Suppressor of Cytokine Signaling 3 Is a Physiological Regulator of Adipocyte Insulin Signaling*

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Many proinflammatory cytokines and hormones have been demonstrated to be involved in insulin resistance. However, the molecular mechanisms whereby these cytokines and hormones inhibit insulin signaling are not completely understood. We observed that several cytokines and hormones that induce insulin resistance also stimulate SOCS3 expression in 3T3-L1 adipocytes and that SOCS3 mRNA is increased in adipose tissue of obese/diabetic mice. We then hypothesized that SOCS3 may mediate cytokine- and hormone-induced insulin resistance. By using SOCS3-deficient adipocytes differentiated from mouse embryonic fibroblasts, we found that SOCS3 deficiency increases insulin-stimulated IRS1 and IRS2 phosphorylation, IRS-associated phosphatidylinositol 3-kinase activity, and insulin-stimulated glucose uptake. Moreover, lack of SOCS3 substantially limits the inhibitory effects of tumor necrosis factor-α to suppress IRS1 and IRS2 tyrosine phosphorylation, phosphatidylinositol 3-kinase activity, and glucose uptake in adipocytes. The ameliorated insulin signaling in SOCS3-deficient adipocytes is mainly due to the suppression of tumor necrosis factor-α-induced IRS1 and IRS2 protein degradation. Therefore, our data suggest that endogenous SOCS3 expression is a key determinant of basal insulin signaling and is an important molecular mediator of cytokine-induced insulin resistance in adipocytes. We conclude that SOCS3 plays an important role in mediating insulin resistance and may be an excellent target for therapeutic intervention in insulin resistance and type II diabetes.

Insulin resistance is a fundamental aspect of the etiology of type II diabetes, a prevalent and serious metabolic disorder worldwide (1). Insulin resistance is also commonly seen with obesity (1). One of the major links between the two disorders is proinflammatory cytokines (1). Increased production of proinflammatory cytokines in obesity has been causally linked to insulin resistance (2). TNFα* is a major cytokine linked to insulin resistance in obesity (3). Increased production of TNFα within adipose tissue has been reported in obese humans and animal models, and this has been linked to insulin resistance (4, 5). Although the role of proinflammatory cytokines in mediating insulin resistance has drawn extensive attention, the molecular mechanisms whereby most cytokines inhibit insulin signaling are incompletely understood.

Many cytokines stimulate the tissue-specific expression of suppressor of cytokine signaling proteins (SOCSs), a group of signaling proteins characterized by their ability to down-regulate cytokine signaling (6). The SOCS protein family includes eight members (CIS and SOCS1 to SOCS7), and each member contains a central SH2 domain and a conserved C-terminal SOCS box (7–9). Cytokine binding to its receptor activates the JAK-STAT signaling pathway, leading to induction of SOCS mRNA and protein. Induced SOCS proteins in turn inhibit cytokine signaling (10). SOCS proteins bind directly via their SH2 domains to tyrosine-phosphorylated JAK or activated cytokine receptors to suppress cytokine signaling (10). An additional mechanism by which SOCS proteins inhibit signaling involves targeted proteasomal degradation of signaling proteins, via a SOCS box-mediated ubiquitination complex (11, 12).

Previous studies from our laboratory have established SOCS3 as a negative regulator of leptin signaling. Leptin induces SOCS3, which in turn inhibits leptin signaling (13, 14), suggesting that SOCS3 may play a potential role in regulating leptin sensitivity. Recent reports have also demonstrated that SOCS3 is capable of blocking insulin signaling (15–17), at least partially through SOCS3-mediated proteasomal degradation of IRS1 and IRS2 (18).

Prior studies demonstrating SOCS3 inhibition of insulin signaling employed forced SOCS3 expression using transfection or adenoviral vectors, so little is known regarding the physiological role of SOCS3 in insulin signaling in insulin-sensitive tissues, such as adipocytes. Accordingly, this study was designed to determine the role of endogenous SOCS3 in setting the level of insulin signaling and mediating adipocyte insulin resistance in response to external factors. In this study, we established an Socs3-deficient adipocyte model, using adipocytes differentiated from mouse embryonic fibroblasts (MEFs) from wild-type or Socs3-deficient embryos. We found that SOCS3 deficiency increases insulin-stimulated IRS1 and IRS2 phosphorylation, enhances downstream PI 3-kinase activity, and leads to increased insulin-stimulated glucose uptake. Moreover, SOCS3 deficiency blocks TNFα-induced inhibition of insulin signaling, and this is largely attributed to the suppression of TNFα-induced IRS1 and IRS2 protein degradation. Taken together, these data demonstrate that the level of SOCS3 expression is a determinant of insulin signaling and is a mediator of cytokine-induced insulin resistance in adipocytes.

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The abbreviations used are: TNFα, tumor necrosis factor-α; SH, Src homology; MEFs, mouse embryonic fibroblasts; PI, phosphatidylinositol; WT, wild type; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; RT, reverse transcriptase; DIO, diet-induced obesity; GH, growth hormone; IFNγ, interferon-γ; AT II, angiotensin II; IL, interleukin; SOCS, suppressor of cytokine signaling.

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Because SOCS3 plays an important role in mediating both leptin and insulin resistance, it could be a unique mediator of both obesity and the associated metabolic syndrome.

EXPERIMENTAL PROCEDURES

Antibodies and Reagents—The rabbit SOCS3 antiserum was generated as described previously (13), and guinea pig anti-SOCS3 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit polyclonal anti-IR, IRS-1, and IRS2 antibodies were kindly provided by Dr. Ronald Kahn or purchased from Upstate Biotechnology, Inc. (Lake Placid, NY). Protein A-agarose, rabbit polyclonal anti-peroxisome proliferator-activated receptor-γ antibody, and goat polyclonal anti-α-actinin antibody were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-GLUT4 antibody was purchased from Chemicon International (Temecula, CA). Mouse monoclonal anti-phosphotyrosine 4G10, rabbit polyclonal anti-p85, rabbit polyclonal anti-Akt1, and rabbit monoclonal anti-phospho-Akt1 (Ser-473) antibodies were obtained from Upstate Biotechnology, Inc. TNFα, IFNγ, interleukin-6, growth hormone, 3-isobutyl-1-methylxanthine, dexamethasone, insulin, and lactacystin were all purchased from Sigma. 2-Deoxy-3H-glucose was from Amersham Biosciences.

Cell Culture—Murine 3T3-L1 preadipocytes were propagated in DMEM (Invitrogen) containing 10% FBS (growth medium). Confluent preadipocytes were induced to differentiate with DMEM containing 10% FBS supplemented with 0.5 μM 3-iodo-l-thyronine, 1 μM dexamethasone, and 1 μg/ml insulin (differentiation medium). Cells were incubated in this differentiation medium for 2 days and then cultured in growth medium containing 200 μg/ml insulin for another 2 days. Subsequently, cells were maintained in growth medium throughout out the adipocyte stage and cultures were refed every 3 days.

Primary MEFs for three Socs3 genotypes (Socs+/−, −/−, and −/−) were generated from 10- to 12-day embryos by crossing heterozygous Socs3 mice, according to a modified 3T3 protocol (19). Briefly, embryos were dissected, and the heads were removed and used for genotyping. The remaining bodies whose visceral organs had been removed were minced and digested in trypsin-EDTA buffer (2.5 g of trypsin, 0.4 g of EDTA, 7 g of NaCl, 0.3 g of Na3HPO4, 0.24 g of KH2PO4, 0.37 g of KCl, 1 g of d-glucose, 3.0 g of Tris-base, 1 ml of phenol red, and H2O added to 1 liter, pH 7.6) at 37 °C with occasional shaking for 30 min. Dissociated cells from individual embryos were collected by centrifugation and cultured in DMEM containing 10% FBS in 75 flask. Eighty hours later, adherent cells were split into 100-mm plates at a density of 1.2 × 10^6/plate. Cells were then cultured in DMEM containing 10% FBS supplemented with 0.1% SDS. Cell lysates were added to scintillation vials with 5 ml of scintillation fluid. Reactions of real-time PCR for standards and unknown samples were performed according to the manufacturer’s instructions (Echelon, Salt Lake City, UT).

Glucose Transport Assay—MEFs were grown and differentiated in 12-well plates. At least 1 week before the assay, insulin was withdrawn from the medium; 72 h prior to the assay, the glucose concentration was reduced to 5 mM in the medium. MEF adipocytes were incubated overnight in serum-free DMEM with or without 1 μM TNFα. Cells were then incubated in KRH buffer (129 mM NaCl, 4.8 mM KCl, 1.2 mM MgSO4, 1.8 mM CaCl2, and 20 mM HEPES, pH 7.4) with or without 100 μM insulin (for 30 min transport, 1 μCi of [3H]glucose was added to each well and incubated for 10 min. To stop the transport, cells were rinsed with ice-cold KRH buffer three times and lysed with 0.1% SDS. Cells were lysed with scintillation vials with 5 ml of scintillation liquid and counted. One well of cells from each treatment group included 50 μl of cytosol B to determine nonspecific transport, which was subtracted from each sample.

Statistical Analysis—All data are expressed as mean ± S.E. Data were evaluated for statistical significance by one-way analysis of variance, and significantly different group means were then separated by the least significant difference test using SPSS (Chicago). p < 0.05 is considered as significant.

RESULTS

SOCS3 mRNA Is Elevated in Fat Depots of Obese/Diabetic and Diet-induced Obesity (DIO) Mice—To assess the potential relevance of SOCS3 in mediating insulin resistance, we quantitated SOCS3 mRNA in fat depots of obese/diabetic and DIO mice. Fig. 1a shows that SOCS3 mRNA was increased in subcutaneous, epididymal, and mesenteric fat pads of ob/ob and db/db mice, compared with WT control. Similar results were observed in DIO mice (Fig. 1b). These data suggest that SOCS3 mRNA expression is increased in adipose tissue of mice with genetic and acquired obesity.

SOCS3 mRNA Expression Is Increased by Hormones and Cytokines in 3T3-L1 Adipocytes—We tested the effects of several hormones and cytokines, which have been shown to be involved in insulin resistance, on SOCS3 mRNA expression in 3T3-L1 adipocytes. Fig. 2, a and b, shows that several hormones, including insulin, growth hormone (GH), angiotensin II (AT II), and cytokines including TNFα, IL6, and IFNγ, did indeed stimulate SOCS3 mRNA expression in 3T3-L1 adipocytes. Dexamethasone and leptin had no effect (data not shown). Although TNFα and insulin caused a slow but relatively sustained stimulation of SOCS3 expression, IL6, IFNγ, AT II, and GH stimulated a more rapid increase of SOCS3 mRNA than was sustained. These data suggest that up-
regulation of SOCS3 may contribute to the insulin resistance caused by inflammatory cytokines and hormones.

Establishment of a SOCS3-deficient Adipocyte Model—Because deletion of the Socs3 gene in mice results in embryonic lethality at day 13 (21), we utilized MEFs as a cell model to explore further the role of SOCS3 in adipocyte insulin signaling. MEFs can be differentiated at 60–70% as shown by Oil Red O staining (Fig. 3a). Although MEFs of three genotypes (+/H11001/H11001, /H11001/H11002, and /H11002/H11002) underwent similar differentiation, as revealed by lipid accumulation and by the equal expression of adipocyte-phenotypic genes (Fig. 3b), Wt, Het, and Ko MEFs exhibited differential SOCS3 expression (Fig. 3c). Real time RT-PCR measurement showed that SOCS3 mRNA was not detectable in Ko MEFs, whereas SOCS3 expression in Het MEFs was decreased by 50%, compared with that in Wt cells (Fig. 3c, lower panel). Similar results were observed when SOCS3 protein levels were measured by immunoprecipitation followed by Western blot (Fig. 3c, upper panel). Moreover, Het MEFs exhibited less SOCS3 mRNA expression in response to TNFα treatment, compared with Wt MEFs. TNFα treatment caused a 10-fold increase of SOCS3 mRNA in Wt MEFs, whereas TNFα stimulated less SOCS3 expression in Het MEFs, which showed a 5-fold increase (Fig. 3d). Therefore, these MEFs are a useful cell model for studying the role of endogenous SOCS3 in insulin signaling in adipocytes.

SOCS3 Deficiency Increases Insulin-stimulated Glucose Uptake in Adipocytes—To study the physiological consequence of SOCS3 deficiency for insulin signaling in adipocytes, we measured insulin-stimulated glucose uptake in differentiated MEFs. In Wt adipocytes, insulin stimulated 2-deoxy-[3H]glucose uptake by 3-fold, whereas insulin stimulated a 4-fold increase of glucose uptake in Ko adipocytes (Fig. 4). Moreover, lack of SOCS3 attenuated TNFα-induced inhibition of insulin-stimulated glucose uptake. Pretreatment of Wt adipocytes with TNFα for 12 h caused a 50% reduction of insulin-stimulated glucose uptake, whereas TNFα only exerted a 25% inhibition of glucose uptake in Ko adipocytes (Fig. 4). Het adipocytes exhibited an intermediate increase in insulin-stimulated glucose uptake and attenuation on inhibition of TNFα on glucose uptake (Fig. 4). These data demonstrate a gene dosage effect for the relationship between SOCS3 expression and insulin-stimulated glucose uptake in both the presence and absence of TNFα.

Endogenous SOCS3 Levels Regulate Insulin-stimulated IRS1 Phosphorylation—We then investigated how endogenous SOCS3 levels affect insulin signaling in adipocytes. Fig. 5a
versus real time RT-PCR. Data are expressed as mean ± S.E. (n = 6); *, p < 0.05 versus Het. c, differential expression of SOCS3 in differenti- ated Wt, Het, and Ko MEFs. SOCS3 protein from lysates was
immunoprecipitated and immunoblotted with SOCS3 antibody. Adipo-
cytic total RNA was isolated, and SOCS3 mRNA was measured using
real time RT-PCR. Data are expressed as mean ± S.E. (n = 6); *, p < 0.05 versus Het. d, Het adipocytes express less SOCS3 mRNA in re-
sponse to TNFα stimulation. Adipocytes were serum-free for 12 h and
then treated with TNFα (1 nM) for 12 h. Adipocyte total RNA was
isolated, and SOCS3 mRNA was measured using real time RT-PCR.
Data are expressed as mean ± S.E. (n = 5); *, p < 0.001 versus Wt control and Het TNFα; ***, p < 0.05 versus Het control.

shows that insulin-stimulated phosphorylation of IR remains
at the same level among three genotypes, demonstrating that
SOCS3 deficiency does not alter IR phosphorylation in these
cells. However, insulin-stimulated phosphorylation of IRS1
was increased in Ko and Het MEFs by 50 and 20%, respec-
tively, compared with Wt adipocytes (Fig. 5b). This suggests
that IRS might be a major signaling molecule through which
SOCS3 targets insulin signaling in adipocytes.

SOCS3 Is Required for the Action of TNFα to Inhibit Insulin
Signaling in Adipocytes—Fig. 6a (left panel) shows that pre-
treatment of Wt adipocytes with TNFα for 12 h inhibited
insulin-stimulated IRS1 phosphorylation by 80%. This was sub-
stantially attenuated in Ko and Het adipocytes, which showed
approximately a 40% inhibition. In the TNFα treatment
groups, in fact, the absolute levels of insulin-stimulated IRS1
phosphorylation in Het and Ko MEFs were increased by 2–3-
fold, compared with Wt adipocytes. As shown in Fig. 6a (right
panel), TNFα treatment reduced the IRS1 protein level by 80%
in Wt adipocytes, whereas TNFα exerted less inhibitory effects
on IRS1 protein levels in Ko and Het adipocytes, with approxi-
mately a 40% inhibition. These data indicate that IRS1 phos-
phorylation is well matched with IRS1 protein levels in the

TNFα treatment study, suggesting that the protection of
TNFα-induced IRS1 protein degradation by SOCS3 deficiency
may account for the attenuation of inhibition by TNFα of IRS1
phosphorylation in Socs3-deficient cells. To confirm further
this protection of SOCS3 deficiency on IRS1 protein degra-
Fig. 6. SOCS3 deficiency substantially blocks the ability of TNFα to inhibit insulin signaling. a, SOCS3 deficiency substantially blocks TNFα inhibition of IRS1 phosphorylation. Adipocytes were pretreated with TNFα (1 nM) for 12 h and then stimulated with 100 nM insulin for 5 min. IRS1 protein was immunoprecipitated (IP) and immunoblotted (IB) with IRS1 (upper right) and phosphotyrosine (upper left) antibodies. The blot is a representative of three similar experiments. Blots were quantified by densitometry. Lower left panel, data are expressed as mean ± S.E. (n = 3); *, p < 0.05 versus Wt insulin alone; **, p < 0.01 versus Wt insulin plus TNFα. Lower right panel, data are expressed as mean ± S.E. (n = 3); *, p < 0.01 versus Wt insulin plus TNFα. b, SOCS3 deficiency prevents TNFα-induced degradation of IRS1. Adipocytes were serum-free for 12 h, then pretreated with or without lactacystin (25 μM) for 1 h, and incubated with TNFα (1 nM) in a time course as indicated in the figure. Cell lysates were used for immunoblotting with the IRS1 and p85 antibodies. c, SOCS3 deficiency substantially blocks the inhibition of TNFα for IRS2 phosphorylation. All the experiments were done as described in a except using IRS2 antibody. Lower left panel, data are expressed as mean ± S.E. (n = 3); *, p < 0.05 versus Wt insulin alone; **, p < 0.05 versus Wt insulin plus TNFα. Lower right panel, data are expressed as mean ± S.E. (n = 3); *, p < 0.01 versus Wt insulin plus TNFα.

tion, we treated the Ko and Wt adipocytes with TNFα in a time course study. Fig. 6b shows that TNFα treatment caused IRS1 protein degradation from 9 to 48 h in Wt adipocytes, with the lowest levels at 12 and 24 h. In contrast, TNFα was unable to exert this action on IRS1 protein in Ko adipocytes. This effect of TNFα on IRS1 was specific, as TNFα was without effect on the degradation of p85 (Fig. 6b, lower panel). Moreover, pretreatment of adipocytes with lactacystin, a proteasomal inhibitor, completely blocks the effect of TNFα on IRS1 degradation (Fig. 6b, upper left). Similar results were observed on the effect of SOCS3 deficiency on IRS2 phosphorylation and protein levels (Fig. 6c). These data suggest that SOCS3 is required for TNFα-induced IRS1 and -2 protein degradation and subsequent hypophosphorylation, and may therefore be required for TNFα-caused impairment of insulin signaling.

SOCS3 Is Required for Chronic Insulin-induced Hypophosphorylation and Degradation of IRS1—Previous studies (22, 23) showed that long term treatment with high-dose insulin causes proteasomal degradation of IRS1. Our present data demonstrate that insulin stimulates SOCS3 expression in adipocytes, which may potentially mediate the insulin-induced degradation of IRS1. Fig. 7a shows that pretreatment with 200 nM insulin for 16 h greatly inhibited insulin-stimulated IRS1 phosphorylation in Wt adipocytes, with a corresponding reduced IRS1 protein level. This inhibition of IRS1 phosphorylation and protein by long term insulin treatment was substantially prevented in Ko adipocytes. A time course study (Fig. 7b) shows that chronic insulin treatment caused a significant decrease of IRS1 protein in Wt adipocytes at 16 h, and this was substantially prevented in Ko adipocytes. These data suggest that SOCS3 plays a role in IRS1 protein degradation induced by long term insulin exposure, and may play a role in the impairment of insulin signaling and insulin resistance associated with hyperinsulinemia in vivo.

SOCS3 Deficiency Increases Insulin-induced IRS1-associated p85, PI 3-Kinase Activity, and Akt Phosphorylation—We further studied the effect of SOCS3 deficiency on downstream insulin signaling. p85 is a regulatory subunit of PI 3-kinase, and p85 docking to IRS proteins is essential for regulation of PI 3-kinase activity. We therefore evaluated the docking of p85 to IRS1 in adipocytes of the three genotypes upon insulin stimulation. IRS1 was immunoprecipitated, followed by immunoblotting with p85 antibody. Fig. 8a shows that insulin-stimulated binding of p85 to IRS1 was increased by 40% in Ko adipocytes, compared with Wt control. Although pretreatment of Wt adipocytes with TNFα markedly inhibited the association of p85 with IRS1, the SOCS3 deficiency in Ko and Het adipocytes substantially overcame the inhibition of TNFα for p85 docking to IRS1 upon insulin stimulation, with 1–2-fold increases in p85 binding in Ko and Het compared with Wt adipocytes (Fig. 8a, lower panel). We next examined the activity of PI 3-kinase in the adipocytes of three genotypes. Consistent with the data shown above, Fig. 8b demonstrates that insulin caused a 4-fold stimulation of PI-3 kinase activity in Wt adipocytes, whereas insulin exerted a more potent effect on PI-3 kinase activity in Ko and Het adipocytes, with a 5–6-fold increase. Similarly,
TNFα exerted a less potent action to inhibit insulin-stimulated PI-3 kinase activity in Ko adipocytes, with only a 20% inhibition, compared with a 75% inhibition in Wt adipocytes. Similar results were observed on the effect of SOCS3 deficiency on Akt1 phosphorylation (Fig. 8c). These data suggest that lack of SOCS3 results in increased insulin signaling via the IRS-PI 3-kinase pathway.

DISCUSSION

We have demonstrated in the present study that SOCS3 mRNA is elevated in adipose tissue of obese/diabetic mice and that several hormones and cytokines that are known to cause insulin resistance induce expression of SOCS3 in cultured adipocytes. We hypothesized that SOCS3 is a negative regulator of adipocyte insulin signaling and may mediate cytokine- and hormone-induced insulin resistance in this tissue. We confirmed this hypothesis by using an SOCS3-deficient adipocyte model. We demonstrated that the SOCS3 deficiency increases insulin-stimulated IRS1 and IRS2 phosphorylation and enhances p85 association with IRS1 and subsequent PI 3-kinase activity, resulting in increased glucose uptake in adipocytes. Moreover, the lack of SOCS3 substantially blocks the inhibitory effects of TNFα on the phosphorylation of IRS1 and IRS2, p85 binding to IRS1 and PI 3-kinase activity, and glucose uptake in adipocytes. These actions of SOCS3 are dependent on SOCS3 gene dosage. As TNFα induces SOCS3 expression and causes the degradation of IRS1 protein in adipocytes, the ameliorated insulin signaling in Socs3-deficient adipocytes is largely due to the prevention of TNFα-induced IRS1 and IRS2 degradation by the proteasomal pathway. Indeed, the IRS1 and -2 phosphorylation levels after insulin exposure are proportionate to their corresponding protein levels after TNFα treatment. Our observations suggest that SOCS3 is a negative regulator of insulin signaling that mediates cytokine- and hormone-induced insulin resistance in adipocytes. Previous data from our laboratory have established SOCS3 as a negative regulator of leptin signaling (13, 14). Therefore, SOCS3 is a single molecule that plays an important role in mediating both leptin and insulin resistance. The factors controlling the expression and function of SOCS3 may be important determinants of the pathogenesis of obesity and diabetes and could be viewed as...
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excellent targets for therapeutic intervention as well.

Consistent with our data, several reports (15–18) have shown that SOCS3 inhibits insulin signaling in transfected cell lines. These studies have proposed two mechanisms underlying SOCS3 inhibition of insulin signaling, which involve two functional domains of SOCS3, the central SH2 domain and the C-terminal SOCS box. Upon insulin stimulation, SOCS3 binds via its SH2 domain to the phosphorylated tyrosine 960 site of the insulin receptor and prevents the association of IRS1 with the insulin receptor, thereby blocking IRS1 phosphorylation and downstream insulin signaling (15, 16). In addition, Rui et al. (18) recently reported that SOCS3 can inhibit insulin signaling by targeting IRS1 and IRS2 for proteosomal degradation mediated via its SOCS box. In this model, SOCS3 functions as a adapter molecule that recruits IRS1 and IRS2 to form a complex including elongin BC and ubiquitin ligase, promoting their ubiquitination and subsequent degradation by the proteasome (18, 24). These two mechanisms are complementary and both may contribute to the action of SOCS3 on insulin signaling, depending on the cellular level of SOCS3. Our data demonstrate that insulin-stimulated IRS1 and IRS2 phosphorylation is increased in SOCS3-deficient adipocytes, whereas this increased phosphorylation is not accompanied by a significantly increased IRS protein level, suggesting that SOCS3 inhibits IRS phosphorylation without affecting IRS protein levels at basal cellular SOCS3 concentrations. However, upon cytokine (TNFα) or hormone (e.g., chronic insulin exposure) treatment, SOCS3 expression is induced, and this in turn causes IRS protein degradation and subsequently decreases IRS tyrosine phosphorylation, whereas SOCS3 deficiency can substantially block the action of TNFα on IRS protein and phosphorylation. Therefore, SOCS3 may require a threshold concentration to act on IRS protein degradation.

In addition to SOCS3, other members of the SOCS family have also been shown to inhibit insulin signaling. Kawazoe et al. (25) reported that SOCS1 physically associates with IRS1 proteins, and overexpression of SOCS1 reduces insulin-stimulated IRS1 phosphorylation. This action may involve SOCS1-induced degradation of IRS1, as evident in the study by Rui et al. (18). Like SOCS3, SOCS1 has also been shown to bind to and act on the insulin receptor to down-regulate insulin signaling (26). Moreover, evidence suggests that SOCS6 can also inhibit insulin signaling by binding to the insulin receptor, IRS2 or IRS4 (26, 27). Our data suggest that lack of SOCS3 does not completely block TNFα-mediated inhibition of IRS1 and -2 protein levels and phosphorylation. It is possible that TNFα induces other SOCS family members that account for the residual inhibitory effects on insulin signaling. However, a potential physiological role for other SOCS members in regulating insulin signaling requires further exploration.

Obesity is the leading risk factor for insulin resistance/type II diabetes (1). Recent studies (28, 29) showed that increased adiposity alters metabolic and endocrine functions of adipose tissue and results in an abnormal inflammatory response, including macrophage infiltration in adipose tissue. This in turn leads to elevated production of proinflammatory cytokines that may contribute to obesity-induced insulin resistance. Cytokines including TNFα (4, 5), IL6 (30), and IFNy (31) have been demonstrated to cause insulin resistance. TNFα has been shown to activate c-Jun N-terminal kinase causing serine phosphorylation of IRS1, which leads to impaired insulin signaling (32). The mechanisms whereby most cytokines such as IL6 and IFNy inhibit insulin signaling remain uncertain. We have observed that TNFα, IL6, and IFNy stimulate adipocyte SOCS3 expression and hypothesized that up-regulation of SOCS3 contributes to this cytokine-induced insulin resistance. We confirmed this hypothesis by testing the role of SOCS3 in mediating the inhibition by TNFα of IRS signaling. The extent to which this signaling pathway mediates the effects of other cytokines in insulin signaling needs to be further verified. A recent study (33) showed that IL6 impairs adipocyte insulin signaling by reducing IRS1 protein levels but without c-Jun N-terminal kinase activation or IRS1 serine phosphorylation. Whether SOCS3 mediates this IL6-induced reduction of IRS1 protein and inhibition of insulin signaling requires further investigation.

In addition to cytokines, many important metabolic hormones, such as insulin, GH, AT II, and glucocorticoids, have been demonstrated to induce insulin resistance in adipocytes (34–37). We demonstrate here that insulin, GH, and AT II are stimulators of SOCS3 expression in adipocytes, and therefore we propose that SOCS3 is a possible mediator of the insulin resistance induced by these hormones. Previous studies (22, 23) showed that chronic insulin treatment caused IRS1 protein degradation, which was mediated by the proteasomal degradation pathway. In agreement, our data illustrated that chronic treatment with insulin caused IRS1 protein degradation in adipocytes, whereas SOCS3 deficiency substantially blocked these effects, suggesting that SOCS3 may mediate this action of long term insulin treatment to impair insulin signaling. It has been recognized for years that insulin resistance-associated hyperinsulinemia aggravates insulin resistance in vivo. SOCS3 may play an important role in mediating hyperinsulinemia-induced insulin resistance in vivo.

The role of SOCS3 in insulin resistance is likely to be physiologically relevant. We found that SOCS3 mRNA is overexpressed in fat depots of obese/diabetic and DIO models. Consistent with our data, increased SOCS3 mRNA and protein levels have been reported previously (38) in adipose tissue from other obesity models including that due to ventromedial hypothalamic lesions and DIO rats. Recent studies from our group showed that mice with SOCS3 haploinsufficiency (SOCS3-/-) exhibit both a lean leptin-sensitive phenotype and have improved insulin sensitivity (39). This is likely to be a consequence of SOCS3 deficiency in both peripheral and central sites. However, to address the specific role of SOCS3 in adipocyte insulin signaling in vivo, we need to selectively delete and overexpress the SOCS3 gene in adipose tissue by using genetic approaches. Such studies are in progress.

In summary, our data demonstrate that the level of endogenous SOCS3 expression is an important negative regulator of insulin signaling and action in adipocytes. This role for SOCS3 appears to be exerted both in the basal state and in response to hormones and cytokines that have the capacity to induce insulin resistance, most likely through a mechanism whereby SOCS3 expression is induced with obesity and then targets IRS proteins for proteosomal degradation. This raises the apparent paradox that a molecule that is viewed as acting to limit the actions of cytokines by suppressing their capacity to signal may also be necessary for some of the adverse actions of cytokines. Because SOCS3 has the capacity to antagonize both leptin and insulin signaling, and its expression is increased in tissues of obese animals, it is in an excellent position to contribute to the development of both obesity and its metabolic complications.

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