min at 12,000 rpm in an Eppendorf microfuge. Aliquots of the internatant below the fat cake were then diluted 1:15 in detergent-free buffer and 150 μl was mixed with 150 μl of substrate emulsion and the reaction was allowed to proceed at 37°C for 2 h. The final concentration of deoxycholate was held below 0.02% to prevent inhibition of LPL activity. In preliminary experiments it was found that LPL activity in deoxycholate extracts of adipose tissue homogenates assayed in the absence of serum (0–30% of total lipolytic activity) tended to vary among samples from different patients, but not systematically with addition of TNF [range 5–20% in control vs 7–26% TNF]. Preliminary observations also indicate that a similar percentage of LPL activity remains after addition of a rabbit anti-human milk LPL antiserum (see below) which inhibits 100% of LPL activity after heparin-elution. Thus, lipolytic activity in the absence of serum (as a source of apolipoprotein C-II activator) is probably not attributable to LPL activity and LPL activity was calculated by subtracting the lipolytic activity observed in each sample in the absence of serum. One unit of LPL activity is defined as one μmole free fatty acid released per hour (13).

Biosynthetic labeling

Biosynthetic labeling of LPL was carried out on tissue after overnight incubation in the absence or presence of TNF/cachectin, as described above. Aliquots of cultured adipose tissue (300–900 mg) were washed with saline, and placed in methionine-free (or methionine and cysteine-free, as specified) minimal essential medium supplemented with nonessential amino acids (Gibco, Grand Island, NY), the same hormones present during culture in the previous 24 h, and 50 to 100 μCi/ml [^{35}S]methionine and/or [^{35}S]cysteine for 30 to 60 min. At the end of the labeling period, an homogenate (~500 mg tissue/0.5 ml buffer) of the adipose tissue in lysis buffer A (0.5 M NaCl, 0.1 M Na borate, 5 mM EDTA) was prepared in a 1-ml all-glass homogenizer, as described by Price, Olivecrona, and Pekala (4). After centrifugation at 12,000 rpm for 15 min in a microfuge, the internatant below the fat cake was collected. SDS was added so that its final concentration was 1%, and the sample was boiled for 10 min (4). Samples were stored at −80°C and later used for immunoprecipitation. Immunoprecipitation was performed as described by Price et al. (4) using homogenates diluted to 0.1% SDS with buffer A containing 1% Triton-X-100. After preclearing with protein A (Pansorbin, Calbiochem), 20 μl of rabbit anti-human milk LPL or control rabbit serum was added and incubated for 48 h at 4°C. This antiserum was raised against LPL purified to homogeneity from human breast milk and has been shown to be specific (R. Zechner, unpublished results). The immune complex was isolated with Pansorbin, and the pellet was washed 2× with buffer A with 0.1% SDS and 1% Triton-X-100, once with the same buffer with 1% Triton, and once with 10 mM Tris, pH 7.3. After boiling in SDS sample buffer for 5 min, proteins were separated by 10% SDS-PAGE (15). Radioactive bands were identified by fluorography using Enlighting (New England Nuclear). The bands were cut from the gel, digested in 30% H_2O_2 overnight, and radioactivity was quantitated by liquid scintillation counting.

RNA extraction and Northern blotting

Adipose tissue (1–2 g) was homogenized in 5 ml of guanidinium isothiocyanate buffer (1 g/5 ml), centrifuged

for 20 min at 2500 rpm at 4°C, and the clear internatant below the fat cake was used for RNA extraction by a modification of the method of Chirgwin et al. (16) as described by Zechner et al. (8). Total RNA (10–30 μg) was separated by formaldehyde agarose gel electrophoresis and Northern blotting was performed (8). Levels of LPL mRNA were determined by probing blots with a 1.3 kilobase Xba fragment of a mouse genomic LPL clone as described by Zechner et al. (8) except that prehybridization and hybridization were carried out at 32°C. As expected from the high sequence homology between the mouse and human LPL (17, 18), the mouse probe recognized human LPL mRNA. Results were identical when a human LPL cDNA probe (courtesy of Dr. M. C. Schotz) was used. Levels of γ-actin mRNA were determined using a cDNA probe (19). Probes were labeled with ^{32}P by the random primer method using a kit from the Amersham Corporation, (Arlington Heights, IL). For dot blots, total RNA ($\sim$1–4 μg), at three dilutions, was applied to a nylon membrane and probed as described for Northern analysis. After autoradiography, the density of the spots was analyzed by scanning laser densitometry.

Statistical analysis

Comparisons of control and TNF groups were made by paired *t*-test or analysis of variance (for more than one dose) on log transformed values. Systat statistical software for the IBM-AT was used. *P* values less than 0.05 were considered significant.

RESULTS

In agreement with the report of Cigolini and Smith (20), insulin and dexamethasone synergistically increase human adipose tissue lipoprotein lipase activity after 1 week in organ culture. After 6–7 days in culture, average values for heparin-releasable LPL activity (units/g, mean ± SEM, n = 8) were 0.47 ± 0.13 without additions, 3.29 ± 1.3 in the presence of 1 mU/ml insulin, 0.86 ± 0.21 in the presence of 30 nM dexamethasone alone, and 9.7 ± 2.5 with insulin plus dexamethasone (paired *t*-tests indicate all four values differ from each other, $P<0.03$). In preliminary experiments, it was observed that LPL activity declined to very low levels, regardless of hormone supplementation, during the first 1–4 days of culture (not shown). By day 6–7, the combination of the insulin plus dexamethasone induced high and stable levels of heparin-releasable LPL activity, usually higher than those observed in the fresh tissue (+551 ± 254%, n = 6). In agreement with Smith (21), we find that human adipose tissue maintained in organ culture remains viable as indicated by responsiveness to the acute stimulatory effects of insulin on glucose metabolism (not shown). Thus, organ culture presents a suitable model system in which to study the hormonal regulation of human adipose tissue LPL activity.

In order to demonstrate a specific inhibitory effect of TNF on LPL activity, adipose tissue was first cultured in the presence of insulin plus dexamethasone (I + D) for 5 to 7 days to induce high LPL activity, and then various concentrations of TNF, in the presence of I + D, were added. At 50 U/ml TNF, LPL activity declined to an average of 37 ± 11% (mean ± SEM, n = 11) of controls, despite the continuing presence of high doses of insulin and dexamethasone. As shown in **Fig. 1**, incubation with TNF for 20 h produced a dose-dependent inhibition of heparin-releasable LPL activity. Total extractable LPL activity in the tissue, reflecting both intra- and extracellular stores of LPL activity, was decreased to a comparable degree by TNF (**Fig. 2**).

To ascertain whether the presence of dexamethasone was necessary to observe a suppressive effect of TNF on LPL activity, human adipose tissue from abdominal or gluteal subcutaneous adipose tissue was cultured solely in the presence of insulin, or in the presence of insulin plus dexamethasone. Despite much lower control values in the presence of insulin alone compared to the combination of insulin plus dexamethasone, a clear suppressive effect of TNF on LPL activity by TNF was observed under each incubation condition (**Table 1**). In addition, TNF effects were similar in the gluteal and abdominal subcutaneous depots, despite differing initial values. TNF also markedly

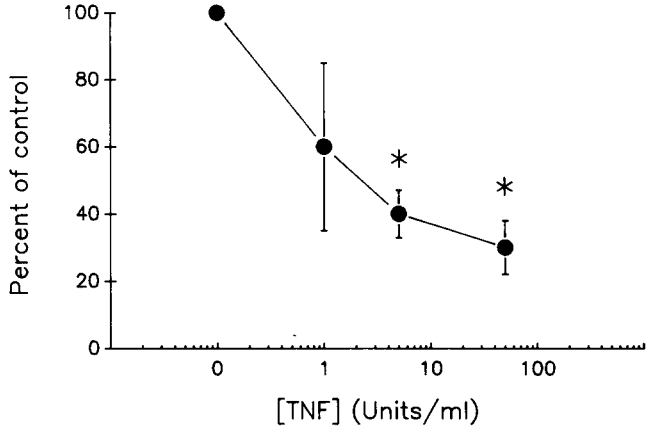

Fig. 1. Inhibition of heparin-releasable LPL activity by rh cachectin/TNF. Adipose tissue fragments were incubated for 6 days in Medium 199 in the presence of 1 mU/ml insulin plus 30 nM dexamethasone and then aliquots were incubated for an additional 20 h in the presence of the same concentrations of insulin and dexamethasone with or without addition of varying concentrations of rh cachectin/TNF. At the end of this period, heparin-releasable LPL activity was measured. Data are presented as mean ± SEM of values calculated as % of control from six patients at doses of 5 and 50 U/ml TNF and three patients studied at the 1 U/ml dose. The x-axis is plotted on a logarithmic scale. LPL activity in the presence of insulin plus dexamethasone was 12 ± 3 (SEM) U/gm, n = 6 and is defined as 100%. Asterisk (*) denotes that effect of TNF was statistically significant ($P<0.01$) by analysis of variance with repeated measures on the actual LPL activities measured at 0, 5, and 50 U/ml. Difference at 1 U/ml did not reach statistical significance.

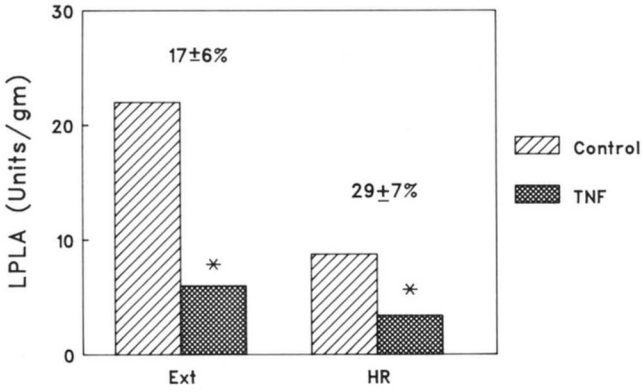

Fig. 2. Comparison of inhibition of total extractable and heparin-releasable LPL activity by TNF in human adipose tissue. Adipose tissue was treated as described in legend to Fig. 1, but the concentration of TNF was 50 U/ml. Both total LPL activity in deoxycholate-treated homogenates as well as heparin-releasable LPL activity were determined in parallel. Data are mean of four independent experiments. Numbers above each set of bars indicates the mean ± SEM of values for samples incubated with TNF compared to controls (100%). $^{*}P<0.01$ vs controls by paired *t*-test on log-transformed values of LPL activities.

suppressed LPL activity in a sample of omental adipose tissue cultured in the presence of insulin plus dexamethasone [heparin-releasable LPL activity: 0.92 (control) vs. 0.25 units/g (50 U/ml TNF)].

The mechanism of the effect of 50 U/ml TNF was investigated. As shown in **Fig. 3**, TNF decreased levels of LPL mRNA measured in Northern blot experiments. This was true when the data were referenced to total RNA or relative to levels of actin RNA. The magnitude of the decrease quantitated by densitometry of dot blots revealed that levels of LPL mRNA were decreased to ~40% of control levels by TNF (**Fig. 4**).

Consistent with the observed decrease in LPL mRNA levels, rates of LPL synthesis were decreased as determined by the incorporation of [^{35}S]methionine plus [^{35}S]cysteine into immunoprecipitable LPL. A fluorograph of a typical experiment is shown in **Fig. 5**. A single

TABLE 1. Inhibition of heparin-releasable LPL activity by TNF/cachectin in human adipose tissue cultured in the presence of insulin

Site	LPL Activity			
	Insulin	Insulin + TNF	I + D	I + D + TNF
	units/g			
Abdominal	0.47	0.17	4.0	2.1
Gluteal	1.76	0.72	9.22	2.4

Data are from one male patient biopsied at two subcutaneous sites, the gluteal and abdominal. Tissue was cultured for 6 days in the presence of 1 mU/ml insulin (I) or 1 mU/ml insulin plus 30 nM dexamethasone (D) and then cachectin/TNF (50 U/ml) was added under the same culture conditions.

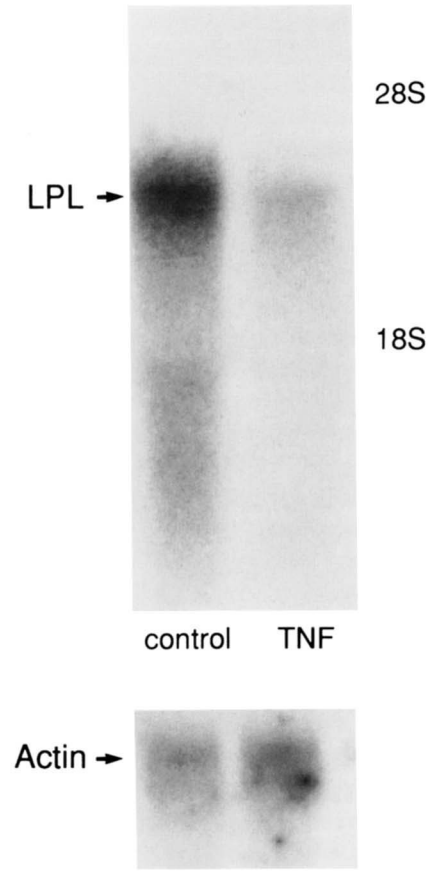

Fig. 3. Northern analysis of LPL mRNA levels. Adipose tissue fragments were cultured as described in the legend to Fig. 1, in the presence or absence of 50 U/ml TNF for the final 20 h. Total RNA (~20 μg) was isolated and electrophoresed on a formaldehyde–agarose gel. LPL mRNA and γ-actin were detected using ^{32}P-labeled mouse genomic LPL fragment or human γ-actin cDNA. Data are from one experiment typical of three performed.

~56 kD protein was immunoprecipitated by the rabbit anti-human LPL antiserum, in agreement with the known molecular weight of human LPL (18). Inspection of the fluorograph clearly shows that TNF decreased rates of LPL synthesis. To quantitate this effect, radioactive bands were cut from the gels and radioactivity was determined by liquid scintillation counting (**Table 2**). In controls, LPL synthesis was 0.02–0.1% of total tissue protein synthesis. A suppressive effect of TNF on rates of LPL synthesis was observed in four independent experiments. The magnitude of the decrease in LPL synthesis was similar using a 30- or 60-min labeling period. Pooling the results from both labeling periods, TNF decreased LPL synthesis to an average of 32 ± 8% (mean ± SEM) of control (n = 4). The inhibitory effects of TNF on LPL were specific, as overall rates of incorporation into total TCA-precipitable protein, calculated per gram of adipose tissue, were slightly enhanced by TNF/cachectin (+20 ± 7%, mean ± SEM, n = 4).

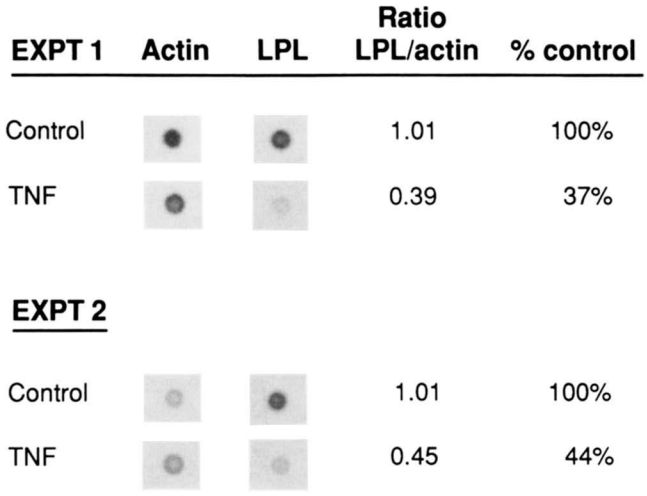

Fig. 4. Dot blot analysis of LPL mRNA levels. Total RNA from adipose tissue fragments was applied to nylon membranes and hybridized as described for Northern analysis. The density of the spots on the autoradiographs was quantitated in arbitrary units by scanning laser densitometry and normalized so that the control was set equal to 1.0 in each experiment. Data from two independent experiments are shown.

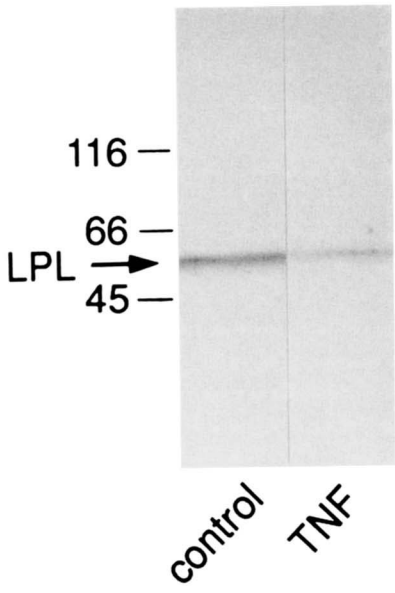

Fig. 5. LPL synthesis. Human adipose tissue was cultured as described in the legend to Fig. 1, in the presence or absence of TNF for 20 h. Tissue was then incubated for 30 min at 37°C in methionine and cysteine-free minimal essential medium plus 100 μCi/ml of [^{35}S]methionine plus 100 μCi[^{35}S]cysteine and the same hormonal additions as present during the final day of culture. After homogenization in the presence of protease inhibitors as described in Materials and Methods, LPL was immunoprecipitated using a specific rabbit anti-human LPL antiserum. Precipitated protein was washed and separated by SDS-PAGE (10% acrylamide), and the gels were fluorographed. No band appeared when preimmune serum was used. Data represent the results of one experiment typical of five performed.

TABLE 2. Effect of cachectin/TNF on LPL synthesis

Patient/ Condition	Labeling Period	CPM	% of Total Protein Synthesis
	min		
1 Control	60	80*	0.025
Cachectin		21	0.006
2 Control	60	96•	0.018
Cachectin		45	0.007
3 Control	30	346	0.040
Cachectin		66	0.006
Control	60	258	0.035
Cachectin		47	0.005
4 Control	30	898	0.103
Cachectin		488	0.050
Control[a]			0.047 ± 0.019
Cachectin[a]			0.017 ± 0.011[b]

Adipose tissue that had been cultured in the presence of 1 mU/ml insulin plus 30 nM dexamethasone for 6 days was then incubated for 20 h in the presence of the same concentration of insulin plus dexamethasone in the absence (control) or presence of 50 U/ml rh cachectin/TNF. At the end of this period, biosynthetic labeling with 50 μCi/ml of [^{35}S]methionine (*) or with 100 μCi/ml of [^{35}S]methionine plus 100 μCi/ml of [^{35}S]cysteine was carried out as described in Methods. After separation of immunoprecipitated proteins by SDS-PAGE, the LPL band was cut out of the gel and counted by liquid scintillation counting. "Background" counts from the same position of nonimmune controls were subtracted. In all cases, equal counts of total protein synthesized determined by TCA precipitation from control and experimental preparations were used for immunoprecipitation.

[a]Mean ± SEM of four patients using pooled data from 30- and 60-min labeling periods, and value from 30-min period for patient #3.

[b]$P < 0.02$ TNF versus control by paired t-test on log-transformed values.

DISCUSSION

These results show that TNF/cachectin, in doses within the physiological range, down-regulates the activity of human adipose tissue LPL in organ culture as it does in 3T3-L1 mouse adipose cells, and in rats, mice, and guinea pigs administered TNF in vivo (4, 5, 6, 8, 9). The range of effective doses of TNF on human adipose tissue LPL activity is similar to that needed to inhibit LPL activity in 3T3-L1 adipocytes (4, 8). The mechanism of the effect of cachectin/TNF on LPL activity in human adipose tissue involves a specific and parallel reduction in levels of mRNA and rates of LPL synthesis. The inhibition of LPL mRNA levels is specific, as actin levels were unchanged in the presence of TNF. Moreover, LPL synthesis was inhibited by TNF despite the fact that TNF caused a 20% increase in overall protein synthesis. The magnitude of the reduction in LPL activity (37%) was quite similar to the decrease in levels of LPL mRNA (~40% of control) and LPL synthesis (32% of controls). Thus, the primary effect of TNF on LPL in human adipose

tissue is apparently at the level of LPL gene expression. In 3T3-L1 adipose cells, the decrease in LPL mRNA levels by TNF is of a similar order of magnitude (8, 9) to that shown here for human adipose tissue and has been shown to involve a decrease in the rate of transcription of LPL mRNA (8). Thus, TNF is a potent inhibitor of human adipose tissue LPL activity and the mechanisms involved are similar to those in other species.

The inhibition of LPL synthesis was similar when measured during a 30- or 60-min labeling period. Thus, it is likely that TNF did not rapidly increase the rate of LPL degradation. This agrees well with data in 3T3-L1 mouse adipocytes (4).

The inhibitory effect of TNF on LPL activity was also present in human adipose tissue cultured solely in the presence of insulin which induces levels of LPL activity 5- to 10-fold lower than the combination of insulin plus dex amethasone. Thus, neither the presence of dexamethasone nor very high initial values of LPL activity are essential to observe a suppressive effect of TNF on LPL activity. It is impossible to assess the effect of TNF on LPL activity in the absence of hormonal inducers or in the presence of dexamethasone alone since, under those conditions, LPL activity rapidly declines to very low levels, usually at the limit of detectability.

The present results also show that organ culture of human adipose tissue by the method of Smith is an appropriate model in which to investigate the molecular mechanisms by which hormones control LPL. Ample adipose tissue for determination of mRNA, biosynthetic labeling, and activity can be obtained from a single needle aspiration. Thus, the method is suitable for studies of LPL regulation in adipose tissue from patients of varying clinical status.

These results are consistent with a role for cachectin/TNF in mediating alterations in lipoprotein metabolism, including hypertriglyceridemia, that occur in patients with infections or cancer (22–24). That TNF plays a role in abnormalities of triglyceride metabolism during infections and cancer is supported by recent reports that documented elevated levels of TNF after endotoxin administration (25), in some cancer patients (26), and in acquired immunodeficiency syndrome (27). Furthermore, infusion of TNF/cachectin produces hypertriglyceridemia in cancer patients (28). Since TNF also stimulates hepatic lipogenesis (29), further investigation will be required to assess the role of the decline in adipose tissue LPL activity in the hypertriglyceridemia associated with these syndromes.

When the immune system is chronically stimulated, cachexia with depletion of fat stores occurs, often despite adequate caloric intake (1, 2, 30). Since exogenous triglycerides are the major source of fatty acids for triglyceride synthesis in human adipose tissue, it is probable that a reduction in adipose tissue LPL activity plays a role in the wasting of fat stores in this syndrome. The lack of effect of TNF on lipolysis in human (31) or rat adipose tissue (32) suggests that the role of TNF in promoting fat wasting in cachexia is primarily by means of its suppression of adipose tissue lipoprotein lipase. Other cytokines may also contribute to the decrease in LPL activity in response to stimulation of the immune system since we have also observed that IL-1α and γ-interferon also decrease human adipose tissue LPL activity (31).

The present results are in contrast to those reported recently by Kern (10) who found no effect of TNF/cachectin on LPL activity or mass in isolated human adipocytes placed in primary culture for 24 h in the absence or presence of 10% serum in the incubation medium. The reason for these discrepant results is not immediately apparent. It is possible that the key difference is the preparation of adipocytes used: tissue fragments in this work compared to isolated adipocytes. However, the source of such a difference is difficult to imagine since LPL is synthesized exclusively by the adipocytes themselves (3) and human adipocytes possess a specific receptor for this cytokine (10). It is possible, though unlikely, that other cell types present in adipose tissue are important in mediating the effect of TNF, or that collagenase, used in the preparation of isolated adipocytes, affects intracellular signalling systems mediating TNF action. The omental adipose tissue used by Kern does not appear to differ from the subcutaneous adipose tissue with regard to responsiveness to TNF effects on LPL activity when a single sample was tested in the organ culture system. A more likely explanation, therefore, lies in the hormonal milieu to which the adipocytes were exposed. In the present work, high levels of LPL activity were first induced by 6–7 days of organ culture in the presence of insulin or insulin plus dexamethasone before addition of TNF for 20 h. Suppressive effects of TNF were seen despite the continuing presence of these hormones, similar to the in vivo situation. In contrast, the human fat cells used by Kern were cultured overnight in the absence of hormones or regulators known to increase LPL activity (10, 20, 33). Thus, it is possible that a reliable TNF/cachectin effect could not be seen against initially low LPL activity and under conditions in which rates of LPL synthesis may have been very low.

In conclusion, we have demonstrated that TNF/cachectin potently suppresses human adipose tissue LPL mRNA levels, rates of LPL synthesis, and LPL activity. Thus, TNF/cachectin may be important in the pathogenesis of hypertriglyceridemia and cachexia associated with infection and malignancy in humans.

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