Multiple Effects of Tumor Necrosis Factor on Lipoprotein Lipase in Vivo*

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A single dose of recombinant murine tumor necrosis factor (TNF) suppressed lipoprotein lipase activity in adipose tissue of fed rats, mice, and guinea pigs for 48 h, even though TNF itself is rapidly metabolized in vivo. Immunoprecipitation of [35S]lipoprotein lipase from fat pads pulse-labeled with [35S]methionine showed a decrease in relative synthesis of the enzyme, which correlated to the decrease in activity. There was no decrease in general protein synthesis and no change in distribution of the enzyme between adipocytes and extracellular locations in the tissue. This is in contrast to fasting in which case there is redistribution of the enzyme within the tissue, decrease in general protein synthesis, but no change in relative synthesis of lipoprotein lipase. TNF did not decrease lipoprotein lipase activity in any tissue other than the adipose but increased the activity in several cases, most markedly in the liver. No [35S]methionine was incorporated into lipoprotein lipase by liver slices from normal or TNF-treated animals. Thus, the increased activity cannot be ascribed to enhanced hepatic synthesis of the enzyme. There was an increase in lipoprotein lipase activity in plasma, which correlated to the increase in liver. Thus, TNF suppresses lipoprotein lipase synthesis in adipocytes, but not in other tissues, and has some as yet undefined effect on lipoprotein lipase turnover in extrahepatic tissues, which results in increased transport of active lipase through plasma to the liver.

Tumor necrosis factor (TNF) is a small protein secreted by macrophages in response to invasive stimuli. TNF was originally identified on the basis of its ability to produce hemorrhagic necrosis of some tumors in vivo and to exert a direct cytotoxic effect on cells in culture (1). It is now clear, however, that TNF mediates other biological effects also (2). It is closely related to, or identical with, cachectin (3), a protein which has profound effects on host metabolism and is believed to be responsible, at least in part, for the cachexia associated with grave infections and neoplastic diseases. One of the effects of cachectin/TNF is to suppress the synthesis (4, 5) and hence the activity of lipoprotein lipase in cultured 3T3-L1 adipocytes (6). With experimentally induced tumors there are changes in lipoprotein lipase activity in several tissues (7), notably the liver (8). These changes are not restricted to tissues directly engaged by the tumor (8), suggesting that they are mediated by humoral factors (9), perhaps TNF.

Fasting is known to decrease lipoprotein lipase activity in adipose tissue (10). An apparent possibility was therefore that TNF and fasting suppress lipoprotein lipase activity by the same mechanism so that in this respect the metabolic state induced by TNF is akin to that during starvation. The aim of this investigation was to explore whether TNF has an effect on lipoprotein lipase activity in a variety of tissues, to compare the effects of TNF with those of fasting, and to explore the mechanisms involved.

EXPERIMENTAL PROCEDURES

Animals—Male guinea pigs (500-700 g), male Sprague-Dawley rats (180-220 g), and male Balb/c mice (20-40 g) were used. For each experiment animals from a single consignment were used. TNF dissolved in phosphate-buffered saline, or saline alone for controls, was injected intraperitoneally without anesthesia. After the injection the animals were returned to their cages with free access to pellets and drinking water. Fasting was for 48 h with access to water only. To obtain plasma, mice and rats were anesthetized with ether, and guinea pigs were anesthetized with Hypnorm Vet* (AB Leo, Helsingborg, Sweden, 1 ml/kg of body weight) and blood was taken from the jugular vein. For all other experiments the animals were killed by a blow on the neck.

Antiserum—The antiserum to guinea pig lipoprotein lipase was the same as that used in previous studies (11, 12). It was raised in a rabbit, using lipoprotein lipase purified from guinea pig milk (13) as antigen. To inhibit lipoprotein lipase in rat and mouse tissues, we used a cross-reaction antiserum raised in a chicken with bovine milk lipoprotein lipase as the antigen (14).

Lipoprotein Lipase Assay—Tissues were homogenized and centrifuged as described (11). The clear solution between a floating fat layer and a sediment was used for assay of lipoprotein lipase activity. Conditions for assay were as described by Peterson et al. (15). The substrate was Intraplipid® (Kabi-Vitrum, Stockholm, Sweden) into which [14C]oleic acid-labeled triolein had been incorporated by sonication (15). Heat-inactivated serum from fasted rats was used as source of activator protein for the lipase (10% by volume of final assay medium for mouse and rat tissues and for guinea pig adipocytes, and 5% for guinea pig tissues). A 20-μl sample was incubated in a total volume of 200 μl at 25 °C, for 1 h or less. Control experiments showed that the assay was linear with the amount of sample and with time over the range used. 1 millilitre = 1 nmol of fatty acid released per min. Adipocytes were prepared by collagenase digestion of epididymal adipose tissue (11). The cells were washed with Krebs-Ringer bicarbonate buffer. The final cell preparation was homogenized and lipoprotein lipase activity was measured as described for tissues. All values in Table 1 represent lipoprotein lipase activities characterized by specific immunoinhibition. For this, a pool of samples from all five animals was prepared for each tissue. Aliquots of these pools were then incubated 2 h on ice with antiserum to lipoprotein lipase or corresponding control serum, before assay of remaining lipase activity (11). In this way we could determine what fraction of the total lipase activity was due to lipoprotein lipase. This factor was then applied to the activities determined for each individual animal.

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† The abbreviations used are: TNF, tumor necrosis factor; SDS, sodium dodecyl sulfate; LPL, lipoprotein lipase.
For heart, lung, and adipose tissue more than 95% of total lipase activity in our assay was inhibited by the antisera. For liver and plasma, where the hepatic, heparin-releasable lipase makes a significant contribution, the percent inhibition by the antisera was less. Most of our studies were in guinea pigs which are previously known to have low levels of the hepatic lipase (16). In this species antisera to lipoprotein lipase inhibited more than 70% of the lipase activity measured in liver homogenates and more than 90% in plasma.

**[^35S]Methionine Incorporation**—To study synthesis of lipoprotein lipase, intact epididymal fat pads from guinea pigs were rinsed at room temperature in medium A (Eagle's minimal essential medium (modified) with Earle's salts and 2 g/liter sodium bicarbonate without glutamine and methionine, supplemented with 5% bovine serum albumin, 1 mM glucose, 10^{-3} M insulin (bovine, Sigma), 0.149 ng/ml methionine, and 1.25 mM Hepes, pH 7.4) (12). The fat pads were then incubated for 30 min at 37 °C, in 4 ml of gassed (95% O_2, 5% CO_2) medium A supplemented with [^35S]methionine (Du Pont-New England Nuclear Scandinavia AB, 1100 μCi/mmol, 45 μCi/ml). To study protein synthesis in adipocytes, the cells were labeled for 15 min under the conditions described above for fat pads. The labeling was stopped by chilling the tubes in crushed ice. Cells and media were separated by centrifugation (30 s at 700 × g). Fat pads, or adipocytes, were homogenized in buffer B (0.025 M ammonia, adjusted with HCI to pH 8.2, containing 5 mM EDTA and, per ml: 8 mg of Triton X-100, 0.4 mg of SDS, 33.3 μg of heparin, 10 μg of leupeptin (Boehringer Mannheim, Federal Republic of Germany), 1 μg of pepstatin (Boehringer Mannheim), and 25 KIU of Trasylol (Sigma)) and immunoprecipitated as described below. Samples of total proteins were processed for electrophoresis as in Ref. 12. To determine total incorporation of [^35S]methionine into proteins, 10 μl of tissue or cell homogenate was applied on a Whatman GF/C filter which was then dropped into ice-cold 10% trichloroacetic acid. The filters were washed and counted in a scintillation counter (12).

**Immunoprecipitation**—Supernatants from homogenates of adipocytes or tissues were made 1% in SDS and heated for 6 min in a water bath at 95 °C. Aliquots were diluted with buffer C (per ml: 3.564 mg of NaHPO_4/2 H_2O, 56 μg of NaN_3, 8.766 mg of NaCl, 10 mg of bovine serum albumin, 1 mg of SDS, 100 μg of heparin, 745 μg of EDTA, and 17.4 μg of phenylmethylsulfonyl fluoride (SERVA Feinbiochemica, Heidelberg, FRG)), pH 7.4, and antisera (or nonimmune control serum) was added (12). The samples were then incubated 15–20 h at 4 °C. Protein A-coated Staphylococcus aureus cells (PANSORBIN, Behring Diagnostics) were added and the precipitates were washed, dissolved, and separated by SDS-polyacrylamide gel electrophoresis as described (12). The gels were either used to obtain fluorograms or to cut the band corresponding to lipoprotein lipase for elution and counting in a liquid scintillation counter (12).

**Materials**—Recombinant mouse tumor necrosis factor was produced in *Escherichia coli* and purified to homogeneity (17). Its specific activity, as measured in a cytotoxicity assay on L929 cells, was shown to be about 7.5 × 10^12 units/mg protein. The endotoxin contamination is 13 ng/mg as detected using a chromogenic LAL assay (Whittaker, M.A. Bioproducts). Bovine serum albumin was from Sigma. Heparin was from Loven, Malmo, Sweden.

**RESULTS**

The first step in this study was to establish dose-response and time curves for the suppression of adipose tissue lipoprotein lipase by TNF in vivo. This is illustrated in Figs. 1 and 2 for guinea pigs. After administration of up to 5 μg of TNF, adipose tissue lipoprotein lipase activity remained unchanged or even increased. 20 μg or more decreased activity to about one-third of control levels. From these experiments we decided to use 30 μg of TNF for subsequent experiments. It is of interest to note that TNF did not, at any dose, decrease adipose lipoprotein lipase activity to the fasting level (compare Table I). The activity in controls varied considerably between different consignments of guinea pigs, as did also the response to TNF. There was, however, no apparent correlation between the activity in controls and the extent of the response.

Fig. 2 shows the time course of the effect. TNF caused a rapid decrease in adipose tissue lipoprotein lipase activity during the first 4 h, and the activity then remained essentially unchanged for 48 h. During this time the animals did not show any symptom of discomfort and continued to eat. Thus, the prolonged effect was not secondary to fasting. From these data we decided to use a 15-h time interval (overnight) for the subsequent experiments. A limited study in which TNF was given intravenously instead of intraperitoneally showed the same time course for suppression of adipose lipoprotein lipase activity as in Fig. 2. In other series of similar experiments, it was noted that lipoprotein lipase activity had returned to essentially normal values after 1 week.

Table I shows lipoprotein lipase activities in tissues of animals treated with TNF and corresponding controls. We used a 4-h time interval for experiments with mice and a 6-h time interval for experiments with rats. The values for guinea pigs are for a 15-h time interval (overnight), since this is the time we used in subsequent experiments on the rate of enzyme synthesis and on the disposition of the enzyme within the tissue. In all three species TNF decreased the lipoprotein lipase activity in adipose tissue. It did not, however, decrease the activity in any other tissue but rather increased it in most cases. In all three species a significant increase was seen in

![Fig. 1. Dose-response curve for the effect of TNF on lipoprotein lipase activity in guinea pig epididymal adipose tissue. A single dose of TNF was injected intraperitoneally to fed animals which were then returned to their cages with free access to food and water. They were killed 15 h later. Data from two identical experiments are shown (•, O). Values are expressed as units/g wet weight and are means of triplicate assays. Two controls, single animals at each dose of TNF.](image)

![Fig. 2. Time course for the effect of TNF on lipoprotein lipase activity in guinea pig epididymal adipose tissue. A single dose of 30 μg of TNF was injected intraperitoneally to fed animals which were then returned to their cages with free access to food and water. Data from two experiments are shown (•, O). Values are units/g wet weight and are from triplicate assays of single samples. Two or three animals at zero time, single animals at subsequent times.](image)
liver (to 200, 290, and 360% of control activity in mice, rats, and guinea pigs, respectively) and in plasma (to 180, 340, and 150% of control activities). In rats and mice TNF also increased lipoprotein lipase activity in heart and lungs.

For comparison, Table I also lists lipoprotein lipase activities in tissues of animals fasted for 48 h. As was previously shown fasting led to a pronounced decrease in adipose tissue lipoprotein lipase activity in rats and guinea pigs. During 48 h of fasting in mice, almost all lipid disappeared from the epididymal adipose tissue which was sampled. The figures in the table are per gram of tissue. Thus, the rather high value for fasted mice resulted from a large decrease in tissue lipoprotein lipase activity but an even larger decrease in tissue lipoprotein lipase activity varied considerably. We have therefore plotted the data in terms of percent of the mean value in the two corresponding controls. The regression equation is: LPL activity = (8.71 ± 13.76) + (0.824 ± 0.365)*(LPL synthesis).

### Table I

|        | Fed     | TNF     | Fasted |
|--------|---------|---------|--------|
| Mice   |         |         |        |
| Heart  | 970 ± 76| 1175 ± 123 | x*     |
| Adipose| 528 ± 222| 115 ± 72  | x       |
| Lung   | 132 ± 10 | 187 ± 52  | xx      |
| Liver  | 17 ± 3.0 | 54 ± 5.8  | xx      |
| Plasma | 3.1 ± 0.7| 5.5 ± 0.9 | xx      |
| Rats   |         |         |        |
| Heart  | 1248 ± 84| 1783 ± 156| xx      |
| Adipose| 429 ± 29 | 174 ± 25  | xx      |
| Lung   | 321 ± 60 | 441 ± 44  | xx      |
| Liver  | 32 ± 0.5 | 94 ± 13   | xx      |
| Plasma | 2.9 ± 0.2| 9.8 ± 2.3 | xx      |
| Guinea pigs |     |         |        |
| Heart  | 1382 ± 220| 1603 ± 116| 1554 ± 434 |
| Adipose| 3365 ± 868| 1182 ± 567| xx      |
| Lung   | 450 ± 165| 375 ± 51  | xx      |
| Liver  | 52 ± 10  | 186 ± 34  | xx      |
| Plasma | 3.5 ± 1.4| 5.2 ± 1.0 | x       |

* x, p < 0.05; xx, p < 0.01 compared to corresponding value in fed controls.

### FIG. 3. Correlation of lipoprotein lipase activity to relative synthesis of lipoprotein lipase in epididymal adipose tissue of guinea pigs given TNF.

A single dose of 30 μg of TNF was injected intraperitoneally to fed animals which were then returned to their cages with free access to food and water. They were killed 16 h later. Six identical experiments were run (●, ○, □, △, ▽, △). In each experiment there were two controls and two TNF-treated animals. Lipoprotein lipase activity was determined in one fat pad. The other pad was incubated in [35S]methionine containing medium, and incorporation of radioactivity into immunoprecipitable lipoprotein lipase and into total proteins was determined. Data are means of triplicate assays on samples from single animals and are expressed as percent of the mean value in the two corresponding controls. The regression equation is: LPL activity = (8.71 ± 13.76) + (0.824 ± 0.365)*(LPL synthesis).

### FIG. 4. SDS-Polyacrylamide gel electrophoresis of 35S-labeled proteins synthesized in vitro by adipocytes from fed or fasted guinea pigs given or not given TNF 15 h before.

Both TNF and fasting changed the pattern of protein synthesis. Some of the changes were similar; for instance, components a and e decreased after TNF (compare lanes 4 and 5 to lanes 2 and 3) as well as after fasting (compare lanes 6 and 7 to lanes 2 and 3). There were, however, also marked differences. Examples are components c and d which increased after TNF (compare lanes 4 and 5 to lanes 2 and 3) and lanes 8 and 9 to lanes 6 and 7 but not after fasting, and component b which was not affected by TNF, but increased after fasting (compare lanes 6 and 7 to lanes 2 and 3).

During fasting lipoprotein lipase activity in adipocytes from guinea pig epididymal adipose tissue decreases 60–80% (11). The activity in the tissue, however, decreases much more, 90–95% (11). Thus, most of the decrease must occur in extracellularly located lipoprotein lipase, as has previously been reported in other species (10). In contrast, TNF caused a commensurate decrease in intracellular and extracellular lipoprotein lipase activity (Table II).
Effects of TNF on Lipoprotein Lipase

A single dose of 30 μg of TNF was injected intraperitoneally to fed animals which were then returned to their cages with free access to food and water. They were killed 15 h later. Fasted animals were given no food for 9 h, then given TNF intraperitoneally, and returned to their cages with water but no food. They were killed 15 h later. Each group contained two animals. Adipocytes were prepared from the epididymal adipose tissue and incubated in [35S]methionine containing medium as detailed under “Experimental Procedures.” Identical amounts of labeled proteins (15,000 cpm) from each animals were separated by SDS-polyacrylamide gel electrophoresis. Lanes 1 and 10, molecular weight markers (phosphorylase b, albumin, ovalbumin, carbonic anhydrase, and lactoglobulin A); lanes 2 and 3, fed; lanes 4 and 5, fed + TNF; lanes 6 and 7, fasted; lanes 8 and 9 fasted + TNF.

TABLE II

Effects of TNF on lipoprotein lipase activity in adipose tissue and in adipocytes from guinea pigs

|                         | Controls | TNF |
|-------------------------|----------|-----|
| Adipocytes              | 452 ± 19 | 234 ± 26 | 4 |
| Tissue homogenate       | 3670 ± 236 | 1575 ± 178 | 4 |

Plasma and in liver in all three species studied. This is illustrated in Fig. 5 for guinea pigs. Similar experiments in rats gave an r-value of 0.79 and in mice an r-value of 0.54. These correlations suggest that lipoprotein lipase in liver is derived from transport through plasma.

DISCUSSION

In this study we demonstrate that TNF changes lipoprotein lipase activity in virtually all tissues in mice and rats, as well as guinea pigs. The view that TNF supresses lipoprotein lipase activity (6) is an oversimplification; this effect is limited to the adipose tissue. It is not clear what the integrated effect of TNF is on total body lipoprotein lipase activity at different doses and times after administration.

Our results demonstrate that TNF suppresses LPL synthesis in adipose tissue in vivo, as it is previously known to do in 3T3-L1 adipocytes (4, 5). Indeed, the presence of specific high affinity TNF receptors has been demonstrated on the membrane of adipocytes (20) as well as on various other cell types (21). It seems possible that the effect is exerted at the DNA level, resulting in decreased formation of LPL message. Lipoprotein lipase is known to be rapidly turned over; half-lives of less than 30 min have been reported both for cell lines (22, 23) and for guinea pig adipocytes (11). Thus, a decreased rate of enzyme synthesis will rapidly manifest itself as decreased enzyme activity.

A single dose of TNF caused suppression of adipose lipoprotein lipase for at least 48 h. The effect was, however, reversible; after 1 week the activity had returned to normal. TNF is rapidly metabolized; the in vivo half-life is about 6 min in rabbits (24). Thus, a relatively short TNF signal may cause a persistent change in adipose gene expression, which is only slowly reversible. This is in contrast with the effects of TNF seen in vitro, e.g. on endothelial cells where a long-lasting effect on HLA antigen expression can be obtained but only in the continuous presence of TNF (25). In other cases fast kinetics have been observed, e.g. induction of H4/18 antigen on endothelial cells (26).

The effects of TNF were not limited to lipoprotein lipase. Relative synthesis of some other proteins in the adipocytes also changed; some increased and others decreased. The effect of TNF on relative synthesis of lipoprotein lipase varied in our experiments, but TNF did not decrease the synthesis by more than 60%. It would appear from Fig. 4 that TNF had more marked effects on expression of some other proteins. It is previously known that TNF induces synthesis of specific proteins in a wide variety of cells (see for instance Refs. 27, 28).

Fasting also changes the pattern of protein synthesis in adipocytes. Some of the changes were the same for TNF and for fasting, but there were many differences. Thus, the overall effect of TNF on adipocytes is not just to put them in a “fasted” state, but the responses to TNF and to fasting are clearly different. In particular, the mechanism by which TNF and fasting lower adipose lipoprotein lipase activity differ in

![Fig. 4. Fluorograph of 35S-labeled proteins in adipocytes from fed and fasted guinea pigs given or not given TNF. Fed animals were given a single dose of 30 μg of TNF and were then returned to their cages with free access to food and water. They were killed 15 h later. Fasted animals were given no food for 9 h, then given TNF intraperitoneally, and returned to their cages with water but no food. They were killed 15 h later. Each group contained two animals. Adipocytes were prepared from the epididymal adipose tissue and incubated in [35S]methionine containing medium as detailed under “Experimental Procedures.” Identical amounts of labeled proteins (15,000 cpm) from each animals were separated by SDS-polyacrylamide gel electrophoresis. Lanes 1 and 10, molecular weight markers (phosphorylase b, albumin, ovalbumin, carbonic anhydrase, and lactoglobulin A); lanes 2 and 3, fed; lanes 4 and 5, fed + TNF; lanes 6 and 7, fasted; lanes 8 and 9 fasted + TNF.](image)

![Fig. 5. Correlation between lipoprotein lipase activities in liver and plasma from guinea pigs given TNF. A single dose of 30 μg of TNF was injected intraperitoneally to fed animals which were then returned to their cages with free access to food and water. They were killed 15 h later. Data from three experiments are shown (●, ○, △). In two experiments there were three controls and three TNF-treated animals, and in one experiment there were two controls and two TNF-treated animals. Data are means of triplicate assays on samples from single animals and are expressed as percent of the means of the three or two corresponding controls.](image)
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several respects. After fasting the activity is rapidly restored by feeding. In contrast, TNF suppressed the activity even though the animals continued to eat, and the decrease was only slowly reversible. At no dose or time did TNF suppress adipose lipoprotein lipase activity in fed guinea pigs to the low levels seen in fasting. In fasting, there is a general decrease in adipocyte protein synthesis, but no decrease in relative synthesis of lipoprotein lipase (12). In addition, there is a disproportionally large decrease in lipoprotein lipase outside adipocytes, indicating some effect on enzyme secretion and/or turnover (10, 11). In contrast, TNF caused a decrease in relative lipoprotein lipase synthesis but no change in general protein synthesis or in the distribution of lipoprotein lipase in the tissue. It is clear that the suppression of adipose lipoprotein lipase activity is mediated by two separate mechanisms; TNF changes the synthesis of lipoprotein lipase, whereas feeding/fasting modulates the transport and turnover of the lipase protein.

In no tissue other than the adipose did we observe a decrease of lipoprotein lipase activity after TNF. It is apparent that the effect to suppress lipoprotein lipase is tissue-specific. In fact, lipoprotein lipase activity increased in most other tissues. The increase was particularly prominent in the liver. The hepatic lipoprotein lipase activity was readily inhibited by our antisera. Thus, had the enzyme been synthesized in the liver we should have been able to immunoprecipitate 35S-labeled lipase molecules in the methionine-incorporation studies. However, none were found before or after TNF. Lipoprotein lipase must therefore have been taken up from plasma. In accord with this, plasma lipoprotein lipase activity was increased, and there was a correlation between plasma and liver lipoprotein lipase activities in TNF-treated animals of all three species. We have previously observed that release of lipoprotein lipase into plasma by heparin or by a fat emulsion leads to increased lipoprotein lipase activity in liver (15). These results support the hypothesis that the increased hepatic lipoprotein lipase activity after TNF is due to increased transport of active lipoprotein lipase in plasma. The question remains how TNF causes this increased transport. It can not be directly related to decreased synthesis of the enzyme in adipose tissue; some other mechanism must operate.

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