Rapid downregulation of adipose tissue lipoprotein lipase activity on food deprivation: evidence that TNF-α is involved

Gengshu Wu,1 Peter Brouckaert,2 and Thomas Olivecrona1

1Department of Medical Biosciences, Physiological Chemistry, University of Umeå, SE-90187, Umeå, Sweden; and 2Department of Molecular Biomedical Research, VIB/Ghent University, B-9052 Ghent, Belgium

Submitted 10 June 2003; accepted in final form 12 December 2003

Wu, Gengshu, Peter Brouckaert, and Thomas Olivecrona. Rapid downregulation of adipose tissue lipoprotein lipase activity on food deprivation: evidence that TNF-α is involved. Am J Physiol Endocrinol Metab 286: E711–E717, 2004.—When food was removed from young rats in the early morning, adipose tissue tumor necrosis factor (TNF)-α activity increased 50% and lipoprotein lipase (LPL) activity decreased 70% in 6 h. There was a strong negative correlation between the TNF-α and LPL activities. Exogenous TNF-α further decreased LPL activity. Pentoxifylline, known to decrease production of TNF-α, had no effect on LPL activity in fed rats but almost abolished the rise of TNF-α and the decrease of LPL activity in rats deprived of food. The specific activity of LPL decreased from 0.92 mU/ng in fed rats to 0.35 and 0.24 mU/ng in rats deprived of food given saline or TNF-α, indicating a shift in the LPL molecules toward an inactive state. Lipopolysaccharide increased adipose tissue TNF-α and decreased LPL activity. Both of these effects were strongly impeded by pretreatment of the rats with pentoxifylline, or dexamethasone. Pretreatment of the rats with actinomycin D virtually abolished the response of LPL activity to food deprivation or exogenous TNF-α. We conclude that food deprivation, like lipopolysaccharide, signals to a gene whose product causes a rapid shift of newly synthesized LPL molecules toward an inactive form and thereby shuts down extraction of lipoprotein triglycerides by the adipose tissue. Adipocytes; cytokines; endothelium; fasting; heparin; lipopolysaccharide; rat; secretion; tumor necrosis factor-α

Lipoprotein lipase (LPL) is a major determinant of triglyceride transport (20, 23, 27). Genetic deficiency of the enzyme results in accumulation of triglyceride-rich lipoproteins in plasma (32). In normal individuals, LPL activity is modulated in a tissue-specific manner (27). This modulation is particularly prominent in adipose tissue. In adipose tissue of fed rats, most of the LPL protein is in a catalytically active form, whereas in fasted rats most of the enzyme protein is inactive (4). There is no evidence that the inactive form can be recruited into the active state. An increase of LPL activity after a meal thus requires synthesis of new, active lipase molecules. We have recently found that the downregulation on fasting is abolished if the rats are pretreated with actinomycin, indicating that a gene is switched on that somehow channels newly synthesized LPL molecules to the catalytically inactive form (5). The physiological purpose of this downregulation is presumably to protect the adipose tissue from unwanted lipolysis of plasma triglycerides during periods when there is net mobilization of intracellular triglycerides from the adipocytes. Further studies showed that the modulation engages the extracellular LPL (39). LPL activity and mass within the adipocytes remain unchanged. The molecular details of this interesting process are not known.

In this study, we asked what the signal(s) is that tells the adipose tissue to rapidly downregulate LPL activity. From the literature, there are two situations when adipose tissue LPL activity is rapidly downregulated. One is food deprivation. Another is trauma/sepsis/lipopolysaccharide (LPS). In the latter case, tumor necrosis factor (TNF)-α has been implicated as a major mediator. A single injection of TNF-α decreases LPL activity in adipose tissue (but not other tissues) of mice, rats, and guinea pigs (35). It was therefore natural to ask whether TNF-α might also be involved in the response to food deprivation. TNF-α has recently attracted much interest as a possible mediator of insulin resistance in adipose tissue (31, 36), and a role for the cytokine in short-term regulation of one of the major pathways of lipid transport was an interesting possibility. The present study provides evidence that TNF-α is in fact involved in the response of adipose LPL to food deprivation and attempts to further dissect the signaling pathway.

Earlier studies had indicated that LPS, presumably acting through TNF-α, downregulates LPL at the transcriptional level (1, 7, 24). Our data indicated that the response to food deprivation involved TNF-α but acted on a posttranscriptional level. We have therefore studied the effects of LPS in the same time frame as the experiments on food deprivation, 6 h. The results show an initial, rapid posttranscriptional response.

Materials and Methods

Animals and treatments. Male Sprague-Dawley rats were bought from Møllegaard Breeding Center (Ejby, Denmark). For most of the experiments, the rats were 23 days old and weighed ~60 g when they arrived in Umeå. They were allowed to acclimate for 7–10 days, by which time they had reached a weight of ~120 g. The rats were kept in a well-ventilated, temperature (21°C)- and humidity (40–45%)-controlled room with free access to a standard laboratory chow (Laktamin, Stockholm, Sweden) and tap water. The light in the room was on between 6:00 AM and 6:00 PM. In experiments where the rats were to be fasted, food was withdrawn from the cages at 6:00 AM, and a grid was placed at the bottom of the cages to prevent coprophagia. The rats were killed by decapitation. The adipose depot used in all experiments was the perirenal depot. In some experiments, the tissue was cut into small pieces and digested with collagenase to isolate the adipocytes as described (34). The extracellular LPL activity and mass were calculated as the difference between tissue total and what was recovered with the adipocytes (“intracellular”; see Ref. 39).
In some experiments, potential inhibitors were administered intraperitoneally. The substances used and the doses in relation to body weight were as follows: Nω-nitro-l-arginine methyl ester (l-NAME), 50 mg/kg (adapted from Ref. 25); pyrrolidine dithiocarbamate (PDTC), 200 mg/kg (adapted from Ref. 22); LPS, 15 mg/kg (adapted from Ref. 24); pentoxifylline, 100 mg/kg (adapted from Ref. 26); dexamethasone, 25 mg/kg (adapted from Ref. 37); and indomethacin, 10 mg/kg (adapted from Ref. 16). All the reagents for injection were dissolved in 0.154 M NaCl. The animal ethics committee in Umeå approved all protocols for animal experiments.

Materials. TNF-α was prepared in Escherichia coli containing an appropriate expression plasmid and purified to apparent homogeneity. The specific activity is 3.3 × 10⁸ IU/mg protein, and the endotoxin content was below the detection limit. PDTC, pentoxifylline, dexamethasone, and LPS were from Sigma (St. Louis, MO). The substrate for the LPL activity assay was [3 H]triolein in Intralipid (10%), kindly prepared by Fresenius-Kabi (Uppsala, Sweden). All other reagents were of the highest commercial grade possible.

Assays. LPL was extracted from tissues by homogenization in a Tris-HCl buffer (pH 8.2) containing detergents and protease inhibitors as described (4, 5). The homogenate was centrifuged for 15 min at 3,000 rpm, after which the intermediate phase (between the floating fat droplets and the pellet) was used for assay of LPL activity and immunoreactivity.

LPL activity was measured as described previously (4). Briefly, 2 µl of tissue homogenate (triplicate samples) were incubated for 60 min at 25°C with substrate in the presence of 10 µl of heat-inactivated serum from fasted rats (as source of apolipoprotein CII) and 6% BSA. The total volume was 200 µl. After termination of lipolysis, the fatty acids were extracted and counted for radioactivity. One milliunit of lipase activity represents one nanomole of fatty acids released per minute.

LPL immunoreactivity was measured with a sandwich ELISA, as described previously (4). Briefly, three different dilutions of tissue homogenate were incubated in microtiter plate wells previously coated with affinity-purified chicken anti-LPL IgG. Detection was mediated via a 5D2 monoclonal antibody (a kind gift by Dr. John Brunzell, University of Washington, Seattle, WA) followed by a peroxidase-conjugated anti-mouse IgG antibody. Absorbance at 490 nm was measured in a Spectramax microplate spectrophotometer (Molecular Devices, Sunnyvale, CA).

TNF-α bioactivity in fed and fasted adipose tissue was measured by using the TNF-α-sensitive cell line WEHI-1640, as described by Morin et al. (21). TNF mass was measured with an ELISA kit for rat TNF-α from Biosource International (Camarillo, CA).

Statistical analysis. Data are presented as means ± SD. There were five rats in each group, unless otherwise specified. Statistical significance between groups was calculated by Student’s t-test.

RESULTS

In our previous studies on modulation of adipose tissue LPL, the rats were fasted overnight for 16 or 18 h (3–5). The first step in the present research was to explore shorter times. Preliminary experiments showed that, when food was removed from the rats in the early morning, LPL activity decreased in 6 h to levels that were almost as low as those seen in rats fasted overnight. For most of these experiments, we used young rats (∼100 g), which are more responsive in modulation of LPL (3), but the response was also seen in mature rats (200 g, Fig. 1). We decided to use this protocol for further studies.

The next question was if the decrease in LPL activity induced by food deprivation for 6 h showed the same characteristics as those after 16 h of fasting. LPL mass decreased, but the decrease was less than that of activity (Table 1), so that the main change was a decrease in specific activity, as seen in 16-h-fasted rats (4). Separation of extracts of adipose tissue on heparin-agarose showed a shift of LPL mass from the form with high affinity to heparin to the form with low affinity, as reported for 16-h-fasted rats (data not shown; see Ref. 4). The downregulation of adipose LPL activity in 16-h-fasted rats engages only the extracellular enzyme, whereas LPL activity and mass within adipocytes remain the same (39). This was also true for rats deprived of food for 6 h (Fig. 1). Extracellular LPL activity, calculated as the difference between tissue total and what was recovered with the adipocytes after digestion of the tissue with collagenase, decreased by a factor of 7, whereas there was no significant change in intracellular LPL activity.

Pretreatment of the rats with actinomycin before food was removed prevented the decrease of LPL activity (see Fig. 5 for data that illustrate this) as found for 16-h-fasted rats (5). All these observations indicate that the decrease of LPL activity in the present 6-h protocol occurred by the same mechanism as in our earlier 16-h protocol, i.e., a shift in the distribution of molecular forms of extracellular LPL in adipose tissue from predominantly active to predominantly inactive in a process that was dependent on synthesis of new mRNA molecules.

We then tested the effect of a single injection of TNF-α. Preliminary experiments showed that the rats ate little or nothing after the injection. We therefore compared three groups, rats with continued access to food and given saline, rats deprived of food but given saline, and rats deprived of food but given TNF-α (Table 1). LPL activity decreased by 70% in the rats deprived of food but by 81% in the rats given TNF-α. This difference was statistically significant. In both of the groups deprived of food, there was a tendency toward a decrease of LPL mass, but this did not reach statistical significance in this experiment. The main change was in the specific activity of LPL, which decreased from 0.92 mU/ng in the fed rats to 0.35 and 0.24 mU/ng in the rats deprived of food given saline or...
Table 1. Effect of TNF-α on adipose tissue LPL activity and mass

<table>
<thead>
<tr>
<th>Food</th>
<th>Treatment</th>
<th>LPL Activity, mU/g</th>
<th>LPL Mass, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Saline</td>
<td>1,724 ± 102</td>
<td>1,880 ± 211</td>
</tr>
<tr>
<td>-</td>
<td>Saline</td>
<td>510 ± 90</td>
<td>1,446 ± 135</td>
</tr>
<tr>
<td>−</td>
<td>TNF-α</td>
<td>321 ± 66*</td>
<td>1,357 ± 141</td>
</tr>
</tbody>
</table>

Values are means ± SD. TNF-α, tumor necrosis factor-α; LPL, lipoprotein lipase. +, with; −, without. Rats were given an ip injection of TNF-α (30 μg/rat) or saline. Food was removed from the cages for two of the groups. Later (6 h), the rats were killed, and LPL activity and mass in adipose tissue were determined. *P < 0.001 comparing animals deprived of food given or not given TNF-α.

Figure 2. Effect of pentoxifylline (PTX) on the response of LPL activity to food deprivation or to TNF-α

Table 2. Effect of food deprivation for 6 h on TNF-α mass in adipose tissue in the presence or absence of pentoxifylline

<table>
<thead>
<tr>
<th>Food</th>
<th>Treatment</th>
<th>TNF-α Mass, μg/g</th>
<th>ng/g fat pad</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>Saline</td>
<td>1,256 ± 245</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>Pentoxifylline</td>
<td>1,235 ± 192</td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>Saline</td>
<td>2,108 ± 103*</td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>Pentoxifylline</td>
<td>1,118 ± 209</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Rats were given ip injections of pentoxifylline or saline and returned to cages with or without food. Later (6 h), the rats were killed, and TNF-α mass in adipose tissue was determined by an ELISA. *P < 0.05 compared with fed rats given saline.

TNF-α, respectively. To explore whether TNF-α is involved in the response to food deprivation, we measured endogenous adipose tissue TNF-α under our experimental conditions. The TNF-α activity was ~40% higher in rats deprived of food for 6 h compared with rats with access to food (Fig. 2). TNF-α mass as determined by an ELISA increased by almost 70% (Table 2). Hence, our protocol was associated with a substantial rise of endogenous TNF-α.

If TNF-α is involved in the response of LPL to food deprivation, blocking TNF-α production should impede the response. To test this, we used pentoxifylline. This is a non-specific inhibitor of phosphodiesterase known to decrease production of TNF-α (13). The inhibitor had no effect on adipose tissue TNF-α in fed rats but almost abolished the rise of TNF-α mass (Table 2) and activity (Fig. 2) in rats deprived of food. Similar results were obtained with theophylline, another phosphodiesterase inhibitor (data not shown). We noted that there was a variation both in TNF-α and in LPL activity between individual rats. This is to be expected from the type of protocol used. When the data for TNF-α and LPL activity were plotted against each other, there was a significant negative correlation (Fig. 3).

From the literature, it seemed possible that the response of LPL to TNF-α might involve nitric oxide (38) or a prostaglandin (9). To test this, rats were pretreated with 1-NAME (an inhibitor of nitric oxide synthetase) or with indomethacin (an inhibitor of cyclooxygenase). Neither of these agents decreased the response of LPL activity to food deprivation or to TNF-α (Table 3).

Dexamethasone impeded the response of LPL activity to food deprivation and TNF-α (Table 3). In this experiment, food deprivation decreased LPL activity from 1,160 to 407 mU/g (65% decrease), and TNF-α caused a further decrease to 306 (74% decrease, P < 0.001 compared with food deprivation only). In rats pretreated with dexamethasone, LPL activity decreased by only 22 and 18% without or with TNF-α, respectively.

The signaling pathway from TNF-α through its main receptor leads to activation of NF-κB (2). If this signaling pathway were involved, an inhibitor for NF-κB action would impede the response of LPL activity to TNF-α and to food deprivation. To test this, rats were pretreated with PDTC, which reversibly suppresses the release of IκB from the latent cytoplasmic form of NF-κB in cells treated with TNF-α (33). This caused a small but statistically significant decrease in the response of adipose tissue LPL activity to food deprivation without or with injection of TNF-α (Table 3).

We then turned to the question whether the downregulation of adipose tissue LPL activity by LPS is mediated by TNF-α (10). Figure 4 shows that LPS caused an increase of adipose tissue TNF-α and a decrease of LPL activity of similar magnitude to that seen in rats given exogenous TNF-α. Both of these effects were strongly impeded by pretreatment of the rats with pentoxifylline, supporting a role for endogenous TNF-α.

TNF-α REGULATES ADIPOSE LPL

AJP-Endocrinol Metab • VOL 286 • MAY 2004 • www.ajpendo.org
in the downregulation of adipose tissue LPL activity by LPS. Experiments similar to those in Fig. 1 showed that the decrease of LPL activity 6 h after LPS engaged the extracellular portion of the enzyme. LPL activity and mass within adipocytes did not change significantly (data not shown). In other experiments, the “heparin-releasable” LPL activity, which presumably reflects primarily the extracellular enzyme, was studied using pieces of adipose tissue. These were incubated for 1 h in the presence of 50 IU heparin/ml medium. The LPL activity extracted was reduced by ∼70% in tissue from rats given LPS 6 h earlier.

Taked together, the above studies indicate that TNF-α is involved in the signaling of both food deprivation and trauma/sepsis/LPS to adipose tissue LPL activity. Our previous studies had indicated that the signaling during fasting involves activation of a gene, separate from the LPL gene (5). We therefore questioned if this gene is up- or downstream of TNF-α. To explore this, rats were pretreated with actinomycin D (Fig. 5). As reported before, this virtually abolished the decrease of LPL activity in rats deprived of food. It also abolished the effect of TNF-α. We conclude that the gene is downstream of TNF-α.

**DISCUSSION**

This study shows that food withdrawal causes rapid responses in adipose tissue. TNF-α, measured as both mass and activity, increased by 40–70% in 6 h. TNF-α is known to be produced in adipocytes and to have autocrine/paracrine effects on adipose tissue metabolism (30). There are direct effects on the expression of a number of genes and indirect effects through impendiment of insulin signaling. Earlier studies had shown that insulin stimulates TNF-α production in adipose tissue (18) and that TNF-α is highly induced in adipose tissue of obese rodents (15) and human subjects (14). In view of that, our present observation of a rise of adipose tissue TNF-α early after food withdrawal is unexpected. The present observations furthermore indicate that the increase of TNF-α has marked physiological effects. Our study focused on the modulation of LPL activity, but TNF-α affects a whole group of enzymes involved in energy metabolism (31), and the response to food withdrawal certainly changes in a number of other signaling substances, both in the adipose tissue and systemically.

A major conclusion from this study is that TNF-α is involved in the physiological short-term modulation of LPL in adipose tissue. Evidence for this is that 1) endogenous TNF-α activity in adipose tissue rose during our 6-h study period; 2) when this rise was prevented by pretreating the rats with pentoxifylline, the decrease of LPL activity was abolished; 3) there was a negative correlation between adipose tissue TNF-α activity and LPL activity; and 4) exogenous TNF-α decreased LPL activity. The process had the same characteristics as previously found for fasting, namely 1) the main change was in the specific activity of the enzyme, indicating a shift from an active to an inactive form; 2) this change engaged only the extracellular LPL, and there was no change in LPL activity or mass within the adipocytes; and 3) the change did not occur if mRNA synthesis was blocked with actinomycin. Hence, the present data indicate that on food deprivation there is a signal via TNF-α to a gene whose product somehow channels extracellular LPL toward a catalytically inactive form.

TNF-α was originally identified on the basis of its ability to downregulate LPL activity in cultured 3T3-L1 adipocytes (6). Early studies with cultured adipocytes indicated that the effect was, at least in part, transcriptional (8), but there were also reports that the effect was posttranscriptional (11). Injection of exogenous TNF-α has been shown to cause downregulation of LPL activity in mice, rats, and guinea pigs (10, 12, 35), and there are reports that trauma/sepsis/LPS causes a decrease of LPL activity in several tissues (1, 24). In the present study, we found a rapid effect of LPS in vivo. This had the same

**Table 3. Effect of some potential inhibitors of TNF-α signaling on the response of adipose tissue LPL activity and mass to TNF-α and to food deprivation**

<table>
<thead>
<tr>
<th>Food</th>
<th>TNF-α</th>
<th>Treatment</th>
<th>LPL Activity, mU/g</th>
<th>LPL Mass, ng/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>–</td>
<td>Saline</td>
<td>1,860 ± 132</td>
<td>1,987 ± 102</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>Saline</td>
<td>521 ± 132*</td>
<td>1,532 ± 114*</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>l-NAME</td>
<td>519 ± 98</td>
<td>1,423 ± 98</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>Indomethacin</td>
<td>499 ± 47</td>
<td>1,435 ± 109</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Saline</td>
<td>391 ± 25†</td>
<td>1,469 ± 101</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>l-NAME</td>
<td>410 ± 37†</td>
<td>1,511 ± 96</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Indomethacin</td>
<td>389 ± 57†</td>
<td>1,541 ± 89</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>Saline</td>
<td>1,160 ± 67</td>
<td>1,753 ± 45</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>Saline</td>
<td>407 ± 54*</td>
<td>1,095 ± 65*</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>Dexamethasone</td>
<td>902 ± 34†</td>
<td>1,944 ± 79</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Saline</td>
<td>306 ± 41†</td>
<td>1,011 ± 52</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Dexamethasone</td>
<td>956 ± 39‡</td>
<td>1,548 ± 69</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>Saline</td>
<td>1,678 ± 81</td>
<td>1,702 ± 105</td>
</tr>
<tr>
<td>–</td>
<td>–</td>
<td>Saline</td>
<td>564 ± 89*</td>
<td>1,412 ± 97*</td>
</tr>
<tr>
<td>–</td>
<td>+</td>
<td>PDTC</td>
<td>801 ± 76†</td>
<td>1,305 ± 111</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>Saline</td>
<td>421 ± 56*</td>
<td>1,225 ± 89</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>PDTC</td>
<td>786 ± 78‡</td>
<td>1,312 ± 108</td>
</tr>
</tbody>
</table>

Values are means ± SD. l-NAME, Nω-nitro-l-arginine methyl ester; PDTC, pyrrolidine dithiocarbamate. Rats were given ip injections of the respective inhibitor or saline and returned to cages with food. Later (30 min), the rats were given a second injection of TNF-α (30 μg/rat) or saline and were returned to cages with or without food as indicated. Later (6 h), the rats were killed, and adipose tissue LPL activity was measured. The doses were 50, 10, and 25 mg/kg body wt for l-NAME, indomethacin, and dexamethasone, respectively. Data are from 3 separate experiments. P < 0.001 compared with rats with access to food (*), compared with rats deprived of food and given saline (†), or compared with rats deprived of food and given TNF-α (‡).
characteristics as the response to food deprivation, i.e., 1) the main change was in the specific activity of the enzyme; 2) the decrease in activity engaged only the extracellular portion of the enzyme, whereas LPL activity and mass within adipocytes remained unchanged; and 3) the downregulation of LPL activity was prevented by pretreatment of the rats with actinomycin D. Hence, TNF-α probably has a biphasic effect, a rapid posttranslational mechanism to quickly suppress LPL, and a more sustained transcriptional response if the stimulus persists.

The decrease of LPL activity after LPS was of similar magnitude to that seen after injection of TNF-α. Hence, food deprivation, injection of exogenous TNF-α, or injection of LPS had similar effects on adipose tissue LPL activity. Pentoxifylline inhibited the rise of adipose tissue TNF-α activity both after LPS and after food deprivation and virtually abolished the decrease in LPL activity. These data strongly suggest that TNF-α is a major mediator not only of the decrease of LPL activity in response to pathological conditions such as trauma/sepsis/LPS but also of the physiological response of LPL activity to food deprivation. We have not studied the origin of the TNF-α but speculate that the response to LPS and other pathological conditions probably is part of a systemic reaction, whereas the response to food deprivation probably is adipose tissue specific and mediated by TNF-α produced within the tissue.

Ranganathan et al. (29) have described translational regulation of adipocyte LPL in response to catecholamines through an RNA-binding protein that interacts with the 3'-untranslated region of LPL mRNA. They recently identified this protein as the catalytic subunit of cAMP-dependent protein kinase (28). The early decrease of LPL activity on food deprivation seen here is presumably a response to a combination of stress and fasting. Both of these conditions are expected to increase adipocyte cAMP levels and might thus trigger inhibition of LPL translation. This is, however, unlikely to be the main mechanism of the downregulation observed here. Food deprivation decreased LPL mass by 20–40%, which may reflect curtailed synthesis. LPL activity decreased more, 60–80%. Hence, reduced synthesis can at most explain part of the decrease in LPL activity. Pentoxifylline is a nonspecific inhibitor of phosphodiesterase and should increase cAMP levels and exaggerate the putative inhibition of LPL translation. In contrast to this prediction, pentoxifylline prevented the decrease of LPL activity.

We questioned how TNF-α signals to LPL. There were reports that suppression of LPL in skeletal muscle by LPS is
mediated by nitric oxide (24) and that an eicosanoid is involved in LPS downregulation of LPL in macrophages (9). These mediators do not appear to be involved in the response of LPL in white adipose tissue to food deprivation. t-NAME, a potent inhibitor of nitric oxide synthetase, and indomethacin, an inhibitor of prostaglandin cyclooxygenase, had no effect on the response to food deprivation. Signaling of TNF-α through its main receptor leads to phosphorylation, ubiquitylation, and ultimately proteolytic degradation of IkB, which frees NF-κB to translocate to the nucleus and activate the transcription of its target genes (19). An indication that this route of signal transduction is used for the downregulation of LPL was that PDTC reduced the response. PDTC is a potent inhibitor that prevents the degradation of IkB and thereby the translocation of NF-κB from the cytoplasm into the nucleus (8). Dexamethasone decreased the response to food deprivation and to exogenous TNF-α. Hence, the effect was downstream of TNF-α and cannot be explained by suppression of TNF-α formation by the glucocorticoid. There is abundant evidence that NF-κB and the glucocorticoid receptor are physiological antagonists. A mechanism with which our present findings would be consistent is a blockage of NF-κB-mediated transcriptional activation by the glucocorticoid receptor (19).

These studies suggest that TNF-α is involved in the adaptation of adipose tissue LPL activity to food supply. This is in line with a study by Kern et al. (17), who have shown that TNF-α is expressed in human adipocytes and correlates inversely to LPL activity. The response to food deprivation requires that a gene, separate from the LPL gene, is switched on. The present data show that, when transcription was blocked by actinomycin, the effect of TNF-α was abolished. Hence, the gene in question is downstream of TNF-α and is probably one of the many genes turned on by NF-κB. A recent study showed that a number of genes in adipose tissue respond rapidly to TNF-α (31).

**GRANTS**

This study was funded by Swedish Medical Research Council Grant 03X-00727. G. Wu was a recipient of Stiftelsen JC Kempes Minnes Stipend.

**References**

11. Gouni I, Oka K, Etienne J, and Chan L. Endotoxin-induced hypertri-
glyceridemia is mediated by suppression of lipoprotein lipase at a post-
15. Hotamisligil GS, Shargill NS, and Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resist-
19. McKay Li and Cidlowski JAJ. Molecular control of immune/inflamma-
21. Morin CL, Ecker RH, Marcel T, and Pagliassotti MJ. High fat diets elevate adipose tissue-derived tumor necrosis factor-alpha activity. Endo-

Downloaded from http://ajpendo.physiology.org/ by 10.220.33.6 on August 27, 2017

AJP-Endocrinol Metab • VOL 286 • MAY 2004 • www.ajpendo.org