TWEAK prevents TNF-α-induced insulin resistance through PP2A activation in human adipocytes

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Vázquez-Carballo A, Ceperuelo-Mallafré V, Chacón MR, Maymó-Masip E, Lorenzo M, Porras A, Vendrell J, Fernández-Veledo S. TWEAK prevents TNF-α-induced insulin resistance through PP2A activation in human adipocytes. Am J Physiol Endocrinol Metab 305: E101–E112, 2013. First published May 7, 2013; doi:10.1152/ajpendo.00589.2012.—Visceral fat is strongly associated with insulin resistance. Obesity-associated adipose tissue inflammation and inflammatory cytokine production are considered key mediators of insulin signaling inhibition. TWEAK is a relatively new member of the TNF cytokine superfamily, which can exist as full length membrane-associated (mTWEAK) and soluble (sTWEAK) isoforms. Although TWEAK has been shown to have important functions in chronic inflammatory diseases its physiological role in adipose tissue remains unresolved. In this study, we explore the molecular mechanisms involved in the modulation of TNF-α-induced effects on insulin sensitivity by sTWEAK in a human visceral adipose cell line and also in primary human adipocytes obtained from visceral fat depots. Our data reveal that sTWEAK ameliorates TNF-α-induced insulin resistance on glucose uptake, GLUT4 translocation and insulin signaling without affecting other metabolic effects of TNF-α such as lipolysis or apoptosis. Co-immunoprecipitation experiments in adipose cells revealed that pretreatment with sTWEAK specifically inhibits TRAF2 association with TNFR1, but not with TNFR2, which mediates insulin resistance. However, sTWEAK does not affect other downstream molecules activated by TNF-α, such as TAK1. Rather, sTWEAK abolishes the stimulatory effect of TNF-α on JNK1/2, which is directly involved in the development of insulin resistance. This is associated with an increase in PP2A binding to sTWEAK and silencing of the PP2A catalytic subunit gene overcomes the dephosphorylation effect of sTWEAK on JNK. The dephosphorylation of the PP2A catalytic subunit gene overcomes the dephosphorylation effect of sTWEAK on JNK. Overall, our data reveal a protective role of TWEAK in glucose homeostasis and identify PP2A as a new driver in the modulation of TNF-α signaling by sTWEAK.

insulin signaling; glucose uptake; TNF; obesity; phosphatases; adipose tissue
fasting glucose and soluble circulating sTWEAK levels, as well as a decrease of sTWEAK in patients with T2D (24). Thus, the effects of TWEAK on insulin sensitivity in human adipose tissue are far from clear.

Insulin signaling is coordinately regulated through a balance between both protein kinases and phosphatases. It is known that some Ser/Thr phosphatases (PPs) participate in cytokine signaling pathways, mainly as negative regulators. In particular, PP2A is a Ser/Thr phosphatase with broad substrate specificity and diverse cellular functions. Here, we have investigated the role and molecular mechanisms by which TWEAK regulates insulin sensitivity in human adipocytes of visceral origin. We demonstrate that TWEAK specifically reverses TNF-α-induced insulin resistance on glucose uptake through the activation of PP2A. Our data reveal, for the first time, that PP2A may be a signaling component of the JNK1/2 cascade involved in relaying the TNF-α signal to promote insulin resistance. Thus, modulation of TWEAK/PP14 axis could be a bona fide pharmacological approach to treat insulin resistance.

MATERIALS AND METHODS

Materials. Insulin, BSA, crystal violet, free glycerol determination kit, cycloheximide, and anti-Lamin and anti-β-actin antibodies were from Sigma-Aldrich (St. Louis, MO). Cell Death Detection ELISA kit was from Roche (Foster City, CA). Soluble human recombinant TWEAK (sTWEAK) and TNF-α were from PeproTech (Rocky Hills, NY). Culture media and sera were from Invitrogen (Paisley, UK). Autoradiographic films were from GE Healthcare (Rainham, UK). 2-Deoxy-D-[1-3H]glucose (11.0 Ci/mmol) was from PerkinElmer. Antibodies against phosphorylated (p)-IRS-1 (Ty632), total IRS-1, GLUT1, and GLUT4 were from Millipore (Bedford, MA). Antibodies against p-ERK1/2 (Thr202/Tyr204), p-Akt (Ser473), total Akt, p-AMPK (Thr172), p-c-Jun NH2-terminal kinase (JNK1/2) (Thr89/Thr183), and p-p38 MAPK (Thr180/Tyr182), and FN14 were from Cell Signaling (Beverly, MA). Antibodies against IkBα (sc-371), IkBβ (sc-945), TRAF2 (sc-876), cavin-1 (sc-894), NF-kB p52 (sc-298), GLUT3 (sc-74399), and TNF-R1 (sc-73195) were from Santa Cruz (Palo Alto, CA). Antibodies against p-TAK1 (Thr182) and TAK1 were from Abcam (Cambridge, UK). NE-PE nuclear and cytoplasmic extraction reagents were from Thermo Scientific (Rockford, IL). A monoclonal antibody to human TNF-R1 (p75/p80) was bought from Hycult Biotech (Plymouth Meeting, PA). An anti-human TNFR1/TNFRSF1A antibody was purchased from R&D Systems (Abingdon, UK).

Cell culture and lysis. L6/Sa-2 cells, kindly provided by Dr. Möller (University of Ulm, Germany), were used as a cellular model of viscer al human adipocytes. This is a stable cell line derived from a poorly differentiated liposarcoma with a pronounced capacity for terminal adipose differentiation (11, 44). Moreover, comparative expression analysis with isolated human adipocytes revealed this cell line to be a robust model for differentiated human adipocytes (41). L6/Sa-2 cells were maintained at 37°C and 5% CO2 in DMEM-F12 (1:1) supplemented with 10% (vol/vol) fetal serum and antibiotics and were induced to differentiate over 10 days in serum-free DMEM-F12 (1:1) supplemented with 10 µg/ml transferrin, 15 mM NaHCO3, 15 mM HEPES, 33 nM biotin, 17 µM pantothenate, 1 nM insulin, 20 µM triiodothyronine, 1 µM cortisol, and antibiotics (11). As previously reported, differentiated human adipose cells show an insulin-sensitive phenotype in terms of both glucose and lipid metabolism (11, 13).

Human visceral primary adipocytes were obtained after differentiation of human preadipocytes isolated from visceral fat depots (Lonzna Iberica, Barcelona, Spain) for 12 days, as recommended by the supplier. Cells were cultured overnight in serum-free, low-glucose (1,000 mg/l) DMEM supplemented with 1% (wt/vol) BSA before initiation of different treatments. sTWEAK-primecl cells were obtained after incubation with sTWEAK (100 ng/ml) for 6 h prior to TNF-α treatment. Cells were lysed in a buffered solution containing 10 mM Tris HCl, pH 7.5, 50 mM NaCl, 100 µM sodium orthovanadate, 50 mM NaF, 30 mM sodium pyrophosphate, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1% (vol/vol) Triton X-100, 10 µg/ml aprotinin, and 10 µg/ml leupeptin. Cell lysates were centrifuged (13,000 rpm, 10 min, 4°C), and the supernatant fractions were stored at −80°C. Protein concentration was determined by the Bradford method using a kit supplied by Bio-Rad Laboratories.

Cell death quantification. Cell viability was determined by crystal violet assay as previously described (15). Apoptosis was measured by determining the rate of cytoplasmic histone-associated DNA fragments (mono- and oligonucleosomes). Cell death was assessed by a photometric enzyme immunoassay, using a Cell Death Detection ELISA kit as described by the manufacturer (Roche).

Measurement of lipolytic rate. The rate of lipolysis in adipocytes was determined through quantification of glycerol release into the medium with an enzymatic method using a free-glycerol determination kit (Sigma-Aldrich) as previously described (11). Results are expressed as the percentage of stimulation over basal (control = 100%). Glucose transport and GLUT4 translocation assays. After treatment, cells were washed with KRP buffer (1.25 mM HEPES, pH 7.4, 120 mM NaCl, 6 mM KCl, 1.2 mM MgSO4, 1 mM CaCl2, 0.4 mM NaH2PO4, and 0.6 mM Na2HPO4) and stimulated with insulin in KRP buffer for 20 min. Then, 2-deoxy-D-[1-3H]glucose (0.25 µCi/ml in 100 µM of unlabeled 2-deoxyglucose) was added, and cells were incubated for 10 min. Next, cells were washed in ice-cold KRP buffer three times and solubilized in 0.05 N NaOH. Samples were removed for protein determination and radioactivity measurements as described (11). Glucose uptake rates were calculated as picomoles of glucose taken up per 10 minutes per milligram of protein, and results were expressed as the percentage of stimulation over basal (control = 100%). Cells were fractionated to obtain the plasma membrane fraction, followed by SDS-PAGE separation and immunoblotting with GLUT4, GLUT3, GLUT1, and caveolin-1 antibodies, as previously described (15).

Immunoprecipitation assays. For immunoprecipitation experiments, cells lysates containing equal amounts of proteins (200–400 µg) were immunoprecipitated with anti-TRAF2, anti-TNFRI, or anti-TNFRII antibodies overnight at 4°C. The immune complexes were washed by incubation with protein A agarose beads or protein G-agarose beads (Sigma-Aldrich) for 4 h at 4°C. The immunoprecipitates were separated by SDS-PAGE. Subsequent immunoblot assays were performed using antibodies to Fn14 or TRAF2.

Immunoblot analysis. Equal amounts of protein were submitted to SDS-PAGE and then transferred to Immobilon membranes and blocked (13). Immunoreactive bands were visualized using the enhanced chemiluminescence (ECL-Plus) Western blot protocol (GE Healthcare).

Extraction of nuclear proteins. Nuclear extracts from differentiated adipocytes treated with sTWEAK were prepared using the Nuclear and Cytoplasmatic Kit following the manufacturer’s specifications (Thermo Scientific, 78835). Lamin A and GAPDH were used as nuclear and cytosolic markers, respectively.

Transient transfection with small interfering RNA. Human PP2A catalytic subunit (PP2A-Co) and control (scrambled) small interfering (si)RNAs were purchased from Dharmacon (Lafayette, CO). Human differentiated adipocytes were transfected with 100 nM of siRNA using Dharmafect 1 reagent as previously described (13). At 48 h posttransfection, adipocytes were treated with TNF-α and/or sTWEAK as shown in respective figures. Cell lysates were collected, and insulin sensitivity on glucose uptake and MAPK phosphorylation status were measured. PP2A-Co protein expression and PP2A phosphatase activity were analyzed to evaluate the effectiveness of siRNA knockdown.

PP2A phosphatase activity. PP2A activity was determined in human adipocytes using a Ser/Thr phosphatase (PPase) assay kit (Promega V22460). Cell lysates were obtained with a phosphatase lysis buffer (20 mM HEPES, pH 7.4, 10% (vol/vol) glycerol, 0.1% (vol/vol) NP-40, 30 mM β-mercaptoethanol, 1 mM EGTA), and activity...
was measured following the manufacturer’s protocol using a PP2A-specific reaction buffer (50 mM imidazole, pH 7.2, 0.2 mM EGTA, 0.03% β-mercaptoethanol, 0.1 mg/ml BSA). Free phosphate generated from a phospho-peptide was quantified by measuring the absorbance (600 nm) of a molybdate-malachite green-phosphate complex.

Data analysis. Results are presented as means ± SE from two to five independent experiments performed in duplicates-quadruplicates (technical replicates), as indicated in the corresponding figure legends. Statistical significance was tested with the unpaired Student’s t-test followed by the protected least significant different test. *P < 0.01 was considered significant.

RESULTS

sTWEAK exhibits beneficial properties in human visceral adipose cells. It has been postulated that TNF-α may act as an important auto/paracrine regulator to limit adipose tissue expansion at the expense of insulin resistance development (20). We explored the potential modulation of TNF-α-mediated responses by sTWEAK during lipolysis, apoptosis, and insulin-dependent glucose uptake in a human visceral adipose cell line with a high capacity to differentiate and an efficient insulin-sensitive phenotype (11, 13). As expected, TNF-α exposure significantly decreased the viability of LiSa-2 cells in the presence of cycloheximide (Fig. 1A). Similarly, sTWEAK induced cell death in differentiated LiSa-2 cells only when protein synthesis was inhibited, but to a lesser extent than TNF-α (Fig. 1A). However, sTWEAK pretreatment did not sensitize cells to the death induced by TNF-α, as described in other cellular models (43). Quantification of nucleosomal release indicated that the type of cell death observed in LiSa-2 cells was apoptosis (Fig. 1B), supporting the previous results of

Fig. 1. sTWEAK [soluble tumor necrosis factor (TNF)-like weak inducer of apoptosis] pretreatment prevents TNF-α-induced insulin resistance on glucose uptake in the human visceral adipocyte cell line LiSa-2 without altering its effects on lipolysis and apoptosis. A: differentiated adipose cells were cultured for 24 h in the absence or presence of 10 mg/ml cycloheximide (CHX), 20 ng/ml TNF-α, and/or 100 ng/ml sTWEAK. sTWEAK-primed cells were obtained after sTWEAK incubation for 6 h prior to TNF-α treatment. Cell viability was measured by crystal violet staining. Results represent means ± SE of 4 independent experiments performed in quadruplicates and are expressed as percent stained cells over basal (control 100). B: adipocytes were cultured as in A and the rate of nucleosomes was measured. Results are means ± SE of 3 independent experiments performed in triplicates and are expressed as percent stimulation over basal (control 100). C: differentiated adipose cells were cultured for 24 h in the absence or presence of 20 ng/ml TNF-α and/or 100 ng/ml sTWEAK. Lipolysis was determined by measuring glycerol release. Results are expressed as percent stimulation over basal from 3 independent experiments performed in triplicates (control = 10.69 ± 0.75 nmol glycerol/mg protein/3 h). D: adipocytes were cultured as in C before stimulation with 50 nM insulin (Ins) for 30 min, and glucose uptake was measured during the last 10 min by incorporation of 2-deoxyglucose into the cells. Results from 5 independent experiments performed in triplicates are expressed as percent stimulation over basal from 3 independent experiments performed in triplicates (control = 7.26 ± 0.53 pmol glucose/mg protein/10 min). E: adipocytes were cultured as in A before stimulation with 50 nM insulin for 20 min and submitted to subcellular fractionation. Plasma membrane proteins were analyzed by Western blot using anti-GLUT1, -GLUT3, -GLUT4, and -caveolin-1 (Cav-1) antibodies. Representative immunoblots and densitometric analysis of 3 independent experiments are shown. *P < 0.01.

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and glucose uptake but also activates JNK1/2, inducing phosphorylation of IRS-1 at the Ser^112 residue. This, in turn, leads to an impairment of insulin-induced glucose uptake (13). Localization of GLUT1, GLUT3, and GLUT4 protein at the plasma membrane was examined by subcellular fractionation. As shown in Fig. 1E, addition of insulin, TNF-α, or TWEAK failed to promote GLUT1 or GLUT3 protein association with plasma membrane. In contrast, TNF-α induced the translocation of GLUT4 to the plasma membrane, which correlates with the increase in basal glucose uptake (Fig. 1D) but impaired insulin-induced GLUT4 translocation, as we previously described (13). Consistent with the improved glucose uptake effect (Fig. 1D) sTWEAK pretreatment restored insulin-stimulated GLUT4 translocation to the plasma membrane in the presence of TNF-α (Fig. 1E). It should be noted that the total cellular levels of GLUT4 were not modified by either TNF-α (13) or sTWEAK treatment (data not shown). Similar to other stress conditions related to insulin resistance (11, 18, 38), TNF-α increases basal glucose uptake but induces detrimental effects on insulin sensitivity by impairing the insulin signaling cascade at the level of IRS-1/Akt phosphorylation through a mechanism dependent on JNK1/2 activation (13). In this respect, we observed that pretreatment of human adipose cells with sTWEAK enhanced insulin-induced activation of both IRS-1 and Akt (Fig. 2). More importantly, insulin-stimulated phosphorylation of the Akt substrate AS160, which controls GLUT4 intracellular retention and release to the cell surface (26), was impaired in cells treated with TNF-α but restored in sTWEAK-primed adipocytes (Fig. 2). The individual metabolic effects of both TNF-α and sTWEAK in differentiated adipose cells correlate with distinguishing intracellular signaling pathways (Fig. 3). TNF-α but not sTWEAK induced IkBα and β degradation, established hallmarks of classical NF-κB pathway activation (Fig. 3A). Proteolytic processing of the NF-κB2 (p100) subunit into p52, which translocates to the nucleus, was also analyzed as a central step for noncanonical NF-κB signaling (37). As shown in Fig. 3B, sTWEAK treatment of LiSa-2 adipocytes induced a significant increase of p52 levels in nuclear fractions. It is known that TWEAK can activate both canonical and noncanonical NF-κB pathways depending on the cellular context (3, 35) and the TWEAK variant (33). Our results reveal that in human visceral adipose cells sTWEAK might exclusively activate a noncanonical NF-κB pathway. In addition, TNF-α but not sTWEAK induced JNK1/2 activation, which is directly involved in serine phosphorylation of IRS-1 and the insulin-resistant state developed by TNF-α, as was previously demonstrated (13).

Overall, these results demonstrate that sTWEAK may provide beneficial effects in human visceral adipose cells, in the setting of obesity, as it restores insulin sensitivity on glucose uptake without affecting the metabolic effects of TNF-α that are directed to limit fat mass, such as lipolysis and apoptosis. sTWEAK pretreatment inhibits TRAF2 association with TNFR1 that signal to insulin resistance. TWEAK triggers multiple cellular responses through the Fn14-TRAF2 axis (35). In our cellular model, coimmunoprecipitation experiments performed with anti-TRAF2 or anti-Fn14 antibodies, followed by immunoblotting with the corresponding antibodies against Fn14 or TRAF2, respectively, showed that both endogenous TRAF2 and Fn14 proteins form a complex under basal conditions that is partly disrupted after TNF-α treatment (Fig. 4A).
In contrast, treatment of LiSa-2 adipocytes with sTWEAK increased significantly (nearly 20%) the amount of Fn14 associated with TRAF2. Interestingly, sTWEAK pretreatment prevented the partial disruption of the complex induced by TNF-α, suggesting that Fn14 may compete with TNFRs for binding to endogenous TRAF2. It should be noted that TNF-α, but not sTWEAK, downregulates TRAF2 protein levels, in line with previous reports (19, 46). Remarkably, the Fn14-TRAF2 complex found in nonstimulated human cells would be in agreement with a TRAF2 constitutively active state under basal conditions, which has been described in noncanonical NF-κB signaling (33).

Under basal conditions, sTWEAK did not modulate TRAF2 association with either TNFR1 or TNFR2. However, sTWEAK pretreatment hampered complex formation between TRAF2 and TNFR1 after TNF-α treatment. Overall, our data indicate that in human differentiated adipose cells both Fn14 and TNFR1 compete to bind to endogenous TRAF2, as reported in other cellular models (35). Although several studies have described that the majority of the effects of TNF-α are mediated by TNFR1, the relative contribution of each TNFR to the role of TNF-α in adipocyte physiology in the context of insulin sensitivity is far from clear. In murine adipocytes, it has been suggested that TNF-α exerts its inhibitory effect on insulin signaling through stimulation of TNFR1 (32), whereas studies in human subcutaneous adipocytes have proposed the participation of both TNFR1 and TNFR2 (21). Since sTWEAK pretreatment inhibits TNF-α-induced association of TRAF2 with TNFR1 (Fig. 4A), we explored insulin sensitivity on glucose uptake in the presence of anti-human TNFR1 and TNFR2 agonist antibodies (23, 32). As shown in Fig. 4B, activation of TNFR1, but not TNFR2, increased basal glucose uptake and induced an insulin-resistant state similar to that induced by TNF-α treatment (13). Our previous studies demonstrated that TNF-α inhibited insulin-induced glucose uptake through a mechanism dependent on JNK1/2 activation (13). Accordingly, p-JNK1/2 was detected only in human adipose cells incubated with the TNFR1 agonist (Fig. 4C). A similar behavior was observed for p38 MAPK activation, whereas ERK1/2 activation, as well as IkBα degradation, was induced by both TNFR1 and TNFR2 agonist treatments (Fig. 4C).
Activation of IRS-1 and Akt was also analyzed to confirm TNFR1-induced insulin resistance. Similar to TNF-α treatment, an anti-human TNFR1 agonist antibody inhibited both insulin-induced IRS-1 tyrosine and Akt serine phosphorylation (Fig. 4D). These data demonstrate for the first time that, in differentiated human adipose cells from a visceral origin, the specific activation of TNFR1 inhibits insulin sensitivity in terms of glucose uptake. The fact that Fn14 competes with adipocytes treated with 20 ng/ml TNF-α and 2 μg/ml TNFR1 or TNFR2 agonist for up to 60 min were analyzed with corresponding antibodies against p-JNK1/2 (Thr183/Tyr185), p-p38 MAPK (Thr180/Tyr182), p-ERK1/2 (Thr202/Tyr204), and p-IκB-α and -β; β-actin was used as a loading control. D: lysates from differentiated LiSa-2 cells cultured for 24 h in the absence or presence of TNF-α or 2 μg/ml TNFR1 agonist before stimulation with 50 nM insulin for 10 min were analyzed by Western blot using antibodies against phosphorylated and total IRS-1 (Tyr612) and Akt (Ser473). A representative experiment of 3 is shown. *P < 0.01.

Fig. 4. TNF-α induces insulin resistance through TNF receptor (TNFR)1, which competes with fibroblast growth factor-inducible 14 (Fn14) for binding to TRAF2. A: total cell lysates from differentiated adipose cells cultured for 24 h in the absence or presence of 20 ng/ml TNF-α, 100 ng/ml sTWEAK, or sTWEAK pretreatment (6 h) were immunoprecipitated with anti-TRAF2, -Fn14, -TNFR1, or -TNFR2 antibodies, and resulting immune complexes were analyzed by Western blot using antibodies against Fn14 and TRAF2. A representative experiment of 3 and densitometric analysis of phosphorylated vs. total protein levels are shown. *P < 0.01. B: differentiated LiSa-2 cells were incubated with specific TNFR1 and TNFR2 agonists (2 μg/ml) and 20 ng/ml TNF-α. After 24 h of treatment, cells were incubated with 50 nM insulin for 30 min, and glucose uptake was measured during the last 10 min by quantification of 2-deoxyglucose incorporation into the cells. Results from 3 independent experiments, each in triplicate, are expressed as percent stimulation over basal (control treatment, cells were incubated with 50 nM insulin for 30 min, and glucose uptake was measured during the last 10 min by quantification of 2-deoxyglucose incorporation into the cells). Thus, we next analyzed intracellular PP2A activity context. Thus, we next analyzed intracellular PP2A activity.
of LiSa-2 adipocytes in response to sTWEAK and TNF-α treatment. As shown in Fig. 6A, sTWEAK but not TNF-α significantly increased PP2A activity after 6 h of treatment. Indeed, PP2A activity decreased upon TNF-α treatment for 24 h (data not shown). Moreover, phosphatase activity remained significantly raised in sTWEAK-primed adipocytes even in the presence of TNF-α (Fig. 6B). To determine whether this PP2A activation by sTWEAK was directly involved in the regulation of JNK1/2, PP2A gene expression was transiently knocked down by a specific siRNA against PP2A catalytic subunit (PP2A-Cx). As shown in Fig. 6C, PP2A-Cx knockdown abolished the inhibitory effect of sTWEAK on TNF-α-induced JNK1/2 phosphorylation. JNK1/2 activation by TNF-α was impaired by TWEAK pretreatment in control cells (RISC-free siRNA transfection), but not in PP2A-Cx siRNA-transfected human visceral adipocytes, where TNF-α-induced JNK1/2 activation remained high regardless of the presence of sTWEAK. The effectiveness of PP2A-Cx siRNA transfection was validated by Western blot analysis (Fig. 6C) together with measurement of PP2A activity (Fig. 6D). Since PP2A-Cx knockdown also increased TNF-α-induced ERK1/2 phosphorylation, we cannot formally exclude a potential role of this phosphatase in the regulation of ERK1/2 activation.

We next confirmed the protective role of sTWEAK on TNF-α-induced insulin resistance on glucose uptake through PP2A activation in differentiated human primary adipocytes obtained from visceral fat-derived preadipocytes. As shown in Fig. 7A, sTWEAK pretreatment of human visceral adipocytes abolished TNF-α-induced JNK1/2 phosphorylation in a similar manner to that found in the human cell line (Fig. 5). Moreover, we also confirmed in these cells that PP2A was involved in the inhibition of JNK1/2 induced by sTWEAK, as PP2A-Cx knockdown prevented the downregulation of TNF-α-induced JNK1/2 activation by sTWEAK pretreatment (Fig. 7B). Finally, to demonstrate that PP2A was directly associated with the protective role of sTWEAK on insulin sensitivity in primary human cells, glucose uptake was analyzed after PP2A-Cx siRNA transfection in visceral adipocytes. As anticipated, TWEAK-pretreatment restored insulin-induced glucose uptake in RISC-free transfected human visceral adipocytes treated with TNF-α but not in those cells where PP2A-Cx had been silenced by specific siRNA transfection (Fig. 7C). Again, measurement of PP2A-Cx protein expression (Fig. 7E) and PP2A phosphatase activity (Fig. 7F) was performed in both control (RISC free siRNA) and PP2A-Cx siRNA-transfected human visceral adipocytes to validate the effectiveness of PP2A-Cx knockdown. Taken together, our results strongly indicate that sTWEAK exerts its beneficial role on insulin sensitivity by preventing TNF-α-induced activation of JNK1/2 through PP2A activation.

DISCUSSION

The multisystem effects of obesity are linked to an imbalance in homeostatic and proinflammatory responses. Obesity triggers inflammatory pathways in adipose tissue, which dysregulate physiological processes that maintain insulin sensitivity. In fact, insulin resistance in adipose tissue is one of the seminal events in the progression toward metabolic dysregulation in obesity. Mediators released by adipocytes and macrophages from this tissue, such as TNF-α family members, have been proposed to impair insulin action in peripheral tissues. In particular, increased levels of TNF-α in the obese state may prevent weight gain through induction of insulin resistance (20).

In the present study, we describe for the first time a positive role for the TWEAK-Fn14 axis in human visceral adipocytes, where it specifically functions to attenuate insulin resistance induced by TNF-α on carbohydrate metabolism without affecting the other metabolic effects of TNF-α directed at limiting fat accretion. Although in vitro studies showed that sTWEAK induced cellular insulin resistance in the liver (10), clinical studies pointed to sTWEAK as a potential novel biomarker of metabolic syndrome with a putative protective effect. In this respect, serum levels of sTWEAK are significantly decreased...
in T2D patients and are negatively correlated with fasting glucose and insulin resistance assessed by HOMA-IR (24). A recent study from our laboratory also revealed that sTWEAK levels are decreased in T1D and are inversely correlated with cardiovascular risk factors (28). In addition, a decrease in sTWEAK might be associated with insulin resistance in pregnancy and could be a predictor of T2D in high cardiovascular risk populations (unpublished results). Under physiological conditions, high levels of TWEAK mRNA have been found in many human tissues such as lymph nodes, pancreas, intestine, heart, brain, lung, ovary, vasculature, and skeletal muscle. However, the real contribution of each tissue to circulating sTWEAK is unknown. The low level of TWEAK mRNA detected in human adipose tissue (42) suggests that adipose tissue contribution to circulating sTWEAK circulation is minor. Interestingly, our unpublished findings suggest that circulating sTWEAK is negatively correlated with fat mass (under review). This situation is the opposite of that found regarding circulating TNF-α in obese individuals. To date, the significance and the mechanisms leading to reduce levels of sTWEAK in pathology associated with a proatherosclerotic profile are not known. One may hypothesize that lower circulating sTWEAK levels in high cardiovascular risk diseases might be a consequence of metabolic derangement in other tissues where TWEAK is usually expressed at higher levels, such as the vasculature and skeletal muscle, which are both involved in the development of these metabolic disturbances.

Our study suggests a potential beneficial role for sTWEAK in the metabolic disturbances associated with T2D and provides strong evidence about the molecular mechanisms through
which the insulin signaling cascade may be improved. No metabolic effects of sTWEAK under basal conditions were observed on either lipolysis or glucose uptake, in agreement with a previous study in human subcutaneous adipocytes (40). However, our results demonstrate for the first time that sTWEAK ameliorates TNF-α-induced insulin resistance on glucose uptake, GLUT4 translocation, and insulin signaling, suggesting that sTWEAK acts as a protective factor in insulin-mediated glucose homeostasis.

sTWEAK has been identified both as a positive and negative regulator of TNF-α signaling, since both TNF-α and TWEAK, through binding to their receptors, signal via similar TNFR-associated factors, including TRAF2. Thus, TWEAK-Fn14 signaling can sensitize tumor cells to TNF-α (43), whereas in nontumoral epithelial cells sTWEAK has been described to inhibit TNFR1 signaling and has the potential to shift the quality of cellular TNF-α responses from inflammation to cell death (46). Similarly, our study indicates that in human visceral adipocytes both Fn14 and TNFR1 compete for binding to endogenous TRAF2. Nevertheless, the analysis of downstream effectors demonstrates that modulation of TNF-α-induced effects on insulin sensitivity by sTWEAK is a consequence, at least in part, of MAPK modulation rather than by competition for shared limiting factors, as has described in other cellular models (29, 46). More specifically, we demonstrate that sTWEAK prevents TNF-α-induced insulin resistance through the direct regulation of JNK1/2 phosphorylation. Furthermore, our study provides compelling evidence that the Ser/Thr phosphatase PP2A is an effector molecule in the TWEAK signaling cascade and that its activation is linked to the protective role of TWEAK during the development of insulin resistance. Until now, PP2A has been mainly described as a negative regulator of cellular processes, such as cell proliferation and cytokine production.
of the insulin signaling pathway as it impairs Akt activation (1). Indeed, in brown adipocytes, TNF-α, acting through a ceramide-dependent mechanism, mediated PP2A activation (39). However, as we show here, TNF-α does not activate PP2A in human white adipocytes and does not induce changes in the protein levels of either the catalytic or the regulatory PP2A subunits, suggesting cell type-specific differences in the effect of PP2A on insulin signaling. Actually, PP2A specificity depends on its regulatory subunits, which may be cell type dependent (16). In this context, studies in murine adipocytes demonstrate that PP2A inhibition triggers insulin resistance as a consequence of mTOR activation and IRS-1 Ser phosphorylation (5). Moreover, particular transduction pathways activ-

Fig. 8. Balance between TNF family members on adipose tissue as a key factor in the pathogenesis of obesity-associated metabolic disorders. A: early stages of obesity/inflammation: elevated levels of sTWEAK may show a protective role against TNF-α-induced insulin resistance on glucose uptake. Although both TNFR1 and Fn14 compete for TRAF2, TWEAK pretreatment does not modulate all TNF-α signaling pathways downstream of TRAF2 (e.g., TAK1). Specifically, PP2A activation by sTWEAK inhibits TNF-α-induced JNK1/2 activation, allowing an adequate response of adipocytes to insulin-induced glucose uptake. B: steady-state obesity/inflammation: decrease in sTWEAK levels (i.e., patients with T2D or obesity) might induce a decrease in insulin responsiveness on glucose uptake as consequence of JNK1/2 activation.

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In summary, we have shown that sTWEAK can prevent obesity-associated insulin resistance brought about by TNF-α signaling through a PP2A-dependent fine tuning of JNK1/2 activation. Further investigation will be required to understand the molecular mechanisms mediating the positive effect of sTWEAK on PP2A activity and, perhaps more importantly, to establish the specific role of the regulatory PP2A subunits in the insulin sensitivity/resistance states. In this scenario, we postulate that elevated sTWEAK levels may have a protective role on insulin resistance development through the induction of PP2A overactivation, supporting the previous hypothesis that identified sTWEAK as a potential novel biomarker of metabolic disturbances such as obesity and T2D. Our results reinforce the idea that an imbalance between the TNF family members on adipose tissue is a key factor in the pathogenesis of the insulin-resistance-associated metabolic disorders (Fig. 8). Contrary to what is observed with TNF-α in obese and T2D patients, circulating sTWEAK appears as a protective element under these conditions. Interestingly, mTWEAK and sTWEAK have been shown to have different effects on signal transduction pathways (33). Since mTWEAK is mainly expressed in activated monocytes, the metabolic effects of TWEAK may therefore differ in cells having contact with monocytes (e.g., adipocytes in an obesity context) and in more distant cells living in a monocyte-free environment. Thus, it is tempting to speculate that the decrease in sTWEAK levels, together with an increase of mTWEAK, may help to maintain the local proinflammatory effect of the TNF-α-driven response. Consequently, the potential use of recombinant TWEAK or Fn14 agonists to manipulate the TWEAK/Fn14 pathway for the treatment of insulin resistance is an exciting prospect that should be explored further.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


REFERENCES


