Effect of tumor necrosis factor administration in vivo on lipoprotein lipase activity in various tissues of the rat

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Abstract When added to murine adipocytes in culture, tumor necrosis factor (TNF) decreases the levels of lipoprotein lipase (LPL). Semb et al (*1987. J. Biol Chem.* **262:** 8390–8394) have shown that administration of murine TNF to rats decreases lipoprotein lipase (LPL) in the epididymal fat pad with maximal inhibition requiring several hours. We have now tested the effects of treatment of rats with TNF on LPL activity in a variety of tissues and find that few show decreases in LPL under conditions that acutely increase serum triglycerides. Ninety minutes after treatment of male rats with human TNF (25 μg/200 g, i.v.), serum triglycerides rose 2.2-fold but there was no decrease in LPL activity in epididymal fat. Sixteen hours after TNF treatment LPL activity had decreased by 44% in epididymal fat, consistent with the previously reported data. In contrast, in female rats, no significant decrease was seen in LPL activity in parametrial adipose tissue at either 90 min or 16 hr after TNF administration despite increases in serum triglycerides (1.8-fold and 1.5-fold, respectively). There was little change in LPL activity in most other adipose tissue sites of male or female rats at either time after TNF treatment. No effect of TNF was seen on heart or diaphragm muscle LPL at any time. TNF treatment of both male and female rats produces consistent increases in de novo hepatic lipogenesis in vivo under conditions that increase serum triglycerides. It is unlikely that the limited effects of TNF on LPL in vivo can account for the rapid and sustained increase in serum triglycerides.—**Grunfeld, C., R. Gulli, A. H. Moser, L. A. Gavin, and K. R. Feingold.** Effect of tumor necrosis factor administration in vivo on lipoprotein lipase activity in various tissues of the rat. *J. Lipid Res.* 1989. **30:** 579–585.

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Infection by micro-organisms often produces profound disturbances in intermediary metabolism (1), including hypertriglyceridemia due to accumulation of very low density lipoproteins (VLDL) (2, 3). Model infections have shown that there is both an increase in hepatic lipid synthesis (4) and a decrease in the metabolism of circulating triglyceride-rich lipoproteins, which is thought to be mediated by decreases in lipoprotein lipase activity (5, 6).

Interest has recently focused on the role of cytokines as the mediators of the hyperlipidemia of infection. Kawakami et al. (7), Pekala et al. (8), and Torti it al. (9) have demonstrated that supernatants from endotoxin-stimulated mouse macrophages contain a factor that decreases the synthesis and storage of lipid in cultured adipose cells by decreasing levels of lipoprotein lipase (LPL) and the lipogenic enzymes. The factor, which they named cachectin, was purified and found to be identical to mouse tumor necrosis factor (TNF) (10).

Purified recombinant TNF has been shown to have the same properties as cachectin against adipose cells in vitro, decreasing the incorporation of acetate into lipid and lowering LPL activity in rodent adipose cells in culture (11–13). The decrease in LPL is mediated by decreased synthesis of the enzyme (12).

There is less information on the effect of TNF treatment of animals in vivo. We have demonstrated that serum triglycerides are acutely elevated within 90 min after TNF treatment in vivo; serum triglycerides remain elevated for up to 17 hr (14). This increase in serum triglycerides could result from decreased clearance of triglyceride due to decreases in LPL activity. Semb et al. (15) have shown that injection of TNF eventually leads to a decrease in LPL in the epididymal fat pad of mice, rats, and guinea pigs.

Abbreviations: TNF, tumor necrosis factor; LPL, lipoprotein lipase; VLDL, very low density lipoproteins; TLC, thin-layer chromatography.

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However, we have previously reported that the increase in serum triglycerides is paralleled by an acute increase in de novo hepatic fatty acid synthesis, which also persists for 17 hr (14). Liver slices from animals treated with TNF in vivo maintain the increase in hepatic lipogenesis when lipid synthesis is asssayed in vitro (14). The acute increase in fatty acid synthesis seen within 2 hr after TNF administration is the result of a TNF-induced elevation of intrahepatic levels of citrate, an allosteric activator of acetyl CoA carboxylase, the key rate-limiting enzyme in fatty acid synthesis (16). By 17 hr after TNF administration, the levels of acetyl CoA carboxylase and fatty acid synthetase are higher than in control animals, which could account for the later increases in hepatic lipogenesis (16). Newly synthesized fatty acid rapidly appears at increased levels in plasma by 1–2 hr after TNF treatment (14). Thus, the observed increase in serum triglycerides that occurs following TNF administration could result from increased synthesis and secretion of VLDL by the liver.

To assess the relative contribution of increased hepatic synthesis versus decreased LPL in the etiology of TNF-induced hypertriglyceridemia, we now examine whether TNF administration influences lipoprotein lipase activity in multiple muscle and adipose tissue sites of the rat under conditions that we have previously shown are optimal for acutely increasing hepatic lipogenesis and serum triglycerides (14).

METHODS

Materials

[^{14}C]Triolein, [26-^{14}C]cholesterol (0.5 mCi/0.33 mg), and [^{14}C]oleic acid (40–60 mCi/mmol) were purchased from New England Nuclear. Tritiated water (1 Ci/g) was purchased from ICN Radiochemicals. Triolein, lecithin, and fatty acid-free bovine serum albumin were from Sigma. TLC polygram Sil G plates were purchased from Brinkmann Instruments. Ultrafluor scintillation fluid was purchased from National Diagnostics. Human tumor necrosis factor alpha with a specific activity of 5×10^7 U/mg produced by recombinant DNA technology was kindly provided by Dr. H. M. Shepard from Genentech, Inc.

Animal procedures

Male and female Sprague-Dawley rats (approximately 200 g) were purchased from Simonsen Animal Vendors. The animals were maintained on a reversed 12 hr light cycle (3 AM to 3 PM dark, 3 PM to 3 AM light) and fed Simonsen Rat Chow and water ad libitum.

Rats were injected via the tail vein with 25 μg of TNF in 0.5 ml of 0.9% saline or saline alone. This dose is approximately one-quarter of that shown to produce tumor necrosis in vivo (17) and is the optimal dose for increasing hepatic lipid synthesis in male rats (14). Because TNF administration induces anorexia (17) all animals were fasted after injection to control for the effects of nutritional status on lipid metabolism.

Serum chemistries

Serum triglyceride levels were measured using Sigma Diagnostic Kit #405 after extraction with Dole's reagent (18). Serum cholesterol levels were measured by using Sigma Diagnostic Kit #351.

Lipoprotein lipase

At the time indicated after TNF administration, rats were killed and adipose tissue from various locations and heart and diaphragm muscle were removed and flash frozen with a Wollenberger clamp cooled in liquid N_2. Lipolytic activity was determined by the method of Pykalisto, Vogel, and Bierman (19), as described previously (20). Briefly, the substrate (unlabeled triolein (200 mg), 4.0 μCi of [^{14}C] triolein, and 3.0 mg of lecithin) was homogenized with 1.2 ml of 10% fatty acid-free bovine albumin (pH 8.2), 0.5 ml of normal plasma (LPL co-factor), and 6.2 ml of 1.0 M Tris-HCl buffer (pH 8.2). An aliquot of the resultant emulsion (0.2 ml) and 0.1 ml of the heparin-extracted medium of the tissue were incubated in a metabolic shaker at 37°C for 60 min. The reaction was stopped by addition of 10 ml of Dole's extraction mixture. Subsequently, the [^{14}C]oleic acid was separated from triolein by sequential alkalinization-reacidification heptane extraction procedure (19) and counted. One unit of lipoprotein lipase activity is defined as equivalents of free fatty acids/g of tissue per hr.

Lipogenesis

Measurement of lipogenesis rates in vivo was performed as described in detail previously (14, 21, 22). In brief, at the time indicated after TNF administration, rats were injected i.p. with tritiated water (50 mCi). One hour later the rats were anesthetized, weighed, and a blood specimen was obtained. The livers were removed, individually weighed, and the lipid was saponified by refluxing overnight in a solution of 45% KOH, water, and 70% ethyl alcohol (2:1:5). After cooling, internal standards of [^{14}C]cholesterol and [^{14}C]oleic acid were added. Nonsaponifiable lipids were extracted and cholesterol was purified by TLC as described previously (14, 21, 22). The saponified material was then acidified to pH less than 2 and fatty acids were extracted three times with petroleum ether. Samples were counted by liquid scintillation spectrometry with windows adjusted so that less than 0.2% of the ^{3}H counts was recorded in the ^{14}C window and approximately 10% of the ^{14}C counts in the ^{3}H window. In

corporation was corrected for spillover of ^{3}H and ^{14}C, for background, and for recovery of internal standard.

An internal standard of [^{14}C]triolein was added to serum samples which were then extracted using the Bligh-Dyer technique (23). To separate phospholipids, free fatty acids, and triglycerides, an aliquot of the lipid extract was applied to TLC plates and developed in diethyl ether–petroleum ether–glacial acetic acid 20:80:1 for approximately 50 min. The band corresponding to triglycerides (R_f 0.7–0.8) was cut from the plate and counted as described above.

The specific activity of tritiated water was determined individually for each animal by measuring the dpm/ml of plasma at the end of the experiment and dividing by mmol of water/ml plasma (52 mmol/ml plasma assuming that plasma is 93% water). The validity of this methodology has been demonstrated previously (21, 22).

Data analysis

Data presented in figures and tables represent the mean ± SEM of single experiments using five rats in each group. The text summarizes the results from multiple experiments. Statistical differences were determined by using a two-tailed Student's *t*-test.

RESULTS

Effect of TNF on adipose tissue lipoprotein lipase in male rats

The effect of TNF administration to intact rats on LPL activity in the epididymal fat pad of males is shown in **Fig. 1**. Ninety minutes after TNF treatment there was no significant change in adipose tissue LPL (Fig. 1, left panel). In three such experiments LPL in TNF-treated animals averaged 91% of controls. By 16 hr after TNF treatment, LPL activity in epididymal fat pad had decreased significantly (Fig. 1, right). In three similar experiments, by 16 hr after TNF treatment LPL was reduced by 44%. Thus, while our data confirmed those of Semb et al. (15) demonstrating that LPL activity is decreased in epididymal fat several hours after TNF administration in vivo, we found no decrease in LPL activity by 90 min after TNF. We have previously demonstrated that TNF significantly increases both serum triglycerides and hepatic lipogenesis at both 90 min and 16 hr after administration of TNF to male rats (14). In the experiments presented here, serum triglycerides were increased by an average of 2.16 ± 0.22-fold within 90 min after TNF administration and by an average of 1.53 ± 0.07-fold by 16 hr after TNF administration in comparison to controls.

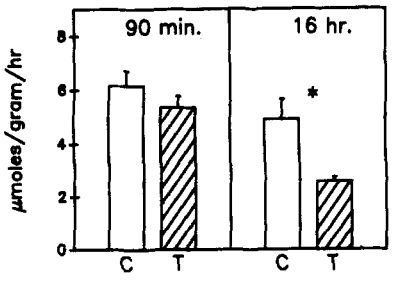

Fig. 1. Male Sprague-Dawley rats were injected with TNF (hatched bars) or saline (open bars), then kept fasting. At the indicated times, epididymal fat pads were removed and LPL activity was measured as described under Methods. Values are the mean ± SEM for five rats; *, $P < 0.02$.

We then examined the effect of TNF administration to intact rats on lipoprotein lipase activity in three other adipose tissue sites in males (**Table 1**). Ninety minutes after TNF administration there was no significant decrease in LPL activity in pericardiac fat. In the experiment presented, LPL activity was slightly decreased in perirenal fat, but in other experiments the decrease was not always significant; LPL values in perirenal fat from TNF-treated rats averaged 86% of controls 90 min after TNF (not significant). LPL activity in subcutaneous adipose tissue was consistently decreased 90 min after TNF administration, showing an average 23% decrease compared to control animals.

In contrast to the acute effects of TNF described above by 16 hr after TNF treatment, LPL activity was not significantly decreased compared to control values in any of these adipose tissues (Table 1).

Effect of TNF on adipose tissue lipoprotein lipase in female rats

We next examined LPL activity in the major parametrial fat pad of female rats. No significant decrease in LPL

TABLE 1. Effect of TNF on LPL in various adipose tissues of male rats

Condition	Time	Pericardiac	Subcutaneous	Perirenal
			μmol/g per hr	
Control	90 min	0.93 ± 0.03	1.47 ± 0.17	3.03 ± 0.03
TNF	90 min	0.77 ± 0.05	1.13 ± 0.16*	2.37 ± 0.12*
Control	16 hr	1.63 ± 0.16	0.54 ± 0.13	3.41 ± 0.44
TNF	16 hr	1.63 ± 0.24	0.51 ± 0.06	2.80 ± 0.14

*, $P < 0.05$.

activity was seen at either 90 min or 16 hr after TNF administration (**Fig. 2**). In three similar experiments, LPL actvity in parametrial fat averaged 74% of control rats 90 min after TNF administration but this apparent decrease was never statistically significant, while 16 hr after TNF treatment, LPL activity averaged 91% of control rats. In the multiple experiments performed here in female rats, serum triglycerides were significantly increased 1.76 ± 0.22-fold 90 min after TNF administration, and at 16 hr after administration, serum triglycerides were increased 1.52 ± 0.29-fold in comparison to controls.

LPL activity in other adipose tissues from female rats was also examined at 90 min or 16 hr after TNF administration. There was no significant decrease in LPL activity from subcutaneous or pericardiac fat 90 min after TNF adminstration (**Table 2**). However, a consistent 29% decrease in LPL activity was seen in perirenal fat at the 90-min point. Sixteen hours after TNF administration no significant decreases were seen in LPL activity from subcutaneous or perirenal fat (Table 2). In contrast, a consistent 20% decrease in LPL activity from pericardiac fat was seen in multiple experiments by 16 hr after TNF treatment of female rats.

Effect of TNF on muscle lipoprotein lipase

In both male and female rats, LPL activity was measured in heart and diaphragm muscle 90 min and 16 hr after TNF administration (**Table 3**). No significant decrease in heart or diaphragm muscle LPL was seen in either male or female rats treated with TNF at either time point.

TNF stimulates hepatic lipogenesis in female rats

In our previous studies of the ability of TNF to stimulate hepatic lipogenesis we used only male rats (14, 16). Therefore we determined the ability of TNF to stimulate de novo fatty acid and cholesterol synthesis in female rats by measuring the incorporation of ^3H_2O in vivo (**Table 4**). Female rats showed a 2.15-fold increase in ^3H_2O incorporation into fatty acid in their livers 1–2 hr after TNF administration. This correlated well with the

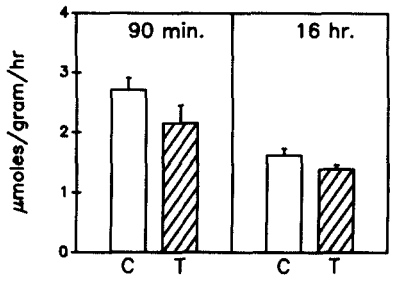

Fig. 2. Female Sprague-Dawley rats were injected with TNF (hatched bars) or saline (open bars), then kept fasting. At the indicated times, parametrial fat pads were removed and LPL activity was measured as described under Methods. Values are the mean ± SEM for five rats.

average 1.76-fold increase in serum triglycerides. As seen with male rats (14), there was no acute increase in cholesterol synthesis in livers from female rats (Table 4). Serum samples were analyzed for the appearance of newly synthesized triglyceride by extraction and chromatography as described in Methods. TNF treatment led to a significant increase in the acute appearance of label in serum triglycerides (TNF 0.97 ± 0.15; control 0.20 ± 0.07 μmol ^3H_2O per ml serum, $P < 0.01$).

The increase in hepatic fatty acid synthesis was sustained for up to 17 hr. ^3H_2O incorporation into fatty acid in vivo was increased 1.56-fold by 16–17 hr after TNF administration. Again there was a good correlation between the increase in fatty acid synthesis and the average 1.52-fold increase in serum triglycerides at this time.

As seen with male rats (14), by 16–17 hr after TNF administration, hepatic cholesterol synthesis was also increased in TNF-treated female rats (Table 4).

DISCUSSION

In this study we treated rats with human TNF at a dose (25 μg/200 g) that leads to significant increases in serum triglyceride levels. We had previously demonstrated that TNF rapidly increases serum triglycerides with the peak occurring 1–2 hr after administration in male rats (14). The increase in serum triglycerides is sustained for over

TABLE 2. Effect of TNF on LPL in various adipose tissues of female rats

Condition	Time	Pericardiac	Subcutaneous	Perirenal
			μmol/g per hr	
Control	90 min	0.86 ± 0.06	1.60 ± 0.28	2.36 ± 0.15
TNF	90 min	0.74 ± 0.09	1.39 ± 0.33	1.68 ± 0.22*
Control	16 hr	0.53 ± 0.02	0.45 ± 0.08	0.82 ± 0.08
TNF	16 hr	0.32 ± 0.04**	0.38 ± 0.05	0.73 ± 0.04

*, $P < 0.01$; **, $P < 0.05$.

TABLE 3. Effect of TNF on muscle LPL

Condition	Time	Sex	Activity	
			Diaphragm	Heart
			μmol/g per hr	
Control	90 min	M	1.14 ± 0.08	6.35 ± 0.76
TNF	90 min	M	1.04 ± 0.10	5.61 ± 0.45
Control	16 hr	M	2.25 ± 0.16	4.88 ± 0.13
TNF	16 hr	M	2.00 ± 0.17	5.39 ± 0.29
Control	90 min	F	2.61 ± 0.37	4.26 ± 0.24
TNF	90 min	F	2.37 ± 0.26	4.27 ± 0.23
Control	16 hr	F	1.86 ± 0.14	4.27 ± 0.08
TNF	16 hr	F	2.19 ± 0.19	4.22 ± 0.08

17 hr (14), despite the rapid clearance of TNF from the circulation. We now find that the same dose of TNF rapidly increases serum triglycerides in female rats and that this is also sustained for at least 17 hr.

Based on data in the literature, there are two potential mechanisms by which TNF could increase serum triglycerides. First, TNF has been shown to decrease the activity of LPL in cultured adipose cells (11–13) and in vivo (15). A decrease in LPL in vivo could produce a decrease in the clearance of triglyceride-rich lipoproteins resulting in an increase in serum triglycerides. Second, TNF administration produces a rapid and sustained increase in hepatic lipogenesis, with increased levels of newly synthesized fatty acid appearing in serum soon after TNF administration (14). Increased production and secretion of VLDL by the liver could produce an increase in serum triglycerides.

To determine the relative contributions of TNF-induced increases in synthesis of triglycerides by the liver versus TNF-induced inhibition of LPL, we have examined the effect of treatment of rats with TNF on serum triglycerides, hepatic lipogenesis, and LPL activity under identical experimental conditions. In order to draw broad conclusions about the role of TNF-induced decrease in adipose tissue LPL in promoting hypertriglyceridemia, it is necessary to look at multiple adipose tissue sites. There was little decrease in LPL activity in these experiments. Ninety minutes after TNF administration, when serum triglycerides were acutely stimulated, no decrease was seen in LPL activity from epididymal or pericardiac fat, and heart or diaphragm muscle of male rats or parametrial, subcutaneous, or pericardiac fat and heart or diaphragm muscle of female rats. A consistent 23% decrease in LPL from subcutaneous adipose tissues of male rats and a 29% decrease in LPL activity in perirenal fat from female rats was seen 90 min after TNF administration. A trend toward the 13% decrease in LPL activity from perirenal fat was also seen in male rats. It is unlikely that these slight decreases in LPL activity in only a few adipose tissue sites could account for the rapid increase in serum triglycerides (2.2-fold in males and 1.8-fold in females) seen by 90 min after TNF administration.

By 16 hr after treatment with TNF, LPL activity in epididymal fat of male rats had decreased by 44% but there was no sustained decrease in LPL activity in pericardiac, perirenal or subcutaneous fat and heart or diaphragm muscle in male rats. By 16 hr after the injection of TNF in female rats there was no decrease in LPL activity in parametrial fat, subcutaneous fat, or perirenal fat, and heart or diaphragm muscle in female rats. In contrast, LPL was reduced by 40% in pericardiac fat 16 hr after TNF administration to female rats. Thus, only one of many tissues examined in either male or female rats showed a decrease in LPL activity by 16 hr after TNF administration.

Our results should be compared to recent data from Semb et al. (15). These authors used murine TNF (20 μg/200 g rat) which is 5–10 times more potent against rodent cells in vitro than human TNF (24, 25). Therefore, the doses of human TNF that we utilized in our studies to produce hypertriglyceridemia may be significantly (⅕ to 1/10) lower, if similar species specificity occurs in vivo. Additionally, Semb et al. (15) allowed control and TNF-treated animals full access to food. Because TNF induces acute anorexia, we fasted both groups of animals after in-

TABLE 4. Effect of TNF on hepatic lipogenesis in female rats

Condition	Time	Incorporated	
		Fatty Acid	Cholesterol
		μmol ^3H_2O/liver per hr	
Control	1–2 hr	4.06 ± 1.33	24.5 ± 5.70
TNF	1–2 hr	8.74 ± 1.45*	27.0 ± 5.59
Control	16–17 hr	7.30 ± 0.47	5.48 ± 0.79
TNF	16–17 hr	11.38 ± 0.65**	22.65 ± 1.58**

*, $P < 0.05$ compared to control; **, $P < 0.001$ compared to control.

jection in order to control for the influence of nutritional factors on adipose tissue LPL. Semb et al. (15) found a 59% decrease in LPL activity in rat epididymal fat pad 6 hr after TNF administration. Some of the decrease in LPL seen by Semb et al. (15) may have been due to the decrease in food intake rather than direct effects of TNF. These authors studied the time course of TNF action in guinea pigs that received 30 μg/600 g. LPL was decreased 66% by 4.5 hr after TNF, with an average 29% decrease occurring at 100–110 min after TNF. Serum triglyceride levels were not determined (15). Under our conditions which acutely increased serum triglyceride levels (90 min post-human TNF administration), we found no decrease in LPL activity in the epididymal fat pad, but we also found that by 16 hr after TNF administration, LPL activity was decreased by 44% in the epididymal fat pad. However, TNF administration did not have similar effects in other fat pads. For example, in the parametrial fat pad of females there was no decrease at either time point. In addition, multiple other adipose tissues and heart and diaphragm muscle showed little or no decrease in LPL activity under these conditions. Thus, while our data confirm those of Semb et al. (15) in the epididymal fat pad, we have now demonstrated that the TNF-induced rise in serum triglycerides precedes the decrease in LPL in epididymal fat. Further, TNF-induced decreases in LPL activity appear limited to only a few adipose tissue sites. Recent data indicate that treatment of cultured human omental adipocytes with human TNF does not produce a decrease in lipoprotein lipase levels (26).

It is interesting to note that Semb et al. (15) found an increase rather than a decrease in LPL from post-heparin plasma of TNF-treated rats. At the higher doses of TNF that they used, LPL activity in rat heart, lung, and liver actually increased many hours after TNF administration. In the rat, LPL in muscle is not coordinately regulated with LPL in adipose tissue; for example, adipose LPL decreases with fasting while muscle LPL has been reported to either stay constant or increase (27–29). During fasting, clearance of chylomicrons is not decreased, but uptake is greater in muscle and lower in adipose tissue compared to fed rats (28). Thus, muscle LPL plays a key role in the clearance of triglyceride under many conditions. Under the conditions reported in this study, we found no effect of TNF on muscle LPL (Table 3).

On the other hand, we have previously demonstrated that TNF causes an acute and sustained increase in hepatic lipogenesis under these conditions. The time course of increased hepatic lipogenesis in male rats parallels the increase in serum triglycerides, with maximum increase at 1–2 hr but with an increase in lipogenesis sustained over the next 17 hr (14). Newly synthesized fatty acids appear in the serum at acutely increased levels 1–2 hr after TNF treatment of animals (14). In this study we have demonstrated that TNF treatment of female rats also produces a similar acute increase in hepatic lipogenesis which is sustained for up to 17 hr. In female rats, newly synthesized triglycerides were shown to acutely appear in serum at increased levels 1–2 hr after TNF treatment of animals. As observed with male rats, in female rats TNF administration does not lead to an acute increase in hepatic cholesterol synthesis, although after 17 hr hepatic cholesterolgenesis is increased in TNF-treated animals of both sexes.

Thus, when hepatic lipogenesis and LPL activity in peripheral tissues are measured under conditions during which TNF acutely raises serum triglycerides, there is an acute and sustained increase in hepatic lipogenesis but only minimal decreases in LPL activity. It is unlikely that these minimal decreases in LPL activity in a few adipose sites can be responsible for the rapid and sustained increase in serum triglycerides that was seen. In this respect the hyperlipidemia induced by TNF treatment may resemble that of diabetes mellitus, where VLDL production is usually increased and there is only a weak correlation between hypertriglyceridemia and LPL activity (29–33). Thus, our data suggest that a plausible mechanism by which TNF increases serum triglyceride levels is by stimulation of hepatic lipogenesis and VLDL production in vivo.

Note added in proof: We have recently demonstrated that TNF administration to rats has no effect on the clearance of chylomicrons from the circulation (34) but does produce an increase in VLDL production (35).

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