

Overexpression of Suppressor of Cytokine Signaling 3 in Adipose Tissue Causes Local but Not Systemic Insulin Resistance

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In adipocytes, suppressor of cytokine signaling (SOCS)3 deficiency increases insulin-stimulated insulin receptor substrate (IRS)-1 and -2 phosphorylation, IRS-associated phosphatidylinositol 3 kinase activity, and insulin-stimulated glucose uptake. Moreover, SOCS3 is required for tumor necrosis factor- α full inhibition of insulin-stimulated IRS-1 and -2 phosphorylation, phosphatidylinositol 3 kinase activity, and glucose uptake. Whether SOCS3 also inhibits adipocyte insulin signaling in vivo and whether this action further affects systemic insulin sensitivity is not clear. We therefore generated a transgenic mouse (aP2-SOCS3 mouse) overexpressing SOCS3 in adipose tissue. Overexpression of SOCS3 in adipocytes decreases IRS1 protein levels and subsequent insulin-stimulated IRS-1 and -2 phosphorylation, decreases p85 binding to IRS-1, and leads to decreased insulin-stimulated glucose uptake in adipocytes. This impaired insulin signaling in adipose tissue of aP2-SOCS3 mice causes decreased lipogenesis and blocks insulin's antilipolytic action. However, because of decreased energy partitioning in adipose tissue, aP2-SOCS3 mice are resistant to diet-induced obesity and are protected against systemic insulin resistance caused by a high-fat diet. Therefore, overexpression of SOCS3 in adipocytes causes local adipocyte insulin resistance, but it is not sufficient to cause systemic insulin resistance. *Diabetes* 55:699–707, 2006

Insulin resistance, defined as a subnormal response of tissues to insulin action, is a central feature of the pathophysiology of type 2 diabetes, a prevalent metabolic disorder worldwide (1). Obesity is the most important factor contributing to insulin resistance. The physiological mechanisms linking obesity to insulin resistance are complex but, at least in part, involve the production of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 within adipose

tissue (1,2). However, the molecular mechanisms whereby cytokines inhibit insulin signaling are incompletely understood.

Many cytokines stimulate the expression of suppressor of cytokine signaling (SOCS) proteins (cytokine-inducible SH-2-containing protein [CIS] and SOCS1–SOCS7) (3). This family of proteins is characterized by their ability to cause feedback inhibition of cytokine signaling (3). Cytokines binding to their receptors activates janus kinase (JAK)–signal transducer and activator of transcription (STAT) signaling, which then induces SOCS gene expression. SOCS proteins in turn suppress cytokine signaling by directly inhibiting tyrosine-phosphorylated JAK or activated cytokine receptors (4) and/or by targeting signaling proteins for proteasomal degradation (5,6). In addition to cytokines, SOCS proteins can also antagonize metabolically important hormones such as leptin (7,8).

There is also evidence that the SOCS family of proteins can antagonize insulin signaling (9–12). Several reports have shown that SOCS3 can inhibit insulin signaling when overexpressed in transfected cell lines (9–11). SOCS3 has been reported to bind to the insulin receptor and prevent the coupling of IRS-1 with the insulin receptor, thereby inhibiting IRS-1 phosphorylation and downstream insulin signaling (9,10). In addition, SOCS3 has been shown to inhibit insulin signaling by targeting IRS-1 and -2 for proteasomal degradation (11). Several cytokines, including TNF- α , interleukin-6, and γ -interferon, stimulate SOCS3 expression in adipocytes (10,12), an insulin-sensitive cell type. SOCS3 expression is also increased in adipose tissue of mice with various syndromes of obesity (10,12). Using a SOCS3-deficient adipocyte model, we found that SOCS3 deficiency increases insulin-stimulated IRS-1 and -2 phosphorylation, enhances downstream phosphatidylinositol 3 kinase activity, and leads to increased insulin-stimulated glucose uptake. Moreover, SOCS3 deficiency limits TNF- α 's ability to inhibit insulin signaling, which is largely attributed to the suppression of TNF- α -induced IRS-1 and -2 protein degradation (12). Taken together, these data demonstrate that SOCS3 expression is a determinant of basal insulin signaling, a potential mediator of cytokine-induced insulin resistance, and a potential mediator of adipose insulin resistance in obesity.

Despite these observations, it is unclear whether SOCS3 is capable of inhibiting adipocyte insulin signaling in vivo. In addition, whether inhibition of insulin signaling in adipocytes by SOCS3 would affect systemic insulin sensitivity is not known. To address these important issues, we generated transgenic mice (aP2-SOCS3 mice) overexpressing SOCS3 specifically in adipose tissue.

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ELISA, enzyme-linked immunosorbent assay; FAS, fatty acid synthase; GPDH, glycerol-3-phosphate dehydrogenase; GTT, glucose tolerance test; HA, hemagglutinin; IRS, insulin receptor substrate; ITT, insulin tolerance test; PPAR, peroxisome proliferator-activated receptor; SOCS, suppressor of cytokine signaling; SREBP, sterol response element-binding protein; TNF- α , tumor necrosis factor- α .

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RESEARCH DESIGN AND METHODS

Rabbit SOCS3 antiserum was generated as previously described (8). Rabbit polyclonal anti-insulin receptor, -IRS-1, and -IRS-2; mouse monoclonal anti-phosphotyrosine 4G10; and polyclonal anti-p85 antibodies were kindly provided by Dr. Ronald Kahn's laboratory (Joslin Diabetes Center, Boston, MA) or purchased from Upstate (Lake Placid, NY). Protein A agarose, rabbit polyclonal anti-hemagglutinin (HA) antibody, and goat polyclonal anti-SOCS3 antibody was purchased from Santa Cruz Biotech (Santa Cruz, CA). NADH, NADPH, acetyl-CoA, and malonyl-CoA were all purchased from Sigma (St. Louis, MO). ^{14}C -deoxy-glucose was from Amersham Pharmacia Biotech (Piscataway, NJ).

Generation of transgenic aP2-SOCS3 mice. The 5.4-kb aP2 promoter/enhancer was used to drive fat-specific expression of the mouse SOCS3 transgene (13). Mouse SOCS3 cDNA with a 3' sequence encoding two HA tags and a sequence for the bovine GH polyadenylation signal were derived by PCR, using pcDNA 3.0 SOCS3 expression vector (7) as a template with a 5' primer sequence including an *Sma*I site and a Kozak consensus sequence, paired with a 3' primer sequence including an *Sal*I site and a stop codon. The PCR product was ligated into the *Sma*I and *Sal*I sites of the 5.4-kb aP2 promoter. The aP2-SOCS3-HA construct was linearized, using *Not*I and *Sal*I digestion and injected into pronuclei of fertilized eggs from FVB and C57BL/6 mice. Two independent lines with SOCS3 overexpression in adipose tissue were obtained.

Animal study and metabolic analysis. This study was approved by the institutional animal care and use committee of the Beth Israel Deaconess Medical Center. Animals were weaned at age 3 weeks and housed individually in our animal facilities on a 12-h light/dark cycle. We placed 5-week-old aP2-SOCS3 transgenic mice and their nontransgenic littermates on a high-fat/high-sucrose diet (TD-88137; Harlan Tekland, Madison, WI) or standard chow diet with water ad libitum, and body weights and food intake were measured weekly. Resting metabolic rate and locomotor activity were measured, using a comprehensive laboratory animal monitoring system (CLAMS; Columbus Instruments, Columbus, OH) (14). Glucose tolerance tests (GTTs) and insulin tolerance tests (ITTs) were performed as previously described (8). At the end of study, all mice were killed by CO_2 , and blood was obtained via cardiac puncture for insulin, leptin, adiponectin, TNF- α , and other measurements of metabolic parameters. Fat pads were dissected, immediately weighed, frozen in liquid nitrogen, and stored at -80°C . For adipose tissue histology, subcutaneous fat pads were fixed in Bouin's solution (Sigma), imbedded in paraffin for multiple sectioning, and stained with hematoxylin/eosin. The average area size of adipocytes was measured, using ImageJ image analysis software (available from <http://rsb.info.nih.gov/ij/>).

Blood glucose was measured with a OneTouch Ultra glucose meter (Lifescan, Mulpitas, CA). Serum insulin and leptin levels were measured, using rat insulin enzyme-linked immunosorbent assay (ELISA) and mouse leptin ELISA kits (Crystal Chem, Downers Grove, IL), respectively. Serum adiponectin and TNF- α levels were determined, using a mouse adiponectin ELISA kit (Linco Research, St. Charles, MO) and a mouse TNF- α ELISA kit (R&D Systems, Minneapolis, MN). Serum triglycerides, free fatty acids, and cholesterol levels were measured, using an L-type TG H kit, NEFA C kit, and Cholesterol E kit (Wako Chemicals, Richmond, VA), respectively.

Fatty acid synthase (FAS) and glycerol-3-phosphate dehydrogenase (GPDH) activity was determined spectrophotometrically in crude cytosolic extracts of mouse adipose tissue (15). Lipolysis assay was conducted as previously described (16).

Total RNA extraction, quantitative RT-PCR, and standard RT-PCR. Adipocyte total RNA was extracted, using an RNeasy Mini Kit (Qiagen, Valencia, CA). Mouse adipocyte mRNA expression was quantitatively measured, using an Mx 4000 Multiplex quantitative PCR system (Stratagene, Cedar Creek, TX) with a Brilliant Single-Step quantitative RT-PCR kit (Stratagene), as previously described (17). The sequence of primers and probes for the mouse adipocyte genes will be provided on request.

For regular RT-PCR detection of the SOCS3 transgene, total RNA was reverse transcribed to the first-strand cDNA with a primer designed in the HA-tag region, and it was then amplified by PCR.

Ex vivo and in vivo insulin signaling studies and glucose transport in isolated adipocytes. For ex vivo insulin signaling experiments, epididymal fat pads were dissected, minced, and cultured in DMEM medium containing 1% fetal bovine serum and 1% BSA for a 4-h recovery, and then they were incubated in Krebs-Ringer bicarbonate buffer containing 1% BSA with or without 100 nmol/l insulin for 5 min. Adipose tissue was then harvested for immunoprecipitation and immunoblotting analysis of insulin signaling protein, as previously described (12).

In vivo insulin signaling experiments were performed on mice after a 16-h fast. Mice were intravenously injected with 10 units/kg body wt of human insulin (Eli Lilly) or saline. At 5 min after injection, epididymal fat pads were

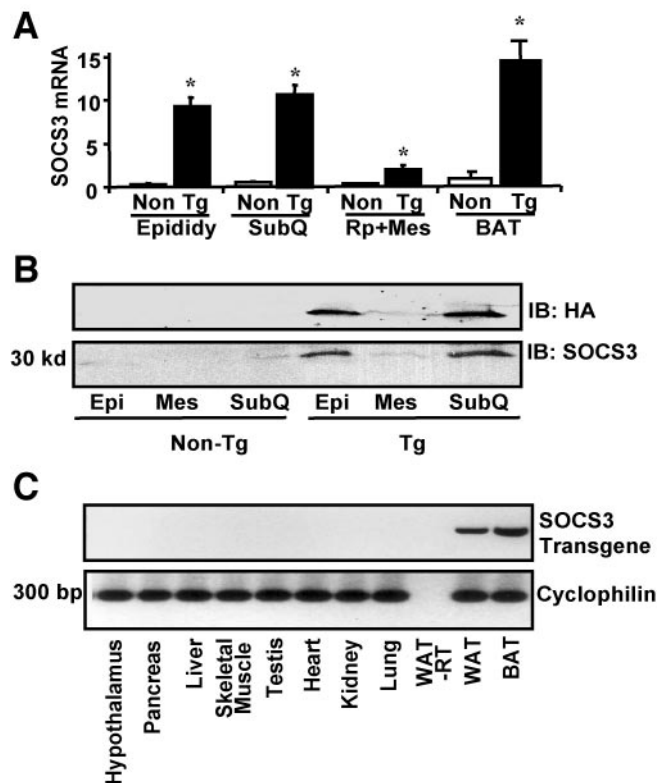


FIG. 1. Generation of transgenic (Tg) mice overexpressing SOCS3 specifically in adipose tissue. **A:** SOCS3 mRNA levels are increased in fat depots of aP2-SOCS3 transgenic mice. Adipose tissue total RNA was isolated and SOCS3 mRNA measured using real-time RT-PCR as described in the RESEARCH DESIGN AND METHODS. * $P < 0.05$ vs. nontransgenic control ($n = 3$ per group). **B:** SOCS3 protein levels are increased in fat depots of aP2-SOCS3 mice. Adipose tissue lysates were used for immunoblotting with antibodies against SOCS3 and HA tag, respectively. **C:** SOCS3 transgene mRNA is specifically expressed in adipose tissue. Total RNA isolation and RT-PCR were performed as described in the RESEARCH DESIGN AND METHODS. BAT, brown adipose tissue; Epi, epididymal; Epi, epididymal; Mes, mesenteric; Rp, retroperitoneal; SubQ, subcutaneous; WAT, white adipose tissue.

dissected and frozen in liquid nitrogen for immunoprecipitation and immunoblotting analysis of insulin signaling proteins.

Adipocytes were isolated from epididymal fat pads by collagenase digestion (15), and glucose transport assays were conducted as previously described (18).

Statistical analysis. All data are the means \pm SE. Data were evaluated for statistical significance by one-way ANOVA, and significantly different group means were then separated by the least significant difference test, using SPSS (Chicago, IL). $P < 0.05$ is considered significant.

RESULTS

Generation of transgenic mice overexpressing SOCS3 specifically in adipose tissue. We generated a transgenic mouse (aP2-SOCS3 mouse) overexpressing SOCS3 specifically in adipose tissue under control of the 5.4-kb aP2 promoter/enhancer. Two independent lines overexpressing the SOCS3 transgene were obtained. aP2-SOCS3 mice were born at the expected Mendelian frequency and showed normal behavior and fertility. All mice used in this study were hemizygous for the SOCS3 transgene or were their nontransgenic littermates on the inbred FVB strains.

To assess the overexpression of the SOCS3 transgene in adipose tissue, we measured the SOCS3 mRNA by real-time RT-PCR. Figure 1A shows that SOCS3 mRNA expression was increased in epididymal, subcutaneous, and brown fat by >10 -fold, whereas SOCS3 mRNA was in-

creased to a lesser extent in mesenteric and retroperitoneal fat. Similar results were observed when SOCS3 protein levels were measured by immunoblotting (Fig. 1B). Using an anti-SOCS3 antibody, the SOCS3 protein was barely detectable in epididymal and subcutaneous fat and not detectable in mesenteric fat of nontransgenic littermates. However, the SOCS3 protein was significantly increased in epididymal and subcutaneous fat and detectable in mesenteric fat of aP2-SOCS3 mice (Fig. 1B, lower panel). Using an anti-HA tag antibody, the transgene-encoded protein was detectable in fat depots of transgenic mice but not in their nontransgenic littermates (Fig. 1B, upper panel). Moreover, using RT-PCR, we detected SOCS3 transgene mRNA exclusively in white and brown adipose tissues at 25 cycles of PCR (Fig. 1C).

Phenotypic characterization of aP2-SOCS3 mice. We first monitored the body weight development of transgenic and nontransgenic littermates fed either a standard chow or high-fat/high-sucrose diet. Body weights of aP2-SOCS3 and nontransgenic mice on a chow diet slightly diverged after 10 weeks, with aP2-SOCS3 mice gaining less weight compared with nontransgenic littermates (Fig. 2A). When challenged with a high-fat/high-sucrose diet, the aP2-SOCS3 mice exhibited resistance to diet-induced weight gain, with an 11% reduction compared with nontransgenic littermates at age 18 weeks (Fig. 2A). Consistent with the body weight, decreased fat mass was also observed in aP2-SOCS3 mice. Although transgenic mice on a chow diet showed a slightly decreased fat mass, aP2-SOCS3 mice on a high-fat diet exhibited a significantly decreased mass of epididymal, mesenteric, and subcutaneous fat, with a 40% reduction of total fat mass, compared with nontransgenic littermates (Fig. 2B and C). In addition, histological examination of white adipose tissue revealed that fat tissue from aP2-SOCS3 mice on chow or high-fat diets contained smaller adipocytes than their nontransgenic littermates (Fig. 2D). These data show that aP2-SOCS3 mice are resistant to diet-induced obesity.

We also measured food intake and parameters of energy expenditure in these aP2-SOCS3 mice. On a chow diet, cumulative food intake over 40 h was decreased by 29% in transgenic mice compared with wild-type littermates (Fig. 3A). Locomotor activity was monitored during a 24-h light/dark cycle. aP2-SOCS3 mice exhibited a tendency toward increased nocturnal activity, although the increase was not statistically significant (Fig. 3B). The metabolic rate was determined by measuring oxygen consumption and carbon dioxide production. Analysis of oxygen consumption revealed no alteration in transgenic animals (Fig. 3C). However, the transgenic mice showed a trend toward decreased respiratory quotient (respiratory exchange ratio) compared with wild-type mice, although this decrease did not reach statistical significance (Fig. 3D).

We assessed the blood metabolic profile of aP2-SOCS3 mice. Serum triglycerides were decreased by 50% in transgenic mice on a high-fat diet, although free fatty acids and cholesterol levels were unchanged in these mice (Table 1). Consistent with the decreased fat mass, transgenic mice on a high-fat diet showed lower serum leptin levels (Table 1). Although serum TNF- α did not change, serum adiponectin levels were increased by 80% in transgenic mice on a high-fat diet compared with nontransgenic littermate controls (Table 1). These data suggest that along with resistance to a high-fat diet, aP2-SOCS3 mice show improved metabolic parameters.

Although aP2-SOCS3 mice on a chow diet did not show

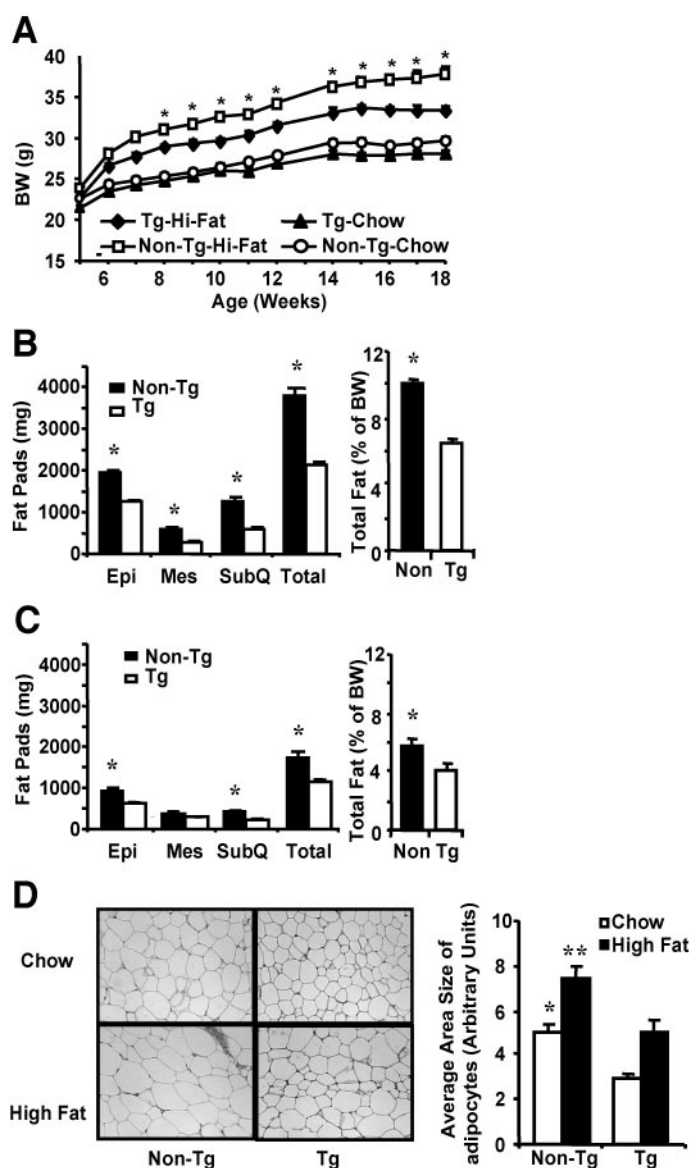


FIG. 2. Body weight development and fat mass of aP2-SOCS3 mice. **A:** Body weight development of male aP2-SOCS3 mice on a high-fat/high-sucrose diet or chow diet. * $P < 0.05$ vs. transgenic (Tg) high-fat diet ($n = 8$ per group). **B and C:** Decreased fat mass in aP2-SOCS3 mice. Mice ($n = 8$ per group) were killed at age 22 weeks. * $P < 0.01$ vs. transgenic high-fat diet (**B**); * $P < 0.05$ vs. transgenic chow (**C**). **D:** Decreased adipocyte size in white adipose tissue of aP2-SOCS3 mice. Epididymal (Epi) fats were dissected and used for histology. Average area size of adipocytes on histological slides was measured, using ImageJ (right panel). * $P < 0.05$ vs. transgenic chow; ** $P < 0.05$ vs. transgenic high-fat diet. BW, body weight; Hi, high; Mes, mesenteric; SubQ, subcutaneous.

any alterations in fed or fasting glucose and insulin levels, transgenic mice on a high-fat diet exhibited lower fasting glucose and insulin levels and lower fed insulin levels compared with nontransgenic mice (Table 1). These data suggest that aP2-SOCS3 mice are protected against insulin resistance associated with diet-induced obesity. To further confirm this, we performed glucose and ITTs on mice fed chow or high-fat diets. GTTs and ITTs showed no difference between aP2-SOCS3 and nontransgenic mice on a chow diet (Fig. 4A and B). However, the hyperglycemic response to intraperitoneal injection of glucose during the GTT was attenuated in transgenic mice on a high-fat diet compared with controls (Fig. 4C). Consistent with this

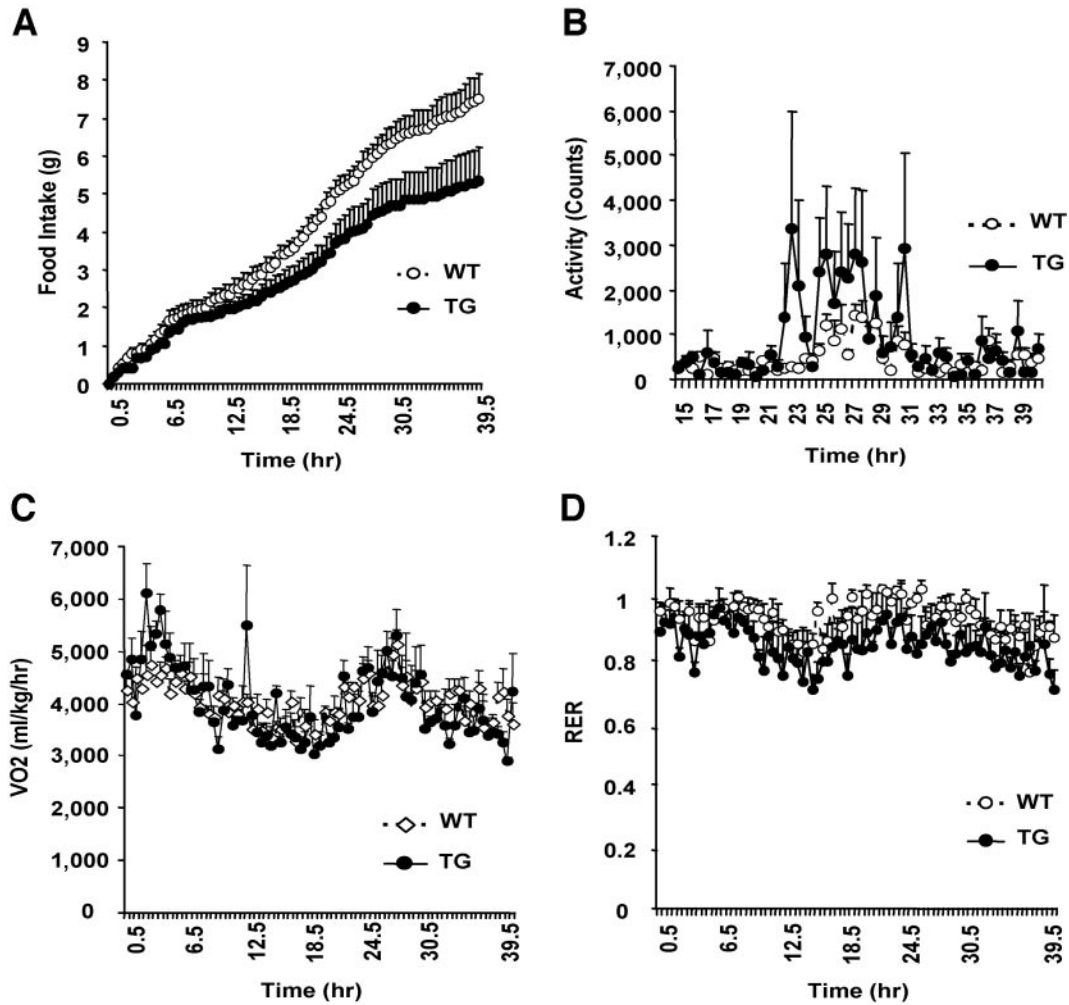


FIG. 3. Food intake, locomotor activity, and metabolic rate of aP2-SOCS3 mice. Animals ($n = 4$ per group) on a chow diet were individually housed. *A*: Food intake of 16-week-old male aP2-SOCS3 mice on a chow diet measured over 40 h. *B*: Locomotor activity of 16-week-old aP2-SOCS3 mice monitored over 24 h in the comprehensive lab animal monitoring system. VO_2 (*C*) and respiratory quotient (respiratory exchange ratio) (*D*) of 16-week-old aP2-SOCS3 mice measured over 40 h, using an open-circuit Oxymax system. TG, transgenic; WT, wild type.

finding, the hypoglycemic response to intraperitoneal injection of insulin during an ITT was greater in transgenic mice on a high-fat diet (Fig. 4D). Therefore, along with resistance to diet-induced obesity, aP2-SOCS3 mice are protected against insulin resistance associated with diet-induced obesity.

aP2-SOCS3 mice show local insulin resistance in adipose tissue. To determine the physiological consequence of adipocyte-specific overexpression of SOCS3 on adipocyte insulin action, we examined adipocyte insulin signaling in vivo. Mice were fasted overnight and injected with human insulin (10 units/kg body wt i.v.) or saline. As

TABLE 1
Metabolic parameters in aP2-SOCS3 transgenic mice

	Chow		High-fat diet	
	Nontransgenic	Transgenic	Nontransgenic	Transgenic
Fasting glucose (mg/dl)	111.75 ± 7.52	106.67 ± 15.10	141.00 ± 6.10	118.57 ± 8.14*
Fasting insulin (ng/ml)	0.26 ± 0.06	0.20 ± 0.02	0.49 ± 0.06	0.29 ± 0.08*
Fed glucose (mg/dl)	176.57 ± 9.28	159.80 ± 15.09	198.85 ± 8.05	179.28 ± 5.95
Fed insulin (ng/ml)	0.92 ± 0.20	0.91 ± 0.22	1.76 ± 0.30	1.08 ± 0.25*
Leptin (ng/ml)	5.05 ± 0.72	3.62 ± 0.44	16.46 ± 1.82	10.96 ± 1.68*
TNF- α (pg/ml)	39.50 ± 1.13	40.00 ± 1.21	41.37 ± 1.08	44.12 ± 2.68
Adiponectin (μ g/ml)	11.91 ± 0.65	16.55 ± 0.73	9.89 ± 0.51	16.05 ± 0.72*
Triglyceride (mg/dl)	132.60 ± 19.09	98.40 ± 32.25	202.89 ± 37.83	100.25 ± 15.77*
Cholesterol (mg/dl)	189.38 ± 5.10	182.61 ± 10.03	363.67 ± 11.22	330.64 ± 10.76
Free fatty acids (mmol/l)	0.48 ± 0.05	0.59 ± 0.02	0.61 ± 0.06	0.66 ± 0.05

Data are means ± SE ($n = 8$). All measurements were conducted as described in RESEARCH DESIGN AND METHODS. * $P < 0.05$ vs. high-fat diet nontransgenic.

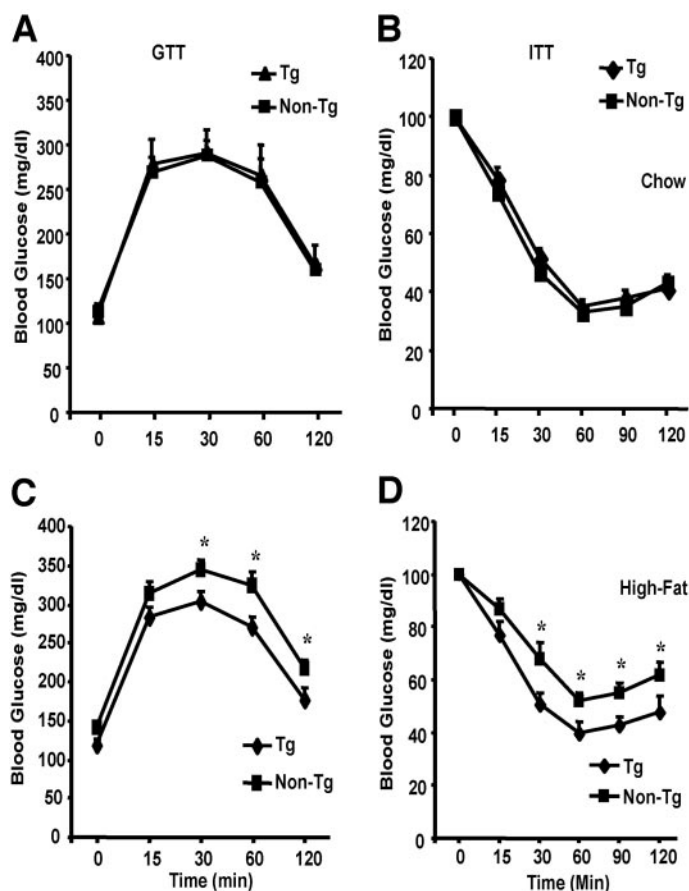


FIG. 4. GTTs and ITTs in aP2-SOCS3 and control mice. GTTs for standard chow (A) and high-fat diet (B) were performed on awake mice after a 16-h fast, whereas ITTs for standard chow (C) and high-fat diet (D) were performed on awake mice after a 6-h fast. Mice were injected intraperitoneally with 1 g/kg body wt glucose for the GTT or 1 unit/kg body wt human insulin for the ITT. * $P < 0.05$ vs. high-fat-fed nontransgenic mice ($n = 8$ per group). TG, transgenic.

shown in Fig. 5A, insulin-stimulated phosphorylation of the insulin receptor did not change in adipose tissue of aP2-SOCS3 mice. Although insulin injection markedly stimulated phosphorylation of IRS-1 in adipose tissue of nontransgenic mice, this signaling event was attenuated in aP2-SOCS3 mice (Fig. 5C, upper panels). The decreased insulin-stimulated phosphorylation of IRS-1 in transgenic mice was accompanied by reduced IRS-1 protein levels (Fig. 5B). Quantitation of the blots showed a 50% reduction of both IRS-1 phosphorylation and protein levels in adipose tissue of aP2-SOCS3 mice (Fig. 5B and C), suggesting that the IRS-1 degradation caused by SOCS3 overexpression may account for the decreased IRS-1 phosphorylation and impaired insulin signaling in adipose tissue of aP2-SOCS3 transgenic mice. This is consistent with data we and others have observed that SOCS3 targets IRS proteins for proteasomal degradation (11,12). Similar results were observed in IRS-2 phosphorylation and protein levels (Fig. 5D).

To determine whether the defect of insulin signaling caused by overexpression of SOCS3 in adipocytes is intrinsic to adipose tissue or caused by the systemic metabolic milieu, we examined insulin signaling in primary-cultured adipose tissue derived from aP2-SOCS3 mice and nontransgenic controls. Figure 6A shows that insulin-stimulated phosphorylation of insulin receptor was

slightly decreased in adipose tissue of transgenic mice. Moreover, insulin-stimulated phosphorylation of IRS-1 was decreased in transgenic mice, with a correspondingly decreased IRS-1 protein level (Fig. 6B). Furthermore, insulin-stimulated association of p85 with IRS-1 was reduced in transgenic mice (Fig. 6D). A similar result was observed in insulin-stimulated phosphorylation of IRS-2 (Fig. 6C). To determine the physiological consequence of this impaired insulin signaling on downstream insulin actions, we measured insulin-stimulated glucose uptake in isolated adipocytes from aP2-SOCS3 mice and nontransgenic controls. Insulin-stimulated glucose uptake was markedly decreased in isolated adipocytes of transgenic mice at different doses of insulin from 0.1–100 nmol/l, although basal glucose transport was not altered in transgenic mice (Fig. 6E).

Decreased lipogenesis and increased lipolysis in aP2-SOCS3 mice. To investigate the physiological consequence of impaired insulin signaling on adipocyte lipid metabolism, we measured the activity of two lipogenic enzymes, FAS and GPDH. The expression and activity of FAS, a key enzyme involved in de novo lipogenesis, have been demonstrated to be upregulated by insulin signaling (19). Figure 7A and B shows that FAS and GPDH activity were decreased by 40–50% in transgenic mice on a high-fat diet compared with nontransgenic controls. On the other hand, because insulin signaling has an antilipolytic effect, we determined whether the impaired insulin signaling caused by SOCS3 overexpression would attenuate insulin's antilipolytic action in the adipose tissue of transgenic mice. Figure 7C shows that although isoproterenol stimulated lipolysis by 3.5-fold in adipose tissue from nontransgenic mice on a high-fat diet, pretreatment with 100 nmol/l insulin inhibited isoproterenol-stimulated lipolysis by 40%. However, pretreatment of adipose tissue from aP2-SOCS3 mice with 100 nmol/l insulin was not able to inhibit isoproterenol-stimulated lipolysis. Similar results were observed in mice fed a chow diet (Fig. 7D). These data suggest that the impaired insulin signaling caused by SOCS3 overexpression inhibits de novo lipogenesis and blocks insulin's antilipolytic effects, and these changes may contribute to impaired energy partitioning and decreased fat mass in aP2-SOCS3 mice.

Gene expression in adipose tissue of aP2-SOCS3 mice. Using real-time RT-PCR, we measured the expression of genes involved in adipocyte differentiation, glucose and lipid metabolism, and secretory factors in white adipose tissue (Table 2). Expression of several genes whose expression is characteristic of mature adipocytes such as peroxisome proliferator-activated receptor (PPAR) γ , aP2, and GLUT-4 did not change in aP2-SOCS3 mice, suggesting that adipose tissue development was not affected by overexpression of SOCS3. FAS and sterol response element-binding protein 1c (SREBP1c) mRNA, encoding two genes involved in lipogenesis, were decreased in white adipose tissue of transgenic mice. This is consistent with the decreased FAS and GPDH activity in adipose tissue of aP2-SOCS3 mice and may contribute to the decreased de novo lipogenesis in transgenic mice. In agreement with the serum adiponectin protein level, adiponectin mRNA was also increased in white fat of transgenic mice. Increased adiponectin protein levels may increase the insulin sensitivity of skeletal muscles and liver and contribute to increased systemic insulin sensitivity.

In addition, we measured the expression of genes that act on fatty acid oxidation and utilization in skeletal

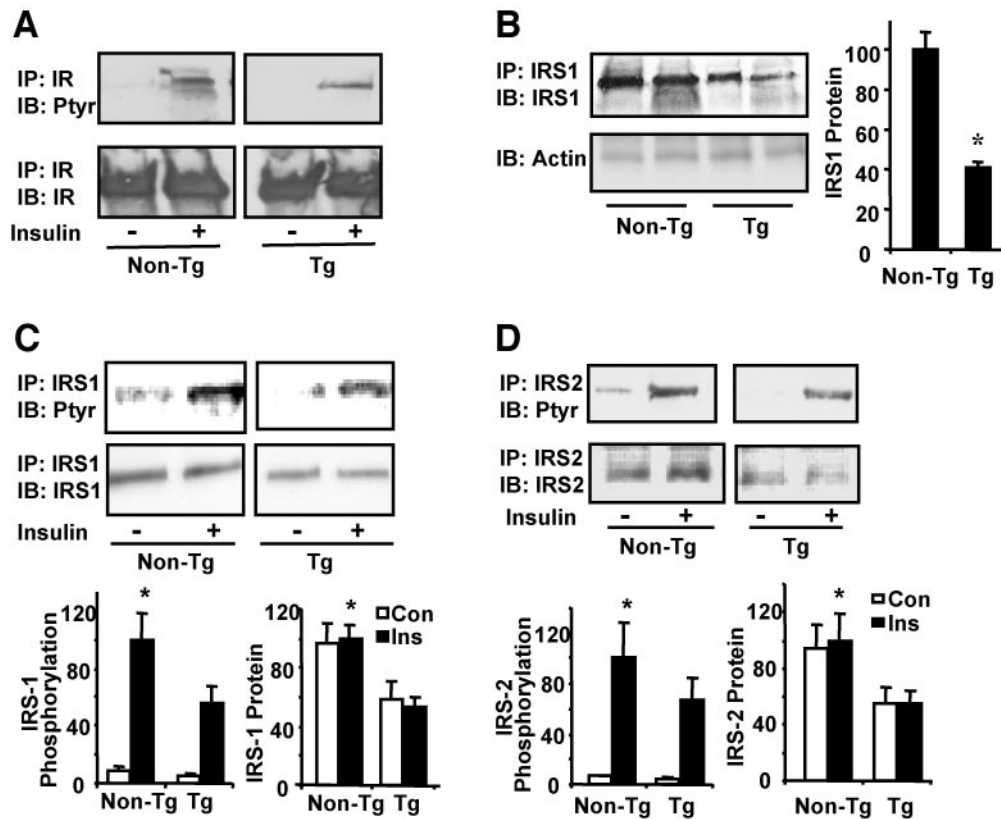


FIG. 5. Insulin signaling is impaired in adipose tissue of aP2-SOCS3 mice in vivo. The 16-week old mice on a chow diet were fasted for 16 h and injected intravenously with 10 units/kg body wt human insulin or saline. At 5 min after injection, epididymal fat pads were dissected and saved for immunoprecipitation (IP) and immunoblotting (IB) analysis of insulin signaling molecules. Adipose tissue lysates were subjected to immunoprecipitation with anti-insulin receptor, anti-IRS-1, or anti-IRS-2 antibodies and immunoblotted with anti-phosphotyrosine (Ptyr) antibodies or antibodies as indicated. Blots were quantified using densitometry. * $P < 0.05$ vs. transgenic (Tg) insulin ($n = 3$).

muscle. The mRNA levels of CPT1 (carnitine palmitoyl transferase-1), ACO (fatty acyl-CoA oxidase), and PPAR α were increased in skeletal muscle of aP2-SOCS3 mice on a high-fat diet. The increased expression of fatty acid oxidative genes in skeletal muscle may be caused by the increased adiponectin levels in fat and circulation.

DISCUSSION

We have previously observed in vitro that SOCS3 deficiency increases insulin-stimulated IRS-1 and -2 phosphorylation and enhances downstream phosphatidylinositol 3 kinase activity, leading to increased insulin-stimulated glucose uptake in adipocytes. Moreover, lack of SOCS3 blocks TNF- α 's inhibition of insulin signaling in adipocytes, which is largely attributed to the suppression of TNF- α -induced IRS-1 and -2 protein degradation (12). We therefore hypothesized that adipocyte-specific overexpression of SOCS3 would cause impaired insulin signaling in adipose tissue in vivo. To test this hypothesis, we generated transgenic mice that overexpress SOCS3 specifically in adipose tissue. We demonstrate that overexpression of SOCS3 in adipocytes decreases IRS protein levels and insulin-stimulated IRS phosphorylation and causes decreased insulin-stimulated glucose uptake in adipocytes. This is consistent with data we and others have previously observed in vitro (11,12). Therefore, overexpression of SOCS3 in adipocytes causes insulin resistance in adipose tissue in vivo.

Although adipocyte-specific overexpression of SOCS3 induces insulin resistance in adipose tissue, whole-body

glucose homeostasis and systemic insulin sensitivity are not impaired. Instead, aP2-SOCS3 mice have improved systemic insulin sensitivity and reduced adiposity when exposed to a high-fat diet. How can this paradox be explained? aP2-SOCS3 mice have reduced adipocyte cell size and total body fat mass. It has been recognized for years that excessive fat (obesity) is causally associated with insulin resistance and is the leading risk for the development of type 2 diabetes (20–22). Likewise, leanness is generally associated with enhanced systemic insulin sensitivity. There are several interesting examples wherein leanness is associated with increased systemic insulin actions, despite insulin resistance within the adipocyte. The most striking example is the adipose-specific insulin receptor knockout (FIRKO) mouse (23). These mice have reduced fat mass and are protected against systemic insulin resistance associated with obesity, despite a complete loss of insulin action and abolished insulin-stimulated glucose uptake in adipocytes (23). A similar phenotype has also been observed in a transgenic mouse overexpressing the transmembrane TNF- α specifically in adipose tissue (18). These transgenic mice have decreased adipocyte cell size and whole-body adipose mass. Although overexpression of transmembrane TNF- α in adipocytes causes local insulin resistance, these mice show a tendency to improved systemic insulin sensitivity in GTT and ITT studies (18). Another example is the heterozygous PPAR γ -deficient mouse model, which shows protection against high-fat diet-induced adipocyte hypertrophy and systemic insulin resistance, although PPAR γ

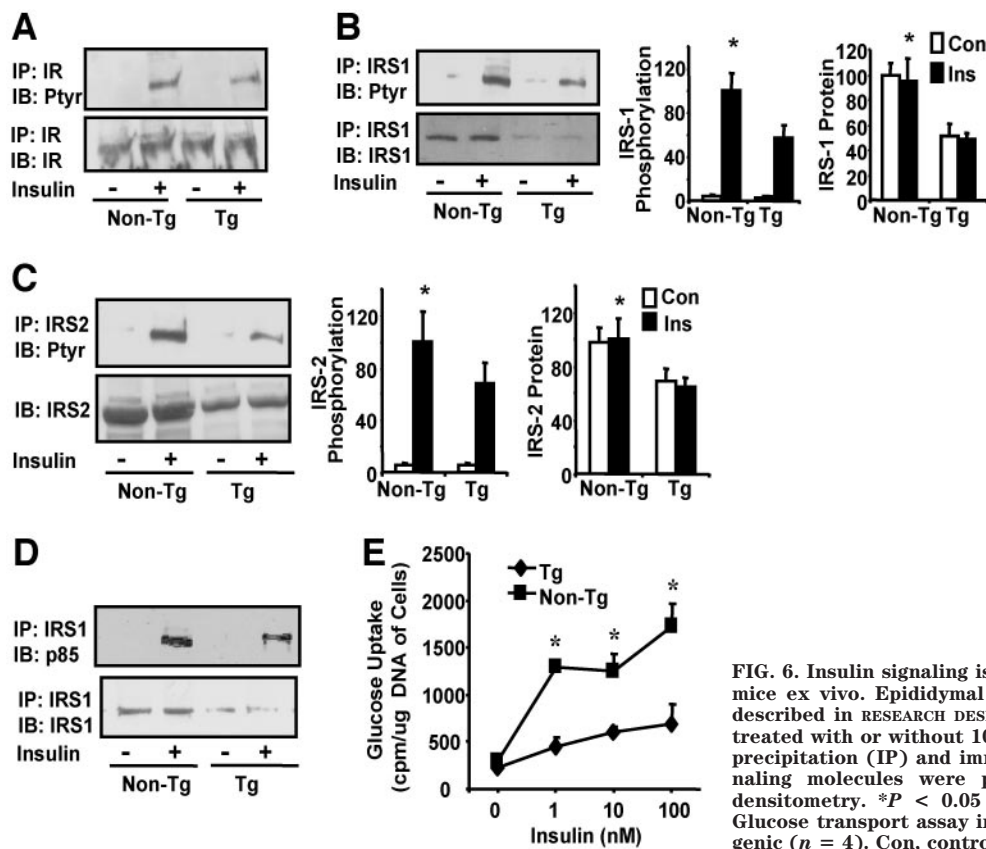


FIG. 6. Insulin signaling is impaired in adipose tissue of aP2-SOCS3 mice ex vivo. Epididymal fat pads were dissected and cultured as described in RESEARCH DESIGN AND METHODS. *A–D*: Adipose tissue was treated with or without 100 nmol/l insulin (Ins) for 5 min. Immunoprecipitation (IP) and immunoblotting (IB) analyses of insulin signaling molecules were performed. Blots were quantified using densitometry. * $P < 0.05$ vs. transgenic (Tg) insulin ($n = 3$). *E*: Glucose transport assay in isolated adipocytes. * $P < 0.05$ vs. transgenic ($n = 4$). Con, control; P_{tyr}, phosphotyrosine.

deficiency is expected to decrease insulin sensitivity (24). Taken together, these studies support a notion that a moderate reduction in adipocyte size and fat mass can, under certain circumstances, exert a dominant effect on systemic insulin sensitivity, overcoming local adipocyte

insulin resistance and decreased glucose uptake in adipose tissue.

Indeed, regarding glucose uptake in vivo, adipose tissue only accounts for ~10% of whole-body glucose uptake, whereas muscle accounts for 80–90% (1). Therefore, insu-

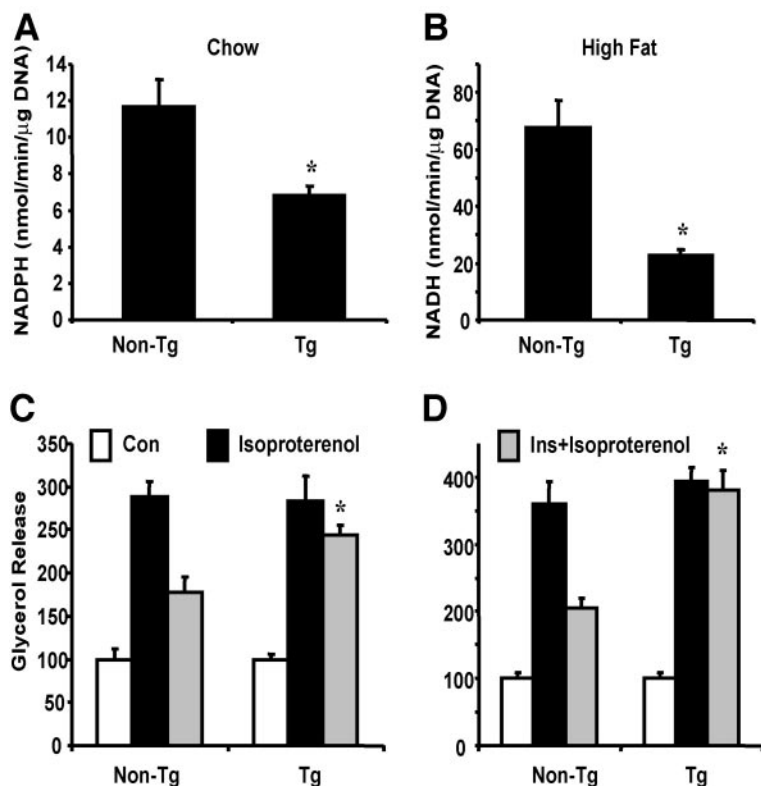


FIG. 7. Overexpression of SOCS3 in adipocytes inhibits lipogenesis and blocks insulin's antilipolytic action. FAS (*A*) and GPDH (*B*) activity is decreased in adipose tissue of aP2-SOCS3 mice. * $P < 0.05$ vs. nontransgenic ($n = 8$). *C* and *D*: Overexpression of SOCS3 in adipocytes blocks insulin's antilipolytic action. Glycerol released into the medium was used as an indicator of lipolysis. * $P < 0.05$ vs. nontransgenic insulin plus isoproterenol ($n = 8$). Con, control; Tg, transgenic.

TABLE 2
Gene expression in adipose tissue and skeletal muscle of aP2-SOCS3 mice

	Chow		High-fat diet	
	Nontransgenic	Transgenic	Nontransgenic	Transgenic
White adipose tissue				
FAS	6.22 ± 1.30	6.29 ± 0.95	12.05 ± 0.33	6.72 ± 0.34*
SREBP1C	4.39 ± 0.65	4.61 ± 0.96	8.45 ± 0.70	4.76 ± 0.60*
PPAR γ	7.82 ± 1.21	8.91 ± 1.26	6.37 ± 0.97	5.59 ± 1.30
aP2	11.37 ± 2.28	12.01 ± 0.90	10.84 ± 0.56	11.24 ± 0.73
GLUT4	6.53 ± 1.05	7.45 ± 1.14	4.06 ± 0.77	4.78 ± 0.48
UCP2	1.66 ± 0.15	1.60 ± 0.18	2.98 ± 0.40	3.06 ± 0.31
Adiponectin	8.29 ± 1.63	14.42 ± 2.15	4.58 ± 1.14	9.77 ± 1.36*
TNF- α	7.27 ± 1.50	6.99 ± 0.74	19.30 ± 1.77	17.1 ± 2.61
Brown adipose tissue				
UCP1	1.00 ± 0.06	1.03 ± 0.10	1.18 ± 0.09	1.20 ± 0.26
UCP2	0.41 ± 0.07	0.50 ± 0.08	0.87 ± 0.12	0.83 ± 0.15
UCP3	1.12 ± 0.05	1.06 ± 0.09	1.19 ± 0.09	1.12 ± 0.05
PGC1 α	0.42 ± 0.09	0.44 ± 0.06	0.51 ± 0.19	0.39 ± 0.13
PPAR α	1.10 ± 0.10	1.11 ± 0.09	1.45 ± 0.13	1.32 ± 0.08
Skeletal muscle				
ACO	1.18 ± 0.06	1.52 ± 0.03	1.20 ± 0.09	1.81 ± 0.07*
CPT1	0.66 ± 0.05	0.96 ± 0.10	0.68 ± 0.04	1.04 ± 0.05*
PPAR α	0.71 ± 0.06	1.08 ± 0.14	0.73 ± 0.04	1.21 ± 0.11*
UCP2	0.59 ± 0.07	0.58 ± 0.06	1.06 ± 0.09	1.13 ± 0.11
UCP3	0.74 ± 0.07	0.91 ± 0.07	1.02 ± 0.06	1.38 ± 0.08

Data are means \pm SE ($n = 8$). All measurements were conducted as described in RESEARCH DESIGN AND METHODS. * $P < 0.05$ vs. high-fat diet nontransgenic. ACO, acyl-CoA oxidase; CPT1, carnitine palmitoyl transferase-1; UCP, uncoupling protein.

lin resistance in adipose tissue may not necessarily cause systemic insulin resistance. In contrast, molecules secreted by adipose tissue may play a more important role in systemic insulin sensitivity than adipose tissue's direct capability for glucose disposal. These secretory molecules can act on muscle and liver to either enhance or decrease glucose uptake, and they thereby override the capacity for glucose uptake by adipose tissue itself. Adipocyte size and fat mass may determine the levels of molecule secretion, and, as we have stated above, a moderate reduction in adipocyte size and fat mass may improve systemic insulin sensitivity by increasing the secretion of molecules such as adiponectin.

How might adipocyte insulin resistance induced by SOCS3 confer protection against adipocyte hypertrophy? Adipocyte hypertrophy characterized by increased storage of triglyceride may result from increased de novo lipogenesis and/or decreased lipolysis. Insulin, a potent lipogenic and antilipolytic hormone, plays a crucial role in triglyceride storage and adipocyte hypertrophy through coordinated control over lipogenesis and lipolysis. Insulin stimulates lipogenesis by inducing the expression and activity of key lipogenic enzymes, including FAS (25). Moreover, insulin stimulation of FAS is mediated by SREBP1c (26). Indeed, one consequence of impaired insulin signaling in adipocytes of aP2-SOCS3 mice is the downregulation of FAS expression and activity as well as reduced SREBP1c expression. On the other hand, insulin inhibits lipolysis by activating phosphodiesterase 3B, mediated by the downstream insulin signal protein kinase B (27). We also observed an attenuation of the antilipolytic action of insulin in adipose tissue of aP2-SOCS3 mice. Therefore, decreased lipogenesis coupled with increased lipolysis, both caused by impaired insulin signaling, may contribute to the protection against adipocyte hypertrophy in aP2-SOCS3 mice on a high-fat diet. These data indicate

that insulin signaling in adipose tissue is permissive for increased energy storage in adipose tissue in response to high-fat diet and that SOCS3 expression in fat can resist energy storage.

The limited flow of energy to adipose tissue in aP2-SOCS3 mice may therefore regulate whole-body energy homeostasis through a feedback circuit. Indeed, our data show that food intake is decreased in aP2-SOCS3 mice, whereas physical activity is slightly increased in these transgenic mice. This suggests that energy equilibrium is reestablished in these transgenic mice to accommodate the restricted deposition of energy in adipocytes. Although the mechanisms underlying this deficit of energy balance are not entirely clear, the alteration of adipocyte insulin signaling and lipid metabolism caused by SOCS3 overexpression in adipocytes most likely affects systemic energy balance through molecules secreted by adipocytes.

Adipocyte hypertrophy alters the metabolic and endocrine function of adipose tissue and results in abnormal secretion of hormones and cytokines, one of which is the adipocyte-derived hormone adiponectin. Adiponectin has been shown to increase systemic insulin sensitivity by increasing fatty acid oxidation and reducing triglyceride content in muscle as well as by inhibiting gluconeogenesis in liver (28,29). Expression and circulating levels of adiponectin correlate inversely with obesity and insulin resistance (30). Indeed, aP2-SOCS3 mice have elevated adiponectin expression in adipose tissue and a corresponding increase in serum adiponectin levels. Increased adiponectin levels may act on muscle and liver to enhance insulin action in these tissues and may therefore account, at least in part, for the improved systemic insulin sensitivity of aP2-SOCS3 mice on a high-fat diet, despite the insulin-resistant glucose uptake in adipocytes.

In conclusion, SOCS3 expression is induced in adipose tissue in obesity, and here we have used a transgenic

approach to clarify the biological consequence of this overexpression. SOCS3 overexpression in fat suppresses several elements of insulin signaling in fat, suppresses insulin-stimulated glucose uptake in fat, and reduces fat storage, at least in part, by suppressing lipogenesis and increasing lipolysis. aP2-SOCS3 mice resist obesity induced by high-fat diets, and, despite insulin-resistant glucose uptake in adipocytes, systemic glucose tolerance is enhanced, as is systemic insulin sensitivity. The latter could be attributable to increased adipocyte expression and circulating levels of adiponectin.

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