Tumor Necrosis Factor-α Stimulates Lipolysis in Differentiated Human Adipocytes Through Activation of Extracellular Signal–Related Kinase and Elevation of Intracellular cAMP

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Tumor necrosis factor-α (TNF-α) stimulates lipolysis in human adipocytes. However, the mechanisms regulating this process are largely unknown. We demonstrate that TNF-α increases lipolysis in differentiated human adipocytes by activation of mitogen-activated protein kinase kinase (MEK), extracellular signal–related kinase (ERK), and elevation of intracellular cAMP. TNF-α activated ERK and increased lipolysis; these effects were inhibited by two specific MEK inhibitors, PD98059 and U0126. TNF-α treatment caused an electrophoretic shift of perilipin from 65 to 67 kDa, consistent with perilipin hyperphosphorylation by activated cAMP-dependent protein kinase A (PKA). Coincubation with TNF-α and MEK inhibitors caused perilipin to migrate as a single 65-kDa band. Consistent with the hypothesis that TNF-α induces perilipin hyperphosphorylation by activating PKA, TNF-α increased intracellular cAMP ~1.7-fold, and the increase was abrogated by PD98059. Furthermore, H89, a specific PKA inhibitor, blocked TNF-α–induced lipolysis and the electrophoretic shift of perilipin, suggesting a role for PKA in TNF-α–induced lipolysis. Finally, TNF-α decreased the expression of cyclic-nucleotide phosphodiesterase 3B (PDE3B) by ~50%, delineating a mechanism by which TNF-α could increase intracellular cAMP. Cotreatment with PD98059 restored PDE3B expression. These studies suggest that in human adipocytes, TNF-α stimulates lipolysis through activation of MEK-ERK and subsequent increase in intracellular cAMP. *Diabetes* 51: 2929–2935, 2002

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DMEM, Dulbecco's modified Eagle's medium; ECL, enhanced chemiluminescence; ERK, extracellular signal–related kinase; FBS, fetal bovine serum; FFA, free fatty acid; HSL, hormone-sensitive lipase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; PDE3B, phosphodiesterase 3B; PKA, protein kinase A; TNF-α, tumor necrosis factor-α.

Obesity and type 2 diabetes are associated with increased concentrations of circulating free fatty acids (FFAs) that are thought to elicit systemic insulin resistance (1). The flux of FFA is primarily dependent on lipolysis of triacylgycerol in adipocytes. Tumor necrosis factor-α (TNF-α) is known to increase adipocyte lipolysis (2–6), and its expression in adipose tissue of obese subjects is increased (7–10). This increased expression of TNF-α may enhance adipocyte lipolysis and hence increase concentrations of circulating FFAs. Indeed, adipose tissue interstitial levels of TNF-α and FFA are positively correlated (11). Infusion of TNF-α in rodents (12) and humans (13,14) results in increased plasma FFAs and systemic insulin resistance. TNF-α knockout mice exhibit lower circulating FFAs and are protected from insulin resistance of obesity (15). Furthermore, rosiglitazone, an antidiabetic agent, blocks the lipolytic effect of TNF-α in 3T3-L1 adipocytes (16), delineating a mechanism by which rosiglitazone may improve insulin resistance. In light of the accumulating data linking adipocyte lipolysis to insulin resistance, it is physiologically relevant to investigate the mechanism(s) by which TNF-α stimulates lipolysis, which to date is largely unknown.

TNF-α is a potent activator of mitogen-activated protein kinases (MAPKs), including extracellular signal–related kinase (ERK)-1 and -2 (p42/p44), c-Jun NH_2-terminal kinase, and p38 kinase (17). Activation of the MAPK pathway affects many physiological processes. Of particular interest, the mitogen-activated protein kinase kinase (MEK) 1/2-ERK1/2 pathway has been shown to mediate TNF-α–induced serine phosphorylation of insulin receptor substrate-1, another possible mechanism by which TNF-α induces insulin resistance (18). Moreover, the MEK1/2-ERK1/2 pathway has recently been shown to regulate lipolysis in 3T3-L1 adipocytes by phosphorylating hormone-sensitive lipase (HSL), a rate-limiting enzyme in hormone-regulated lipolysis (19). We hypothesize that the MEK1/2-ERK1/2 pathway also regulates TNF-α–induced lipolysis in human adipocytes.

Perilipins are phosphoproteins that are located at the surface of lipid droplets in adipocytes and may act as a barrier to reduce the access of lipase(s) to intracellular lipids (20–25). Phosphorylation of perilipins by cAMP-

dependent protein kinase (PKA) allows access of lipase(s) to the droplets by a modification of lipid surfaces, resulting in lipolysis (23,24,26,27). Activation of PKA has been associated with TNF-α–induced lipolysis in 3T3-L1 adipocytes and the mechanism may involve TNF-α–induced downregulation of cyclic-nucleotide phosphodiesterase 3B (PDE3B), an enzyme that hydrolyzes cAMP, which leads to increased intracellular cAMP (28). We hypothesize that TNF-α stimulates lipolysis in human adipocytes through a similar cAMP/PKA mechanism.

In this paper, we investigated the mechanisms by which TNF-α stimulates lipolysis in human fat cells. As a cell culture system, we used differentiated human adipocytes that offer prolonged survival compared with mature, freshly isolated adipocytes. First, we examined the potential role of the MEK/2-ERK1/2 pathway. We then examined the involvement of perilipin A, the predominant form of the perilipins in human adipocytes. Finally, we studied the association of intracellular cAMP, PDE3B, and PKA with TNF-α–induced lipolysis.

RESEARCH DESIGN AND METHODS

Chemicals and reagents. All chemicals were from Sigma Chemical (St. Louis, MO) unless otherwise indicated. BSA (fraction V) was from Intergen (Purchase, NY), fetal bovine serum (FBS) was from Hyclone (Logan, UT), PD98059 was from Calbiochem (San Diego, CA), and U0126 was from Promega (Madison, WI). Tissue culture reagents were purchased from GIBCOBRL (Rockville, MD) and Hyclone; type I collagenase was from Worthington Biochemical (Lakewood, NJ); enhanced chemiluminescence (ECL) Western blotting detection reagents, horseradish-linked anti-rabbit and anti-mouse IgG were from Amersham Life Sciences (Arlington Heights, IL); [^{125}I]-Protein A was from PerkinElmer Life Sciences (Boston, MA); and rosiglitazone (BRL 49653) was kindly provided by GlaxoSmithKline (from Stephen A. Smith and Thomas Leonard; King of Prussia, PA).

Cell culture. Subcutaneous adipose tissues were obtained from male or female patients undergoing abdominal plastic surgery in accordance with the approval of the Tufts New England Medical Center Human Investigation Review Committee. Patients ranged in age from 24 to 64 years and body weight from 65 to 80 kg; all were fasted overnight and underwent surgical anesthesia. None of the patients had diabetes or severe systemic illnesses. Preadipocytes were isolated using the method of Rodbell (29) with modifications. In brief, freshly diced tissue pieces were digested in Hank's balanced salt solution (HBSS) containing 2 g/l type I collagenase and 4% BSA for 1 h in a 37°C water bath and shaken at 100 cycles/min. The disrupted tissue was filtered through double-layered cotton mesh and centrifuged at 250*g* for 5 min.

Preadipocytes were resuspended in erythrocyte-lysing buffer (154 mmol/l NH_4Cl, 5.7 mmol/l K_2HPO4, and 0.1 mmol/l EDTA, pH 7.0) and incubated for 10 min to remove contaminating erythrocytes. After centrifugation at 150*g* for 5 min, preadipocytes were resuspended in Dulbecco's modified Eagle's medium (DMEM):F12 medium containing 10% FBS and inoculated into 24-well plates at a density of ~50,000 cells/cm^2. After overnight incubation, preadipocytes were washed in HBSS and cultured in DMEM:F12 containing 3% FBS, 100 nmol/l human insulin, 1 μmol/l dexamethasone, and for the first 3 days of culture, 0.25 mmol/l 3-isobutyl-1-methylxanthine and 5 μmol/l rosiglitazone. Medium was changed every 2–3 days, and cells were used after 2–3 weeks of incubation. As judged by microscopy, >80% of the cells were differentiated.

Differentiated human adipocytes from seven healthy female patients (aged 25–60 years, BMI <25 kg/m^2) were obtained from Zen-Bio (Research Triangle, NC). Cells cultured by our laboratory and Zen-Bio responded similarly to treatments despite variations in body weight and age of patients. Therefore, data were pooled.

Measurement of lipolysis. Differentiated human adipocytes were incubated in serum-free DMEM containing 5 mmol/l glucose and 2% BSA for 24 h before treatments. Glycerol content in the incubation medium was used as an index for lipolysis and was measured using a colorimetric assay (GPO-Trinder; Sigma). Results were corrected for cellular proteins, which were quantified using the bicinchoninic acid protein assay kit (Pierce, Rockford, IL) and were expressed as micromoles of glycerol per milligrams of protein or percentage of control.

Western analyses. Cells were washed briefly with PBS and collected with boiling lysis buffer containing 62.5 mmol/l Tris-HCl (pH 6.8), 6% SDS, 0.5 mmol/l Na_3VO_4, and 0.5 μmol/l dithiothreitol. After boiling at 95°C for 10 min, cells were briefly sonicated. Equal amounts of protein were subjected to ECL-based immunoblotting as described (6). A rabbit polyclonal anti-perilipin antibody was generated using peptide MSMNKGPTLLDGDLPEQENVL and used at 1:3,000. Rabbit antibodies specific for individual Gα subunits $G\alpha_s$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$ were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and used at 1:1,000 for immunoblotting of plasma membrane proteins. The expression of PDE3B was examined by subjecting equal amounts of plasma membrane proteins to SDS-PAGE, immunoblotting with a rabbit polyclonal antibody to PDE3B generated using a COOH-terminal peptide (QWLEAEEDNDTESGDDEDG), and as a loading control, immunoblotting with an antibody to $G\alpha_s$. Protein expression was detected by ECL and for densitometry by reaction with [^{125}I]-protein A. Binding of [^{125}I]-protein A to rabbit IgG was detected by autoradiography at −80°C with Kodak XRP film and a DuPont Lightening Plus intensifying screen. Quantification of the autoradiograms was by scanning densitometry.

MAPK assays. Activation of ERK kinase was determined by measuring the phosphorylation state of ERK1/2 (30). Aliquots (50 μg protein/sample) of cellular proteins were resolved by SDS-PAGE. ERK1/2 phosphorylation was detected by immunoblotting using a 1:1,000 dilution of rabbit polyclonal phospho-MAP kinase-specific antibody (Cell Signaling Technology, Beverly, MA) with a 1:5,000 dilution of horseradish peroxidase–conjugated anti-rabbit IgG as secondary antibody. Quantitation of ERK 1/2 phosphorylation was performed using ChemiImager 4000 (Alpha Innotech, San Leandro, CA). To assess ERK1/2 expression, an equal amount of proteins (50 μg protein/sample) was used for immunoblotting using a 1:1,000 dilution of rabbit polyclonal anti-ERK1/2 IgG (Cell Signaling Technology).

Measurement of intracellular cAMP concentrations. After 24 h treatment, intracellular cAMP concentrations in differentiated human adipocytes were measured using an enzyme immunoassay kit from Amersham Pharmacia Biotech. Results were corrected for cellular protein content and expressed as femtomoles of cAMP per milligrams of protein.

Isolation of membrane fractions. After 24 h treatment, plasma membranes were prepared as described (31). Equal amounts of proteins were used for Western analyses of G proteins and PDE3B.

Statistical analysis. Results were expressed as means ± SE. One-way ANOVA was used for statistical analyses. P values <0.05 were considered statistically significant.

RESULTS

All experiments were performed with differentiated human adipocytes. After 2–3 weeks in culture, >80% of the preadipocytes were differentiated as assessed by microscopic lipid accumulation.

TNF-α stimulates lipolysis in differentiated human adipocytes. To investigate the mechanism(s) by which TNF-α regulates lipolysis in human adipocytes, we first examined the concentration dependency of TNF-α on lipolysis. TNF-α increased lipolysis in a dose-dependent manner beginning at 10 ng/ml. The lipolytic effect was evident only after 6 h, suggesting that this effect does not occur via a direct activation of the lipolytic machinery (Fig. 1). This finding is consistent with that of Hauner et al. (4) in human adipocytes. At 100 ng/ml, TNF-α (at 24 h) increased lipolysis by ~1.7-fold. Because of the potentially high concentrations of adipose-derived TNF-α in the adipose interstitial fluid in obese subjects (7–10), we used 100 ng/ml in subsequent experiments.

TNF-α activates ERK1/2. As a first step to determine whether the MAPK pathway is involved in TNF-α–mediated lipolysis, the time course of the effects of TNF-α on ERK1/2 activation was examined. TNF-α treatment increased ERK1/2 phosphorylation, whereas it did not affect the expression of total ERK (Fig. 2). The increase was maximal at 15–30 min, and the effect remained detectable for 24 h.

MEK1/2 inhibitors PD98059 and U0126 inhibit TNF-α–induced lipolysis. ERK1/2 activity is regulated by the upstream kinase MEK1/2, which phosphorylates ERK1/2

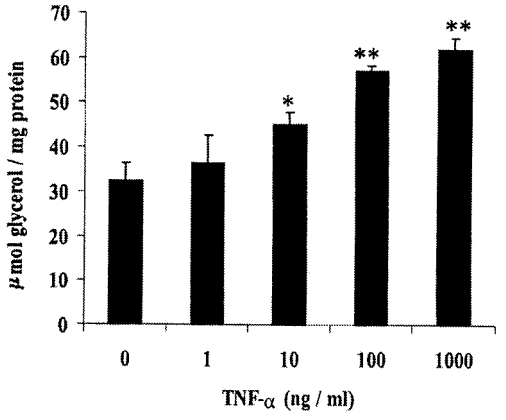

FIG. 1. Stimulation of lipolysis by TNF-α in differentiated human adipocytes. Differentiated human adipocytes were treated with the indicated concentrations of TNF-α for 24 h. Conditioned medium was collected and assayed for glycerol. Results are means ± SE of triplicate measurements and are representative of three dose-response experiments. Significantly different from untreated controls by one-way ANOVA: *$P < 0.05$, **$P < 0.01$.

on threonine and tyrosine residues (32,33). To study the possible role of the ERK1/2 pathway in lipolysis, two specific MEK1/2 inhibitors, PD98059 (34) and U0126 (35), were used to block activation of the MEK/ERK pathway. PD98059 inhibits MEK activation by binding to inactive MEK and preventing the phosphorylation by upstream kinases such as Raf-1. U0126 inhibits the catalytic activity of the active enzyme MEK1/2. As shown in Fig. 3*A*, treatment with PD98059 (6 and 24 h) inhibited TNF-α–induced and basal ERK1/2 phosphorylation without altering total ERK protein expression. Concomitant with its inhibitory effect on ERK activation, PD98059 treatment decreased lipolysis in TNF-α–treated cells to below control levels, and in non–TNF-α–treated cells (PD98059 alone), to ~50% of control (Fig. 3*B*).

Treatment with U0126 resulted in more potent inhibitory effects on ERK1/2 phosphorylation and lipolysis. It decreased lipolysis in both TNF-α–treated and non–TNF-α–treated cells (U0126 alone) to 9.4 and 13.6% of control, respectively (Fig. 3*C*) and reduced ERK1/2 phosphorylation to an undetectable level, as assessed by Western blotting (Fig. 3*D*).

MEK1/2 inhibitors block the TNF-α–induced electrophoretic shift of perilipin and decrease the TNF-α–induced increase in intracellular cAMP. TNF-α has been shown to induce lipolysis, in part, by decreasing perilipin expression in 3T3-L1 adipocytes (6,23). To determine whether TNF-α stimulates lipolysis in human adipocytes by a similar mechanism, Western blotting was performed. As shown in Fig. 4*A* and *B*, TNF-α treatment did not significantly decrease perilipin expression but caused an electrophoretic shift of perilipin from 65 to 67 kDa, consistent with PKA activation and hyperphosphorylation of perilipin. Coincubation with PD98059 or U0126 diminished this effect of TNF-α and resulted in perilipin migrating as a single 65-kDa band. These results led us to hypothesize that the MEK inhibitors PD98509 and U0126 inhibit TNF-α–induced lipolysis, at least in part, by inhibiting hyperphosphorylation of perilipin.

The rate of lipolysis in adipocytes, in general, is critically dependent on the intracellular concentration of cAMP. To examine the possibility that TNF-α–induced lipolysis could involve the regulation of intracellular cAMP, differentiated human adipocytes were treated in the absence or presence of TNF-α for 24 h. TNF-α treatment resulted in an ~1.6-fold increase in intracellular cAMP; incubation with PD98059 abolished this effect of TNF-α while decreasing basal intracellular cAMP by ~26% (Fig. 5*A*). These effects of TNF-α and PD98059 were consistent with their effects on lipolysis, i.e., stimulation by TNF-α and inhibition by PD98059.

The PKA inhibitor, H89, blocks TNF-α–induced lipolysis and hyperphosphorylation of perilipin. To further verify whether TNF-α–stimulated lipolysis is mediated by activation of cAMP-dependent PKA, cells were incubated in the presence or absence of TNF-α and the PKA inhibitor H89 (20 μmol/l) for 24 h. Treatment with H89 abrogated TNF-α–induced lipolysis (Fig. 5*B*), and in non–TNF-α–treated cells (H89 alone), it reduced lipolysis to ~50% of control. Concomitant with this effect, H89 reversed the TNF-α–induced electrophoretic shift of perilipin and caused it to migrate as a single 65-kDa perilipin band, suggesting that this protein was not hyperphosphorylated by PKA in the presence of H89 (Fig. 5*C*).

TNF-α decreases expression of PDE3B. In adipocytes, intracellular concentration of cAMP is determined by its synthesis controlled by adenylyl cyclase and its degradation, regulated by the hydrolytic activity of cyclic nucleotide phosphodiesterases (PDEs), especially PDE3B. To determine the mechanism(s) by which TNF-α increases intracellular cAMP in differentiated human adipocytes, Western analyses of G protein expression were performed. In rat adipocytes, TNF-α has been suggested to stimulate lipolysis by decreasing the expression of $G_{i,}$ which inhibits adenylyl cyclase and, hence, the production of cAMP (31). Treatment of differentiated human adipocytes with TNF-α, however, did not significantly decrease expression of $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$, nor did it have significant effect on $G\alpha_{s}$ expression (data not shown). We next examined the expression of PDE3B by Western analyses using $G\alpha_{s}$ as a loading control and both ECL- and [^{125}I]-protein A–based detection systems (Fig. 6). Densitometry using [^{125}I]-protein A detection indicates that TNF-α treatment for 24 h decreased expression of PDE3B by ~50%. Cotreatment with PD98059 abrogated this decrease, and treatment with PD98059 alone increased PDE3B by ~30%. Thus, the TNF-α–induced increase in intracellular cAMP is likely to be mediated by downregulation of PDE3B expression, and the regulation is downstream of ERK1/2 activation.

DISCUSSION

In the present study, we show that TNF-α–increased lipolysis in human adipocytes correlates with ERK activa-

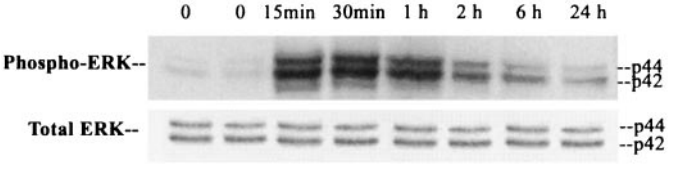

FIG. 2. Activation of ERK1/2 by TNF-α. Differentiated human adipocytes were treated with TNF-α (100 ng/ml) for the times indicated. Cell extracts were immunoblotted for phospho-ERK and total ERK as described in the RESEARCH DESIGN AND METHODS section.

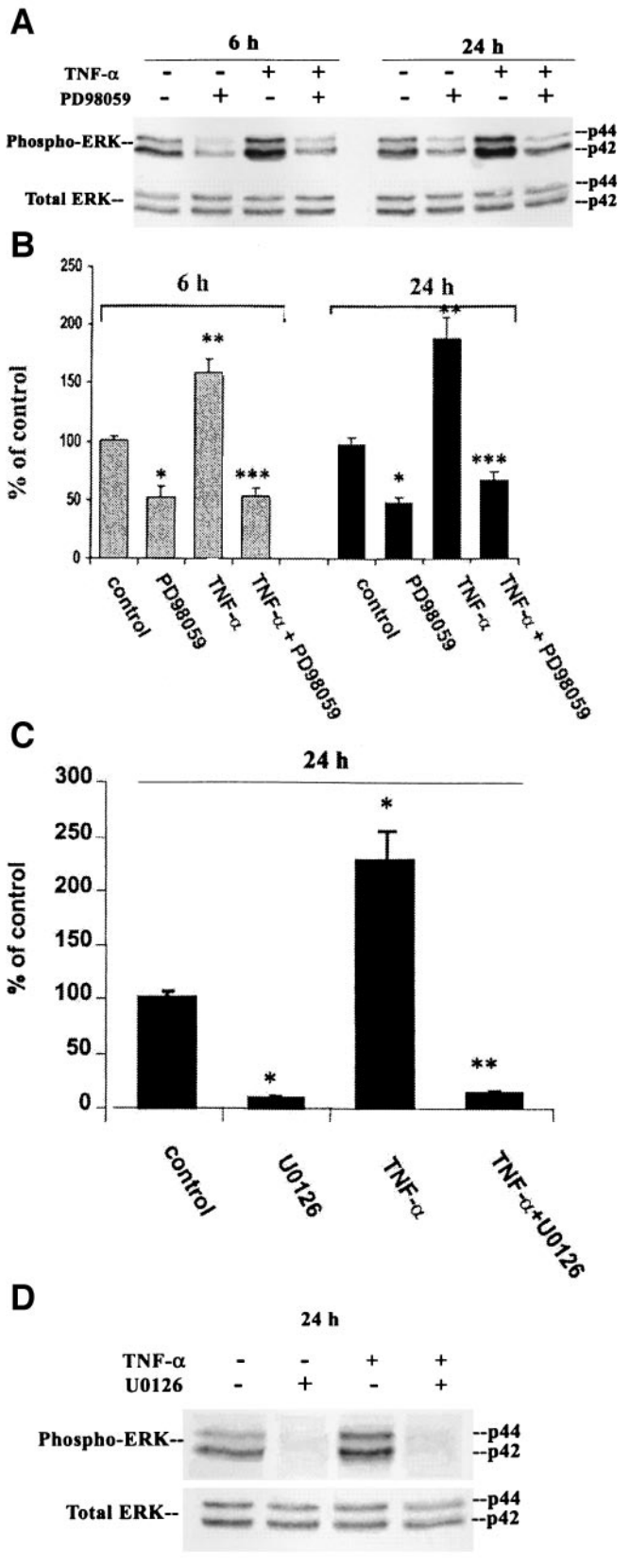

FIG. 3. Effect of MEK inhibitors on TNF-α–induced ERK1/2 activation and lipolysis. Differentiated human adipocytes were incubated with and without TNF-α in the presence or absence of PD98059 or U0126 for 6 and 24 h. Cell extracts were immunoblotted for phospho-ERK and total ERK, and glycerol content in conditioned medium was measured as described in the RESEARCH DESIGN AND METHODS section. *A*: Effect of PD98059 on ERK1/2 activation. *B*: Effect of PD98059 on lipolysis. Results are expressed as percentages of control and are means ± SE of three individual experiments. Significantly different from untreated control: **P* < 0.05, ***P* < 0.001. Significantly different from TNF-α–treated sample: ****P* < 0.001. *C*: Effect of U0126 on lipolysis. Results are means ± SE of three individual experiments. Significantly different from untreated control: **P* < 0.001. Significantly different from TNF-α–treated sample: ***P* < 0.001. *D*: Effect of U0126 on ERK1/2 activation.

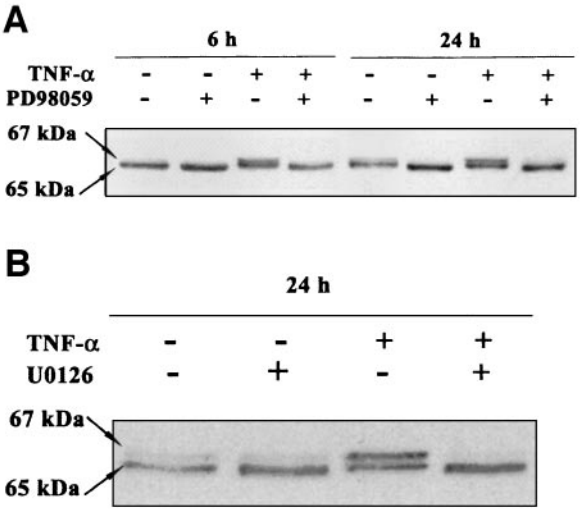

FIG. 4. Effects of MEK inhibitors on the TNF-α–induced electrophoretic shift of perilipin. Differentiated human adipocytes were incubated with or without TNF-α (100 ng/ml) in the presence or absence of PD98059 or U0126 for 24 h. Cell extracts were immunoblotted for perilipin as described in the RESEARCH DESIGN AND METHODS section. Data shown are representative of three experiments. *A*: Effects of PD98059 and TNF-α on perilipin migration. *B*: Effects of U0126 and TNF-α on perilipin migration.

tion. The data are the first demonstration to link TNF-α activation of ERK to stimulation of PKA, resulting in increased adipocyte lipolysis.

The MEK1/2 inhibitors PD98059 and U0126 inhibited TNF-α–induced lipolysis. The reduction in lipolysis paralleled the reduction in ERK1/2 activation. In addition, PD98059 and U0126 reduced basal ERK1/2 activation and basal lipolysis, indicating that MEK1/2-ERK1/2 regulate TNF-α–induced as well as basal lipolysis in these cells. In many cells, activation of ERK triggers its translocation into the nucleus, where it phosphorylates and activates transcription factors (36). In turn, these activated transcription factors regulate the expression of specific genes. For example, the ERK pathway has been shown to mediate phosphorylation of peroxisome proliferator–activated receptor-γ, a major transcription factor in adipocytes, and reduce its transcriptional activity (37). In our study, TNF-α required 6 h to stimulate lipolysis, but the peak of ERK activation occurred within 15–30 min. The temporal discrepancy suggests that the mechanism of ERK1/2 action is more complicated than direct phosphorylation of HSL (19) and that alterations in gene and protein expression may be involved. Concomitant with the increase in lipolysis, TNF-α decreased PDE3B protein expression and increased intracellular cAMP. Both effects were restored by cotreatment with the MEK1/2 inhibitor PD98059, indicating that TNF-α may decrease PDE3B expression and thereby increase cAMP concentrations and lipolysis. We propose that TNF-α increases ERK activation within 15–30 min, which subsequently results in the alteration of gene

and protein expression over the course of hours, leading to decreased PDE3B protein expression, increased cAMP levels, and ultimately increased lipolysis. Our hypothesis is supported by previous observations that in 3T3L1 adipocytes, treatment of 3T3-L1 adipocytes with TNF-α for 24 h reduced PDE3B mRNA and protein expression and concurrently increased lipolysis (28).

TNF-α has been shown to stimulate lipolysis in isolated rat adipocytes by decreasing the expression of G_i protein (31). In our present study, we did not observe a significant effect of TNF-α on expression of immunoreactive G_i (data not shown). The discrepancy between the two studies may be due to the differential effects of TNF-α in rat versus human adipocytes.

Increases in intracellular cAMP and resulting activation of PKA are the major pathway by which lipolytic agents stimulate lipolysis, which occurs at the surface of the intracellular lipid droplets where perilipins are specifically located (21,38). In our study, treatment with TNF-α resulted in an increase in the molecular weight of perilipin in SDS-PAGE, as evidenced by the electrophoretic shift from 65 to 67 kDa. The increase in perilipin migration was concomitant with the increase in intracellular cAMP and lipolysis. The involvement of cAMP in TNF-α–mediated lipolysis was further evidenced by the fact that H89, a selective and potent cAMP-dependent PKA inhibitor, inhibited TNF-α–induced lipolysis and reversed the TNF-α–induced electrophoretic shift of perilipin, causing it to migrate as a 65-kDa band. In view of the fact that hyperphosphorylation of perilipin by PKA facilitates lipase-mediated lipolysis (24), we speculate that in human adipocytes, TNF-α increases intracellular cAMP levels and PKA activation, resulting in hyperphosphorylation of perilipin and ultimately increased lipolysis. Activated PKA is known to simultaneously phosphorylate perilipin A, and the major lipase in adipocytes, HSL (39–43). Whether, the TNF-α–induced increase of intracellular cAMP in our study leads to phosphorylation of HSL awaits further study.

The TNF-α–induced electrophoretic shift of perilipin and stimulation of lipolysis were abrogated by PD98059 and U0126, suggesting that regulation of the two events is downstream of the activation of the ERK1/2-MEK1/2 pathway. In contrast to the finding of Souza and colleagues (6,23), that TNF-α increased lipolysis in 3T3-L1 adipocytes by decreasing perilipin expression, we did not observe significant reduction of perilipin by TNF-α. The difference between the two studies may reflect the differential regulatory effects of TNF-α on perilipin(s) in the two cell types, with downregulation of perilipin expression in 3T3-L1 adipocytes and the hyperphosphorylation of perilipin in human adipocytes. Both effects can result in reduced perilipin coating of the lipid droplet, leading to increased access of lipase(s) to the lipid droplet surface and increased lipolysis.

Based on our findings, we suggest a model for TNF-α–stimulated lipolysis in human adipocytes, which includes the activation of the MEK1/2-ERK1/2 pathway, resulting in downregulation of PDE3B expression with a subsequent increase in intracellular cAMP and activation of PKA, which hyperphosphorylates perilipin, leading to a modification of the lipid droplet surface and allowing lipase(s) to hydrolyze triacylglycerol. The model may represent only one of the pathways by which TNF-α stimulates lipolysis in human adipocytes, but certainly in our cell culture system, this is a major mechanism.

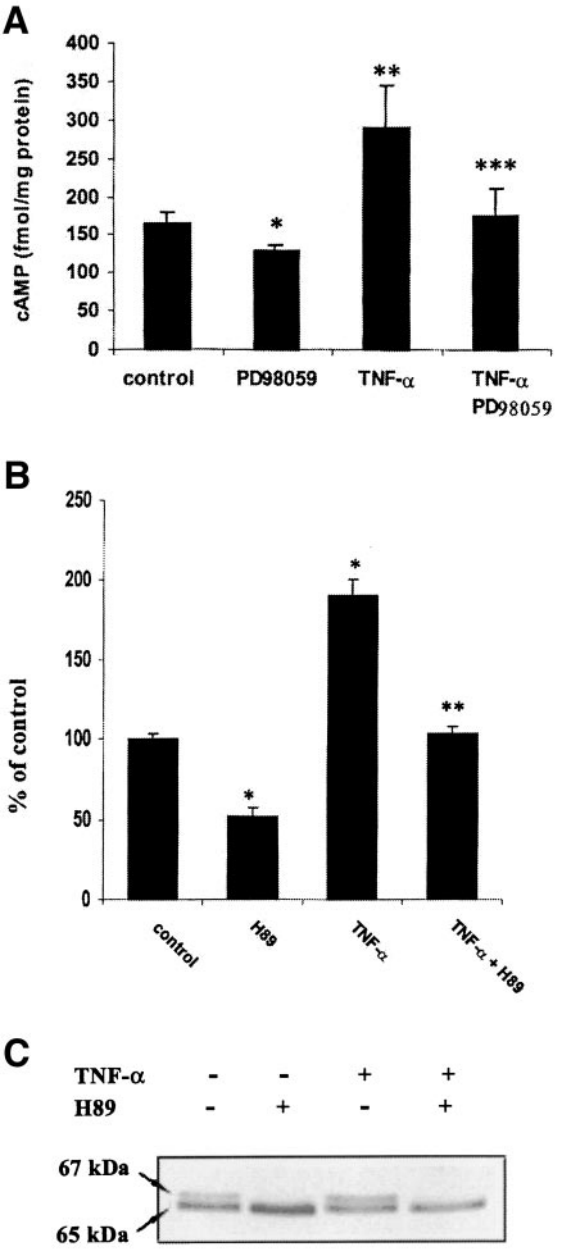

FIG. 5. *A*: Effects of MEK inhibitor and TNF-α on intracellular cAMP concentrations. Differentiated human adipocytes were incubated with or without TNF-α (100 ng/ml) in the presence or absence of PD98059 for 24 h. Intracellular cAMP concentrations were assayed as described in the RESEARCH DESIGN AND METHODS section. Results are the means ± SE of three independent experiments. Significantly different from control: *$P < 0.05$, **$P < 0.001$. Significantly from TNF-α–treated cells: ***$P < 0.01$. *B* and *C*: Effect of PKA inhibitor on TNF-α–induced lipolysis and phosphorylation of perilipin. Differentiated human adipocytes were incubated with or without TNF-α (100 ng/ml) in the absence or presence of H89 (20 μmol/l) for 24 h. Conditioned medium was collected for measurement of lipolysis, and cell extracts were immunoblotted for perilipin. *B*: Effect of H89 on lipolysis. Results are the means ± SE of three individual experiments. Significantly different from control: *$P < 0.001$. Significantly different from TNF-α–treated cells: **$P < 0.001$. *C*: Effect of H89 on TNF-α–induced hyperphosphorylation of perilipin. Data are representative of three experiments.

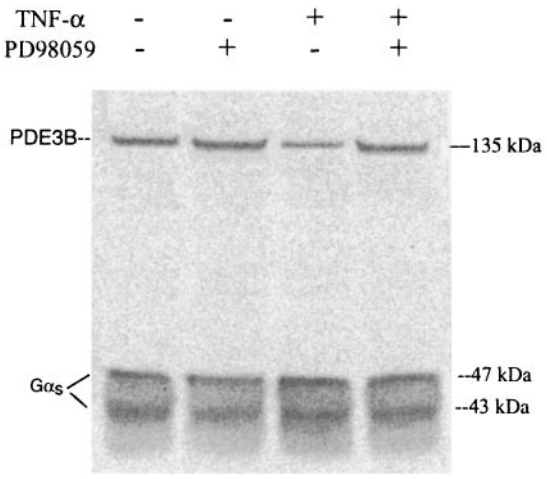

FIG. 6. Effect of TNF-α on PDE3B expression. Differentiated human adipocytes were incubated with or without TNF-α in the presence or absence of PD98059 for 24 h. Isolated plasma membranes were immunoblotted for PDE3B and as a loading control, for $G\alpha_s$. Protein expression was detected by ECL. Data are representative of three independent experiments.

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