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 depot. 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 activity upon sTWEAK treatment. Silencing of the PP2A catalytic subunit gene overcomes the dephosphorylation effect of sTWEAK on JNK inactivation. 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

INSULIN SIGNALING IS AN IMPORTANT cellular regulator of metabolic pathways involved in maintaining the correct balance between the organism’s energy expenditure and storage capacity. Visceral obesity is considered a key contributor to the dysregulation of insulin transduction pathways and is also a major risk factor for the development of insulin resistance and type 2 diabetes (T2D). Adipose tissue, a central player in the mild inflammatory state that is characteristic of obese patients, is one of the first tissues affected by insulin resistance. Several mediators released from adipocytes and infiltrating macrophages have been described to alter glucose homeostasis through inhibiting insulin-stimulated GLUT4 translocation to the plasma membrane by tissue-specific mechanisms (14). These include the activation of proinflammatory and stress kinases as well as protein tyrosine phosphatases such as PTP1B that reduce tyrosine phosphorylation of the insulin receptor (IR) and insulin receptor substrates (IRSs) (12). In this context, our previous studies with human adipocytes demonstrated that TNF-α inhibits insulin-induced glucose uptake in human visceral but not in subcutaneous adipocytes by impairing insulin signaling at the level of IRS-1 in a JNK1/2-dependent manner (13).

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) is a recently identified member of the TNF superfamily, whose biological activities in the immune system continue to be unraveled. Although TWEAK can be expressed as a membrane-bound form (mTWEAK), it is mainly processed as a soluble variant (sTWEAK), resulting from proteolytic cleavage of mTWEAK (47). Both isoforms can bind to fibroblast growth factor-inducible 14 (Fn14), which signals via the recruitment of several TNFR-associated factors (2, 17, 33). Recently, sTWEAK has been revealed as a potential new player in the development of insulin resistance in hepatocytes (10). TWEAK/Fn14 signaling mediates unique and context-dependent pleiotropic effects. In a manner similar to TNF-α, sTWEAK activates proinflammatory signaling pathways, including both canonical and non canonical NFκ-B pathways (35), as well as Akt, ERK1/2, and JNK1/2 (9, 25). However, several lines of evidence also show that sTWEAK may modulate the cellular responses to TNF-α by competition at a shared limiting factor (29, 46). Although TWEAK and its receptor F1n4 are expressed in a variety of organs, including adipose tissue (7), the physiological and patho-physiological roles of this cytokine in an obesity-associated insulin-resistant state remains largely unknown. TWEAK has been described to inhibit adipogenesis (40) and we have recently shown that TWEAK may have a proinflammatory function in human subcutaneous adipocytes (42). However, clinical studies have shown a negative association between

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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/Fn14 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 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 (Tyrosine 612), p-IRS-1 (Thr183/Tyr185), p-p38 MAPK (Thr180/Tyr182), and Fni4 were from Cell Signaling (Beverly, MA). Antibodies against IκBα (sc-371), IκBβ (sc-945), TRAF2 (sc-876), cavedin-1 (sc-494), NF-κB p52 (sc-298), GLUT3 (sc-74399), and TNF-R1 (sc-73195) were from Santa Cruz (Palo Alto, CA). Antibodies against p-TAK1 (Thr205) and TAK1 were from Abcam (Cambridge, UK). NE-Per nuclear and cytoplasmic extraction reagents were from Thermo Scientific (Rockford, IL). A monoclonal antibody to human TNF-R1 (p75) was from Becton Dickinson (Plymouth Meeting, PA). An anti-human TNFR1/TNFRSF1A antibody was purchased from R&D Systems (Abingdon, UK).

Cell culture and lysis. LiSa-2 cells, kindly provided by Dr. Möller (University of Ulm, Germany), were used as a cellular model of visceral human adipocytes. This is a stable cell line derived from a visceral human adipose. This is a stable cell line derived from a visceral human adipose. This is a stable cell line derived from a visceral human adipose. This is a stable cell line derived from a visceral human adipose. This is a stable cell line derived from a visceral human adip...
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
reduced cell viability. We also evaluated lipolysis by measuring glycerol release. As shown in Fig. 1C, treatment of differentiated adipocyte cells with sTWEAK did not increase glycerol release to the medium, and TNF-α-induced lipolysis was not changed in sTWEAK-primed cells (Fig. 1C). Since it has been described that sTWEAK induces insulin resistance in human hepatocytes (4), insulin sensitivity of carbohydrate metabolism was also analyzed. As shown in Fig. 1D and following from our previous results, TNF-α increased basal glucose uptake and induced insulin resistance in differentiated LiSa-2 cells (13). In contrast, sTWEAK did not modulate glucose uptake either under basal conditions or upon insulin stimulation, in agreement with the effects described for adipocytes of subcutaneous origin (40). Surprisingly, however, sTWEAK pretreatment decreased the TNF-α-induced glucose uptake and impaired TNF-α-induced insulin resistance on glucose uptake (Fig. 1D). Similar results were obtained when we analyzed the modulation of TNF-α metabolic responses upon cotreatment with sTWEAK (data not shown).

TNF-α produces paradoxical effects on glucose uptake in human visceral adipocytes. It increases GLUT4 translocation and glucose uptake but also activates JNK1/2, inducing phosphorylation of IRS-1 at the Ser312 residue. This, in turn, leads to impaired glucose uptake and induced insulin resistance in differentiated LiSa-2 cells (13). In contrast, sTWEAK did not modulate glucose uptake either under basal conditions or upon insulin stimulation, in agreement with the effects described for adipocytes of subcutaneous origin (40). Surprisingly, however, sTWEAK pretreatment decreased the TNF-α-induced glucose uptake and impaired TNF-α-induced insulin resistance on glucose uptake (Fig. 1D). Similar results were obtained when we analyzed the modulation of TNF-α metabolic responses upon cotreatment with sTWEAK (data not shown).

Fig. 2. sTWEAK pretreatment ameliorates TNF-α-induced insulin resistance at IRS-1/Akt/AS160 level. Lysates from differentiated LiSa-2 cells cultured for 24 h in the absence or presence of TNF-α, sTWEAK, or sTWEAK pretreatment (6 h) before stimulation with 50 nM insulin for 10 min were analyzed by Western blot using antibodies against phosphorylated and total IRS-1 (Tyr612), Akt (Ser473), and AS160 (Thr642). A representative experiment is shown together with densitometric analysis of phosphorylated vs. total proteins (3 independent experiments). *P < 0.01.
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). In line with the TNF-α-induced disassociation of Fn14-TRAF2 complex (Fig. 4A), treatment of human adipose cells with TNF-α significantly increased TRAF2 association to TNFR1, but not to TNFR2, which in fact was reduced (Fig. 4A). Under basal conditions, sTWEAK did not modulate TRAF2 association with either TNFR1 or TNFR2. However, TWEAK 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 IκBα 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 TNFR1 for TRAF2 association may explain the inhibitory effect of sTWEAK on TNF-α-induced insulin resistance. However, TWEAK pretreatment specifically modulates the effect of TNF-α on insulin-induced glucose uptake, but not on other TNF-α effects such as lipolysis and apoptosis, also mediated via TNFR1 (4, 34), suggesting that TRAF2 association with TNFR1 in TWEAK-primed cells is sufficient to signal. Therefore, the molecular mechanism involved in the protective mechanism proposed here should occur downstream of TRAF2.

TWEAK-Fn14 signaling specifically abrogates TNF-α-induced insulin resistance by preventing JNK1/2 activation through a mechanism dependent on PP2A activity. We next explored the potential modulation of TNF-α signaling by sTWEAK, focusing on the NF-κB and MAPK transduction pathways, since they are involved in TNF-α-mediated insulin resistance (6, 30, 48). Although both Fn14 and TNFR1 compete for the binding to endogenous TRAF2, sTWEAK pretreatment did not impair the activation of TAK1 (Fig. 5), a known TNF-α-induced TRAF2 effector that regulates both MAPK and NF-κB signaling pathways (25, 31). However, sTWEAK pretreatment abolished the stimulatory effect of TNF-α on JNK1/2 activation that is directly involved in the development of insulin resistance (13). A decrease in both p38 MAPK and ERK1/2 activation was also detected (Fig. 6), whereas IκB-α and -β degradation were not significantly modulated by sTWEAK. Therefore, our data suggest that sTWEAK pretreatment would ameliorate insulin resistance by acting at the level of JNK1/2 rather than competing for shared limiting factors, as has been previously described in other cellular models (29, 46). In this regard, it is known that a regulated balance between kinases and phosphatases is crucial for normal cell function. MAPK pathways are negatively regulated by dephosphorylation by multiple phosphatases. Primarily, the PP2 Ser/ThrPPase family can catalyze initial deactivation, whereas at later time points dual-specificity phosphatases would contribute to the downregulation of MAPK signals (22, 45). It seemed reasonable to hypothesize that PP2A, which has been described to modulate JNK1/2 activation in pancreatic cells (27), could be regulated by sTWEAK in our cellular 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-C). As shown in Fig. 6C, PP2A-C 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) but not in PP2A-C siRNA-transfected human visceral adipocytes (Fig. 6D). Since PP2A-C 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-Cα 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-Cα 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-Cα had been silenced by specific siRNA transfection (Fig. 7C). Again, measurement of PP2A-Cα protein expression (Fig. 7E) and PP2A phosphatase activity (Fig. 7F) was performed in both control (RISC free siRNA) and PP2A-Cα siRNA-transfected human visceral adipocytes to validate the effectiveness of PP2A-Cα 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/H9251-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 events.
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 activated by adiponectin, a well-known insulin-sensitizing adipokine, are elicited by PP2A activation (8). Our study in human adipocytes reveals that PP2A controls JNK1/2 phosphorylation and is in agreement with previous studies performed in pancreatic cells (27). In addition to PP2A, other PP2A-related Ser/Thr phosphatases such as PP4 have also been described to be involved in the regulation of TNF-α-induced activation of JNK1/2 (49). Although we clearly demonstrate here the positive role of sTWEAK on PP2A activation in human visceral adipocytes in the context of TNF-α-induced insulin resistance, additional effects of alternative phosphatases such as PP2C, which are also involved in the inhibition of insulin sensitivity by TNF-α, should not be rule out (36).

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.
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

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