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# Regulation of lipoprotein lipase activity and mRNA content in rat epididymal adipose tissue *in vitro* by recombinant tumour necrosis factor

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Tumour necrosis factor (TNF) has previously been shown to decrease lipoprotein lipase (LPL) activity and mRNA levels in 3T3-L1 cells and in adipose tissue from rats and guinea pigs when injected *in vivo*, but not to alter LPL activity in human adipocytes incubated *in vitro*. The effect of recombinant human TNF on LPL activity and mRNA levels in rat epididymal adipose tissue incubated *in vitro* was examined. LPL activity and mRNA levels fell in adipose tissue taken from fed rats and incubated in Krebs–Henseleit bicarbonate medium with glucose. The addition of insulin and dexamethasone prevented these falls. TNF (400 ng/ml) produced a fall of approx. 50% in LPL activity after 2 h of incubation and of approx. 30% in LPL mRNA levels after 3 h. TNF did not decrease LPL activity in isolated adipocytes. These results demonstrate that rat adipose tissue incubated *in vitro* is responsive to TNF whereas isolated adipocytes are not.

## INTRODUCTION

Lipoprotein lipase (LPL) hydrolyses triacylglycerol on chylomicrons and very-low-density lipoproteins and is a key enzyme in determining the plasma concentration of triacylglycerol and the provision of fatty acids to extrahepatic tissues, mainly muscle and adipose tissue. The enzyme has a short half-life, variously reported as from 22 min to 5 h [1], and therefore the rates of enzyme synthesis, translocation and degradation are important for determining the activity at the endothelial cell wall. Marked changes in LPL activity and protein synthesis are produced by changes in nutritional state [2], hormones [3] and drugs [4]. More recently, alterations in LPL mRNA content have been observed [5–7].

Chronic infections and malignancies produce alterations in lipid metabolism including hypertriacylglycerolaemia [8]. This is believed to be caused in part by the decreased LPL activity which has been observed in these conditions [8,9]. Various monokines have been implicated as the agents producing the lipid alterations, and those shown to alter LPL activity include tumour necrosis factor (TNF) [7], interleukin-1 [10] and $\gamma$-interferon [11].

There is agreement that injection of TNF *in vivo* results in the lowering of LPL activity in the epididymal adipose tissue of rat, mouse and guinea pig [12–16]. TNF also decreases adipose tissue LPL synthesis and mRNA content *in vivo* [7]. Effects of TNF have also been observed *in vitro*. The addition of TNF to 3T3-L1 cells resulted in decreased LPL activity and mRNA content [15,17]. However, in contrast with the above findings with 3T3-L1 cells, Kern [18] did not find an effect of TNF when it was added to human adipocytes incubated *in vitro*.

## EXPERIMENTAL

### Adipose tissue incubations

Porton–Wistar rats were fed *ad libitum* on a diet consisting of (by weight) 51.6% carbohydrate, 22.7% protein and 4.3% fat (the residue was non-digestible material; Special Diet Services, Witham, Essex, U.K.). The rats had free access to drinking water, and were maintained at an ambient temperature of $20 \pm 2$ °C with a 14 h light/10 h dark cycle (lights on from 07:00 h). Where rats were fasted, food was removed at 09:00 h, 24 h before killing. Three groups of rats were studied, with weight ranges 180–200 g, 200–250 g and 250–280 g. Epididymal adipose tissue was removed at 09:00 h. Whole adipose tissue was incubated in Krebs–Henseleit bicarbonate buffer, pH 7.4 [19], containing 2% defatted albumin, 10 mM-glucose, 0.5% casein hydrolysate and, as indicated, 40 nM-insulin and 0.75 $\mu$M-dexamethasone phosphate. The temperature of incubation was either 27 °C or 37 °C as indicated. Acetone/ether-dried powders were made from the adipose tissue and medium [20] and were homogenized in 0.25 M-sucrose/1 mM-EDTA/10 mM-Hepes, pH 7.4 [21]. Adipocytes were prepared by the method of Rodbell [22] and incubated in the above medium (final lipocrit of 7.5%), except that the buffer was Krebs–Ringer containing 20 mM-Hepes, and the defatted albumin concentration was increased to 4% [23]. At the end of the incubations, the cells and medium were homogenized in an equal volume of homogenizing buffer (20 mM-Hepes/10 mM-EDTA and, per ml, 8 mg of Triton X-100, 0.4 mg of SDS and 5 units of heparin) [24]. The homogenates were centrifuged at 3000 *g* for 10 min at 4 °C, and 0.5 ml of the clear solution between a floating fat layer and a sediment [24] was taken for assay of LPL. LPL activity was measured with Intralipid as substrate in the presence of heparin (0.5 units/ml) as previously described [25], and is expressed as $\mu$mol of non-esterified fatty acid (NEFA) released/h per g wet weight of tissue or, for fat-cell activity, per g wet wt. of fat pad equivalent.

### RNA analysis

Total cellular RNA was prepared by the method of Auffray & Rougeon [26]. For Northern analysis, 10 $\mu$g of total RNA was separated by electrophoresis in 1.2% agarose/2.2 M-formaldehyde gels and transferred to Hybond-N (Amersham) [27]. Filters were pre-hybridized at 42 °C for 2 h in 50% (v/v) formamide/5 × SSPE (0.9 M-NaCl/0.05 M-$NaH_2PO_4$/5 mM-EDTA, pH 8.0), containing 0.5% SDS, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone and 0.1% Ficoll.

Abbreviations used: LPL, lipoprotein lipase; TNF, tumour necrosis factor; NEFA, non-esterified fatty acids.

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Hybridizations were carried out overnight under the same conditions in the presence of cDNA probes ($1 \times 10^6$ c.p.m./ml), $^{32}$P-labelled with a Random Primed kit (Boehringer Mannheim). After hybridization, filters were washed as described in [27] and then subjected to autoradiography by using pre-flashed Hyperfilm-MP (Amersham) with intensifying screens for various lengths of time at −70 °C to ensure linear film response. LPL cDNA hybridized to four mRNAs: one major band at 3.6 kb and three minor bands at 3.3, 2.7 and 2.2 kb [28]. The relative intensity of the major band on the autoradiograph was quantified with a scanning densitometer (Model 1650, Bio-Rad). Filters were first probed with LPL cDNA, and were then reprobed with α-tubulin cDNA following washing at 65 °C in 5 mM-Tris/HCl (pH 8.0)/2 mM-$Na_2EDTA$ containing 0.002% of each of bovine serum albumin, Ficoll and polyvinylpyrrolidone [27] and prehybridization as above. Values for mRNA are expressed as the ratio of LPL mRNA to α-tubulin mRNA. The LPL cDNA probe was a gift from M. C. Schotz, Wadsworth Medical Center, Los Angeles, CA, U.S.A. and the α-tubulin probe was a gift from P. Middleton, University of Newcastle upon Tyne.

## Materials

Recombinant human TNF was a gift from Dr. P. Wingfield, Biogen-SA, and had a specific activity of $1 \times 10^7$ units/mg of protein. Dexamethasone phosphate was a gift from Merck, Sharp and Dohme (Hoddesdon, Herts., U.K.). Bovine insulin was from Sigma and [α-$^{32}$P]dCTP was from Amersham.

Results were analysed using Student's *t* test and are expressed as means ± S.D.

## RESULTS

### Effects of TNF on adipose tissue LPL activity and mRNA levels in the absence of other hormones

In the first series of experiments, the effects of TNF were investigated in the absence of other hormones. A concentration of TNF of 150 ng/ml was chosen as being higher than the dose having a maximal effect on LPL content in cell culture systems [17]. Epididymal adipose tissue was incubated in the presence or absence of TNF for 4 and 5 h. The addition of TNF resulted after 4 h in a decrease in LPL activity compared with that in pads incubated in the absence of hormones (Table 1). However, after 4 h of incubation LPL activity had fallen in the control incubations as compared with the activity at zero time (Table 1).

**Table 1. Effect of TNF on LPL activity**

Adipose tissue was removed from rats (body wt. 180–200 g) fasted for 24 h; one fat pad was incubated in the absence of TNF, and the paired fat pad was incubated in the presence of 150 ng of TNF/ml. After incubation, acetone/ether-dried powders were prepared for the measurement of LPL. Fat pads were also taken for the preparation of acetone/ether-dried powders immediately after removal from the animal at 0 h. Results are means ± S.D. ($n = 5$). (LPL activity in fed rats of the same weight was 86 ± 7 μmol of NEFA/h per g; $n = 6$.)

| Incubation period (h) | LPL activity (μmol of NEFA/h per g) | |
|---|---|---|
| | Control | +TNF |
| 0 | 37 ± 12 | – |
| 4 | 16 ± 6 | 9 ± 3 |
| 5 | 15 ± 6 | 10 ± 3 |

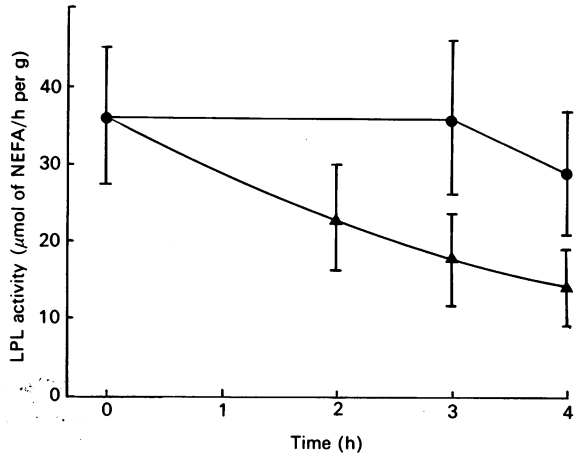


**Fig. 1. Effect of insulin and dexamethasone on LPL activity**

Fat pads were removed from fed rats (200–250 g) and incubated in the presence (●) or absence (▲) of 40 nM-insulin and 0.75 μM-dexamethasone phosphate. After the appropriate time, acetone/ether powders of the tissue were made. The results are means ± S.D..

**Table 2. Effect of TNF on LPL activity in the presence of insulin and dexamethasone**

Epididymal adipose tissue was removed from fed rats (body wt. 200–250 g) and paired fat pads were incubated in the presence of 40 nM-insulin and 0.75 μM-dexamethasone phosphate and the presence or absence of 400 ng of TNF/ml. At appropriate times pads were removed for preparation of acetone/ether-dried powders for measurement of LPL activity. The results are mean values ± S.D. with the numbers of observations in parentheses. Values that are significantly different from the controls (Student's *t* test) are indicated: $^*P < 0.01$. (LPL activity in fat pads from rats of similar weight fasted for 24 h was 13 ± 1 μmol of NEFA/h per g; $n = 6$)

| Incubation period (h) | LPL activity (μmol of NEFA/h per g) | |
|---|---|---|
| | Control | +TNF |
| 0 (5) | 33 ± 9 | – |
| 1 (3) | 35 ± 5 | 33 ± 2 |
| 2 (3) | 33 ± 5 | 19 ± 1* |
| 3 (5) | 34 ± 9 | 18 ± 8* |
| 4 (5) | 24 ± 11 | 14 ± 7 |

There was no further fall in LPL activity in the control pads between 4 and 5 h of incubation.

Incubation of adipose tissue in the presence of TNF for 5 h produced a fall in LPL mRNA content. These levels, relative to α-tubulin mRNA, fell to 69 ± 12% ($P < 0.02$, $n = 4$) of the value in control adipose tissue incubated in the absence of TNF. However, LPL mRNA levels had also fallen in the control incubations to 54 ± 4% ($P < 0.01$, $n = 3$) of the value at zero time. Tubulin mRNA levels did not alter in the control between 0 and 5 h, but were slightly decreased in the presence of TNF.

### Effects of insulin and dexamethasone on LPL activity and mRNA levels

Because of the fall in LPL activity and mRNA levels observed above, further experiments were carried out to determine whether similar falls occurred in fat pads from fed rats and to investigate the effects of the addition to the incubations of insulin and

**Table 3. Comparison of effects of TNF on LPL activity in adipose tissue and isolated adipocytes**

Adipocytes were prepared from the adipose tissue of two rats and incubated in quadruplicate. Rat weights: Experiment 1, 200–250 g; Experiment 2, 180–200 g. Other procedures were as described in Table 2 and the Experimental section. Results are means ± S.D. ($n = 4$). Values that are significantly different from controls (Student's $t$ test) are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$.

| Temperature of incubation | LPL activity (μmol of NEFA/h per g) in: | | | |
|---|---|---|---|---|
| | Cells | | Adipose tissue | |
| | Control | +TNF | Control | +TNF |
| Experiment 1 | | | | |
| 37 °C | 10.8±0.9 | 11.9±1.2 | 52±10 | 35±7* |
| 27 °C | 20.6±0.2 | – | 78±14 | – |
| Experiment 2 | | | | |
| 27 °C | 19.1±0.4 | 19.7±0.6 | 92±9 | 63±4** |

**Table 4. Effect of TNF on LPL activity and mRNA levels**

One fat pad from each of two rats (200–250 g) was incubated together for 3 h in the presence of 40 nM-insulin and 0.75 μM-dexamethasone phosphate and 400 ng of TNF/ml. The paired fat pads were incubated in the absence of TNF. After incubation the paired fat pads from the same animal were taken for measurement of LPL activity and the paired fat pads from the other rat were taken for measurement of mRNA. Results are given as the values in the presence of TNF expressed as a percentage of the control values and are means ± S.D. LPL mRNA levels are expressed relative to tubulin mRNA levels ($n = 4$). Values that are significantly different from controls by Student's $t$ test are indicated: $^{*}P < 0.02$, $^{**}P < 0.01$, $^{***}P < 0.001$.

| Incubation period (h) | LPL activity (% of control) | LPL/tubulin mRNA (% of control) |
|---|---|---|
| 3 | 55±9*** | 73±7*** |
| 4 | 56±16*** | 68±13** |
| 5 | 65±12* | 66±13** |

dexamethasone, which increase LPL activity in pads from fasted animals [3]. As shown in Fig. 1, with no additions LPL activity fell during a 4 h incubation, but the activity was maintained in the presence of insulin and dexamethasone. Neither substance alone was able to maintain enzyme activity at the zero-time value (M. P. Rogers, unpublished work). With no additions, LPL mRNA levels had fallen at 3 h to 79±14% ($n = 6$) of the value at zero time. The addition of 40 nM-insulin and 0.75 μM-dexamethasone phosphate prevented this fall, and increased the levels to 112±22% ($n = 6$) of the value at zero time. LPL mRNA levels were significantly higher ($P < 0.05$) after 3 h of incubation with insulin and dexamethasone than in their absence.

### Effect of TNF on LPL activity in the presence of insulin and dexamethasone

The time course of the effect of TNF on LPL activity was investigated first. The addition of TNF produced no change in LPL activity after 1 h of incubation, but activity was significantly decreased to a maximum of 54% of the control value by 3 h (Table 2). Lower concentrations of TNF were also effective in decreasing LPL activity. After 3 h, TNF at 5 and 50 ng/ml lowered LPL activity to 63±2% and 63±5% ($n = 3$) respectively. LPL activity was not inhibited when TNF (400 ng/ml) was added directly to the assay.

Whereas the maintenance of LPL activity by insulin and dexamethasone was reproducible, the response to TNF was variable and was not observed in adipose tissue from rats larger than 250 g. Thus the LPL activity in adipose tissue from rats of 250–280 g incubated for 3 h in the presence of insulin and dexamethasone was 43±8 μmol of NEFA/h per g and 43±6 μmol of NEFA/h per g ($n = 6$) in the presence and absence respectively of TNF (400 ng/ml).

### Comparison of effect of TNF on LPL activity in adipose tissue and adipocytes

Kern [18] was unable to observe any change in LPL activity on incubation of human adipocytes with TNF, and concluded that human adipose tissue is unresponsive to TNF. In order to determine whether the lack of response was due to species differences or to the use of isolated adipocytes, the effect of TNF on rat adipocytes was investigated. As the incubations with adipose tissue had been carried out at 37 °C, the first experiment was at this temperature. TNF had no effect on LPL activity in the isolated adipocytes, although it significantly decreased activity in the whole adipose tissue (Table 3). The half-life of LPL in isolated adipocytes is greater at 27 °C than at 37 °C [1] and enzyme activity in control incubations was found to be higher at 27 °C than 37 °C (Table 3). Therefore further experiments were carried out at 27 °C. The effect of TNF was determined in three different cell preparations incubated at 27 °C, but no alteration in LPL activity was observed (control, 19.2±1.4 μmol of NEFA/h per g; TNF, 19.2±1.2 μmol of NEFA/h per g). TNF significantly decreased LPL activity in whole adipose tissue incubated at 27 °C (Table 3).

### Effect of TNF on LPL activity and mRNA levels in the presence of insulin and dexamethasone

Since Zechner *et al.* [17] found that, in 3T3-L1 cells, higher concentrations of TNF were required to decrease LPL mRNA levels than to decrease LPL activity, and that activity changes were observed before changes in mRNA levels, further experiments to determine the effect of TNF on LPL mRNA levels were carried out with a concentration of TNF of 400 ng/ml for 3–5 h.

Incubation of adipose tissue in the presence of TNF resulted in a fall in LPL mRNA levels, with no change in tubulin mRNA levels. For ease of comparison, Table 4 gives the results expressed as a percentage of the control value. The TNF-induced fall in LPL activity at 3 h was accompanied by a significant 27% fall in LPL mRNA levels. Incubation with TNF for longer periods produced no further significant change in LPL mRNA levels.

## DISCUSSION

Our results show that LPL in rat epididymal adipose tissue, but not that in isolated adipocytes, is responsive to TNF when incubated *in vitro*. The maximum decrease in activity of 46% is not as great as the 95% decrease observed in 3T3-L1 cells incubated *in vitro* [17] but is similar to the findings after injection of TNF *in vivo*. LPL activity decreased by 60% in epididymal adipose tissue and by 51% in parametrial adipose tissue of fed rats [12,16], by 44% in fasted rats [13] and by 65% in guinea pig adipose tissue [12]. The decrease in LPL mRNA levels of around 30% reported here is again equivalent to the 20% decrease observed in guinea pig tissue *in vivo* [7], although it is much less than the 75% and 60% decreases observed for 3T3-L1 cells

[15,17]. Our findings of a greater effect of TNF on LPL activity than on mRNA levels are consistent with all reports in which both parameters have been measured [15,17,18].

Zechner *et al.* [17] found that LPL activity decreased before any change in LPL mRNA levels had occurred. Because in our study the decrease in LPL mRNA levels observed after 3 h was 30%, it was not realistic to try to determine the time course of the changes.

There are a number of explanations as to why an effect of TNF is observed in whole adipose tissue but not in isolated cells. First, TNF may not be the active factor but it could stimulate the production of some other mediator in endothelial cells. Recent studies have shown synergism between TNF and interleukin-1 in 3T3-L1 cells [29]. Second, changes in LPL activity brought about by hormones are usually less in isolated adipocytes than in whole adipose tissue [1]. Third, there is increasing evidence that, in isolated hepatocytes, transcription controls are blocked but mRNA is stabilized by hormones [30]. A similar situation may arise in isolated adipocytes so that the normal control processes do not occur.

Kern [18] was unable to observe any change in LPL activity on incubation of human isolated adipocytes with TNF. Further investigations will have to be carried out to determine whether human adipose tissue is sensitive to TNF.

Our findings that insulin and dexamethasone prevent the fall in LPL activity and mRNA levels that otherwise occur are consistent with the observations of other workers in related systems. Speake *et al.* [3] found that both insulin and dexamethasone are required for a maximal increase in LPL activity in fat pads taken from starved rats and incubated *in vitro*. Ong *et al.* [6] found a doubling of LPL mRNA levels in rat adipocytes incubated with insulin alone. However, the fall in LPL activity in pads from fed animals may be due in part to increased degradation of the enzyme, and insulin and dexamethasone may be decreasing degradation, although this was not observed by other workers [3,6].

The observed increase in LPL mRNA could be due either to increased transcription or to increased stability of the mRNA. In view of the findings described above in hepatocytes, it will be important to determine whether insulin and dexamethasone affect mRNA stability in adipocytes or whole adipose tissue and whether the changes relate only to a system *in vitro*.

A further debate concerns the role played by changes in LPL activity produced by TNF in the production of hypertriacylglycerolaemia. Recently, Grunfeld *et al.* [13] reported that, at 90 min after administration of TNF to male rats, the serum triacylglycerol concentration had risen, although there was no decrease in adipose tissue LPL activity at this time. By 16 h adipose tissue LPL activity had decreased by 44%. These authors found a consistent increase in hepatic lipogenesis *de novo*. Chajek-Shaul *et al.* [14] also suggested that the main cause of hypertriacylglycerolaemia was increased secretion of triacylglycerol by the liver. However, Evans & Williamson [31], on studying tissue-specific effects of rapid tumour growth on lipid metabolism, found no change in rates of lipogenesis but did observe decreased oxidation of oral $^{14}$C-labelled lipid to $^{14}CO_2$, decreased parametrial adipose tissue LPL activity and increased plasma lipids. These workers also found increased plasma triacylglycerol concentrations, decreased adipose tissue LPL activity and decreased disposal of an oral [$^{14}$C]triolein load in rats injected with TNF [16]. Given that a decrease in epididymal adipose tissue LPL activity has always been observed, the role of LPL in the onset and maintenance of hypertriacylglycerolaemia following TNF injection or tumour growth may be largely determined by the response of muscle. TNF has been reported to increase [12,14], to decrease [32] or not to change [13,15,16] LPL activity in muscle.

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