Tumor Necrosis Factor-α-induced Insulin Resistance in 3T3-L1 Adipocytes Is Accompanied by a Loss of Insulin Receptor Substrate-1 and GLUT4 Expression without a Loss of Insulin Receptor-mediated Signal Transduction*

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A number of studies have demonstrated that tumor necrosis factor-α (TNF-α) is associated with profound insulin resistance in adipocytes and may also play a critical role in the insulin resistance of obesity and non-insulin-dependent diabetes mellitus. Reports on the mechanism of TNF-α action have been somewhat contradictory. GLUT4 down-regulation has been implicated as a possible cause of insulin resistance as has been the reduced kinase function of the insulin receptor. Here we examine the effects of tumor necrosis factor on the protein components thought to be involved in insulin-stimulated glucose transport in adipocytes, namely the insulin receptor, its major substrate IRS-1, and the insulin responsive glucose transporter GLUT4. Prolonged exposure (72–96 h) of 3T3-L1 adipocytes to TNF-α causes a substantial reduction (>80%) in IRS-1 and GLUT4 mRNA and protein as well as a lesser reduction (>50%) in the amount of the insulin receptor. Nevertheless, the remaining proteins appear to be biochemically indistinguishable from those in untreated adipocytes. Both the insulin receptor and IRS-1 are tyrosine-phosphorylated to the same extent in response to acute insulin stimulation following cellular TNF-α exposure. Furthermore, the ability of the insulin receptor to phosphorylate exogenous substrate in the test tube is also normal following its isolation from TNF-α-treated cells. These results are confirmed by the reduced but obvious level of insulin-dependent glucose transport and GLUT4 translocation observed in TNF-α-treated adipocytes. We conclude that the insulin resistance of glucose transport in 3T3-L1 adipocytes exposed to TNF-α for 72–96 h results from a reduced amount in requisite proteins involved in insulin action. These results are consistent with earlier studies indicating that TNF-α reduces the transcriptional activity of the GLUT4 gene in murine adipocytes, and reduced mRNA transcription of a number of relevant genes may be the general mechanism by which TNF-α causes insulin resistance in adipocytes.

Insulin resistance is defined as the inability of cells or tissues to respond to physiological levels of insulin and is a characteristic condition of early stage non-insulin-dependent diabetes mellitus (1). Insulin resistance has also been described in a number of disease conditions including cancer, sepsis, endotoxemia, trauma, and alcoholism. These latter situations are known to cause altered levels of cytokine expression whose actions, in part, may lead to the establishment of the pathophysiological state (2). In particular, tumor necrosis factor-α (TNF-α) secretion from activated macrophages has been recognized as one of the initial responses in these disease states. Evidence from both whole-animal and cell culture studies indicate that TNF-α alters protein and lipid metabolism in adipose tissue and in skeletal muscle, the insulin target tissues whose lack of hormonal response would result in insulin resistance (2, 3). Studies in cultured cells have demonstrated that many cytokines can regulate glucose transport (4, 5) and TNF-α, in particular, causes the decreased expression of the insulin-sensitive glucose transporter GLUT4 (6). However, it is not completely clear whether this accounts for organismal insulin resistance, since muscle GLUT4 levels are normal in insulin-resistant rodents and humans (7–9).

On the other hand, numerous recent studies have implicated the involvement of TNF-α in insulin resistance in adipocytes in culture as well as in whole-animal models. As noted above, TNF-α treatment of cultured murine adipocytes (3T3-L1s) for several days results in a significant repression of GLUT4 transcription and expression, resulting in a condition of insulin resistance without a depletion of lipid content or a change in other fat-specific genes such as lipoprotein lipase (6). In vivo studies have demonstrated that the adipose tissue of obese insulin-resistant rodents (10) and obese humans (11, 12) has a significant increase in TNF-α production and that neutralization of TNF-α in insulin-resistant rodents results in an increase in the peripheral uptake of glucose in response to insulin (10).

Thus, a role for TNF-α in some types of insulin resistance appears quite likely, and therefore understanding its mechanism of action will be important. One mechanism that has been suggested for TNF-α-induced insulin resistance is inhibition of signaling from the insulin receptor (13, 14). Both a defect in the ability of the insulin receptor to autophosphorylate and a loss of its ability to phosphorylate, on tyrosine residues, its major substrate, insulin receptor substrate-1 (IRS-1) has been reported (13, 14). More recently, TNF-α has been reported to induce serine phosphorylation of IRS-1 which, in turn, inhibits the insulin receptor from phosphorylating this substrate (15).

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1 The abbreviations used are: TNF-α, tumor necrosis factor-α; TNF, tumor necrosis factor; IRS-1, insulin receptor substrate-1; DMEM, Dulbecco's modified Eagle's medium.
precedent for this mechanism comes from studies of the phosphatase inhibitor okadaic acid, which has been shown to cause serine phosphorylation of IRS-1 and a subsequent inhibition of insulin receptor signaling (16). This biochemical mechanism makes sense for the rapid regulation of insulin signaling by TNF-α (17, 18) and okadaic acid (16), because phosphorylation/dephosphorylation are rapid (seconds to minutes) events. However, the effect of chronic TNF-α treatment (96–120 h) would seem less likely to utilize such a mechanism. Moreover, although GLUT4 is significantly down-regulated after 96 h of adipocyte TNF-α exposure, there is still some residual insulin-stimulated glucose transport (6) (see Table II).

Thus, in the present study, we have used insulin-sensitive glucose uptake as an assay for insulin-dependent signal transduction to examine the development of TNF-α-induced insulin resistance. We see no evidence for any changes in the overall tyrosine phosphorylation states of the insulin receptor or its major substrate, IRS-1. On the other hand, TNF-α-induced insulin resistance appears to involve down-regulation of several of the known component proteins required for insulin-stimulated glucose transport. These include GLUT4, IRS-1, and to a lesser extent the insulin receptor.

EXPERIMENTAL PROCEDURES

Materials—Recombinant human TNF-α was from Biogen. Murine TNF-α was purchased from Quality Control Biochemicals. All experiments were performed with both human and murine TNF-α, and identical results were obtained with both the murine and human cytokine. Dulbecco’s modified Eagle’s medium (DMEM) was purchased from Bio-Whittaker, Inc. Bovine and fetal bovine serum were obtained from Whittaker, Inc. Bovine and fetal bovine serum were obtained from Hyclone and Life Technologies, Inc., respectively. Wheat germ agglutinin-affine chromatography was purchased from E. Y. Labs. [γ-32P]ATP and [3H]2-deoxyglucose were acquired from Dupont NEN. All other chemicals were purchased from Sigma unless otherwise noted.

Cell Culture—Murine 3T3-L1 preadipocytes were cultured, maintained, and differentiated as described previously (19). Briefly, cells were plated and grown for 2 days postconfluence in DMEM supplemented with 10% calf serum. Differentiation was then induced by the medium to DMEM supplemented with 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, 1 mM dexamethasone, and 1.75 μg/ml insulin. After 48 h, the differentiation medium was replaced with maintenance medium containing DMEM supplemented with 10% fetal bovine serum. The maintenance medium was changed every 48 h until the cells were used for experimentation. Human or murine TNF-α was dissolved in phosphate-buffered saline containing 0.1% fatty acid-free and growth factor-depleted bovine serum albumin (Sigma) and was added to the cell culture media 7 days after the induction of adipocyte differentiation. The cells were harvested at 3 days after the initiation of adipocyte differentiation, rinsed, and collected according to the protocol of Clancy and Czech (20) into plasma membranes, cytosol, and whole-cell fractions. Membrane and cytosolic fractions were divided and immediately stored at −70°C. Both Buffer A and Buffer B were used to collect the membrane and cytosolic fractions that were fractionated according to the protocol of Clancy and Czech (20) into plasma membrane, intracellular membranes, and a nuclear/mitochondrial fraction as we have previously described (6). Membrane and cytosolic fractions were divided and immediately stored at −70°C. Both Buffer A and Buffer B contain the following protease and phosphatase inhibitors: 1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 50 trypsin inhibitor milliunits of aprotinin, 1 mM 1,10-phenanthroline, 1 μM pepstatin, 2.5 mM benzamidine hydrochloride, 2 mM sodium fluoride, 1 mM sodium vanadate, 30 mM sodium pyrophosphate, and 1 mM sodium molybdate. The protein content for all fractions was determined with a BCA kit (Pierce) according to the manufacturer’s instructions.

Gel Electrophoresis and Immunoblotting—Proteins were separated in 7.5 or 12% polyacrylamide (acylamide from National Diagnostics) gels containing sodium dodecyl sulfate (SDS) according to Laemmli (21) and transferred to nitrocellulose (Bio-Rad) or Immobilon-P (Millipore) in 25 mM Tris, 192 mM glycine, and 20% methanol. Following transfer, the membrane was blocked in 4% nonfat milk for 1 h at room temperature. Results were visualized with horseradish peroxidase-conjugated secondary antibodies (Sigma) and an enhanced chemiluminescence kit (Pierce).

The antibody used for detection of the insulin receptor (R1064) was generated against the C terminus deduced from the insulin receptor (22). The anti-phosphotyrosine monoclonal antibody, 4G10, was purchased from Upstate Biotechnology, Inc. and used to detect insulin receptor phosphorylation. The anti-phosphotyrosine monoclonal antibody, PY20, was purchased from Upstate Biotechnology, Inc. and used to detect IRS-1 phosphorylation. A monoclonal antibody for GLUT4 (IFS8), was used for detection of the insulin-sensitive glucose transporter (23). Western blots employing a monoclonal antibody were analyzed using goat antimouse antibody (Sigma) coupled to horseradish peroxidase followed by chemiluminescence using the Renaissance system (Dupont NEN). Blots using polyclonal antibodies were exposed to goat anti-rabbit (Sigma) coupled to horseradish peroxidase and visualized with chemiluminescence.

Exogenous Kinase Activity Assays—The exogenous kinase activity of the insulin receptor following its solubilization and partial purification from cell extracts was determined as described previously (24). Briefly, the insulin receptor was purified from total membranes by wheat germ agglutinin-affine chromatography in the presence of the protease inhibitors and phosphatase inhibitor added above. Bound receptor was eluted with 0.3 M N-acetylglucosamine in 20 mM HEPES (pH 7.4) containing 0.1% Triton X-100. The receptor was incubated with 50 μM ATP (100 μCi/ml [32P]ATP), 10 mM MgCl2, 8 mM MnCl2, and 2.5 mg/ml of poly(Glu:Tyr) (4:1) as a phosphate acceptor. Following a 10-min incubation, the reaction was stopped by adding EDTA to a final concentration of 67 mM. The reaction mixture was loaded on a 3 × 3-cm piece of 3 μ filter paper which was extensively washes with 10% trichloroacetic acid and 100 mM pyrophosphate. Radioactivity incorporated into poly(Glu:Tyr) was determined as Cerenkov radiation in a scintillation counter. For the final results, radioactivity was normalized by the amount of the insulin receptor, which was determined by Western blot as described previously (24).

RNA Isolation and Analysis—Total RNA was isolated from the cells by extraction with Trizol (Life Technologies, Inc.) and was prepared according to the manufacturer’s instructions. Poly(A)+ RNA was selected using a poly(A)+ tract kit (Stratagene). For Northern blot analysis, 5 μg of poly(A)+ RNA were separated by electrophoresis in 1.3% agarose, 2% formaldehyde gels and transferred to Gene Screen Plus (Dupont NEN). After ultraviolet cross-linking, filters were prehybridized, hybridized, and subjected to analysis as described previously (19). The cDNAs utilized in these studies were as follows: GLUT4, the insulin-sensitive glucose transporter (25); IRS-1, a generous gift from Dr. Morris White (26); and β-actin, 1.9-kilobase HindIII fragment obtained from Dr. D. W. Cleveland (27).

Determination of 2-Deoxy-D-Glucose—The assay of [γ-32P]2 deoxyglucose uptake was performed as described previously (19). Prior to the assay, the cells were deprived of serum for 2–4 h. Uptake measurement were made in triplicate under conditions when hexose uptake was linear, and the results were corrected for nonspecific uptake and absorption determined by [γ-32P]2 deoxyglucose uptake in the presence of 5 μM cytochalasin B. Nonspecific uptake and absorption was always less than 10% of the total uptake.

RESULTS

Effect of TNF-α on Insulin-sensitive Glucose Uptake—In hepatocytes, it has been reported that TNF-α induces a defect in insulin signaling in a time frame of less than 1 h (16, 17). Thus, we examined the effect of relatively low (250 μM) and high (1 mM) doses of TNF-α treatment on fully differentiated 3T3-L1 adipocytes over a 24-h time course. As shown in Table I, low doses of TNF-α had essentially no effect on basal or insulin-stimulated glucose uptake. Further, insulin-stimulated uptake was unaffected by 1 μM TNF-α during a 24-h treatment for 1 and 6 h (Table I). There may be a slight decrease in insulin-stimulated glucose uptake in adipocytes exposed to the high doses of TNF-α for 24 h (7.8-fold stimulation versus 9.2-fold, untreated). However, at this time, GLUT4 protein is slightly diminished (data not shown), and the basal uptake is slightly elevated (Table I). Thus, we observe no
significant compromise in insulin-stimulated glucose transport in adipocytes exposed to relatively short treatments of TNF-α, and therefore, no lesion in insulin-dependent signal transduction.

We also performed a longer time course of TNF-α treatment under conditions previously shown to cause insulin-resistant glucose uptake in fully differentiated 3T3-L1 adipocytes (28). As in the cited study, maximal inhibition of insulin-sensitive glucose uptake was not achieved until after 96 h of TNF-α exposure, thus indicating there is minimal synergistic action of TNF-α with insulin when serum is supplemented with a high insulin concentration (Table II). Exposure to TNF-α in untreated cells (Table II) further, there is no insulin receptor autophosphorylation unless cells had not been exposed to insulin. Subsequent measurement of representative Northern blot of IRS-1, GLUT4, and β-actin mRNA expression in untreated adipocytes and in cells treated with 250 pm TNF-α for 96 h. As expected and as normalized against β-actin mRNA expression, IRS-1 and GLUT4 mRNA from TNF-α-treated adipocytes are less than 20% of the levels in untreated adipocytes.

The Effect of TNF-α on Insulin Receptor Phosphorylation and Kinase Activity—It has been suggested that prolonged TNF-α treatment (96 h or more) results in diminished insulin receptor phosphorylation and kinase activity in adipocytes (13, 14). Accordingly, we examined insulin receptor amount and its ability to undergo autophosphorylation in cells following 96 h of adipocyte exposure to various TNF-α doses. Fig. 3 shows that the insulin receptor content diminishes slightly with exposure to 250 pm TNF (see also Fig. 1) and is more dramatically down-regulated at higher cytokine doses (Fig. 3, bottom). However, the tyrosine phosphorylation state of the insulin receptor from TNF-α-treated cells was essentially unchanged (Fig. 3, top). There is no insulin receptor autophosphorylation unless cells are exposed to insulin, and when they are, they respond completely normally as determined by phosphotyrosine blotting (Fig. 3, top). The autophosphorylation signal precisely corresponds to the amount of receptor, and they are diminished in parallel in response to TNF-α treatment.

Fig. 4 depicts another experiment in which insulin receptors were isolated from untreated and TNF-α-treated adipocytes by wheat germ agglutinin chromatography (see under “Experimental Procedures”) and then examined for insulin receptor content and phosphorylation state (top) by Western blotting. We observed a small decrease in both receptor amount and phosphorylation state (top) from the TNF-α-treated cells (see also Fig. 3). We used the partially purified receptor to phosphorylate substrate (bottom) in the test tube, and because slightly less receptor was isolated from the TNF-α-treated cells (top), slightly lower substrate phosphorylation was seen (bottom). When normalized to receptor amount, there is no difference in the in vitro kinase activity or autophosphorylation state of receptors isolated from untreated and TNF-α-treated adipocytes. We also performed an experiment using receptors purified from untreated and TNF-α-treated (96 h) adipocytes that had not been exposed to insulin. Subsequent measurement of
FIG. 1. TNF-α-treated adipocytes have reduced insulin receptor, IRS-1, and GLUT4 expression. Total membrane and cytosolic fractions were prepared from fully differentiated 3T3-L1 adipocytes treated with 250 pM TNF-α for 0, 24, 72, or 96 h. Proteins (75 μg/ lane) were separated by SDS-polyacrylamide gel electrophoresis, transferred to Immobilon-P, and subjected to Western blot analysis with the appropriate antibody. The detection system was horseradish peroxidase-conjugated secondary antibodies and a chemiluminescence substrate kit. The molecular mass of each protein is indicated to the left of the blot in kilodaltons. The autoradiogram displayed is representative of an experiment performed three times with independent cell preparations, all of which gave similar results.

FIG. 2. IRS-1 and GLUT4 mRNA are down-regulated in TNF-α-treated adipocytes. Poly(A)+ RNA was isolated from fully differentiated 3T3-L1 adipocytes that were not treated or were exposed to 250 pM TNF-α for 96 h. Five μg of poly(A)+ RNA was electrophoresed and subjected to Northern blot analysis. The same blot was sequentially hybridized, stripped, and rehybridized with the indicated cDNA probes. The IRS-1 blot was exposed for 6 days, GLUT4 for 6 h, and β-actin for 12 h. The sizes of the mRNAs are shown in kilobases (kb) to the left of each panel. The autoradiogram displayed is representative of an experiment performed twice with independent RNA preparations.

FIG. 3. TNF-α causes a dose-dependent decrease in insulin receptor amount but no change in the overall phosphorylation state. A total membrane fraction was isolated from adipocytes that were chronically (96 h) TNF-α-treated with the indicated doses of TNF-α followed by a 10-min exposure to 100 nM insulin. Following electrophoresis, the membrane preparations were examined for insulin receptor amount and phosphorylation state using an anti-receptor and anti-phosphotyrosine antibody, respectively. Detection was as described in Fig. 1. The molecular mass markers to the left of the blot are in kilodaltons. The autoradiogram displayed is representative of experiments performed three times with independent cell preparations.

autophosphorylation and exogenous kinase activity gave identical results for receptors from TNF-α-treated and untreated cells (data not shown).

The Effect of TNF-α on IRS-1 Tyrosine Phosphorylation—From the previous figures, only the amount of protein and mRNA for the various components of insulin-stimulated glucose uptake were shown to diminish in response to TNF-α. However, the function of IRS-1 has also been suggested to be altered in TNF-α-induced insulin resistance in adipocytes (13–15). Therefore, we examined the insulin-dependent tyrosine phosphorylation of IRS-1 in chronically TNF-α-treated adipocytes performed three times with independent cell preparations.

FIG. 4. The insulin receptor isolated from TNF-α-treated adipocytes phosphorylates poly(GluTyr) identically to receptor from untreated cells. Insulin receptors were partially purified by wheat germ agglutinin chromatography as described under “Experimental Procedures” from untreated and TNF-α-treated (96 h) adipocytes exposed to insulin (+) or not (−) for 10 min. Receptor was then used for Western blotting (A) as in the previous figure and was also used to phosphorylate substrate (B) in the test tube. In B, the open bars represent basal levels of phosphorylation and the solid bars represent insulin stimulation. The exogenous kinase assay was performed as described under “Experimental Procedures.” The results displayed are representative of an experiment performed three times with independent cell preparations.

cytes (250 pM for 96 h). As also shown in Fig. 1, Fig. 5 shows that TNF-α treatment results in significant loss of IRS-1 protein (>80%, top). IRS-1 tyrosine phosphorylation is diminished to the same extent (bottom). Thus, as was the case for insulin receptor autophosphorylation (Fig. 3), TNF-α diminishes the amount of IRS-1, yet the remaining IRS-1 protein is tyrosine-phosphorylated in a normal fashion in response to insulin.

Effect of TNF-α on GLUT4 Translocation—The prior data (Figs. 1–5) coupled with the transport data of Table II suggests no obvious lesion in insulin signaling in adipocytes due to TNF-α exposure except for a decreased amount of the component proteins of the signaling pathway. As a further verification of this, we performed a GLUT4 translocation assay on adipocyte membrane fractions (Fig. 6) on insulin-sensitive (untreated) and insulin-resistant (96 h of TNF-α exposure) 3T3-L1 adipocytes. The cells were exposed or not to insulin stimulation (100 nM for 8 min) and then fractionated into membrane compartments. In insulin-treated adipocytes, there is an increase in GLUT4 at the plasma membrane (PM), and a corresponding decrease in internal membrane (IM) GLUT4, a result indicative of transporter translocation to the plasma membrane (31). The level of GLUT4 protein present in the plasma membrane of untreated cells is higher than expected from the transport data of Table II but is consistent with many published studies that indicate membrane fractionation of 3T3-L1 adipocytes is less precise than that of rat adipocytes (e.g. see Ref. 20). In any case, following 96 h of TNF-α exposure, GLUT4 levels were markedly diminished in both plasma membrane and internal membrane fraction (top) as would be expected from Figs. 1 and 2 and from previous studies (6, 10, 28). Nevertheless, when enough protein was loaded onto SDS-polyacrylamide gels and immunoblotted for GLUT4 protein (bottom), an increase in the transporter at the plasma membrane is caused by insulin exposure of TNF-α-treated adipocytes as is a depletion of GLUT4 from the internal membranes. Thus, GLUT4...
TNF-α-induced Insulin Resistance in 3T3-L1 Adipocytes

In general, the biochemical changes in cells brought about by cytokines such as TNF-α are thought to occur as result of altered gene expression, often due to activation of the STAT (signal transducers and activators of transcription) family of transcription factors (32, 33). Thus, the recent results from several groups indicating that TNF-α may cause rapid (17, 18) or slow (13–15) alterations in the ability of insulin to phosphorylate its receptor in a matter of minutes (17, 18). In adipocytes, acute TNF-α treatment (15 min) or pretreatment of TNF-α followed by insulin stimulation results in an enhancement of IRS-1 tyrosine phosphorylation and promotes its association with phosphatidylinositol 3-kinase (37). In the course of our studies, we have also observed a very small enhancement of IRS-1 tyrosine phosphorylation in adipocytes exposed to prolonged TNF-α treatment (data not shown). However, it has not been demonstrated that IRS-1 is a mediator of TNF-α signal transduction in any cell type.

Another question raised by our data concerns the nature of the TNF receptor that mediates the responses we observed in adipocytes. Very recently, Peraldi et al. reached the same conclusion based on the same protocol of comparing human and murine TNF-α actions in 3T3-L1 cells (41). In summary, we conclude that TNF-α-induced insulin resistance in 3T3-L1 adipocytes is not accompanied by a defect in insulin receptor phosphorylation, IRS-1 tyrosine phosphorylation, or the ability of the insulin receptor to recruit GLUT4 to}

Translocation to the plasma membrane appears normal even after prolonged exposure of fat cells to TNF-α, a result consistent with the glucose uptake data (Table II).

DISCUSSION

In general, the biochemical changes in cells brought about by cytokines such as TNF-α are thought to occur as result of altered gene expression, often due to activation of the STAT (signal transducers and activators of transcription) family of transcription factors (32, 33). Thus, the recent results from several groups indicating that TNF-α may cause rapid (17, 18) or slow (13–15) alterations in the ability of insulin to phosphorylate its receptor in a matter of minutes (17, 18). In adipocytes, acute TNF-α treatment (15 min) or pretreatment of TNF-α followed by insulin stimulation results in an enhancement of IRS-1 tyrosine phosphorylation and promotes its association with phosphatidylinositol 3-kinase (37). In the course of our studies, we have also observed a very small enhancement of IRS-1 tyrosine phosphorylation in adipocytes exposed to prolonged TNF-α treatment (data not shown). However, it has not been demonstrated that IRS-1 is a mediator of TNF-α signal transduction in any cell type.

Another question raised by our data concerns the nature of the TNF receptor that mediates the effects we observe. There are two cell surface TNF-α receptors, a type I receptor of M, 55,000 and a type II species of M, 75,000, that are present in all cell types (38). Most biological actions of TNF-α are mediated by the type I receptor (39), which in the case of murine cells is the only receptor isotype that binds human TNF-α (40). As indicated under “Experimental Procedures,” we performed all our experiments with TNF-α from both species, and we obtained identical results, thus indicating that the type I receptor is mediating the responses we observed in adipocytes. Very recently, Peraldi et al. reached the same conclusion based on the same protocol of comparing human and murine TNF-α actions in 3T3-L1 cells (41).
the plasma membrane. Our data indicate that the primary mechanism of TNF-α action is likely to be at the level of regulation of gene expression, the general mode of action for cytokines (32, 33). This hypothesis is supported by previous studies which demonstrate that short (2 h) exposure of 3T3-L1 adipocytes to TNF-α results in a ~90% inhibition of GLUT4 transcription in the absence of protein synthesis (28). These conclusions differ dramatically from other investigators (13–15, 17, 18), and further studies are warranted to resolve both the current discrepancies and the mechanism of action of TNF in adipocytes.

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