Profiling Gene Transcription In Vivo Reveals Adipose Tissue as an Immediate Target of Tumor Necrosis Factor-α

Implications for Insulin Resistance

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Despite extensive studies implicating tumor necrosis factor (TNF)-α as a contributing cause of insulin resistance, the mechanism(s) by which TNF-α alters energy metabolism in vivo and the tissue specificity of TNF-α action are unclear. Here, we investigated the effects of TNF-α infusion on gene expression and energy metabolism in adult rats. A 1-day TNF-α treatment decreased overall insulin sensitivity and caused a 70% increase ($P = 0.005$) in plasma levels of free fatty acids (FFAs) and a 46% decrease ($P = 0.01$) in ACRP30. A 4-day TNF-α infusion caused insulin resistance and significant elevation of plasma levels of FFAs and triglycerides and reduction of ACRP30. Plasma glucose concentration was not altered following TNF-α infusion for up to 4 days. As revealed by oligonucleotide microarrays, TNF-α evoked major and rapid changes in adipocyte gene expression, favoring FFA release and cytokine production, and fewer changes in liver gene expression, but favoring FFA and cholesterol synthesis and VLDL production. There was only a moderate repressive effect on skeletal muscle gene expression. We demonstrate that TNF-α antagonizes the actions of insulin, at least in part, through regulation of adipocyte gene expression including reduction in ACRP30 mRNA and induction of lipolysis resulting in increased plasma FFAs. TNF-α later alters systemic energy homeostasis that closely resembles the insulin resistance phenotype. Our data suggest that blockade of TNF-α action in adipose tissue may prevent TNF-α–induced insulin resistance in vivo. ***Diabetes*** **51:3176–3188, 2002**

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CPT, carnitine palmitoyltransferase; EST, expressed sequence tags; FFA, free fatty acid; HSL, hormone-sensitive lipase; IL, interleukin; IPT, isoproterenol; LAL, lysosomal acid lipase; NF, nuclear factor; PPAR, peroxisome proloferator–activated receptor; RXR, retinoid X receptor; TGH, triacylglycerol hydrolase; TNF, tumor necrosis factor.

Insulin plays a pivotal role in the regulation of energy homeostasis including the storage, mobilization, and utilization of free fatty acids (FFAs) and glucose. Loss of insulin responsiveness in insulin-target tissues evokes major metabolic consequences such as hyperinsulinemia, impaired glucose tolerance, and dyslipidemia. Once β-cell function is compromised and is no longer able to maintain adequate insulin secretion, plasma glucose levels increase and type 2 diabetes develops (1,2). Thus, understanding the mechanisms driving the development of insulin resistance is critical given the therapeutic potential of improving insulin sensitivity in vivo.

Since 80% of the patients with type 2 diabetes are obese, and obesity with or without overt hyperglycemia is associated with insulin resistance (3,4), attention has focused on identification of the mediator(s) that links obesity to insulin resistance. Tumor necrosis factor (TNF)-α has been widely recognized as a candidate for such a link, because TNF-α is highly expressed in adipose tissues of obese animals (5) and human subjects (6) and induces most aspects of insulin resistance in both cell culture (7) and experimental animals (8). Furthermore, the absence of either TNF-α (9) or its receptors (10) improves the actions of insulin in mice, indicating an essential role of TNF-α in energy metabolism. Although many factors may precipitate the development of obesity-linked insulin resistance in humans, TNF-α–regulated autocrine or paracrine pathways in adipose tissue may mediate, at least in part, the functional consequences of obesity to cause decreased insulin sensitivity. Supporting this notion, TNF-α has been shown to stimulate lipolysis in adipose tissue (11). The resulting increased concentration of circulating FFAs contributes to the development of the insulin-resistant phenotype, including excessive rates of hepatic glucose production, lipid synthesis, and VLDL secretion and decreased glucose disposal. In addition, we (12) and others (13,14) have previously demonstrated that TNF-α represses the mRNA levels of an adipocyte-derived hormone, ACRP30, which inhibits gluconeogenesis in the liver (15), and decreases plasma levels of glucose (16) and

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FFAs (17). Thus, the increased circulating FFA concentrations and/or reduced ACRP30 protein levels could mediate some of the delayed effects of TNF-α on whole-body energy metabolism.

At the molecular level, several mechanisms have been suggested to explain the direct effects of TNF-α on insulin sensitivity in insulin-responsive tissues, including inhibition of insulin-stimulated glucose transport (7) and the tyrosine kinase activity of the insulin receptor (7). Alternatively, TNF-α–induced insulin resistance may result from changes additional to insulin signaling, such as downregulation of protein levels of peroxisome proliferator–activated receptor (PPAR)-γ (17), CEBP-α (18), GLUT4 (19), insulin receptor substrate (IRS)-1 (19), and perilipin (20); inhibition of lipoprotein lipase activity in adipocytes (21); and alterations of the global gene expression pattern in adipocytes (12). To date, the mechanisms by which TNF-α attenuates insulin sensitivity and alters energy metabolism and the tissue specificity of TNF-α actions in vivo are not fully understood.

Since TNF-α regulates gene transcription in a variety of cell types and is highly induced in adipose tissues from obese individuals, we hypothesized that TNF-α may have major effects on adipocyte gene expression in vivo and, if so, may affect secreted protein and/or lipid factors that act as systemic mediators of TNF-α action and antagonize the actions of insulin. To test this, we infused TNF-α into adult male Wistar rats for 1 and 4 days. Using an oligonucleotide microarray approach, we found that TNF-α infusion caused major gene expression changes in adipose, liver, and muscle tissues. The potential functional consequences predicted from our microarray data were confirmed by plasma measurements of markers of insulin resistance.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar rats (Charles River) weighing 250–300 g were housed individually under controlled light (12/12 h) and temperature conditions and had free access to regular chow and water. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Animal Subjects Committee of the University of California, San Diego.

TNF-α infusion using the osmotic pump. Animals underwent surgery to implant an osmotic pump using standard sterile techniques. General anesthesia consisted of ketamine (50 mg/kg; Aveco, Fort Dodge, IA), xylazine (4.8 mg/kg; Lloyd, Shenandoah, IA), and acepromazine (1 mg/kg; Aveco) given intramuscularly. Through a small midline laparotomy, a mesenteric vein was isolated and catheterized (Micro-Renathane MRE-033, 0.033 OD and 0.014 ID; Braintree Scientific, Braintree, MA). An osmotic pump (Alzet; Alza, Palo Alto, CA) was connected to the catheter and placed inside the abdomen, and the abdominal wall and skin was sutured closed. The pumps delivered TNF-α or vehicle for 24 h (Alzet, Model 2001D; 34.6 mg/ml or 0.27 mg/h) or for 4 days (Alzet, Model 2ML1; 28.6 mg/ml or 0.27 mg/h). These infusion rates equated to $\sim$1 mg · kg^{-1} · h^{-1} for an average 275 kg per rat. TNF-α (Genzyme, Cambridge, MA) was dissolved in PBS containing 0.1% BSA.

Insulin tolerance test. Whole-body insulin sensitivity was assessed using the insulin tolerance test 24 h or 4 days following pump implantation. The rats were fasted for 5 h, and a basal blood sample (1 ml) was collected by nicking the tail-tip. The blood was immediately analyzed for glucose (5 ml) while the rest was centrifuged and the resultant plasma frozen for later determination of FFA, triglyceride, total cholesterol, ACRP30, and C-reactive protein. Insulin was then injected intraperitoneally at $t = 0$ (0.6 units/kg, Novolin R; Novo Nordisk, Copenhagen), and subsequent blood samples (5 ml) were collected at 15, 30, 60, and 90 min for glucose determination.

Plasma measurements assays. Blood glucose concentration was measured with a B Glucose Analyzer (HemoCue, Mission Viejo, CA). Plasma FFAs were determined using an Acyl-CoA oxidase-based colorimetric kit (NEFA C; Wako, Richmond, VA). Plasma triglyceride and total cholesterol levels were measured according to standard procedures. C-reactive protein was measured by the biochemistry laboratory at the University of California at San Diego Medical Center. Plasma ACRP30 was measured using a double-antibody radioimmunoassay kit (Mouse adiponectin; Linco, St. Charles, MO).

Microarray data collection and analysis. Total RNA was isolated from rat tissues, converted to biotin-labeled cRNA targets, hybridized to RG34A oligonucleotide arrays (Affymetrix, Santa Clara, CA), and scanned on Affymetrix scanners essentially as described previously (12). Each rat RG34A array contains oligonucleotide sequences representing $\sim$7,000 known genes and $\sim$1,400 expressed sequence tags (ESTs). We collected 12 data sets of steady-state mRNA levels in adipose tissue, liver, and skeletal muscle from rats infused with TNF-α for 1 or 4 days and from rats infused with saline for 1 or 4 days as matching controls. We used six rats for each experimental group; therefore, each data set included six independent scans for the specified experimental condition.

The average of P-calls (P-calls, according to Affymetrix algorithm) for adipose tissue, liver, and skeletal muscle arrays was 44.0 ± 0.9, 36.2 ± 0.9, and 30.5 ± 0.7% (mean ± SE, $n = 24$), respectively. We used A-calls and P-calls to define the expressed genes by requiring a minimum of four P-calls out of six measurements for each data set. An arbitrary value of 50 was allocated for genes whose expression levels were undetectable or scan readouts $<$50 to facilitate calculation of the fold changes compared with the control condition. Array measurements for all samples were normalized with arrays hybridized with cRNA targets prepared from the appropriate control rats by using the median of the hybridization signals of all genes with P-calls as a scaling factor.

P values were calculated for all genes by comparing the array measurements from saline-treated rats with those from TNF-α–treated rats, using the paired Student's t test assuming unequal variances between the two groups. Fold changes were calculated by averaging the measurements of each data set and determining the ratio of the average values from the two data sets. The potential TNF-α–affected genes (known genes and ESTs) were determined by selecting those with a P value $<$0.05 and with an average of 1.5-fold or greater change in expression levels. We then performed hierarchical clustering (Fig. 2) to sort these genes according to their correlation with the control (saline infusion) versus TNF-α treatment, using Cluster and TreeView software (22). We assigned each of the TNF-α–affected known genes into functional categories according to the putative biological functions of the encoded protein, as determined by searches of public databases (Tables 3–5 and supplemental materials). The results of oligonucleotide microarrays were validated by semiquantitative RT-PCR analysis of mRNA levels of several known TNF-α–regulated genes (data not shown).

Lipolysis assay. Fully differentiated 3T3-L1 adipocytes were incubated with 50 μmol/l [^{3}H]oleic acid (oleic acid:BSA = 1:1) for 3 h at 37°C. Then the cells were washed exhaustively with PBS, and the radioactivity of the last wash was checked to be at the background level. The cells were incubated with fresh serum-free media containing 150 μmol/l BSA. At each indicated time point, an aliquot of media was taken and its associated radioactivity was determined by scintillation counting.

RESULTS

TNF-α increases plasma concentrations of FFAs and reduces ACRP30 levels and later leads to significant insulin resistance. To determine whether TNF-α can acutely antagonize the actions of insulin in vivo, we first measured the plasma levels of several markers and/or potential mediators of insulin resistance after TNF-α treatment (Table 1). One day after TNF-α infusion, plasma concentrations of FFAs increased by 70% ($P = 0.005$, $n = 6$), with a concomitant 46% decrease in ACRP30 protein levels ($P = 0.01$, $n = 6$). Plasma glucose concentration was not changed 1 day after TNF-α treatment, and body weight and the levels of triglyceride, total cholesterol, and C-reactive protein were not altered.

Plasma FFA levels continued to increase by 150% 4 days after TNF-α infusion ($P = 0.001$, $n = 6$), whereas ACRP30 concentrations remained 51% that of the controls ($P = 0.03$, $n = 6$). In addition, the plasma concentration of triglyceride was increased 55% by a 4-day TNF-α infusion ($P = 0.05$, $n = 6$). No significant differences were observed in body weight, plasma levels of glucose, total cholesterol, or C-reactive protein 4 days after TNF-α treatment.

We next assessed the effect of TNF-α infusion on overall

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TABLE 1
Body weight and plasma concentrations of some molecular markers of insulin resistance after TNF-α treatment

	Weight (g)	Glucose (mg/dl)	FFA (mmol/l)	Total chol (mg%)	Triglycerides (mg%)	C-reactive protein (mg/dl)	ACRP30 (μg/ml)
1-day saline	281.1 ± 4.8	105 ± 4	1.08 ± 0.14	79 ± 7	96 ± 6	1.9 ± 0.2	10.5 ± 1.4
1-day TNF-α	273.5 ± 4..6	101 ± 2	1.84 ± 0.18	75 ± 5	106 ± 6	2.0 ± 0.2	5.7 ± 0.7
Student's t test	NS	NS	$P = 0.005$	NS	NS	NS	$P = 0.01$
4-day saline	280.4 ± 8.1	104 ± 4	0.98 ± 0.14	56 ± 4	88 ± 10	2.7 ± 0.3	8.1 ± 1.4
4-day TNF-α	273.3 ± 3.1	105 ± 5	2.48 ± 0.27	64 ± 4	136 ± 21	2.8 ± 0.3	4.1 ± 0.4
Student's t test	NS	NS	$P = 0.001$	NS	$P = 0.05$	NS	$P = 0.03$

Data are means ± SEM. There were six rats in each experimental group. ACRP30, adipocyte complement related protein of 30 kDa. P values indicate probability level of random difference by paired Student's t test. Chol, cholesterol; NS, not significant.

in vivo insulin sensitivity using the insulin tolerance test (Fig. 1). In rats infused with saline for 1 day, insulin injection caused a 17% decrease ($P < 0.01$, $n = 5$) in plasma glucose concentrations within 30 min and a 40% ($P < 0.001$, $n = 5$) and a 36% ($P < 0.01$, $n = 5$) decrease 60 and 90 min after insulin administration, respectively (Fig. 1*A*). In rats treated with TNF-α for 1 day, however, the drop in the plasma glucose levels in response to the injected insulin was significantly slower and smaller than that of control rats, indicating decreased insulin sensitivity (Fig. 1*A*). The hypoglycemic effects of insulin were further diminished 4 days after TNF-α infusion, indicating the development of significant insulin resistance (Fig. 1*B*), consistent with earlier reports (23). The kinetics of insulin's glucose-lowering effects were not different in the 1-day and 4-day saline-infused groups, indicating unchanged insulin sensitivity. Thus, TNF-α infusion reduced systemic insulin sensitivity in a time-dependent manner,

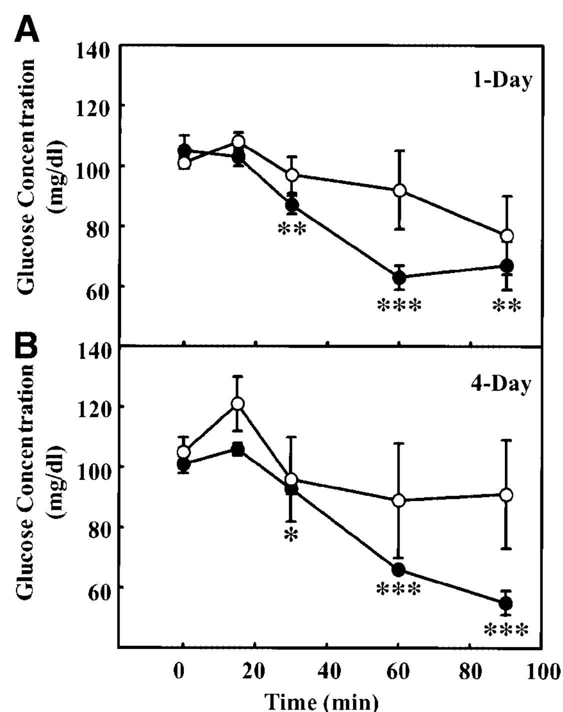

FIG. 1. Insulin tolerance test. Adult Wistar rats were treated with saline or TNF-α for 1 day (*A*) and 4 days (*B*), and the insulin tolerance test was performed as described in RESEARCH DESIGN AND METHODS. Plasma samples were taken 10, 30, 60, and 90 min after insulin administration. Data shown are means ± SE ($n = 6$ for TNF-α groups and $n = 5$ for saline groups). ●, saline infusion; ○, TNF-α infusion. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. basal plasma glucose concentrations.

which is associated with a progressive elevation of plasma FFA concentrations and a significant drop in ACRP30 levels.

Oligonucleotide microarrays reveal that TNF-α provokes major and sustained changes in gene transcription in adipose tissue and liver, but exhibits moderate repressive effects on skeletal muscle gene expression. To determine whether gene transcription would be significantly altered during TNF-α treatment, we assessed the expression profiles of ~7,000 known rat genes in major insulin-responsive tissues 1 and 4 days after TNF-α infusion. First, we filtered the normalized gene expression data to exclude genes showing minimal changes across the various groups of sample being analyzed, as described in RESEARCH DESIGN AND METHODS. Then, we performed hierarchical clustering to sort these genes by their correlation with the control versus TNF-α groups. Figure 2 shows that TNF-α evoked major and rapid changes in adipocyte gene expression and fewer changes in liver. There was only a moderate repressive effect on skeletal muscle gene expression. The TNF-α–affected genes form distinct clusters before and following the development of insulin resistance (Fig. 2), with clusters of genes in adipose tissue and the liver being most metabolically relevant (see below). Among them, the mRNA levels of 187 (2.7% of the 7,000 genes assayed) and 208 (3.0%) known adipocyte genes increased at least 1.5-fold ($P < 0.05$) after 1-day and 4-day TNF-α infusion, respectively. In parallel, the expression of 77 (1.1%) and 122 (1.7%) known adipocyte genes were repressed at least 1.5-fold ($P < 0.05$) by TNF-α after 1 and 4 days. In the liver, however, TNF-α induced 79 (1.1%) and 44 (0.6%) known genes and repressed 41 (0.6%) and 28 (0.4%) known genes at least 1.5-fold ($P < 0.05$) after 1- and 4-day infusions, respectively. In contrast, TNF-α infusion for up to 4 days did not significantly induce gene expression in skeletal muscle, but repressed the mRNA levels of 82 (1.2%) and 30 (0.4%) known genes at least 1.5-fold ($P < 0.05$) after 1- and 4-day infusions (Table 2).

Analysis of the tissue-specific responses to TNF-α infusion for up to 4 days revealed that TNF-α affected a unique subset of genes in each insulin-sensitive tissue, with only a small number of the 7,000 known genes tested regulated by TNF-α in the three different tissues (Fig. 3). Notably, the mRNA levels of 1.3% (91 known genes) of the adipocyte genes that are affected by 1-day TNF-α treatment remained altered after 4 days of TNF-α infusion. Noteworthy, only 0.3% (21 known genes) and 0.03% (2 known genes) of the 7,000 genes tested showed sustained changes

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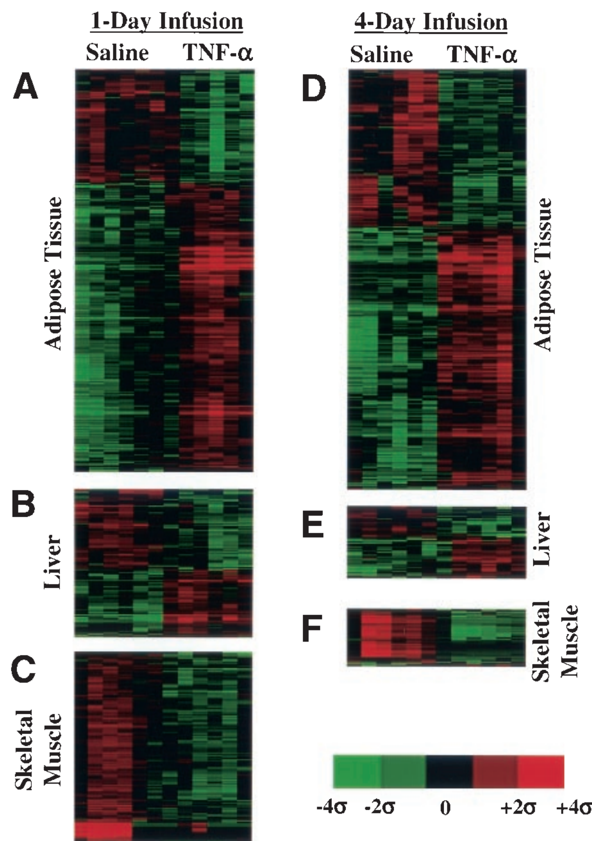

FIG. 2. Hierarchical clustering analysis of TNF-α–regulated gene expression in major insulin-responsive tissues. The expression profiles of adipose tissue, liver, and skeletal muscle isolated from rats infused with saline or TNF-α were analyzed as described in RESEARCH DESIGN AND METHODS. The TNF-α–affected genes (known genes and ESTs) were determined by selecting those with a *P* value <0.05 and with an average of 1.5-fold or greater change in expression levels. Each rat is represented by a single column. Saline-infused rats (*n* = 6) are shown on the left, and TNF-α–infused animals (*n* = 6) are on the right. Each gene is represented by a single row of colored bars. Red indicates high level expression, and green denotes low level expression. The color scale at the bottom indicates relative expression in SDs from the median. *A–C*: One-day infusion: adipose tissue (*A*), liver (*B*), and skeletal muscle (*C*). *D–F*: Four-day infusion: adipose tissue (*D*), liver (*E*), and skeletal muscle (*F*).

in mRNA levels in liver and muscle, respectively, throughout the 4-day period of TNF-α infusion. These data demonstrate that TNF-α induced major and sustained changes in adipocyte gene expression and caused distinct early and late responses in liver and muscle, suggesting that the delayed responses in liver and muscle might result from effects secondary to TNF-α infusion. The entire gene lists as well as the full data sets for the 7,000 known genes (raw data) are available in an online appendix at http://diabetes.diabetesjournals.org.

TNF-α represses adipocyte genes involved in the uptake and storage of FFA and glucose. To identify the potential correlation of changes in adipocyte gene expression with adipocyte energy metabolism and biological function, we further analyzed the TNF-α–affected adipocyte genes and assigned each gene into functional categories according to the biological functions of their encoded proteins. As shown in Table 3, TNF-α caused profound changes in adipocyte gene expression.

TNF-α repressed the mRNA levels of retinoid X receptor-γ (RXR-γ, −1.5-fold after 4 days) and PPAR-γ (−1.3-fold after 1 day), spot 14 (−1.6-fold after 1 day, −2.2-fold after 4 days), and CCAAT binding transcription factors NF1-A1 and NF1-X1 (−1.6-fold after 4 days), but induced nuclear factor (NF)-κB p105 (+1.3-fold after 1 day, +2.0-fold after 4 days), hypoxia inducible factor 1 (+2.6-fold after 1 day, +1.6-fold after 4 days), high-mobility group protein 2 (+1.6-fold after 1 day), and fos-related antigen (Fra, +1.5-fold after 1 day). While PPAR-γ, RXR-γ, and spot 14 (24) are essential for adipogenesis and lipogenesis, the accumulation of mRNAs encoding NF-κB, and Fra-1 inhibits adipocyte differentiation (25,26). Thus, TNF-α may negatively regulate adipocyte differentiation by reducing adipogenesis in vivo and restoring levels of preadipocyte transcription factors.

TNF-α repressed the expression of many genes involved in the uptake and storage of FFAs in adipocytes. Among them, the mRNA level of lipoprotein lipase was repressed by 1.4- and 1.9-fold (Fig. 4*C*) after 1 and 4 days of TNF-α infusion, respectively (Table 3). Since lipoprotein lipase is the major enzyme that hydrolyzes triglycerides in triglyceride-rich lipoproteins and initiates FFA uptake by adipose tissue, downregulation of lipoprotein lipase gene expression may result in an elevation of plasma triglyceride. The expression of many proteins involved in the utilization and storage of FFAs were also downregulated by TNF-α, such as genes encoding adipocyte fatty acid binding protein (−1.7-fold after 4 days), acyl CoA binding protein (−1.6-fold after 4 days), long-chain fatty acyl CoA synthase (−1.7-fold after 1 day, −2.3-fold after 4 days; Fig. 4*D*), diacylglycerol acyltransferase (−1.5-fold after 4 days), and perilipin A (−2-fold after 1 day) (Table 3). The overall changes in the mRNA levels of these proteins, while statistically significant, are relatively small, but their encoded proteins act in series in a common pathway of FFA metabolism and, therefore, the moderate changes of each protein aggregated together will have a significant effect,

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TABLE 2
Genes affected by TNF-α in major insulin-responsive tissues after 1-day and 4-day infusions

	1-day infusion			4-day infusion		
Tissue	Induced known genes	Repressed known genes	Total affected known genes	Induced known genes	Repressed known genes	Total affected known genes
Adipose	187 (2.7)	77 (1.1)	264 (3.8)	208 (3.0)	122 (1.7)	330 (4.7)
Liver	79 (1.1)	41 (0.6)	120 (1.7)	44 (0.6)	28 (0.4)	72 (1.0)
Skeletal muscle	0 (0)	82 (1.2)	82 (1.2)	3 (0)	30 (0.4)	33 (0.5)

Data are *n* (%). The number of known genes induced or repressed >1.5-fold with a *P* value <0.05 after either 1-day or 4-day TNF-α infusion were determined as described in RESEARCH DESIGN AND METHODS. In each tissue, the number of genes changed significantly was divided by 7,000 (the approximate total number of known genes on RG34A array) to get the percentage of genes changed under each condition.

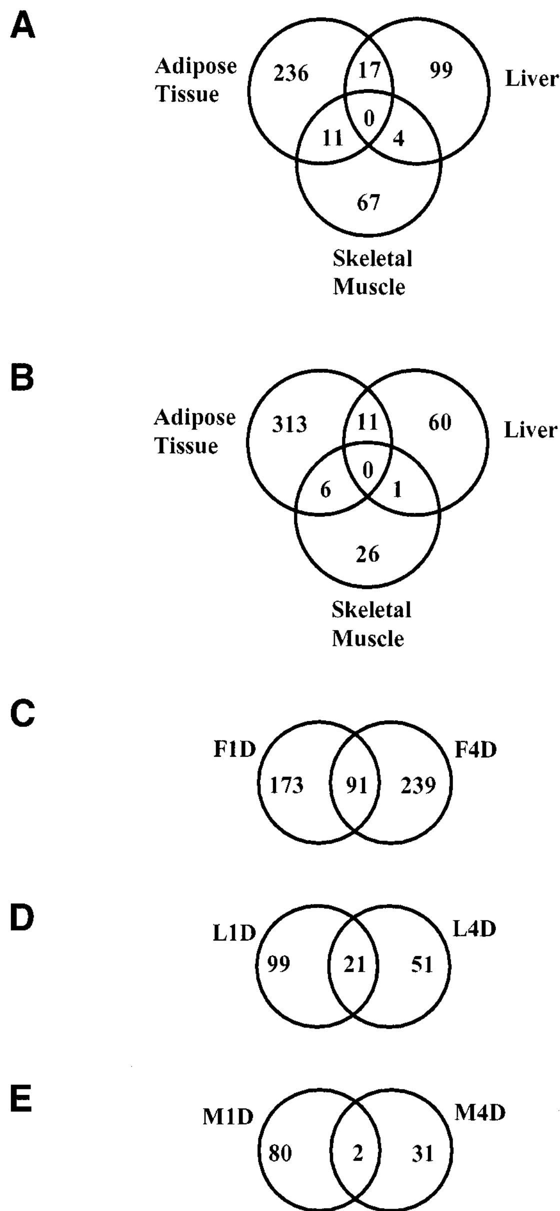

FIG. 3. Common and unique genes regulated by TNF-α in major insulin-sensitive tissues. The overlapping region of the Venn diagram indicates the number of common genes, and the nonoverlapping region denotes the number of unique genes regulated by TNF-α in adipose tissue, liver, and skeletal muscle following a 1-day (*A*) or a 4-day (*B*) TNF-α infusion. The number of common genes and unique genes affected by TNF-α after 1-day and 4-day infusions in adipose tissue (*C*), liver (*D*), and skeletal muscle (*E*) are shown in the overlapping and nonoverlapping regions of the Venn diagram, respectively. F1D, fat tissue, 1-day TNF-α infusion; F4D, fat tissue, 4-day TNF-α infusion; L1D, liver, 1-day TNF-α infusion; L4D, liver, 4-day TNF-α infusion; M1D, muscle, 1-day TNF-α infusion; M4D, 4-day TNF-α infusion. All numbers are relative to the 7,000 known genes assayed on the rat chip.

favoring fatty acid release while preventing fatty acid uptake by adipose tissue.

To ascertain whether TNF-α can directly stimulate FFA release from adipocytes, we treated 3T3-L1 adipocytes with TNF-α and determined the kinetics of TNF-α–stimulated FFA release. As shown in Fig. 5*A*, TNF-α did not acutely stimulate FFA release from adipocytes. Rather, TNF-α slightly increased FFA release after a lag period of 4 h (28%, $P < 0.04$), but resulted in a profound increase in FFA release after 8 h (2.4-fold, $P < 0.001$) and remained at 2.3-fold 24 h after TNF-α addition ($P < 0.001$, Fig. 5*B*). In contrast, isoproterenol (IPT), which rapidly activates hormone sensitive lipase, caused a 49% increase ($P < 0.01$) in FFA release as early as 10 min of incubation. The IPT-stimulated fatty acid release peaked at 30 min of incubation (3.0-fold, $P < 0.04$) and remained at 2.6-fold ($P < 0.01$), 2.2-fold ($P < 0.001$), and 1.7-fold ($P < 0.01$) that of control cells after 1, 2, and 4 h, respectively, of IPT treatment. The stimulatory effect of IPT diminished after 8 h of incubation (Fig. 5*A* and *B*), indicating a rapid, transient, and saturable nature of IPT-stimulated FFA release in adipocytes. Thus, the distinct time courses of TNF-α and IPT action suggest that different mechanisms are involved in stimulation of FFA release in fat cells.

In addition to affecting genes involved in FFA metabolism, TNF-α had profound effects on the expression of adipocyte genes involved in glucose uptake and utilization. One-day TNF-α infusion downregulated the mRNA levels of GLUT4 (−2-fold, Fig. 4*E*) and fatty acid synthase (−4.3-fold, Fig. 4*B*), as it did in 3T3-L1 adipocytes (12). In addition, since glucose provides the three-carbon backbone for triglyceride synthesis in adipose tissue, the lack of lipogenic substrates may also favor FFA release from adipocytes. Furthermore, oligonucleotide microarrays revealed that TNF-α had significant impact on the expression of many genes involved in energy metabolism, including glycolysis (Fig. 4*F*), the citric acid cycle, and the pentose phosphate pathway (Table 3). Taken together, the coordinate changes in gene expression induced by TNF-α may result in enhanced catabolism in adipose tissue and thus antagonize the anabolic effects of insulin.

TNF-α induces the expression of adipocyte-secreted factors and adhesion molecules. The expression in adipocytes of several mRNAs whose overexpression has been reported in type 2 diabetes was also increased by TNF-α infusion, such as adrenomedullin, plasminogen activator inhibitor 1, plasminogen activator inhibitor 2, fibronectin, and ceruloplasmin. Thus, TNF-α may contribute to the induction of the molecular markers associated with obesity-linked type 2 diabetes. In addition, TNF-α upregulated the mRNA levels of several genes encoding adhesion molecules in adipose tissue (Table 3).

TNF-α induces genes implicated in inflammatory responses as well as many chemokines, cytokines, and their receptors and complement components in adipose tissue. It has been increasingly recognized that adipose tissue is capable of producing a variety of chemokines and cytokines (27). Consistent with this notion, our microarray results indicate that many genes involved in inflammatory responses, as well as a number of chemokines, cytokines, and their receptors, and many proteins involved in cytokine production are strongly induced by TNF-α in adipose tissue. Examples of inflammatory mediators include 5-lipoxygenase activating protein (1.7-fold at 1 day, 1.9-fold at 4 days), prostacyclin synthase (1.5-fold at 1 day), phospholipase A2 precursor (1.7-fold at 4 days), and leukotriene A4 hydrolase (1.5-fold at 4 days) (online materials, http://diabetes.diabetesjournals.org). Genes involved in immune response include CXC chemokine LIX, CXC chemokine receptor, tumor growth factor (TGF)-β1, TNF-α converting enzyme, interleukin (IL)-1β converting

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TABLE 3
Principle adipose genes affected by 1-day and 4-day TNF-α infusions

GenBank accession no.	Gene name	Fold change	
		1 Day	4 Day
Cell adhesion			
U72660	Ninjurin	1.8	2.2
X63722	VCAM-1	2.3	2.5
U09361	Tenascin	3.1	NS
D00913	ICAM-1	2.3	2.6
X53517	CD37	NS	2.5
M61875	CD44	NS	2.7
X65036	Integrin-α chain,H36-α7	−1.6	−4.8
X52140	Integrin-α-1	−1.4	NS
Chemokine, cytokine, and receptors			
X99337	Glycoprotein 55	1.6	1.6
X52498	TGF-β 1	2.9	2.5
U68272	Interferon γ receptor	1.6	3.8
S79263	IL3 receptor β subunit	1.5	1.6
M55050	IL2 receptor β chain	2.3	NS
Z22812	IL 1 receptor type 2	4.3	1.8
D78591	Cardiotrophin-1	−1.5	NS
U03491	TGF-β3	−1.8	NS
Cytokine synthesis			
U14647	IL-1β converting enzyme	1.8	1.6
AJ012603	TNF-α converting enzyme	1.7	2.4
Metabolism (glucose)			
X58865	Phosphofructokinase	1.8	NS
U20643	Aldolase A	1.8	4.8
M31788	Phosphoglycerate kinase	1.6	NS
S63233	Phosphoglycerate mutase type B	1.5	NS
X02610	Enolase	1.5	1.5
X07467	G6P dehydrogenase	1.4	NS
AA818951	Pyruvate kinase	1.6	NS
L01793	Glycogenin	1.8	2
X04069	Glycogen phosphorylase	NS	1.7
AA893663	N-glycan-α 2,8-sialyltransferase	NS	1.5
Z12158	Pyruvate dehydrogenase E1α	NS	1.8
AA799452	Transaldolase	NS	1.5
AI059508	Transketolase	NS	2
M60322	Aldehyde reductase 1	−1.5	NS
D10655	Dihydrolipoamide acetyltransferase	−1.4	NS
D28561	GLUT4	−2	NS
U07181	Lactate dehydrogenase B	−1.6	NS
U32314	Pyruvate carboxylase	−2.1	−2.4
U10357	Pyruvate dehydrogenase kinase 2	−1.6	NS
U03120	Sodium/glucose cotransporter	NS	−1.5
AA892314	NADP-dependent isocitrate dehydrogenase	NS	−1.5
AA892828	Pyruvate dehydrogenase β	NS	−1.5
Metabolism (fatty acid)			
S69874	Cutaneous-Fatty acid binding protein	1.6	NS
D88890	Acyl-CoA hydrolase	1.4	1.6
AB012933	Acyl-CoA synthetase 5	1.4	NS
L07736	Carnitine palmitoyltransferase I	1.4	NS
S81497	Lysosomal acid lipase	1.7	2.2
AI230354	Phosphatidate phosphohydrolase type 2	2.2	NS
M25758	Phosphatidylinositol transfer protein	1.5	NS
AA893280	Adipose differentiation-related protein	NS	1.7
L07114	ApoB editing protein	NS	2.2
J02844	Carnitine octanoyltransferase	NS	1.6
AA799326	Fatty acid translocase	NS	2.9
AB005743	Fatty acid transporter	NS	2.3
E12286	GM2 activator protein	NS	2.6
L34262	Palmitoyl protein thioesterase	NS	1.8
AB005143	Uncoupling protein 2	NS	1.5
AI044900	Long chain acylCoA synthetase	−1.7	−2.3
S76779	ApoE	−1.8	1.6
M76767	Fatty acid synthase	−4.3	−2.2
X13527	Fatty acid synthase-acyl carrier protein	−3.6	NS
L03294	Lipoprotien lipase	−1.4	−1.9

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TABLE 3
Continued

GenBank accession no.	Gene name	Fold change 1 Day	Fold change 4 Day
M26594	Malic enzyme	−2.2	NS
U10697	Microsomal carboxylesterase	−1.5	NS
L26043	perilipin	−2	NS
AI169612	Adipocyte fatty acid binding protein	NS	−1.7
AA859529	Diacylglycerol acyltransferase	NS	−1.5
AI010581	Acyl-CoA binding protein	NS	−1.6
U40001	Hormone sensitive lipase	NS	−2.2
AA800220	Lysophospholipase	NS	−1.5
D21132	Phosphatidylinositol transfer protein	NS	−1.4
L15556	Phospholipase C	NS	−2.3
Secreted protein			
U82612	Fibronectin	1.8	7.3
L33869	Ceruloplasmin	1.6	2.6
M64733	Clusterin	1.6	NS
K01933	Haptoglobin	8.4	NS
AF072892	Versican V3 precursor	4.7	NS
D15069	Adrenomedullin	5	NS
M24067	PAI-1	1.8	NS
X64563	PAI-2	1.7	NS
M29866	Complement component C3	3.5	7.3
X52477	Pre-pro-C3	NS	7.6
X71127	Complement component C1q, beta chain	NS	1.8
M29866	Complement component C1q, c chain	NS	7.3
AI639117	Complement component factor B	NS	1.6
AI639534	properdin	NS	2.0
AJ005394	Collagen α1 type V	−1.4	NS
Z78279	Collagen α1 type I	−1.5	−1.5
U27562	Extracellular matrix protein 2	−2.3	−2.6
AF097723	Hematooietic lineage switch 2 related protein	−1.4	NS
U24489	Tenascin-X	−2.4	NS
J04035	tropoelastin	−1.6	−2.4
AA946313	Osteonectin	NS	−1.5
D38036	Truncated form of TSH receptor	NS	−1.4
U32681	Ebnerin	NS	−2
U07619	Coagulation factor III	NS	−1.6
U06230	Protein S	NS	−1.8
U42719	Complement component C4	NS	−1.6
M92059	Adipsin	NS	−1.6
M12112	angiotensinogen	NS	−2.3
Transcription Regulation			
D84418	High mobility group protien 2	1.6	NS
U34932	Fos-related antigen	1.5	NS
M34253	Interferon regulatory factor 1	2	NS
AJ010828	RDC-1	1.5	NS
M95791	Transcription factor EF1 (A)	1.6	NS
D78308	Calreticulin	1.5	NS
S77528	C/EBP related transcription factor	1.9	NS
Y09507	Hypoxia-inducible factor 1	2.6	1.6
U83883	NF-κB p105 subunit	1.3	2
AF055292	STAT 6	NS	1.6
AA859952	Early development regulator 2	NS	1.6
U71293	Hairless protein	−1.5	NS
U17254	Immediate early gene transcription factor NGF1-B	−1.4	NS
AB011365	PPAR-γ	−1.3	NS
K01934	Spot 14	−1.6	−2.2
D12769	BTE binding protein	NS	−1.6
M64986	High mobility group 1	NS	−1.4
AB012234	NF1-X1	NS	−1.7
X84210	NFI-A1	NS	−1.6
AF016387	Retinoid X receptor γ	NS	−1.5
M31322	Sperm membrane protein (YWK-II)	NS	−1.4

The TNF-α–affected genes and their fold changes were determined as described in RESEARCH DESIGN AND METHODS, and were assigned into functional categories based on the putative biological functions of the encoded proteins as determined by searches of public databases. A selected subset of genes induced or repressed >1.5-fold with a P value <0.05 (Student's paired t test) at either 1 day or 4 days are included in this list and are discussed in the text. The entire list of genes in which >1.5-fold change in expression at either time point was detected is presented as online appendixes at http://diabetesjournals.diabetes.org.

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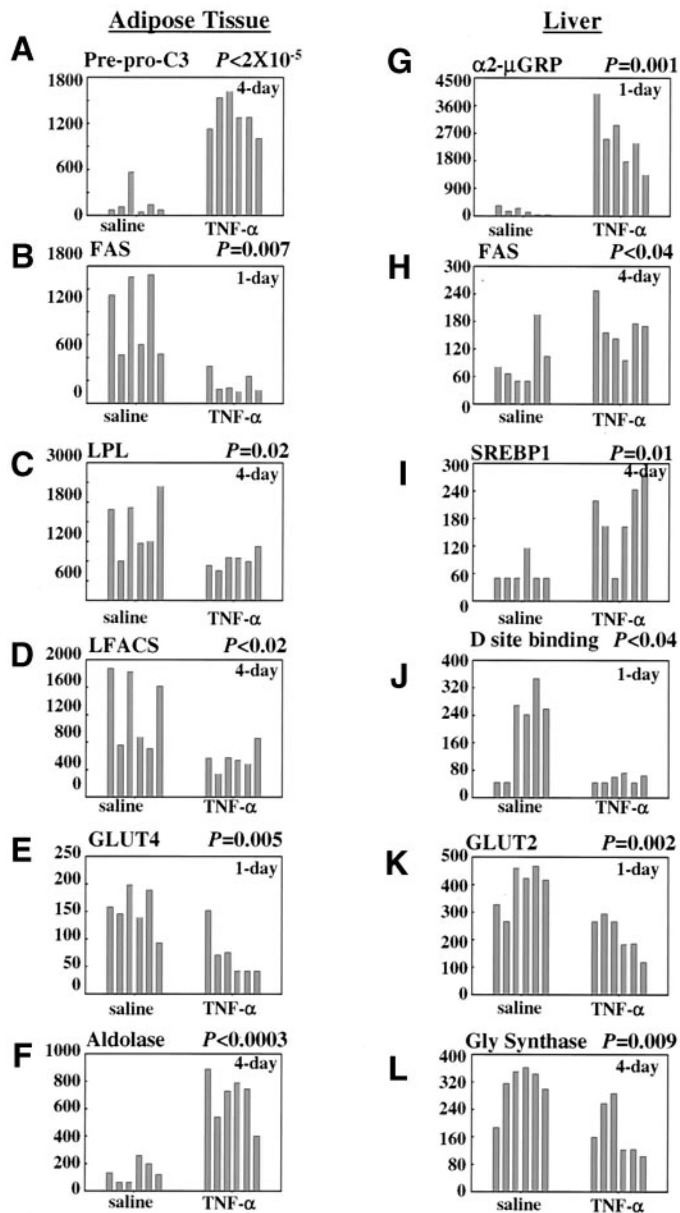

FIG. 4. Selected gene expression following saline or TNF-α infusion. *A–F*: Gene expression in adipose tissue. *G–L*: Gene expression in the liver. The duration of TNF-α or saline treatment is indicated in each panel. Each bar represents an individual rat; saline-infused rats ($n = 6$) are shown on the left and TNF-α–infused animals ($n = 6$) are on the right. The expression levels are raw data (average difference, according to Affymetrix) obtained from Affymetrix GENECHIP output file after normalization as described in RESEARCH DESIGN AND METHODS. *P* values (comparison of saline- and TNF-α–treated groups) are shown on top of each panel. C3, complement component prepro-C3; FAS, fatty acid synthase; LPL, lipoprotein lipase; LFACS, long-chain fatty acyl CoA synthase; SREBP1, sterol regulatory element binding protein 1; D site binding, D site albumin promoter binding protein; Gly Synthase, glycogen synthase; α2-μGRP, α2-μ globulin-related protein.

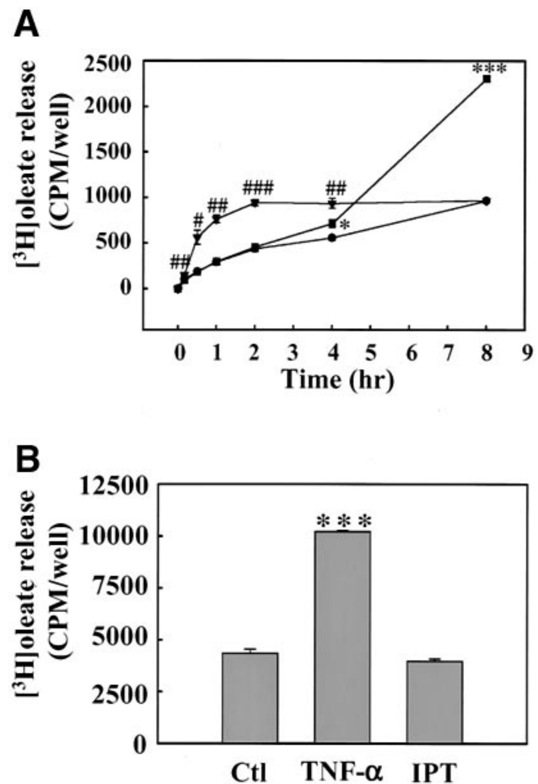

FIG. 5. TNF-α– and IPT-induced FFA release from 3T3-L1 adipocytes. The lipolysis assay was performed as described in RESEARCH DESIGN AND METHODS. *A*: [^{3}H]oleate release after the indicated stimulation. ●, control cells; ▲, IPT-treated cells; ■, TNF-α–treated cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. control cells at each time point. *B*: [^{3}H]oleate release after 24 h of incubation with the indicated reagents. ***$P < 0.001$ vs. control cells. The results shown are means ± SE ($n = 3$) and are representative of two independent experiments.

enzyme, IL-1 receptor type II, and interferon-γ receptor (Table 3). Notably, many components of the complement pathway, including C1q, C3 (Fig. 4*A*), factor B, and properdin, were also induced by TNF-α infusion (Table 3). These secreted molecules can not only regulate adipocyte metabolism and function, but may contribute to the systemic immune response as well.

TNF-α elicits distinct immediate, sustained, and delayed changes in gene expression in the liver. TNF-α induced rapid and significant changes in the expression of genes involved in energy metabolism and major physiological functions of the liver. As shown in Table 4, TNF-α repressed liver mRNA levels of several genes involved in glucose uptake and metabolism, such as GLUT2 (−1.8-fold after 1 and 4 days, Fig. 4*K*) and glycogen synthase (−1.7-fold after 1 day, −1.8-fold after 4 days; Fig. 4 *L*), and genes involved in fatty acid oxidation including carnitine palmitoyltransferase (CPT)-I (−1.6-fold after 1 day, −1.4-fold after 4 days), and CPT-II (−1.3-fold after 4 days). In addition, genes encoding transcription factors essential for hepatocyte function, such as D site albumin promoter binding protein (Table 4 and Fig. 4*J*), and genes involved in amino acid metabolism (online materials) are repressed by TNF-α. Meanwhile, TNF-α infusion induced many secreted proteins (Fig. 4*G*) and caused a significant increase in the mRNA levels of transcription factor SREBP/ADD1 (Fig. 4*I*), fatty acid translocase/CD36, as well as many genes involved in de novo cholesterol synthesis (Table 4).

Several genes involved in the de novo synthesis of fatty acids (fatty acid synthase, +1.8-fold, Fig. 4*H*) as well as genes involved in VLDL production (apolipoprotein B, +twofold) were induced by TNF-α, but only after 4 days of infusion, indicating that these effects of TNF-α may be secondary to its effects on adipose tissue or other peripheral tissues. Notably, the mRNA levels of enzymes involved in gluconeogenesis, such as phosphoenolpyruvate

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TABLE 4
Principle liver genes affected by 1-day and 4-day TNF-α infusions

GenBank accession no.	Gene name	Fold Change	
		1 Day	4 Days
Metabolism (Cholesterol)			
D45252	2,3-oxidosqualene:lanosterol cyclase	1.6	NS
AB016800	7-dehydrocholesterol reductase	1.7	1.8
X52625	HMGCoA synthase	1.7	2.3
M89945	Farnesyl diphosphate synthase	1.7	1.5
AF003835	Isopentenyldiphosphate:dimethylallyl diphosphate isomerase	2.2	NS
AA963449	Lanosterol 14-demethylase	1.8	2.4
E12625	Protein assocaited with nerve injury	2.3	NS
AI172293	RANP-1	2.1	NS
D37920	Squalene epoxidase	2.4	NS
M95591	Squalene synthetase	1.6	2.4
AI180442	Testis-specific farnesyl pyrophosphate synthetase	1.7	NS
X55286	HMG CoA reductase	NS	1.5
X91234	17-β hydroxysteroid dehydrogenase type 2	NS	−1.6
Metabolism (fatty acid and lipid)			
AA946368	Fatty acid translocase/CD 36	1.7	2.4
M76767	Fatty acid synthase	NS	1.8
U53873	ApoB	NS	2
D50580	Carboxylesterase 2	−1.6	NS
X65296	Carboxylesterase 3	−1.6	NS
J02844	Carnitine otanoyltransferase	−1.6	NS
L07736	Carnitine palmitoyltransferase I	−1.6	−1.4
J05470	Carnitine palmitoyltransferase II	NS	−1.3
AB009372	Lysophospholipase	−1.6	NS
U89905	Methylacyl-CoA racemase α	−1.7	NS
U10697	Microsomal carboxylesterase	−1.5	NS
D00729	Delta3, delta2-enoyl-CoA isomerase	NS	−1.5
AI170568	Mitochondrial 3-2trans-enoyl-CoA isomerase	NS	−1.5
AA892864	Monoglyceride lipase	NS	−1.4
U08976	Peroxisomal enoyl hydratase-like protein	NS	−1.7
Metabolism (glucose)			
X07467	G6P dehydrogenase	NS	1.8
AA891797	Phosphoprotein enriched in astrocytes	NS	1.7
L28135	GLUT2	−1.8	−1.8
J05446	Glycogen synthase 2	−1.7	−1.8
AF080468	Glucose-6-phosphatase	−1.5	NS
Secreted protein			
V01216	α-1 acid glycoprotein	1.7	1.7
M23566	α-2 macroglobulin	7.8	10.9
AA946503	α2u globulin-related protein	15	5.0
X86561	α-fibrinogen	1.5	1.5
X07648	Amyloidogenic glycoprotein	1.7	NS
L33869	Ceruloplasmin	1.8	2.0
Z50051	Complement component 4 binding protein α	1.5	NS
U55765	RASP 1	1.5	NS
M35300	Serine protease inhibitor	3.8	NS
L22190	Serum amyloid A protein	4.5	NS
AF016503	Type 1 procollagen C proteinase enhancer protein	1.5	NS
AA891576	Complement C1q C polypeptide	NS	1.5
M63991	Thyroxine-binding globulin	NS	2.5
M22360	Plasma proteinase inhibitor α-1 inhibitor III	−1.7	NS
Transcription regulation			
L16995	SREBP1/ADD1	NS	3.1
X54686	pJunB	NS	1.6
D12769	BTE binding protien	−1.5	−1.4
J03179	D site albumin promoter binding protein	−3.6	−2.6
AA891422	Hypoxia induced gene 1	NS	−1.5
D86580	Small heterodimer partner homolog	NS	−1.5
M96548	Zinc finger protein (kid 1)	NS	−1.8

See notes in Table 3.

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TABLE 5
Principle muscle genes affected by 1-day and 4-day TNF-α infusions

GenBank accession no.	Gene name	Fold change	
		1 Day	4 Days
Acute-phase protein			
K01933	Haptoglobin	−2.4	−3.3
AA945589	Hydroxysteroid sulfotransferase	−2.3	NS
Cell adhesion			
X65036	Integrin H36-α7	−1.6	NS
S58644	Integrin β5	−1.6	NS
X89963	Thrombospondin 4	−1.7	NS
U44845	Vitronectin	−1.7	NS
Cell cycle progression			
S55427	Growth arrrest specific 3 homolog	−1.6	NS
Cell structure and cytoskeleton			
V01227	α-tubulin	−2.5	NS
Metabolism			
X15512	Apolipoprotein C 1	NS	−2.5
S76779	Apolipoprotein E	NS	−3.8
M10934	Retinol-binding protein	NS	−2.8
Mitochondrial Function			
AA875531	Cytochrome B	−2.3	NS
AA945573	Cytochrome P450	NS	−2.5
AA945585	ATPase inhibitor	NS	−3.1
Muscle contraction regulation			
U15138	LIC-2 dynein light intermediate chain 53/55	−1.8	NS
S77900	Myosin regulator light chain isoform C	−1.6	NS
Protein degradation			
X63446	Fetuin	NS	−2.9
J03524	α-1 inhibitor III	NS	−4.5
X16273	Serine protease inhibitor-like protein	NS	−4.4
AI010453	α-1 protease inhibitor	NS	−3.8
L07281	Carboxypeptidase E	−1.8	NS
X82396	Cathepsin B	−1.5	NS
U36580	Serine proteinase rPC7 precursor	−1.5	NS
Protein synthesis			
U05014	PHAS-1	NS	−2
X96426	Eukaryotic elongation factor 2 kinase	−1.5	NS
AA819338	Signal sequence receptor δ	−1.5	NS
Secreted protein			
Z78279	Collagen α1 type I	−2.1	NS
AI639233	decorin	−2.1	NS
X05834	fibronectin	−2.2	NS
AA945169	Transthyretin	NS	−3.3
AA945569	α-1 macroglobulin	NS	−2.2
M62642	Hemopexin	NS	−3.9
M35601	α-fibrinogen	NS	−2.9
M12450	Vitamin D binding protein	NS	−3.5
M27434	α-2u globulin	NS	−4.5
V01216	α-1 acid globulin	NS	−4.2
U44845	vitronectin	NS	−2.1
AI176456	Metallothionein	NS	−2.3
Transport			
X67948	Aquaporin 1	−2.2	NS
D38380	transferrin	NS	−4.3
Other			
U31866	Nclone 10	NS	−4.3

See notes in Table 3.

carboxykinase and pyruvate carboxylase, were not affected by TNF-α infusion up to 4 days. Thus, TNF-α–induced changes in hepatocyte gene expression appear to favor cholesterol and lipid synthesis and VLDL production, and reduce glucose uptake and conversion to glycogen in the liver.

TNF-α exhibits moderate effects on gene expression in skeletal muscle. In contrast to the effects in adipose tissue and the liver, TNF-α caused only moderate changes in gene expression in skeletal muscle (Table 5). We could not determine whether TNF-α represses the expression of essential insulin signaling molecules in muscle because

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the hybridization signals of the insulin receptor, IRS-1, 2, and 3, PI-3 kinase and AKT were too weak to be considered significant. On the other hand, the mRNA levels of many genes involved in the uptake and utilization of glucose and FFAs, such as GLUT4, hexokinase, glycogen phosphorylase, glucose-6-phosphatase, long-chain fatty acyl-CoA synthase, CPT-I, and CPT-II, were not affected by TNF-α infusion up to 4 days. Thus, TNF-α exhibits tissue-specific effects on gene transcription, and the decreased glucose uptake in skeletal muscle is likely due to covalent modifications that impair insulin signaling or other systemic mediators generated in response to TNF-α.

DISCUSSION

We report three major findings, which taken together describe how TNF-α alters energy metabolism in vivo as well as the tissue specificity of TNF-α actions. First, we demonstrated that TNF-α caused major changes in gene expression in adipose tissue and the liver, which may mediate, at least in part, the development of systemic insulin resistance. Second, we found that TNF-α did not cause changes in plasma glucose levels for up to 4 days of infusion. Rather, TNF-α administration for 1 day increased the plasma concentration of FFAs and reduced circulating levels of ACRP30, with significant insulin resistance and elevation of plasma triglyceride concentration occurring after 4 days of infusion. Third, we found that some of the major effects of TNF-α on gene transcription in liver occurred only after 4 days of TNF-α infusion, indicating that these changes are likely to be secondary.

The TNF-α–induced changes in adipocyte gene expression in vivo are broadly consistent with our previous observations in 3T3-L1 adipocytes (12) and favor FFA release. Indeed, TNF-α caused a profound increase in plasma levels of FFAs (Table 1) and stimulated lipolysis in cultured adipocytes with delayed kinetics compared with IPT (Fig. 5), indicating that TNF-α–induced changes in adipocyte gene expression have direct functional consequences in adipose tissue and on systemic energy homeostasis.

Since the balance between cellular triglyceride synthesis, FFA re-esterification, and triglyceride hydrolysis determines the amount of FFA released from adipocytes (28,29), aberrations of any of these cellular pathways may contribute to increased FFA production in adipose tissue. As shown in Table 3 and Fig. 4*B*–D, TNF-α significantly repressed many adipocyte-abundant genes, including lipoprotein lipase, long-chain fatty acyl-CoA synthetase, diacylglycerol acyltransferase, pyruvate carboxylase, transaldolase, and perilipin. These proteins act together in a common pathway of cellular triglyceride synthesis and storage, and the decreases in their expression levels when aggregated together will favor FFA release and inhibit FFA uptake and their conversion to triglyceride in adipocytes. Interestingly, a 4-day TNF-α infusion also downregulated the mRNA level of hormone-sensitive lipase (HSL, Table 3). However, the HSL-mediated triglyceride hydrolysis is mainly regulated by the phosphorylation-state of HSL rather than HSL protein mass and, thus, the repression of HSL mRNA levels may not contribute substantially to the amount of FFA released from fat cells. Alternatively, TNF-α may stimulate FFA release by inducing other members of the triglyceride hydrolase family. Table 3 shows that TNF-α increased the mRNA levels of lysosomal acid lipase (LAL) 1.7- and 2.2-fold after 1-day and 4-day infusions, respectively. LAL is a lysosomal enzyme that hydrolyses cholesteryl esters and triglycerides and, thus, may regulate cellular triglyceride content. Another attractive candidate is the triacylglycerol hydrolase (TGH) originally described by Lehner and colleagues (30–32). TGH was initially identified as a microsomal lipase that mobilizes cytoplasmic triglyceride stores in hepatocytes, and it is likely to play a significant role in adipocyte metabolism as well. However, we did not locate the TGH gene on the rat RG34A chip, and in this study we could not determine its expression level in fat cells and its regulation by TNF-α. Nevertheless, these triglyceride hydrolases may contribute, at least partially, to the regulation of FFA release from adipocytes, and further investigation is required to explore the role of the triglyceride hydrolase family in adipocyte biology and metabolism.

It is widely accepted that elevated plasma concentrations of FFAs can cause insulin resistance by impairing the ability of insulin to stimulate skeletal muscle glucose uptake and to inhibit hepatic glucose production. Although the mechanisms for FFA-induced insulin resistance are incompletely understood, they involve decreased function and/or activity of key insulin signaling molecules in muscle (33) and liver (34). Thus, elevated plasma concentrations of FFAs appear to mediate some of the delayed systemic effects of TNF-α to decrease insulin sensitivity.

Additionally, TNF-α may induce insulin resistance, in part, by decreasing the circulating levels of ACRP30. ACRP30 is a hormone synthesized exclusively in adipose tissue, and it is immediately downregulated by TNF-α infusion (Table 1). The mechanisms by which TNF-α represses plasma levels of ACRP30 may include inhibition of ACRP30 gene transcription, reduction of ACRP30 mRNA half-life, repression of ACRP30 protein synthesis, and enhancing ACRP30 protein degradation and clearance. Although ACRP30 mRNA was not included in the ~7,000 known genes on the RG34A array, we (12) and others (13,14) have previously demonstrated that TNF-α represses the steady-state levels of ACRP30 mRNA in cultured adipocytes in a dose- and time-dependent manner. Moreover, ACRP30 mRNA and protein levels are significantly reduced in patients with type 2 diabetes. Thus, TNF-α–mediated downregulation of ACRP30 mRNA is likely to contribute, at least partially, to the decrease in circulating levels of ACRP30 in vivo as well. Although the signaling pathway, as well as the mechanisms of ACRP30 action remain uncertain, ACRP30 has been shown to sensitize liver to the actions of insulin (15,16) and reduce plasma FFA concentrations (35). Therefore, TNF-α may further antagonize the actions of insulin by repressing the plasma levels of ACRP30.

Leptin is another adipocyte-derived hormone that regulates systemic energy metabolism, food intake and adiposity, and insulin responsiveness (36–38). Several groups showed that TNF-α increased circulating levels of leptin (39,40) in both experimental animals and human subjects, and that TNF-α stimulation of leptin secretion in 3T3-L1 adipocytes is independent of protein synthesis (41). Our

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microarray data revealed that the mRNA levels of leptin were not altered in the adipose tissues from rats infused with TNF-α for up to 4 days, consistent with a previous report (41), supporting the notion that post-translational regulation of leptin production by TNF-α is likely to be involved. Further elucidation of the molecular mechanisms by which TNF-α regulates the secretion of adipocyte-derived hormones may advance our understanding of how TNF-α causes systemic insulin resistance and thereby facilitate the development of therapeutic interventions that will block these pathways in vivo.

TNF-α also caused distinct immediate and delayed changes in liver gene expression favoring FFA, triglyceride, and cholesterol synthesis (Table 3). At present, we could not determine whether TNF-α directly induces or represses the transcription of these genes in the liver, because the changes in gene expression could result from increased plasma FFA levels and/or decreased plasma ACRP30 protein content, or some other factors regulated by TNF-α.

As revealed by the insulin tolerance test, 1-day TNF-α treatment decreased insulin sensitivity, whereas TNF-α infusion for 4 days caused a significant decrease in overall in vivo insulin sensitivity (Fig. 1). The impaired glucose-lowering effect of injected insulin can be due to decreased insulin-stimulated glucose disposal, impaired inhibition of hepatic glucose production, or both. Since skeletal muscle is responsible for ~80% of overall in vivo insulin-mediated glucose uptake, a significant defect in in vivo glucose uptake indicates the development of insulin resistance in muscle. Interestingly, infusion of TNF-α for 4 days did not repress muscle genes such as GLUT4 and hexokinase, which are essential for glucose uptake and utilization. Similarly, many liver genes involved in gluconeogenesis, including phosphoenolpyruvate carboxykinase and pyruvate carboxylase, were not affected by TNF-α infusion. Thus, our data suggest that impaired activity of the insulin signaling transduction pathways, rather than changes in gene expression, may play a significant role in the development of insulin resistance in muscle and liver after 4 days of TNF-α infusion. Furthermore, the defects in insulin signaling following exposure to TNF-α may result from the time-dependent mediators including FFA, ACRP30, or some other unidentified factors regulated by TNF-α.

In summary, we demonstrated that TNF-α induces rapid and major changes in adipocyte gene expression and that it is sufficient to trigger the development of systemic insulin resistance in vivo. Given that TNF-α is overexpressed in adipose tissues of obese individuals, either TNF-α–induced defects in insulin signal transduction in adipocytes or TNF-α–dependent changes in adipocyte gene expression could be essential to elicit the insulin resistance phenotype in humans. Inhibition of TNF-α–induced changes in gene expression in cultured adipocytes, using retroviral delivery of a dominant-negative inhibitor of NF-κB, could distinguish between these alternatives (12). Importantly, the obligatory role of NF-κB in TNF-α induction of insulin resistance could not have been predicted from various studies using gene knockout mice (9,10). Taken together, our study provides information to identify the direct target tissue and molecular mediators of TNF-α and provides a molecular basis for testing pharmacological interventions of insulin resistance in humans.

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