Glucocorticoids antagonize tumor necrosis factor-α-stimulated lipolysis and resistance to the antilipolytic effect of insulin in human adipocytes

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Lee MJ, Fried SK. Glucocorticoids antagonize tumor necrosis factor-α-stimulated lipolysis and resistance to the antilipolytic effect of insulin in human adipocytes. Am J Physiol Endocrinol Metab 303: E1126–E1133, 2012. First published September 4, 2012; doi:10.1152/ajpendo.00228.2012.—High concentrations of TNF within obese adipose tissue increase basal lipolysis and antagonize insulin signaling. Adipocytes of the obese are also exposed to elevated levels of glucocorticoids (GCs), which antagonize TNF actions in many cell types. We tested the hypothesis that TNF decreases sensitivity to the antilipolytic effect of insulin and that GCs antagonize this effect in differentiated human adipocytes. Lipolysis and expression levels of lipolytic proteins were measured after treating adipocytes with TNF, dexamethasone (DEX), or DEX + TNF for up to 48 h. TNF not only increased basal lipolysis, it caused resistance to the antilipolytic effects of insulin in human adipocytes. DEX alone did not significantly affect lipolysis. Cotreatment with DEX blocked TNF induction of basal lipolysis and insulin resistance by antagonizing TNF stimulation of PKA-mediated phosphorylation of hormone-sensitive lipase (HSL) at Ser563 and Ser660 and perilipin. TNF did not affect perilipin, HSL, or phosphodiesterase-3B mass but paradoxically suppressed adipose tissue triglyceride lipase expression, and this effect was blocked by DEX. The extent to which GCs can restrain the lipolytic actions of TNF may both diminish the potentially deleterious effects of excess lipolysis and contribute to fat accumulation in obesity.

dexamethasone; obesity; perilipin; adipose triglyceride lipase; hormone-sensitive lipase

Obesity, particularly visceral and upper body subcutaneous obesity, is associated with high rates of fatty acid (FA) turnover in vivo, increasing risk for obesity-related comorbidities including type 2 diabetes, and cardiovascular diseases. Adipocytes from obese individuals exhibit increased basal lipolysis (18) due, at least in part, to elevated levels of inflammatory cytokines, especially tumor necrosis factor-α (TNF) (5). Unlike the acute effect of adrenergic agonists on lipolysis, the lipolytic effects of TNF require at least several hours (33, 45). Studies in newly differentiated human adipocytes indicate that the lipolytic effect of TNF is mediated through an increase in cAMP, with subsequent phosphorylation of perilipin and hormone-sensitive lipase (HSL) (45). TNF is also known to impair insulin signaling (2), thereby decreasing the ability of insulin to stimulate glucose transport in adipocytes (14). Although insulin also has potent antilipolytic effects, the hypothesis that TNF impairs this major insulin action in adipocytes has rarely been studied. Porter et al. (28) found that prior treatment of rat adipocytes with 100 ng/ml TNF overnight does not affect the ability of insulin to inhibit lipolysis. However, only one very high concentration of insulin was tested in their study, so the question of whether TNF affects sensitivity to insulin antilipolysis remains open.

The prereceptor activation of cortisone to cortisol via 11β-hydroxysteroid dehydrogenase (HSD1) is upregulated in obese adipose tissue (20, 31, 37). Thus, adipocytes of obese people are exposed to relatively high levels of cortisol along with high levels of cytokines. Although it is well known that glucocorticoids (GCs) and TNF antagonize each other’s actions in many cell types (1), and that GCs suppress TNF production in human subcutaneous (sc) adipose tissue (6, 15, 27), the interactions of these two factors in the regulation of lipolysis in adipocytes have not been addressed. In the present study, we used newly differentiated human adipocytes in culture to assess how TNF and GCs interact to regulate lipolysis and sensitivity to the antilipolytic effects of insulin. To mimic the physiological situation, differentiated cells were maintained with insulin and then cotreated with varying concentrations of TNF and/or GCs. To elucidate the mechanisms through which GCs and TNF regulate lipolysis, we assessed the expression levels of key proteins involved in this process: perilipin, a lipid droplet protein that regulates both basal and β-adrenergically stimulated lipolysis (40), and adipose tissue triglyceride lipase (ATGL) and HSL, lipases that hydrolyze triglycerides (TG) to diacylglycerol (DAG) and DAG to monoglyceride, respectively (46). We also assessed the expression of cyclic nucleotide phosphodiesterase-3B (PDE3B), which mediates the antilipolytic effect of insulin by hydrolyzing cAMP (29).

METHODS

Subjects. Adipose tissues were obtained during elective abdominal surgeries, mainly abdominoplasties in weight-reduced subjects following bariatric surgeries. By medical history, all were free of diabetes and endocrine or inflammatory diseases. All subjects were weight stable for at least 1 mo prior to surgery. Adipose tissues from six females and one male (mean age 46 ± 4.5 yr and BMI 34 ± 3.4 kg/m2) were used. All subjects gave informed consent as approved by institutional review boards of the University of Maryland at Baltimore and Boston University.

Human adipose tissue handling and isolation of stromal vascular cells (adipose stem cells). Adipose tissues were transferred to the laboratory in room temperature Medium 199. Stromal vascular cells were obtained by collagenase digestion (22). Briefly, minced adipose tissue was subjected to collagenase digestion (type I Worthington; 1 mg/ml in Hank’s balanced salt solution) for 2 h, filtered through a 250-μm mesh, and centrifuged to obtain cell pellets. After lysing of red blood cells with erythrocyte lysis buffer, cells were pelleted and resuspended in growth medium (α-MEM (5.5 mM glucose) supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin). After overnight attachment, cells were grown till 70–80% confluent and frozen down in 10% DMSO supplemented growth medium or subcultured up to five passages.

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Cell culture. Two days after reaching confluence, medium was switched to adipogenic differentiation medium (DMEM-F12 (17.5 mM glucose) with 500 μM IBMX, 100 nM insulin (Humulin), 100 nM dexamethasone (DEX), 1 μM rosiglitazone, 2 nM triiodothyronine (T3), 10 μg/ml transferrin, 33 μM d-biotin, and 17 μM pantothenic acid (22)). After induction with complete differentiation media for 7 days, cells were kept in maintenance medium (DMEM-F12 with 10 nM insulin and 10 nM DEX). Adipocytes were maintained with the combination of insulin and DEX, as we have find that the combination of these two factors best maintains adipocyte gene signatures (21).

Adipose tissue culture. Minced abdominal sc adipose tissues were placed in organ culture in the conditions of control (insulin 0.7 nM), DEX alone (10 nM), TNF (3 ng/ml), or DEX + TNF for 24 h, as previously described (21).

Lipolysis. Glycerol accumulation in culture medium during treatments was measured as an indicator of lipolysis. To provide a more robust assessment of changes in lipolytic capacity after treatments, we also performed acute incubations in Krebs-Ringer bicarbonate buffer (KRB) with 5 mM glucose and 4% BSA (KRB + 4% BSA). Adenosine deaminase (ADA) and phenylisopropyl adenosine (PIA) were used to standardize any potential variations in adenosine levels. Basal lipolytic rates were defined as ADA (1 U/ml) / PIA 20 nM (11) and stimulated lipolysis were measured with isoproterenol (10–6 M) and 8-bromo-cAMP (1 mM). The acute insulin antilipolysis (0, 30, 120, and 600 pM) was measured against 8-bromo-cAMP after 4-h insulin starvation. The ED50 values for antilipolytic actions of insulin in newly differentiated sc human adipocytes were around 10 –30 pM, similar to the isolated adipocytes ex vivo (11). Glycerol concentration in culture media was measured with a standard enzymatic fluorometric assay. For samples containing albumin, glycerol was assayed in neutralized perchloric acid extracts (11).
RNA extraction and gene expression. Total RNA was extracted using TRIzol (Invitrogen). Total RNA (0.5–1 μg) was reverse transcribed using a Transcriptor First Strand cDNA synthesis kit (Roche). qPCR was performed on a LightCycler 480 (Roche) with Taqman probes (Applied Biosystems). 18S rRNA and cyclophilin A (PPIA) were used as reference genes. Relative expression levels were calculated using LightCycler 480 software. Data were similar whether 18S or PPIA was used as a reference gene; relative expression levels compared with PPIA were presented.

Western blotting. After a wash with ice-cold PBS, cells were scraped into cell lysis buffer (Cell Signaling) supplemented with 5% SDS, protease inhibitor cocktail (Pierce), and 50 μM DTT. Cell lysates were incubated in a 37°C water bath for 1 h with vortexing every 5–10 min to extract lipid droplet proteins and then centrifuged at 14,000 g for 10 min to obtain clear lysates. Protein (5–10 μg) was resolved in 10% Tris·HCl gels and transferred to PVDF membranes. After blocking, blots were probed for perilipin (a gift from Dr. A.S. Greenberg, Tufts University), phospho- (Ser563, Ser565, Ser566) and total HSL (Cell signaling), PDE3B (Santa Cruz Biotechnology), ATGL (a gift from Dr. D.W. Gong, University of Maryland), phospho-NF-κB (Ser326), fatty acid-binding protein-4 (FABP4), and loading controls [α-tubulin, heat shock protein-90 (HSP90), and RNA Pol II, Santa Cruz Biotechnology]. Chemiluminescence images were captured using Luminescent Image Analyzer (LAS4000, Fuji) and band densities were quantitated using Multi Gauge Image software.

Statistics. Data are expressed as means ± SE. The effects of different treatments were determined by analysis of variance with repeated measures and post hoc t-tests when main effects or interactions were significant (P < 0.05) (Prism, GraphPad).

RESULTS

DEX antagonized TNF stimulation of lipolysis. Twenty-four-hour treatment of human adipocytes with TNF (1 or 10 ng/ml) increased glycerol accumulation in the culture media as expected (Fig. 1A). DEX alone did not consistently affect basal lipolysis (Fig. 1B). Cotreatment with DEX, however, significantly blocked the TNF-stimulated lipolysis. Although 1 nM DEX was not effective (not shown), higher concentrations of DEX (10 and 1,000 nM) mitigated TNF-induced lipolysis. Time course studies revealed that TNF (3 ng/ml) increased glycerol accumulation in as early as 3 h (Fig. 1C). Cotreatment with DEX (10 nM) blocked the TNF-stimulated lipolysis after 24 and 48 h, but not at 3 h. DEX or TNF treatments for up to 48 h did not affect degree of differentiation, as determined by the lack of change in FABP4 protein levels (Fig. 1D).

To better assess whether TNF and DEX affect the lipolytic capacity of adipocytes, both basal and β-adrenergically stimulated lipolysis were measured during acute 2-h incubation in KRB buffer containing 4% albumin as a fatty acid acceptor. TNF at 1 ng/ml was as effective as 10 ng/ml in increasing basal lipolysis (Fig. 2A), and we chose an intermediate concentration of 3 ng/ml TNF for subsequent studies. Overnight TNF pretreatment increased basal lipolytic rates 2 ± 0.4-fold without affecting the maximally stimulated (isoproterenol, 10−6 M) lipolytic rates (Fig. 2B). Responsiveness to isoproterenol (fold over basal) was lower in TNF-pretreated cells (4.7 ± 1.0-fold control vs. 1.9 ± 0.4-fold TNF treatment, P < 0.05, n = 4). DEX, compared with the control, did not significantly affect lipolysis. However, because DEX tended to lower basal lipolysis while tending to increase stimulated rates, the fold stimulation by isoproterenol was higher after DEX treatment (4.7 ± 1.0-fold control vs. 7.5 ± 2.4-fold DEX treatment, P < 0.05, n = 4). Cotreatment with DEX completely blocked the TNF

![Fig. 2. Dex antagonized TNF stimulation of basal lipolytic rates. A: after overnight treatment with TNF (1 or 10 ng/ml), lipolytic rates were measured in KRB + 4% BSA in basal and isoproterenol (Iso, 10−6 M)-stimulated conditions, n = 2. B: after overnight pretreatments with media alone (control), Dex (D, 10 nM), TNF (3 ng/ml), or TNF + D, acute lipolysis was measured in KRB + 4% BSA in basal and Iso (10−6 M)-stimulated conditions. TNF effects: *P < 0.05; DEX effects: #P < 0.05, n = 4.](http://ajpendo.physiology.org/)

To verify that DEX antagonizes the lipolytic effect of TNF in another system, we used human adipose tissue organ culture. TNF stimulation about threefold, and DEX suppressed its prolipolytic effect (Fig. 1E). In addition, DEX tended to suppress basal lipolysis in human adipose tissue (P = 0.07, n = 3).

TNF pretreatment compromised the ability of insulin to suppress lipolysis, both sensitivity and responsiveness to insulin; DEX mitigated this effect. We tested whether TNF would impair the antilipolytic actions of insulin in human adipocytes. In the control condition, insulin acutely suppressed lipolysis with an ED50 of 12 ± 2 pM (Fig. 3A). Pretreatment with TNF (3 ng/ml overnight) caused a rightward shift in the dose-response curve, increasing the ED50 to 36 ± 8 pM (P < 0.05, n = 4). Responsiveness to insulin, calculated as percent suppression, was lower in TNF-pretreated cells (Fig. 3B). Thus, TNF compromised both sensitivity and responsiveness to insulin antilipolysis in cultured human adipocytes.

Pretreatment with DEX alone did not significantly affect sensitivity and responsiveness to insulin’s antilipolytic effect compared with the control. However, cotreatment with DEX + TNF blocked TNF-induced impairment in both responsiveness and sensitivity to insulin antilipolysis, decreasing the ED50 from 36 ± 8 to 20 ± 2 pM (P < 0.05, n = 4).
DEX blocked TNF stimulation of perilipin and HSL phosphorylation and TNF suppression of ATGL expression. To understand the mechanisms through which TNF and DEX interact to regulate lipolysis, we analyzed expression levels of key proteins in the lipolytic cascade (PDE3B, HSL, perilipin, and ATGL) after overnight treatments. Although TNF increased basal lipolysis, it did not affect protein mass of HSL, perilipin, and PDE3B. However, TNF increased HSL phosphorylation at protein kinase A (PKA) sites (Ser563 and Ser660) without affecting Ser 565 phosphorylation (AMP-dependent protein kinase (AMPK) site (38); Fig. 4). TNF also increased perilipin phosphorylation, as judged by the appearance of higher-molecular-weight bands (45). As expected, TNF increased phosphorylation of NF-κB (Ser536). DEX, however, did not affect either basal or TNF-stimulated NF-κB phosphorylation. Paradoxically, TNF decreased ATGL protein levels by 32% whereas DEX alone increased ATGL protein mass by 37%. DEX alone did not alter HSL, perilipin, or PDE3B protein mass. DEX, however, completely blocked the TNF-stimulated phosphorylation of HSL and perilipin and the TNF-suppression of ATGL protein levels.

Time course studies showed that after 24 and 48-h treatment TNF increased phosphorylation of HSL (Ser563 and Ser660) and perilipin without any significant effects at 3 h (Fig. 5). Similarly, TNF suppressed ATGL protein expression after 24 h without significantly affecting the total mass of HSL, perilipin, or PDE3B for up to 48 h. DEX antagonized TNF stimulation of perilipin and HSL phosphorylation and its suppression of ATGL protein. DEX increased ATGL protein levels after 24 h. However, DEX did not alter protein levels of PDE3B, HSL, and perilipin for up to 48 h (representative blots are shown in Fig. 5, n = 4).

To determine whether DEX and TNF affected ATGL, perilipin, and HSL gene expression, mRNA levels were measured (Fig. 6). There were no effects after 3 h, but after 24 h DEX increased and TNF decreased ATGL mRNA levels, consistent with their effects on protein levels. Cotreatment with DEX partially mitigated the TNF suppression of ATGL mRNA. TNF decreased perilipin mRNA expression after 24 and 48 h, and this was partially antagonized by DEX at 48 h. DEX and TNF did not significantly affect HSL mRNA expression levels for up to 48 h.

DISCUSSION

In obesity, adipocytes are subjected to conflicting signals. Both TNF, a proinflammatory and prolipolytic signal, and GCs, an anti-inflammatory and prolipogenic signal, are simultaneously elevated in obese adipose tissue and our results imply that the balance of GCs and TNF determines lipolytic rates. Our study is the first to demonstrate that, in addition to stimulating basal lipolysis, TNF causes resistance to the antilipolytic effect of insulin in human adipocytes. Moreover, coinubcation with DEX, a type II GC receptor agonist, blocks these TNF actions on lipolysis, at least in part, via changes that result in altered PKA signaling. Thus, locally, high GCs in obese adipose tissue not only promote the uptake and storage of FA by increasing lipoprotein lipase (10) but may also promote fat deposition by limiting TNF-induced lipolysis. Interactions of cortisol and TNF may be especially important in...
visceral compared with subcutaneous adipose tissue, because it expresses higher levels of proinflammatory cytokines (4, 9) and is differentially responsive to some GCs’ actions (10, 34).

Our data with regard to the mechanism by which TNF increases spontaneous (basal) lipolysis are consistent with those of Zhang et al. (45), who showed that 24-h treatment with TNF stimulates lipolysis in human adipocytes through changes that result in activation of the PKA signaling pathway. Notably, this mechanism differs from that in 3T3-L1 adipocytes, where TNF stimulates lipolysis by downregulating perilipin expression at the mRNA level and in parallel with protein levels (39). It remains unclear how TNF leads to an increase in cAMP and thus PKA activity over time. Unlike Zhang et al. (45), we did not find any significant effects of TNF on PDE3B protein levels nor total intracellular cAMP levels up to 48 h (not shown), suggesting that other mechanisms are involved. TNF is also known to affect expression of many components in the lipolytic cascade, including β-adrenergic receptors, GTP-binding protein G_i, and fat-specific protein-27 (12, 13, 30). In contrast to the 24- and 48-h time points, TNF increased lipolysis at 3 h without any detectable alterations in HSL and perilipin phosphorylation. TNF-induced phosphorylation of MAPKs and NF-κB at 3 h is one possible mechanism (19, 33), and indeed we observed this as expected (M.J. Lee, unpublished observation).

We and others previously observed that DEX or cortisol suppresses basal lipolysis in intact adipose tissue fragments in culture (24, 41), consistent with the current results. Here, we show that DEX also inhibits the stimulatory effect of TNF on basal lipolysis at the level of the adipocyte (primary culture). The suppressive effect of DEX on basal lipolysis in tissue fragments may be in part related to a decrease in endogenous TNF. However, in contrast to tissue fragments, in newly differentiated human adipocytes we found very low, barely detectable expression levels of TNF mRNA (unpublished observation). We therefore conclude that GCs restrain lipolysis in the setting of human adipose tissue, at least in part through a direct action on the adipocytes, and not solely via suppression of proinflammatory cytokine production in nonadipose cells present within the tissue.

DEX antagonized TNF-stimulated lipolysis by suppressing TNF-induced PKA activation and phosphorylation of HSL and perilipin without affecting their protein levels. In both basal and TNF-stimulated conditions, the DEX suppression of lipolysis did not involve alterations in ATGL protein, which was paradoxically increased by DEX. Notably, the reintroduction of DEX after a starvation period did not have a general effect on adipocyte differentiation, as judged by protein levels of FABP4 or gene expression of PPARγ (not shown). With regard to signaling mechanisms, DEX did not mitigate TNF stimulation of NF-κB; thus, we do not believe this mechanism is involved in DEX antagonism of the prolipolytic actions of TNF. The mechanism through which DEX antagonizes the TNF stimulation of the PKA pathway needs further investigation.

Our in vitro findings address the controversial question of whether GCs increase or suppress “basal” lipolysis and potential relevance to in vivo circumstances (26). Although infusion of fairly high concentrations of GCs increases systemic free fatty acids and glycerol within several hours in overnight-fasted humans (7, 8, 35, 36), cortisol infusion actually decreases adipose tissue lipolysis as measured by
venous-arterial differences for FAs across abdominal subcutaneous adipose tissue (35). Results from long-term (days to weeks) infusion of GCs (3, 42–44) are complicated by compensatory hyperinsulinemia, which may chronically promote higher FA flux via induction of insulin resistance and activation of the sympathetic nervous system, and the well-known ability of GCs to increase expression of β-adrenergic receptors and responsiveness to β-adrenergic agonists (16, 17, 23, 32). Consistent with this possibility, we found that DEX increased the magnitude (fold stimulation) of the β-adrenergic activation of lipolysis.

In summary, our results suggest that the antilipolytic and anti-inflammatory actions of GCs balance the prolipolytic and proinflammatory actions of TNF through direct actions on adipocytes. In addition, GCs are known to suppress inflammatory cytokine expression in other cells within adipose tissue (6, 15, 27) and a recent study demonstrated that GCs inhibit macrophage infiltration into mouse adipose tissue (25). GCs also increase lipogenesis and lipid storage (21, 44). Taken together, these data lead us to conclude that GCs act at multiple levels to mitigate the potentially deleterious metabolic effects of excess adipose tissue inflammation,
contributing to increased fat accumulation under pathophysiologically hypercortisolemic conditions as observed in Cushing’s syndrome.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.-J.L. and S.K.F. conception and design of research; M.-J.L. performed experiments; M.-J.L. analyzed data; M.-J.L. and S.K.F. interpreted results of experiments; M.-J.L. prepared figures; M.-J.L. and S.K.F. drafted manuscript; M.-J.L. and S.K.F. edited and revised manuscript; M.-J.L. and S.K.F. approved final version of manuscript.

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