# Recombinant human tumor necrosis factor does not inhibit lipoprotein lipase in primary cultures of isolated human adipocytes

**Philip A. Kern**

Department of Medicine, Cedars-Sinai Medical Center/University of California at Los Angeles, Los Angeles, CA 90048

**Abstract** Previous studies have demonstrated that cachectin/tumor necrosis factor (TNF) inhibits lipoprotein lipase (LPL) activity in cultures of 3T3-L1 cells. To determine whether TNF also inhibits LPL in human adipocytes, primary cultures of isolated human adipocytes were exposed to a spectrum of concentrations of recombinant human TNF. TNF concentrations up to 1000 pM had no effect on either LPL activity or LPL immunoreactive mass in the human adipocytes. Specific binding of $^{125}$I-labeled TNF was demonstrated in human adipocytes, and a TNF concentration of 100 pM competed for approximately 50% of the $^{125}$I-labeled TNF binding sites. In contrast, the same TNF in the same concentrations progressively inhibited LPL activity and immunoreactive mass in 3T3-L1 cells. Thus, human adipocytes respond to TNF in a different manner than 3T3-L1 cells. TNF may not cause the cachexia of cancer or chronic infection by directly inhibiting LPL in adipose tissue. — **Kern, P. A.** Recombinant human tumor necrosis factor does not inhibit lipoprotein lipase in primary cultures of isolated human adipocytes. *J. Lipid Res.* 1988. **29:** 909–914.

**Supplementary key words** 3T3-L1 cells • cachexia

A contributing factor to mortality from cancer and chronic infection is cachexia. Recent studies on the mechanism of cachexia have focused on the enzyme lipoprotein lipase (LPL) (1–3). LPL is synthesized and secreted by adipocytes, and then bound to glycosaminoglycans on the capillary endothelium, where the enzyme hydrolyzes the triglyceride core of chylomicrons and very low density lipoproteins (4). LPL is important for fat cell lipid accumulation, as adipocytes synthesize little lipid de novo and instead rely on LPL-mediated hydrolysis of circulating triglyceride (5). Thus, any tumor- or infection-related substance that inhibits LPL may generally impair storage of adipose tissue lipid, and thus lead to the clinical condition of cachexia.

In studies over the last few years, endotoxin-stimulated macrophages were found to secrete a substance that inhibited LPL in 3T3-L1 adipocytes (6). The 17,000 dalton protein that was responsible for inhibition of LPL was called cachectin, and was subsequently found to be identical to tumor necrosis factor (TNF) (7). Subsequent studies have demonstrated that the injection of TNF into animals inhibited LPL (8), and caused a syndrome compatible with endotoxemia or cachexia (9).

A central thesis in this hypothesis is the inhibition of LPL by TNF. 3T3-L1 adipocytes respond to TNF in a manner that is very predictable, such that this cell line has been used as a bioassay for cachectin (10). However, 3T3-L1 cells are a heteroploid cell line derived originally from embryonic mouse fibroblasts (11), and may not be representative of the response of LPL in primary cultures. Thus, the experiments described herein were undertaken to determine the effects of recombinant human TNF on LPL in primary cultures of human adipocytes.

## METHODS

### Cells and tissue culture

Human isolated adipocytes were prepared and cultured as described previously (12). In brief, omental adipose tissue was obtained from healthy patients undergoing elective abdominal surgery. The study subjects consisted of 14 women and 1 man between the ages of 24 and 54 years (mean age 38). Subjects were all close to ideal body weight (mean body mass index: 22.4 kg/m$^2$; range: 18.4 to 25.4 kg/m$^2$), did not have malignancies, and were undergoing procedures such as cholecystectomy, hysterectomy, or other gynecologic surgery. The adipose tissue was promptly minced and digested with collagenase II (Cooper Biomedical) and the adipocytes were placed into Medium 199 (Irvine Scientific) containing 10% fetal bovine serum (Hyclone). TNF was then added in the same medium and

Abbreviations: LPL, lipoprotein lipase; TNF, tumor necrosis factor/cachectin; HR, heparin-releaseable LPL activity; EXT, extractable LPL activity.

LPL was measured 24 hr later. The TNF was recombinant human TNF-alpha with a specific activity $2.5 \times 10^7$ units/mg (13), and was a generous gift from Dr. Phillip Koeffler.

Preadipocytes were grown and differentiated as described previously (10). 3T3-L1 cells were passed into 8-$cm^2$ six-well clusters (Costar). After growing to confluence, the preadipocytes were differentiated by the addition, for 48 hr, of Dulbecco's Modified Eagle's medium (DME) containing 10% fetal bovine serum, 10 $\mu$g/ml insulin, 0.5 mM isobutylmethylxanthine, and 1.0 $\mu$M dexamethasone. Cells were then maintained in DME containing 10% serum and 50 ng/ml insulin for 7–10 days. TNF was then added in the same medium to four different wells of the cluster, using two wells as controls. LPL was then measured 24 hr later.

### LPL activity and immunoreactive mass

LPL activity was measured against an emulsified [$^{14}$C]triolein substrate as described previously (12). The heparin-releasable (HR) fraction was prepared by incubating cells in phosphate-buffered saline containing 13 $\mu$g/ml heparin (Fisher Scientific, Los Angeles, CA) for 45 min at 37°C. The extractable (EXT) fraction was prepared subsequent to the preparation of HR by disrupting cells in 0.5% deoxycholate, 0.02% NP-40, 0.73% sucrose, 0.1% albumin, 125 $\mu$g/ml heparin, and 25 mM Tris, pH 8.3. After separating the lipid cake by centrifugation, the aqueous layer was recovered. An aliquot of each sample was incubated with substrate for 45 min at 37°C and the reaction was then stopped by the addition of Belfrage-Vaughn extraction mixture (14). The liberation of $^{14}$C-labeled free fatty acids was quantitated and LPL activity was expressed as nEq of free fatty acids released/min per $10^6$ cells.

LPL immunoreactive mass was measured in an enzyme-linked immunosorbent assay (ELISA) that used affinity-purified antibodies specific for LPL (15, 16). In brief, affinity-purified anti-bovine LPL antibodies (raised in chicken #2271) were bound to microtiter wells, and sample or bovine LPL standards were then added in buffer containing 1 M NaCl, 0.1% Triton X-100 and 0.1% albumin. The wells were then washed, and biotinylated affinity-purified antibody (raised in chicken #2272) was added, followed by streptavidin-peroxidase (Bethesda Research Labs). The peroxidase reaction was developed by adding peroxidase substrate (1 mg/ml *o*-phenylenediamine, 0.012% hydrogen peroxide, in 0.1 M citrate, pH 4.5). The reaction was stopped with 25 $\mu$l of 4 M sulfuric acid, and was read in an ELISA plate reader (Bio-Tek Instruments).

### $^{125}$I-Labeled TNF binding

Recombinant human TNF (Amgen) was iodinated to a specific activity of 46 $\mu$Ci/$\mu$g using the iodogen method, as described previously (17). After culturing cells overnight in Medium 199 containing 10% fetal bovine serum, TNF binding was measured by placing cells into 1 ml of Medium 199 containing 1% albumin and 100,000 cpm of $^{125}$I-labeled TNF at 16°C. Cells were separated from medium and the binding reaction was terminated by rapidly centrifuging triplicate 300-$\mu$l aliquots of cell suspension in microtubes containing 100 $\mu$l of silicone oil, as described previously (18). To control for nonspecific binding, $^{125}$I-labeled TNF binding was carried out in the presence of an excess of unlabeled TNF. As shown in Fig. 2, maximal competition for $^{125}$I-labeled TNF binding was achieved with a TNF concentration of 40 nM. Thus, this level of tracer binding (19% of total binding) was assumed to be nonspecific.

### Statistics

The two-tailed, paired *t*-test was used. Data were expressed as the mean ± SEM.

## RESULTS

Human adipocytes were prepared from 14 different subjects and cultured as described previously (12). To assess the effect of TNF on LPL, a spectrum of TNF concentrations was added to the medium (Medium 199 containing 10% fetal bovine serum) and cells were cultured for 24 hr. Both LPL activity and LPL immunoreactive mass were measured in the heparin-releasable (HR) and extractable (EXT) fractions. As shown in **Fig. 1,** there was no effect of TNF on either LPL activity or mass in human adipocytes. The slight decrease in HR immunoreactive mass at a TNF concentration of 100 pM was inconsistent and not statistically significant. In addition, a higher concentration of TNF (1000 nM) produced no further effect on LPL (Fig. 1). In two experiments, adipocytes were incubated with TNF for 5 hr, to determine whether a shorter exposure to TNF would affect LPL. Again, no change in LPL was observed with a shorter exposure to TNF.

The cells that were used in these experiments were also used in studies reported previously (16), involving regulation of LPL by serum, adenosine, and insulin-like growth factor-I ($IGF_1$). **Table 1** contains the data from three such experiments in which HR activity and mass were measured in adipocytes exposed to these substances as well as to TNF. LPL activity and mass were stimulated by adenosine, $IGF_1$, and 10% serum. However, the addition of TNF to cells cultured in serum-containing medium yielded LPL levels no different from the LPL levels in cells exposed to 10% serum alone. In other experiments, TNF did not inhibit the relatively low level of LPL in cells cultured without serum (data not shown). Thus, these

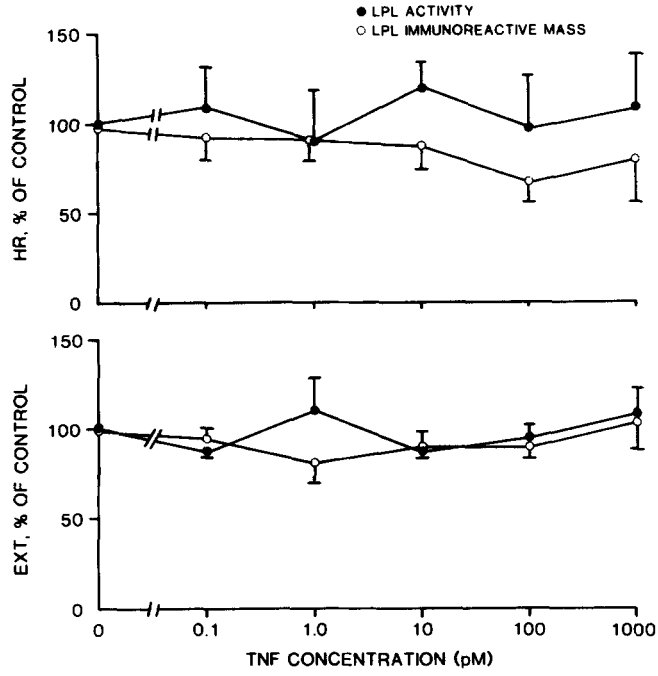


**Fig. 1.** Effect of TNF on LPL in isolated human adipocytes. Cells from 14 subjects were cultured for 24 hr in Medium 199 containing 10% fetal bovine serum and the indicated concentration of TNF. LPL activity and immunoreactive mass were then measured in the HR (A) and EXT (B) fractions as described in Methods. Control LPL activity was 2.06 ± 0.65 and 3.56 ± 1.2 nEq/min per $10^6$ cells for HR and EXT, and control LPL immunoreactive mass was 177 ± 43 and 200 ± 63 ng/$10^6$ cells for HR and EXT, respectively. There was no statistical difference between control and TNF-treated cells.

adipocytes were responsive to the regulatory influences of other substances, but were not affected by TNF.

TNF interacts with cells via specific plasma membrane receptors. To determine that these receptors were present in human adipocytes, the binding of $^{125}$I-labeled TNF to human adipocytes was measured after 24 hr of culture. When the time course of binding was assessed at 16°C, saturation binding occurred by 2 hr (data not shown). Therefore, a 2-hr incubation at 16°C was used for competition-binding experiments.

A tracer concentration of $^{125}$I-labeled TNF was added to approximately $10^6$ cells in the presence of increasing concentrations of unlabeled TNF. Specific binding was determined by subtracting cell-associated radioactivity that was measured in the presence of an excess of unlabeled TNF (see Methods). As shown in **Fig. 2,** increasing concentrations of TNF competed for $^{125}$I-labeled TNF, and 50% of tracer binding was displaced by a TNF concentration of 0.1 nM. Thus, the competition for specific binding suggests the presence of intact TNF receptors.

To determine whether the TNF used in these experiments was indeed bioactive, TNF was added to cultures of 3T3-L1 adipocytes. As shown in **Fig. 3,** progressive inhibition of LPL activity and immunoreactive mass in HR was observed. Only at the highest TNF concentration (100 pM) was there a significant effect on the EXT fraction of 3T3-L1 cell LPL. Thus, the recombinant human TNF employed herein inhibited LPL in 3T3-L1 adipocytes, but had no significant effect on LPL in human adipocytes.

## DISCUSSION

A number of studies have demonstrated effects of TNF and cachectin on various aspects of adipose tissue and lipid metabolism. Both purified cachectin and recombinant human TNF inhibited LPL activity in 3T3-L1 cells (10, 19), and inhibition of LPL synthesis (20) and mRNA level (21) has been demonstrated. Other effects of TNF on 3T3-L1 adipocyte metabolism included inhibition of lipid synthesis (19, 22), a stimulation of hormone-sensitive lipase (19), and inhibition of gene expression of other lipogenic enzymes (23).

Because LPL is important for both adipocyte lipid storage and plasma triglyceride clearance, the inhibition of LPL by cachectin/TNF has been suggested to be part of the etiology of cachexia, and the hyperlipidemia of infections (1–3). This hypothesis was originally based on the effects of TNF in 3T3-L1 cells. More recently, the injection of TNF into rats, mice, and guinea pigs resulted

TABLE 1. Regulation of LPL by other factors

| | Control | | Adenosine[a] | | $IGF_1$[a] | | Serum[a] | | TNF plus Serum[a] | |
|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | $LPL_a$ | $LPL_m$ | $LPL_a$ | $LPL_m$ | $LPL_a$ | $LPL_m$ | $LPL_a$ | $LPL_m$ | $LPL_a$ | $LPL_m$ |
| 1 | 2.95 | 46.4 | 4.02 | 52.4 | 5.13 | 68.7 | 5.42 | 151 | 4.98 | 203 |
| 2 | 0.74 | 44.2 | 1.34 | 129 | | | 2.50 | 114 | 3.68 | 93 |
| 3 | 3.23 | 69.2 | 3.85 | 83.4 | | | 5.13 | 93.0 | 4.32 | 98.1 |

Cells were cultured for 24 hr in Medium 199 in the presence and absence of phenylisopropyladenosine (PIA, a nonmetabolizable analog of adenosine), $IGF_1$, fetal bovine serum, or TNF, as indicated. Cells marked "control" were incubated in medium alone. HR LPL activity ($LPL_a$) and immunoreactive mass ($LPL_m$) were then measured, and expressed as nEq/min per $10^6$ cells and ng/$10^6$ cells, respectively.

[a]Concentrations: adenosine (PIA), 50 nM; $IGF_1$, 6.5 nM; serum, 10%; TNF, 100 pM.

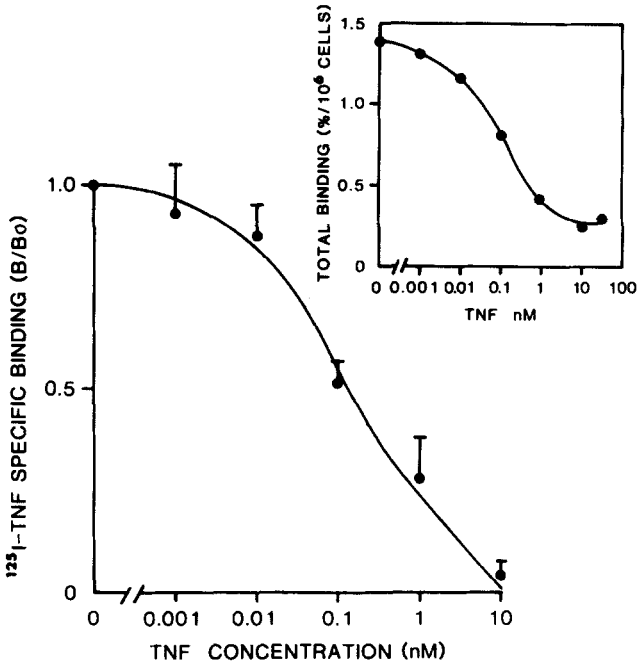


**Fig. 2.** Binding of $^{125}$I-labeled TNF to isolated human adipocytes. Cells were cultured overnight in Medium 199 containing 10% fetal bovine serum. Approximately $10^6$ cells were then exposed to $10^5$ cpm $^{125}$I-labeled TNF in the presence of the indicated concentration of unlabeled TNF for 2 hr at 16°C and cell-associated radioactivity was measured. Specific binding represents total binding minus cell-associated radioactivity in the presence of 40 nM TNF. Data are pooled from three experiments. Insert. Competition for total $^{125}$I-labeled TNF binding from one representative experiment.

in a decrease in adipose tissue LPL activity (8). In another recent study, the TNF gene was transfected into cells and implanted into nude mice, which then developed cachexia, weight loss, and anorexia (24). However, these studies could not determine whether TNF directly inhibited adipose tissue LPL, or whether TNF triggered other events which mediated a decrease in LPL and subsequent adipose tissue wasting. In addition, the anorexia that accompanied TNF-induced cachexia (24) makes interpretation of LPL data difficult, because adipose LPL is sensitive to the level of food intake (5).

Isolated human adipocytes represent a useful in vitro system to test the effects of various substances on LPL at a primary source of enzyme synthesis. In addition, the recent development of a sensitive and specific ELISA for LPL (15, 16) has permitted the study of LPL immunoreactive mass in these cells. Previous studies using this system of adipocytes have demonstrated regulation of LPL by serum, $IGF_1$, glucose, adenosine, and inosine (12, 16, 25), and adipocyte cultures from three different subjects herein demonstrated regulation of LPL by several of these substances. Therefore, there was every reason to expect that these cells would be responsive to TNF in vitro, yet no effect of TNF was observed.

Recombinant human TNF was added to adipocyte cultures from 14 normal subjects. The concentrations of TNF were the same as in previous studies on 3T3-L1 cells (10, 19), in which maximal inhibition of LPL activity occurred with a TNF or cachectin concentration of 100 pM. When human adipocytes were cultured in medium containing TNF concentrations between 0.1 pM and 1000 pM, there was no effect on LPL activity or LPL immunoreactive mass. In contrast, when the same TNF in the same concentrations was added to cultures of 3T3-L1 cells, a dose-response effect was obtained that was similar to that reported by others (10, 19). Thus, TNF was ineffective at inhibiting LPL in this system of human adipocyte primary cultures. In addition to their responsiveness to TNF, human adipocytes and 3T3-L1 cells differed in their relative proportions of HR and EXT activity and immunoreactive mass. Whereas HR and EXT activity and mass were approximately the same in the human adipocytes, EXT activity was higher than HR in the 3T3-L1 cells, yet EXT immunoreactive mass was lower than HR mass (see legends to Figs. 1 and 3). These data suggest that there may be important differences between these two cell types with regard to LPL synthesis, processing, and degradation.

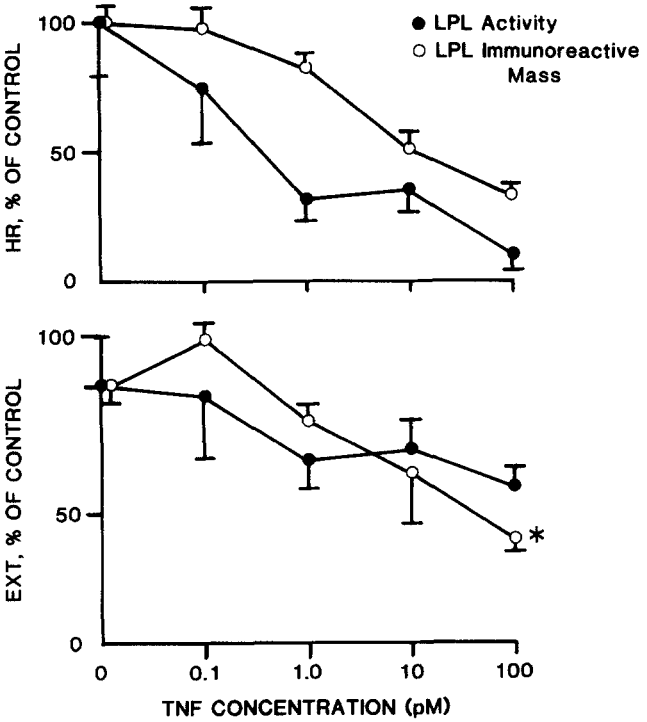


**Fig. 3.** Effect of TNF on LPL in 3T3-L1 adipocytes. Cells were cultured and differentiated as described in Methods. TNF was added in the indicated concentrations for 24 hr, and LPL activity and immunoreactive mass were measured as the HR and EXT fractions. Control LPL activity was 1.12 ± 0.16 and 7.43 ± 0.93 nEq/min per $10^6$ cells, and LPL immunoreactive mass was 501 ± 29 and 203 ± 11.2 ng/$10^6$ cells for HR and EXT, respectively. $^*P < 0.05$ compared to control.

Because human adipocytes are studied 24 hr after a collagenase digestion, TNF receptors may have been damaged. To assess this, $^{125}$I-labeled TNF binding was studied. Specific binding was demonstrated, and the competition for specific binding resembled that reported previously for 3T3-L1 cells (19, 26). Thus, these data suggest that TNF receptors on human adipocytes were intact.

Several recent observations suggest that the etiology of cachexia in humans may involve a number of cytokines, and not only TNF. White et al. (27) have reported that TNF had no effect on LPL in human monocyte-macrophages. In addition, patients who have been treated with TNF have not become cachectic (28). Although plasma TNF concentrations may reach nanomolar concentrations in animal models of sepsis or cachexia (24, 29), patients with malignancies have very low plasma TNF concentrations (30). There are numerous other cytokines that may be important to tumor- and endotoxin-associated cachexia. Both gamma-interferon and interleukin 1 inhibited LPL in 3T3-L1 cells (30, 31), and interferon treatment of cancer patients caused hypertriglyceridemia and decreased postheparin plasma LPL activity (30). Finally, TNF has been shown to stimulate hepatic very low density lipoprotein production (32), thus providing an additional etiology for the hyperlipidemia of endotoxemia. Thus, TNF may affect lipid metabolism in humans in a different manner than in cell lines and in animals.

In summary, primary cultures of human adipocytes were cultured in medium containing recombinant human TNF, in parallel with cultures of 3T3-L1 cells. Whereas TNF inhibited LPL in the 3T3-L1 cells in a dose-response fashion similar to that reported by others, TNF had no effect on LPL in the human adipocytes, in spite of the presence of specific TNF binding. The mechanism of cachexia in humans is likely complex, and may involve inhibition of LPL in adipose tissue by a number of humoral factors. Although TNF may begin a cascade of events that results in a decrease in adipose tissue LPL, these data suggest that TNF does not directly inhibit LPL in human adipocytes.

I wish to thank the many surgeons in the Departments of Surgery and Obstetrics and Gynecology at Cedars-Sinai Medical Center for their cooperation in obtaining adipose tissue. In addition, I thank John Ong for performing the iodination of TNF, and Angela Mandic, Robert Martin, and Joanne Carty for technical assistance. The recombinant human TNF was kindly supplied by Dr. Phillip Koeffler, and the 3T3-L1 cells were supplied by Dr. Robert Williams. The anti-LPL antibodies for the ELISA were supplied by Dr. John Goers. This work was supported by NIH grant AM-HL 37085, and a Career Development Award from the Juvenile Diabetes Foundation.

*Manuscript received 9 September 1987, in revised form 27 January 1988, and in re-revised form 29 February 1988.*

## REFERENCES

1. Beutler, B., and A. Cerami. 1987. Cachectin: more than a tumor necrosis factor. *N. Engl. J. Med.* **316:** 379-385.
2. Bagby, G. J., and P. H. Pekala. 1987. Lipoprotein lipase in trauma and sepsis. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener Publishers, Inc., Chicago. 247-276.
3. Beutler, B., and A. Cerami. 1986. Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature.* **320:** 584-588.
4. Garfinkel, A. S., and M. C. Schotz. 1987. Lipoprotein lipase. *In* Lipoproteins. A. M. Gotto, Jr., editor. Elsevier, Amsterdam. 335-354.
5. Eckel, R. H. 1987. Adipose tissue lipoprotein lipase. *In* Lipoprotein Lipase. J. Borensztajn, editor. Evener Publishers, Inc., Chicago. 79-132.
6. Kawakami, M., P. H. Pekala, M. D. Lane, and A. Cerami. 1982. Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induced mediator from exudate cells. *Proc. Natl. Acad. Sci. USA.* **79:** 912-916.
7. Beutler, B., D. Greenwald, J. D. Hulmes, M. Chang, Y. C. Pan, J. Mathison, R. Ulevitch, and A. Cerami. 1985. Identity of tumour necrosis factor and the macrophage-secreted factor cachectin. *Nature.* **316:** 552-554.
8. Semb, H., J. Peterson, J. Tavernier, and T. Olivecrona. 1987. Multiple effects of tumor necrosis factor on lipoprotein lipase in vivo. *J. Biol. Chem.* **262:** 8390-8394.
9. Tracey, K. J., B. Beutler, S. F. Lowry, J. Merryweather, S. Wolpe, I. W. Milsark, R. J. Hariri, T. J. Fahey III, A. Zentella, J. D. Albert, G. T. Shires, and A. Cerami. 1986. Shock and tissue injury induced by recombinant human cachectin. *Science.* **234:** 470-474.
10. Beutler, B., J. Mahoney, N. Le Trang, P. Pekala, and A. Cerami. 1985. Purification of cachectin, a lipoprotein lipase-suppressing hormone secreted by endotoxin-induced RAW 264.7 cells. *J. Exp. Med.* **161:** 984-995.
11. Green, H., and M. Meuth. 1974. An established pre-adipose cell line and its differentiation in culture. *Cell.* **3:** 127-133.
12. Kern, P. A., S. Marshall, and R. H. Eckel. 1985. Regulation of lipoprotein lipase in primary cultures of isolated human adipocytes. *J. Clin. Invest.* **75:** 199-208.
13. Munker, R., J. Gasson, M. Ogawa, and H. P. Koeffler. 1986. Recombinant human TNF induces production of granulocyte-monocyte colony-stimulating factor. *Nature.* **323:** 79-82.
14. Belfrage, P., and M. Vaughn. 1969. Simple liquid-liquid partition system for isolation of labeled oleic acid from mixture with glycerides. *J. Lipid Res.* **10:** 341-344.
15. Goers, J. F., M. E. Petersen, P. A. Kern, J. Ong, and M. C. Schotz. 1987. Enzyme-linked immunoassay for lipoprotein lipase. *Anal. Biochem.* **166:** 27-35.
16. Kern, P. A., J. M. Ong, J. F. Goers, and M. E. Petersen. 1988. Regulation of lipoprotein lipase immunoreactive mass in isolated human adipocytes. *J. Clin. Invest.* **81:** 398-406.
17. Aggarwal, B. B., T. E. Eessalu, and P. E. Hass. 1985. Characterization of receptors for human tumor necrosis factor and their regulation by gamma interferon. *Nature.* **318:** 665-667.
18. Marshall, S., and J. M. Olefsky. 1979. Effects of lysosomotropic agents on insulin interactions with adipocytes. Evidence of a lysosomal pathway for insulin processing and degradation. *J. Biol. Chem.* **254:** 10153-10160.
19. Patton, J. S., H. M. Shepard, H. Wilking, G. Lewis, B. B.

Aggarwal, T. E. Eessalu, L. A. Gavin, and C. Grunfeld. 1986. Interferons and tumor necrosis factors have similar catabolic effects on 3T3-L1 cells. *Proc. Natl. Acad. Sci. USA.* **83:** 8313–8317.

20. Price, S. R., T. Olivecrona, and P. H. Pekala. 1986. Regulation of lipoprotein lipase synthesis by recombinant tumor necrosis factor: the primary regulatory role of the hormone in 3T3-L1 adipocytes. *Arch. Biochem. Biophys.* **251:** 738–746.
21. Zechner, R., T. C. Newman, B. Sherry, A. Cerami, and J. L. Breslow. 1987. Tumor necrosis factor regulates lipoprotein lipase mRNA levels in 3T3-L1 adipocytes. *Arteriosclerosis.* **7:** 538 (abstract).
22. Pekala, P. H., M. Kawakami, C. W. Angus, M. D. Lane, and A. Cerami. 1983. Selective inhibition of synthesis of enzymes for de novo fatty acid biosynthesis by an endotoxin-induced mediator from exudate cells. *Proc. Natl. Acad. Sci. USA.* **80:** 2743–2747.
23. Torti, F. M., B. Dieckmann, B. Beutler, A. Cerami, and G. M. Ringold. 1985. A macrophage factor inhibits adipocyte gene expression: an in vitro model of cachexia. *Science.* **229:** 867–869.
24. Oliff, A., D. Defeo-Jones, M. Boyer, D. Martinez, D. Kiefer, G. Vuocolo, A. Wolfe, and S. H. Socher. 1987. Tumors secreting human TNF/cachectin induce cachexia in mice. *Cell.* **50:** 555–563.
25. Kern, P. A., A. Mandic, and R. H. Eckel. 1987. Regulation of lipoprotein lipase by glucose in primary cultures of isolated human adipocytes: relevance to the hypertriglyceridemia of diabetes. *Diabetes.* **36:** 1238–1245.
26. Aggarwal, B. B., and T. E. Eessalu. 1987. Effect of phorbol esters on down-regulation and redistribution of cell surface receptors for tumor necrosis factor-alpha. *J. Biol. Chem.* **262:** 16450–16455.
27. White, J. R., A. Chait, S. J. Klebanoff, and J. D. Brunzell. 1987. Regulation of macrophage lipoprotein lipase production by bacterial lipopolysaccharide is independent of tumor necrosis factor (cachectin). *Clin. Res.* **35:** 160 (abstract).
28. Old, L. J. 1987. Polypeptide mediator network. Tumour necrosis factor. *Nature.* **326:** 330–331.
29. Beutler, B. A., I. W. Milsark, and A. Cerami. 1985. Cachectin/tumor necrosis factor: production, distribution, and metabolic fate in vivo. *J. Immunol.* **135:** 3972–3977.
30. Kurzrock, R., M. F. Rohde, J. R. Quesada, S. H. Gianturco, W. A. Bradley, S. A. Sherwin, and J. U. Gutterman. 1986. Recombinant gamma interferon induces hypertriglyceridemia and inhibits post-heparin lipase activity in cancer patients. *J. Exp. Med.* **164:** 1093–1101.
31. Beutler, B. A., and A. Cerami. 1985. Recombinant interleukin 1 suppresses lipoprotein lipase activity in 3T3-L1 cells. *J. Immunol.* **135:** 3969–3971.
32. Feingold, K. R., C. Grunfeld, A. H. Moser, S. R. Lear, and B-J. Huang. 1987. Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. *J. Clin. Invest.* **80:** 184–190.