Persistence of the Hypertriglyceridemic Effect of Tumor Necrosis Factor Despite Development of Tachyphylaxis to Its Anorectic/Cachectic Effects in Rats


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ABSTRACT

The administration of a single injection of tumor necrosis factor (TNF) produces a variety of acute and sustained biological effects, including hyperlipidemia, stimulation of hepatic lipogenesis, decreases in adipose tissue lipoprotein lipase activity, and anorexia with weight loss. Chronic administration of a fixed dose of TNF produces tachyphylaxis to the anorectic/cachectic effects of TNF. We now report that the hyperlipidemic effect of TNF persists during chronic TNF administration in the absence of any cachectic effect of TNF. Sprague-Dawley rats injected with TNF (250 μg/kg) show a significant decrease in weight over the next 24 h which can be accounted for by decreases in food and water intake accompanied by an increase in urinary output. With subsequent daily injections of TNF, treated rats begin eating and rapidly regain weight. Hypertriglyceridemia persists for up to 10 days of daily injections of TNF. After three daily injections of TNF, no decreases were seen in lipoprotein lipase activity in a wide variety of tissues. De novo hepatic lipogenesis remained increased in TNF-treated animals after four daily injections, but by the fifth day hepatic lipogenesis returned to normal. After 5 days of TNF treatment the acute incorporation of labeled glycerol into serum triglycerides remained elevated. These data indicate that hypertriglyceridemia persists during multiple daily injections of TNF and that TNF induced hypertriglyceridemia is not inevitably linked to the syndrome of cachexia.

INTRODUCTION

Infection of the body by microorganisms elicits a complex host response that is directed towards the killing and removal of infectious agents as well as providing immunity against future challenges. Infection can also be accompanied by marked disturbances in intermediary metabolism as well as a syndrome of wasting (1). Infection may produce hyperlipidemia characterized by increases in serum VLDL (2, 3). In model infections, increases in hepatic lipogenesis (4) and inhibition of triglyceride clearance secondary to decreases in lipoprotein lipase (5) have been reported. Recent evidence indicates that a variety of cytokines and, in particular, TNF mediate the immune response and the changes in metabolism (6–8).

Administration of purified recombinant TNF to rats produces a rapid (within 45 min) increase in serum triglycerides, which is sustained for over 17 h (8). In parallel, TNF induces an acute increase in de novo hepatic fatty acid synthesis which is likewise sustained for 17 h (8). Although no initial increase in hepatic cholesterol synthesis is seen, by 7 h after TNF administration serum cholesterol levels increase and there is a parallel increase in hepatic cholesterol synthesis (8). TNF acutely stimulates hepatic lipogenesis by increasing hepatic levels of citrate, an allosteric activator of acetyl CoA carboxylase, the primary rate limiting enzyme in fatty acid synthesis (9). By 16 h after TNF administration the activity of acetyl CoA carboxylase, fatty acid synthetase, and hydroxymethylglutaryl CoA reductase in the liver are increased compared to control animals (8, 9). There is also evidence for an increased efficiency of fatty acid reesterification under these conditions (9). Recent studies of the incorporation of glycerol into lipid in vivo indicate that TNF increases total hepatic synthesis of triglyceride with an accompanying increase in secretion of newly synthesized triglyceride into the circulation (10, 11). The increase in serum triglycerides seen acutely after TNF administration is due to an increase in VLDL.3 Studies in humans receiving TNF as an antineoplastic agent have demonstrated that TNF increases both serum triglycerides and the turnover of fatty acids (12).

Treatment of rats with TNF does not acutely change adipose tissue lipoprotein lipase activity, but after several hours a decrease in lipoprotein lipase is seen in the epididymal fat pad (11, 12). However, lipoprotein lipase activity is not decreased or is increased in a variety of other tissues and an increase in total plasma lipase activity is seen after heparin injection (11, 13).

TNF has also been postulated to mediate the chronic wasting (Cachexia) seen with persistent infection (6). Initial studies with crude supernatants from endotoxin-stimulated macrophages produced a syndrome of chronic weight loss and hypertriglyceridemia (6, 14). Injection of animals with purified recombinant TNF acutely induces acute gastrointestinal inflammation, anorexia, and weight loss (15–18). With repeated injection of TNF, animals develop tolerance to the gastrointestinal side-effects and regain the weight that they have lost (15–18). The tolerance to TNF-induced anorexia and weight loss can be overcome by sequentially escalating the dose of TNF administered (16).

The effect of repeated TNF administration on lipid metabolism has not been studied. We now demonstrate that TNF-induced hypertriglyceridemia persists in the face of development of tachyphylaxis to the anorectic/cachectic effects of TNF. These studies suggest that the hyperlipidemia of cytokine administration or infection is not inevitably linked to the syndrome of cachexia.

MATERIALS AND METHODS

[14C]Triolein (80–120 mCi/mmol), [26-14C]cholesterol (0.5 mCi/0.33 mg), [2-3H]glycerol (200 mCi/mmol), and [14C]oleic acid (40–60 mCi/mmol) were purchased from Du Pont-New England Nuclear. Tritiated water (1 Ci/g) was purchased from ICN Radiochemicals. Triolein,

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4 The abbreviations used are: VLDL, very low density lipoprotein; TNF, tumor necrosis factor; CoA, coenzyme A; TLC, thin-layer chromatography.

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 \( \times 10^8 \) units/mg produced by recombinant DNA technology was kindly provided by Drs. H. M. Shepard and J. Kaumeyer of Genentech, Inc.

Animal Procedures. Male Sprague-Dawley rats (approximately 200 g) were purchased from Simonsen Animal Vendors. The animals were maintained on a reversed 12-h light cycle (3 a.m. to 3 p.m. dark, 3 p.m. to 3 a.m. light) and fed Simonsen Rat Chow and water \textit{ad libitum}.

Rats were injected via the tail vein or intramuscularly as indicated in the text with 50 \( \mu \)g of TNF in 0.5 ml of 0.9% saline or saline alone. This dose, which is the same as that used by others, was chosen to produce tolerance to the anorectic effects of TNF (15), is approximately one-half of that shown to produce tumor necrosis \textit{in vivo} (15) and is twice the dose that produces maximal stimulation of hepatic lipid synthesis in male rats (8). In another experiment rats were injected with 50 \( \mu \)g/200 g twice daily with similar results.

Serum Chemistries. Serum triglyceride levels were measured using Sigma Diagnostic Kit 405 after extraction with Dole’s reagent. Serum cholesterol levels were measured by using Sigma Diagnostic Kit 351.

Adipose Tissue Lipoprotein Lipase Analysis. Two h after the last injection of TNF or saline, rats were killed, adipose tissue and muscle were removed from the sites indicated, and flash frozen with a Wollenberger clamp cooled in liquid \( N_2 \). Lipolytic activity was determined by the method of Pykalisto et al. (19), as described previously (20). Briefly, the substrate [unlabeled triolein (200 mg), 4.0 \( \mu \)Ci of \(^{14} \)Ctriolen, 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 cofactor), and 6.2 ml of 1.0 M Tris-HCl buffer (pH 8.2) using a Branson D-125 sonifier for 3 min (setting 3 and maximal tuning). 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. Triglycerides (triolen) and fatty acids (oleic acid) were extracted from the incubation mixture by three washes with heptane. Subsequently, the oleic acid was separated from triolen by the standard sequential alkalization-reacidification-heptane extraction procedure. The final heptane phase was transferred to counting vials and evaporated under nitrogen. The generated \(^{14} \)Coleic acid was measured by liquid scintillation spectrometry. The standard consisted of 20 \( \mu \)l of the substrate emulsion. This was extracted initially by 5.0 ml of heptane in 2.5 ml water and then by 2.5 ml of heptane. Both extracts were added to the counting vials, evaporated, and measured. One unit of lipoprotein lipase activity is defined as equivalents of free fatty acids/gram of tissue per hour.

Lipogenesis. At the time indicated after TNF administration, rats were injected i.p. with tritiated water (50 \( \mu \)Ci). One h later the rats were anesthetized, weighed, and a blood specimen obtained. Livers were removed, individually weighed, and the lipid saponified by refluxing overnight in a solution of 45% KOH, water, and 70% ethyl alcohol (2:1:5). After cooling, internal standards of \(^{13} \)Ccholesterol and \(^{13} \)Coleic acid were added before extracting the nonsaponifiable material three times with 25 ml of petroleum ether. The petroleum ether extract was dried, dissolved in chloroform, and then applied to TLC plates. After development in ethyl acetate:benzene (1:5), the band corresponding to the cholesterol standard was cut from the plate and counted by liquid scintillation. The window settings of the scintillation counter were adjusted so that less than 0.2% of the tritium counts were recorded in the \(^{14} \)C window and approximately 10% of the \(^{13} \)C counts in the tritium window. Incorporation was corrected for spillover of \(^{14} \)C and \(^{13} \)C, for background, and for recovery of internal standard.

After acidifying the saponified material to pH less than 2 with concentrated hydrochloric acid, fatty acids were extracted three times with petroleum ether. The extract was dried, dissolved in chloroform and an aliquot counted as described above. The specific activity of tritiated water was determined individually for each animal by measuring the disintegrations/min/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 our methodology for measuring lipid synthesis has been demonstrated in earlier publications (21, 22).

Triglyceride Synthesis. The incorporation of glycerol into hepatic and serum lipids was performed as described in detail elsewhere (10). At the time indicated after TNF administration, animals were injected i.p. with \(^{3} \)Hglycerol (25 \( \mu \)Ci). Thirty min later a blood specimen was obtained and the animals were killed. The livers were removed, weighed, and an aliquot of liver homogenized in a 0.2 M KCl solution. An internal standard of \(^{14} \)Ctriolen was added to serum and homogenized liver samples. Livers and serum were extracted using the Bligh Dyer technique as previously described (23). The lipid extract was transferred to scintillation vials and dried. Liquid scintillation counting and calculation of data were performed as described above under “lipogenesis.”

Plasma Free Fatty Acid Levels. Blood for free fatty acid analysis was placed in iced, heparinized glass tubes within 30 s of sampling, centrifuged at 1000 \( \times \) g at 4°C and plasma separated. The free fatty acid content of plasma was determined by a modification of the extraction method of Ko and Rower (24), as previously described (25). After acidification and extraction of the plasma with heptane/isopropyl alcohol containing pentadecanoic acid as an internal standard, the heptane was evaporated and 0.05 ml of 10% BCl3/methanol was added to convert the fatty acids to their respective methyl esters. After the reaction has proceeded for 10 min, two layers are present: an oily film adhering to the walls of the reaction vial and the BCl3/methanol layer. The latter solution, after transfer to another vial, was evaporated with nitrogen. Methylene chloride (0.025 ml) was added to the vial and a sample was injected into a gas chromatograph using a 10′ \( \times 1/4′ \) ID glass column, 1% SP-2330 (Supelco, Inc., Bellefonte, PA), oven temperature 190°C, with an FID detector. The amounts of the individual fatty acids were then determined by comparison with the pentadecanoic acid internal standard and integrated with a 5880 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA). Eight analyses of one sample gave a coefficient of variation of 1.9% in our laboratory. The values for individual fatty acids were summed and expressed as nmol total free fatty acids per milliliter of plasma.

Data Analysis. Data presented in Figs. 1–5 and Tables 1–4 represent the mean ± SEM of experiments using five rats in each group. Statistical differences were determined by using a two-tailed Student’s \( t \) test, except in Fig. 1d where a paired \( t \) test was used.

RESULTS

A Second Injection of TNF Induces a Second Increase in Serum Triglycerides and Hepatic Lipogenesis. We have previously demonstrated that TNF administration induces a rapid increase in serum triglycerides and \textit{de novo} hepatic lipogenesis, with the maximal increase occurring by 1 to 2 h after injection (8). The increase in both serum triglycerides and hepatic lipogenesis persists for over 17 h. We therefore first determined whether a second injection of TNF, 17 h after the first injection, induces a new acute increase in serum triglycerides and hepatic lipogenesis. Two sets of rats were injected with TNF (50 \( \mu \)g/200 g i.v.) on Day 1. Seventeen h later on Day 2, one set of rats received a repeat injection of TNF (50 \( \mu \)g/200 g i.v.) while the others received saline alone. One h later, all animals were injected with 50 \( \mu \)Ci of \(^{3} \)H2O i.p. to measure \textit{de novo} hepatic lipid synthesis. After an additional hour, animals were anesthetized, weighed, a blood sample taken, and their livers excised for measurement of lipid synthesis.

As can be seen in Table 1, a second injection of TNF 17 h after the first injection produced a significant increase in serum triglycerides compared to animals that received a second injection of saline. No change in serum cholesterol levels was seen. The second injection of TNF was also able to induce again an acute increase in the \textit{de novo} synthesis of fatty acids in the liver (Table 1). There was no difference in the rate of hepatic cholesterol synthesis, consistent with the absence of a rapid increase.
in cholesterol synthesis after a single injection of TNF (8).

The quantity of newly synthesized fatty acid in the serum is increased after a second injection of TNF (Table 1), but the amount of labeled cholesterol in serum was not significantly increased with further TNF treatment (Table 1). This pattern of appearance of label in serum 2 h after a second injection of TNF is also similar to that found 2 h after the first injection of TNF (8, 11). Previous studies indicate that newly synthesized fatty acid rapidly appears in serum triglycerides after TNF treatment (11). Thus, with regards to lipid metabolism a single injection of TNF does not induce tachyphyllaxis to a subsequent injection of TNF.

**Metabolic Balance Studies of Repeated TNF Injection.** Animals were placed in individual Nalgene metabolic cages which allow for quantification of water intake, food intake, urine output, and stool output. Animals were weighed each day just prior to injection. Five animals received daily injections of TNF (50 μg/200 gm) starting on Day 1, while five other animals received daily injections of the saline vehicle. The average weight and daily weight changes for these two groups of animals are shown in Fig. 1. The weight of control animals steadily increased during repeated injections of saline (Fig. 1A). The weight of the TNF-treated animals dramatically decreased by 24 h after the first TNF injection. Four out of five animals treated with TNF showed a striking reduction in weight after the first injection (P < 0.05 in a paired t test). Subsequently, TNF-treated animals began to gain weight, with the mean weight approaching that of controls over the next 3 days. Daily weight changes are plotted in Fig. 1B. Control animals gained approximately 5 grams of weight per day. In the 24 h after the first TNF injection, weight loss averaged 11 ± 5.4 g. In the 24-h period after each subsequent TNF injection, treated animals gained weight at rates at least as great as control animals. In an experiment not shown, the weight gain of animals treated with TNF for 10 days was the same as control animals.

Food intake was dramatically decreased in the first 24 h after TNF treatment (Fig. 2A). Food intake subsequently increased and, by the day of the fifth injection, had reached that of control animals. Likewise, water consumption was dramatically decreased in the 24 h after the first injection of TNF but subsequently returned to normal despite repeated injections of TNF (Fig. 2B). In contrast, urine output was increased in the first 24 h after TNF injections, but also returned to normal by the second day of TNF injection (Fig. 2C). There was no difference in fecal weight between control and TNF treated animals (Fig. 2D), although the presence of blood was noted in some feces within the 24 h after the first TNF treatment. These data are consistent with the induction of profound anorexia by TNF, with development of tolerance to anorexia as previously described by others (15–18).

A more detailed analysis of the first 24 h of TNF treatment is of interest. Table 2 presents the mean data for balance studies during this period. While the control animals gained an average of 5.2 g, the TNF-treated animals lost an average of 11 g in weight. This represents a decrease of 16.2 g in weight from what the TNF-treated animals would have been anticipated to gain. As expected, weight gain in control animals represented one-third of their food consumption. TNF-treated animals ate 10 g less of food, which could account for up to approximately 3.3 g of the difference in weight. Water consumption was decreased by 6 ml in TNF-treated animals while urine output was increased by 7.6 ml. Therefore, with regards to fluid balance, TNF treated animals showed a deficit of 13.6 ml. Thus, the difference in weight gain observed between TNF-treated and control animals that could be attributed to both the decrease in food consumption (3.3 g) and the negative fluid balance (13.6 g) equals 16.9 g; this value is comparable to the calculated weight deficit of 16.2 g. Thus, within the limitations of these balance studies, the weight loss acutely induced by TNF appears proportional to changes in food and water consumption and urine output and does not represent tissue wasting. When data from the subsequent 24-h periods are analyzed in a similar

### Table 1 Effect of a second injection of TNF on serum lipids and hepatic lipogenesis

<table>
<thead>
<tr>
<th>Injection</th>
<th>Serum chemistries (mg/dl)</th>
<th>Hepatic lipogenesis (μmol H₂O inc/liver/h)</th>
<th>Labeled lipid in serum (μmol H₂O/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Triglycerides</td>
<td>Cholesterol</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>A.</td>
<td>TNF</td>
<td>Saline</td>
<td>48 ± 3.3</td>
</tr>
<tr>
<td>B.</td>
<td>TNF</td>
<td>TNF</td>
<td>104 ± 5.8*</td>
</tr>
</tbody>
</table>

* P < 0.001 compared to control.
manner, the rapid regaining of weight is due to rapid restoration of water consumption without further diuresis and somewhat slower resumption of food consumption.

Effect of Repeated TNF Injection on Serum Lipids. In three separate experiments, serum triglycerides and cholesterol were determined in animals that had received 3, 4, or 5 days of injections with TNF or saline (Fig. 3). Although detailed metabolic balances were not carried out in each of these experiments, in all three experiments TNF-treated animals lost weight in the first 24 h after TNF injection and rapidly gained weight thereafter. Serum was drawn 3 h after the last injection. As can be seen in Fig. 3A, the elevation in serum triglycerides persisted for up to 5 days of repeated injections with TNF, despite loss of TNF's anorectic effects.

In two other experiments, rats were injected with TNF (50 μg/200 g) daily or twice daily for 10 days. In both experiments the net weight gain in TNF-treated animals was the same as saline-treated controls. Serum triglycerides remained elevated in TNF-treated animals compared to controls. With 10 daily injections serum triglycerides were 121 ± 11.8 mg/dl with TNF treatment and 24 ± 2.5 mg/dl in controls (P < 0.001). With twice daily injection for 10 days, serum triglycerides were 86 ± 4.3 with TNF treatment and 58 ± 4.4 in controls (P < 0.005).

In contrast, serum cholesterol levels were not elevated compared to control animals after 3, 4, or 5 consecutive days of TNF injections (Fig. 3B). Thus, the increase in serum cholesterol seen 17 h after a single injection of TNF compared to saline treated animals does not persist with repeated injections in animals feeding ad libitum.

Lipoprotein Lipase. A single injection of TNF has been shown to produce a decrease in lipoprotein lipase activity in the epididymal fat pad after several hours (11, 13). Either no change or an increase was seen in other tissues. We therefore tested the effect of three daily injections of TNF (50 μg/200 g) under conditions known to produce a chronic increase in serum triglycerides (Fig. 3A) on lipoprotein lipase activity. No significant decrease was seen in lipoprotein lipase activity in epididymal, pericardic, or perirenal fat or in diaphragm or heart muscle from TNF treated rats (Table 3). A small but significant increase in lipoprotein lipase activity was seen in s.c. fat in TNF-treated rats. There was a trend towards an increase in lipoprotein lipase activity in diaphragm muscle of TNF-treated rats, but this did not reach statistical significance. Thus, repeated treatment of normal rats with TNF under these conditions does not lead to chronic suppression of lipoprotein lipase.

De Novo Hepatic Lipogenesis. Two h after the last injection with TNF, rats received 50 mCi of 3H2O i.p. to measure de novo lipogenesis. One h later a serum sample was taken and the livers removed for assessment of hepatic lipogenesis. In contrast to the effects of TNF on lipoprotein lipase, de novo synthesis of fatty acids remained increased in the liver after three and four daily injections of TNF (Fig. 4A). However, by the fifth TNF injection, hepatic fatty acid synthesis returned to normal. De novo cholesterol synthesis was measured after 3, 4, and 5 injections (Fig. 4B). At no time during this period was there a significant increase in hepatic cholesterol synthesis in TNF-treated animals.

Labeled Lipids in Serum. We next assessed the appearance of newly synthesized fatty acid and cholesterol in the serum during studies of tritiated water incorporation in vivo. Significantly increased levels of labeled fatty acid were found in the

Fig. 2. Metabolic balance studies during repeated TNF administration. Data are on the same animals whose weights are shown in Fig. 1. Animals were housed in Nalgene metabolic cages which allow separation of urine from feces for daily weighing as well as accurate measurement of food and water consumption. A, daily food consumption; * P < 0.05. B, daily water consumption; * P < 0.05; C, daily urine output; * P < 0.02; D, daily fecal output.

<table>
<thead>
<tr>
<th>Weight gain (g)</th>
<th>Food consumption (g)</th>
<th>Water consumption (ml)</th>
<th>Urine output (ml)</th>
<th>Fluid balance (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.2 ± 1.9</td>
<td>15.8 ± 1.2</td>
<td>26 ± 1.9</td>
<td>11.6 ± 0.75</td>
</tr>
<tr>
<td>TNF</td>
<td>−11 ± 5.4</td>
<td>5.8 ± 0.8</td>
<td>20 ± 1.6</td>
<td>19.2 ± 2.3</td>
</tr>
<tr>
<td>Difference (control − TNF)</td>
<td>−16.2</td>
<td>10 g</td>
<td>6</td>
<td>7.6</td>
</tr>
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(P < 0.05) (P < 0.05) (P < 0.05) (P < 0.02) (P < 0.001)
PERSISTENCE OF TNF INDUCED HYPERLIPIDEMIA

Fig. 3. The effect of repeated TNF administration on serum lipid levels. In three separate experiments animals received daily injections of saline on TNF (50 µg/200 mg) as described above for 3, 4, or 5 consecutive days. Three h after the last injection serum samples were taken for lipid determination. N = 5 for each group. A, triglycerides (d3, P < 0.01; d4, P < 0.001; d5, P < 0.005). B, cholesterol.

Table 3 Effect of repeated injection of TNF on lipoprotein lipase activity

Animals were injected with TNF (50 µg/200 g) or saline i.v. on Day 1 and intramuscularly on Days 2 and 3. Two h after the third injection animals were anesthetized and tissues prepared for lipoprotein lipase analysis as described in "Materials and Methods." N = 5 for each group. A, 3H2O incorporated into fatty acid (d3, P < 0.01; d4, P < 0.02); fi, 3H2O incorporated into cholesterol.

Fig. 4. Effect of repeated TNF administration on de novo hepatic lipid synthesis. In three separate experiments animals were treated daily with saline or TNF as described above for 3, 4, or 5 consecutive injections. Two h after the last injections animals received 50 mCi of 3H2O i.p. One h later, animals were anesthetized, livers removed, and incorporation of 3H2O into lipid analyzed as described in "Materials and Methods." N = 5 for each group; A, 3H2O incorporated into fatty acid (d3, P < 0.01; d4, P < 0.02); B, 3H2O incorporated into cholesterol.

Free Fatty Acids. We have previously demonstrated that 90 min after injection of TNF, free fatty acid levels are acutely increased, although unlike serum triglycerides, a significant increase in free fatty acid did not persist over the next few hours (9). Therefore, we assessed the effect of three daily injections of TNF on serum free fatty acid levels. There was no increase in serum free fatty acids 2 h after the third injection of TNF in animals that had received three daily injections of TNF, compared to saline treated animals (TNF 0.582 ± 0.045 versus control 0.677 ± 0.060 µmol/ml).

Incorporation of Glycerol into Triglyceride. When labeled glycerol is injected, the glycerol is rapidly taken up by the liver and incorporated into lipid, which is then secreted into the circulation (10). We have previously utilized this technique to demonstrate that TNF acutely (within 90 min) increases the synthesis and secretion of total triglyceride by the liver (10). Therefore we have tested whether this effect of TNF persists after multiple injections. Animals received five daily injections of TNF and/or saline and 60 min after the last injection were given 25 µCi of 1H1glycerol i.p. Thirty min later, livers and serum were obtained and assayed for the presence of labeled lipid. There is a trend towards an increase in glycerol incorporation into lipid in livers from TNF-treated animals but this did not reach statistical significance (Table 4). However, the amount of labeled triglyceride that acutely appears in the serum was significantly increased (Table 4).

DISCUSSION

Infection is often accompanied by hyperlipidemia characterized by increases in serum VLDL (2, 3). Experiments with model infections have found both increases in hepatic lipogenesis (4) and inhibition of triglyceride clearance secondary to decreases in lipoprotein lipase (5). Attention was focused on the cytokines as mediators of the hyperlipidemia of infection when it was discovered that a factor produced by endotoxin stimulated macrophages inhibited lipoprotein lipase activity in cultured fat cells (6). This factor was subsequently found to be tumor necrosis factor (26). Administration of purified TNF to rats acutely increases serum triglyceride levels, an effect which is sustained for more than 17 h (8). In addition, we found that,
weight loss, and lipid metabolism. As seen by others, we found the relationships between chronic administration of TNF, repeated administration of TNF leads to repeated fever spikes and resume weight gain with repeated injections of TNF (15, properties. However, others have now demonstrated that animals become tolerant to the anorectic/cachectic effects of TNF in parallel, TNF acutely stimulated de novo hepatic fatty acid synthesis, an effect which was also sustained for more than 17 h (8). Recent data indicate that TNF increases total hepatic lipogenesis which includes reesterification of fatty acid into triglyceride and results in increased production of VLDL particles (10).

Because cachexia may accompany infections that produce hypertriglyceridemia and because TNF inhibits the storage of triglyceride and stimulates its breakdown in cultured mouse fat cells, hypertriglyceridemia and cachexia were thought to be linked (6). Indeed, the product of endotoxin-stimulated macrophages was initially named “cachectin” because of these properties. However, others have now demonstrated that animals become tolerant to the anorectic/cachectic effects of TNF and resume weight gain with repeated injections of TNF (15, 17, 18). This tolerance can be overcome by significantly escalating the doses of TNF (16). Recent experiments found that repeated administration of TNF leads to repeated fever spikes despite tolerance to anorexia (17). Therefore we have examined the relationships between chronic administration of TNF, weight loss, and lipid metabolism. As seen by others, we found that animals become resistant to the anorectic/cachectic effects of TNF after repeated injections. Although TNF-treated animals lost weight in the first 24 h, they then rapidly regained weight. Detailed metabolic studies analyzing food and water consumption as well as fecal and urinary output indicate that the loss of weight in the first 24 h could be accounted for by the effects of anorexia and increased urine output. Thus, there is little evidence in these studies for true cachexia (tissue wasting). Weight gain resumed with the restoration of eating and drinking and reduction of urine output to normal; TNF-treated animals rapidly restored fluid balance.

Our most important finding is that TNF-induced hypertriglyceridemia persisted despite tachyphylaxis to its anorectic effects. When rats received 50 µg TNF on a daily basis for up to 10 days, they remained significantly hypertriglyceridemic compared to animals receiving saline injections. Hypertriglyceridemia persisted despite resumption of weight gain, even when TNF was given twice daily at a dose of 50 µg/200 g for 10 days.

Each of the known steps in lipid metabolism that have previously been shown to be altered by TNF were studied in rats chronically treated with TNF. Persistent treatment with TNF did not lead to suppression of adipose tissue lipoprotein lipase activity. Previous studies had indicated that only a limited number of tissues show decreases in lipoprotein lipase activity when TNF is administered in vivo (11, 13). In contrast, de novo lipogenesis in the liver was increased after 4 straight days of TNF treatment. By the fifth day of TNF treatment, de novo hepatic fatty acid synthesis returned to control levels. The increased appearance of newly synthesized fatty acid in the serum also persisted during several repeated injections of TNF.

With regards to total triglyceride synthesis, we studied free fatty acid levels and the incorporation of glycerol into triglyceride. Two h after the third daily injection, plasma free fatty acid levels were similar in TNF and saline-treated animals. When glycerol incorporation into serum lipid was used as a measure of newly synthesized hepatic lipoproteins, we found that repeated treatment with TNF led to an increase in the appearance of labeled lipid in serum.

Other cytokines, in addition to TNF, can disturb lipid metabolism (7). This study does not address whether the changes seen in lipid metabolism are direct effects of TNF on target tissues or are mediated by induction of interleukins, interferons, prostaglandins or platelet activating factor. Likewise, it is not known whether purified TNF could induce cachexia if administered with other cytokines, inflammatory mediators or tumor products.

Recent evidence indicates that the acute increase in serum triglycerides seen after TNF administration is due to increases in hepatic synthesis and secretion of triglyceride in the form of VLDL particles (10). TNF induces no decrease in the clearance of triglyceride rich lipoproteins (27) and neither adipose nor muscle tissues show major decreases in lipoprotein lipase after acute TNF administration (11, 13, 27). In this study of chronic repeated administration of TNF, we found that the effects of TNF on hepatic lipid secretion, as measured by glycerol incorporation into circulating lipid, persisted during repeated injection of TNF at times when there was no inhibition of lipoprotein lipase activity.

The persistence of the ability of TNF to induce hypertriglyceridemia in the absence of cachexia or even persistent anorexia is also consistent with an important role for hepatic production and secretion of lipoproteins in the hyperlipidemia of infection as opposed to decreased storage in fat. These data imply that
the hyperlipidemia of infection by itself is not a major cause of cachexia. Our metabolic studies imply that the weight loss seen after acute TNF administration is due entirely to anorexia and increased urine output and not necessarily due to catabolic effects of this cytokine. In this light it is notable that studies of weight loss due to increasing doses of TNF demonstrated that TNF-treated animals consumed 60% as much food as control animals (16). When control animals were pair fed at the 60% level, they showed at least the same if not more weight loss than TNF treated animals with similar food intake. Lipid metabolism was not analyzed during those studies (16).

Our findings now demonstrate that persistent hyperlipidemia induced by chronic administration of purified TNF can be dissociated from the induction of cachexia and weight loss. In addition, because our previous data indicate that the primary site for the rapid hypertriglyceridemia effect of TNF is the liver, the possibility must be considered that cytokine-induced hyperlipidemia is part of the "acute phase response" which is known to be induced by TNF and other cytokines (28, 29). Whether the effects of TNF and other cytokines on lipid metabolism are deleterious or constitute part of the protective response to infection now needs to be explored.

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