Deletion of Skeletal Muscle SOCS3 Prevents Insulin Resistance in Obesity

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Obesity is associated with chronic low-grade inflammation that contributes to defects in energy metabolism and insulin resistance. Suppressor of cytokine signaling (SOCS)-3 (SOCS3) expression is increased in skeletal muscle of obese humans. SOCS3 inhibits leptin signaling in the hypothalamus and insulin signal transduction in adipose tissue and the liver. Skeletal muscle is an important tissue for controlling energy expenditure and whole-body insulin sensitivity; however, the physiological importance of SOCS3 in this tissue has not been examined. Therefore, we generated mice that had SOCS3 specifically deleted in skeletal muscle (SOCS MKO). The SOCS3 MKO mice had normal muscle development, body mass, adiposity, appetite, and energy expenditure compared with wild-type (WT) littermates. Despite similar degrees of obesity when fed a high-fat diet, SOCS3 MKO mice were protected against the development of hyperinsulinemia and insulin resistance because of enhanced basal metabolic rate and control of whole-body insulin sensitivity. Although these data suggest a potentially intriguing role for SOCS3 in regulating muscle function, a major caveat of these studies involving the overexpression of SOCS3 is that, in the absence of overt inflammation, SOCS3 expression in muscle is low. SOCS3 also may play an important role in regulating energy balance because it inhibits leptin activation of Y985 within the leptin receptor (10,11). SOCS3 heterozygous mice (12) or those with SOCS3 deleted in hypothalamic neurons (13) have reduced appetite and are protected from development of diet-induced obesity attributable to enhanced hypothalamic leptin sensitivity within proopiomelanocortin-expressing neurons (11). Like the hypothalamus, we have shown that skeletal muscle also becomes resistant to leptin in obesity (14,15), which is characterized by an impaired ability of leptin to increase fatty acid oxidation via the AMP-activated protein kinase (AMPK) (16). In cultured muscle cells, the overexpression of SOCS3 inhibits leptin activation of AMPK and fatty acid oxidation (17). However, because leptin also activates AMPK in skeletal muscle via hypothalamic circuits (18), it is unknown whether physiological increases in SOCS3 expression in obesity (two-fold to three-fold) may be of biological importance for regulating muscle function and energy balance.

SOCS3 is an important negative regulator of insulin signaling (19). Genetic deletion of SOCS3 from mouse liver results in enhanced insulin signaling because of increased insulin receptor substrate 1 (IRS1) phosphorylation (20,21). However, when mice are fed a high-fat diet (HFD), the enhanced liver insulin sensitivity paradoxically promotes liver lipogenesis, exacerbating the development of nonalcoholic fatty liver disease, systemic inflammation, and the onset of obesity (21). These data, which are in contrast to transient partial reductions in SOCS3 expression using small interfering RNA (5,22), suggest that chronic inhibition of SOCS3 in the liver is not an appropriate treatment for insulin resistance in obesity. In skeletal muscle, SOCS3 has been shown to coimmunoprecipitate with both the insulin receptor (IR) and IRS1 (23); however, in contrast to reports in adipose tissue (24) and liver (5), the overexpression of
SOCS3 in skeletal muscle is not associated with reduced IRS1 signaling or the development of insulin resistance (8).

Given the importance of skeletal muscle in the regulation of energy metabolism and insulin sensitivity, we generated mice with muscle-specific deletion of SOCS3 (SOCS3 MKO). We demonstrate that deletion of SOCS3 in muscle does not alter muscle development, body mass, adiposity, or energy expenditure, but it causes substantial protection against the development of obesity-induced hyperinsulinemia and hyperglycemia attributable to enhanced skeletal muscle IRS1 phosphorylation and glucose uptake.

RESEARCH DESIGN AND METHODS

Animal experimental procedures. All procedures were approved by St. Vincent’s Hospital and McMaster University Animal Ethics Committees. The SOCS3 MKO mice were generated by crossing SOCS3 floxed mice (generated on a C57Bl6 background (25)) with mice expressing Cre-recombinase under the control of the muscle creatine kinase promoter (26) that had been backcrossed to a C57Bl6 background for at least 10 generations. All mice were maintained on a 12 h light/dark cycle with lights on at 0700 h. At 6 weeks, SOCS3 MKO or wild-type (WT) littermates were randomly assigned to one of two diets (ad libitum (control) or diet dispatched (Diet SF04-027; Specialty Feeds, Glen Forrest, West Adelaide, Australia), Body mass was monitored weekly. Metabolic rate and activity levels were measured using a Columbus Instruments Laboratory Animal Monitoring System over 72 h after mice had been on their respective diets for 11 weeks as described (27,28). Leptin/saline injection experiments were conducted in a separate group of HFD fed mice to control for variations in the metabolic cages and daily saline injections at 0700 h for 4 days. On day 1 of the experiment, mice were injected at 0700 h with saline, followed by leptin (3 mg/kg) 24 h later as described (29). Glucose (1 g/kg i.p.) and insulin (0.5 units/kg) tolerance tests were performed 6 h after food as described (21,30). Treadmill running capacity testing was completed in ad libitum chow-fed mice after 2 days of acclimatization as described (28). Tibialis anterior muscle function and cross-sectional area were assessed as described previously (31).

For determination of insulin signaling, control-fed and HFD-fed SOCS3 MKO mice that were fasted overnight were anesthetized and injected with a bolus of insulin (0.5 units/kg) via the descending branch of the inferior vena cava and tissues collected 5 min later as described (21). Basal and insulin-stimulated (100 nmol/L) 2-deoxyglucose uptake was determined in isolated extensor digitorum longus muscles isolated from mice fed ad libitum as described (32). Basal and lepitin-stimulated (10 μg/mL) palmitate oxidation were determined in isolated soleus muscles from mice fed ad libitum as described (14).

In vivo glucose uptake. [14C]Fluorodeoxyglucose (FDG) was synthesized by nucleofection substitution method using an FDG synthesizing instrument (GE Healthcare, Milwaukee, WI) and a cyclotron (Siemens 20/30 gb). Positron emission tomography (PET) was performed using an advance scanner (Philips Mosaic PET Scanner). After fasting for 8 h, mice were restrained and injected with 0.9% saline (control) or insulin (0.5 U/kg), the same dose used in the insulin tolerance test diluted in 0.9% saline for 5 min, and then received intravenous administration of FDG (10.8 ± 1.2 MBq; Hamilton Health Sciences and McMaster University). All mice underwent small-animal PET and microcomputed tomography (CT; γ Medica-Ideas Xspect System, NorthRidge, CA), and whole-body PET images were acquired 30 min later. After injection, the mice were maintained under conscious conditions and warmed using a heating pad. Mice were imaged at exactly 30 min after injection using an acquisition time of 15 min for PET and followed by CT for 5 min. Images were reconstructed using 3D-RAMLA algorithm, with no attenuation correction and no correction for partial-volume effects of the tomograph. Quantification was performed by region-of-interest analysis using AICRA Research Workplace software and FDG tissue uptake was calculated using the mean value of standardized uptake units as described (33). Body composition was calculated from the CT image as recently described (27).

Quantitative RT-PCR. RNA from tibialis anterior or mixed gastrocnemius muscle (as indicated) was isolated using the RNaseasy mini kit (Qiagen, Doncaster, Australia), reverse-transcribed using the Thermoscript RT-PCR system (Invitrogen, Southyorker, Australia) and analyzed via quantitative RT-PCR on the Rotorgene 3000 (Corbett Research, Sydney, Australia) using SOCS3 primers and normalized using 18s ribosomal RNA as described (21,34).

Protein analysis. Frozen gastrocnemius muscles (~50 mg) were prepared in homogenization buffer supplemented with protease and phosphatase inhibitors, and immunoblotting was conducted using antibodies as described (32,34,35).

Mitochondrial enzyme activities. Mitochondria were isolated from fresh (not frozen) gastrocnemius muscle by differential centrifugation, and activities of oxidative phosphorylation complexes were measured on sonicated samples using spectrophotometric assays as previously described (36). Briefly, NADH–ubiquinone oxidoreductase (complex I) activity was measured as the rotenone-sensitive oxidation of NADH using decylubiquinone as electron acceptor; ubiquinol–cytochrome c oxidoreductase (complex III, antimycin-sensitive activity) was measured using reduced decylubiquinone as an electron donor and cytochrome c as an electron acceptor; cytochrome c oxidase (complex IV) activity was measured using reduced cytochrome c as an electron donor (35).

Plasma metabolite, hormones, and cytokines. Whole blood was spun at 7,000 g for 5 min at 4°C, and the serum was removed. Plasma hormone and cytokines were assessed using a Lincoplex mouse serum adipokine panel (35).

Electron microscopy. Transmission electron microscopy was used to determine tibialis anterior muscle and mitochondrial morphology and distribution as recently described (28).

Gene expression profiling. Tibialis anterior muscle from WT and SOCS3 MKO mice fed a chow diet or an HFD for 12 weeks (n = 8 per group) were dissected after a 6 h fast at the start of the light cycle and snap-frozen. RNA extraction and microarray analysis using Affymetrix Mouse Enox 1.0 ST arrays were performed by Almac Diagnostics (Craigavon, Northern Ireland). Total RNA was extracted using Trizol, cDNA was prepared using NuGENs WT-Ovation Pico RNA Amplification Kit, and sense transcript cDNA was prepared using NuGENs WT-Ovation Module V2. Fragmentation, labeling, and hybridization to arrays were performed with NuGENs FL-Ovation cDNA Biotin Module V2. The arrays were stained, washed, and scanned in accordance to the Affymetrix GeneChip whole-transcript sense target labeling assay manual. Raw data from the scanned arrays were further processed and analyzed using ArrayStudio (Omicsoft Corporation, Cary, NC). Data were normalized using Robust Multiarray Average method. One array (from the WT chow-fed group) was identified as abnormal in the quality-control process and excluded from further analysis. Differential expression was assessed on the core transcript (gene) level using a general linear model (model: ~type+diet+type: diet) with an adjustment for multiple test correction (Benjamini-Hochberg). Significance was accepted at adjusted P ≤ 0.05.

Calculations and statistical analysis. All data are reported as mean ± SE. Results were analyzed by t-test or ANOVA procedures and a Bonferroni post hoc test when appropriate. Significance was accepted at P < 0.05.

RESULTS

SOCS3 MKO mice have normal muscle development. A bolus of lipopolysaccharide (2 mg/kg) dramatically increased SOCS3 mRNA (2 h after injection) in WT mice, and this effect was markedly blunted in muscle and heart but not other tissues (liver, epidymidal white adipose, or hypothalamus) of SOCS3 MKO mice (Supplementary Fig. IA). SOCS3 mRNA detected in muscle of SOCS3 MKO mice was most likely attributable to contamination from other tissues. Consistent with the well-documented efficiency of the muscle creatine kinase promoter (26), SOCS3 protein expression was not detected in soleus muscle and was dramatically reduced in the heart of SOCS3 MKO mice after a bolus of lipopolysaccharide (Supplementary Fig. 1B). Given the reported role of SOCS3 in regulating muscle fiber development (8,37), we examined muscle cross-sectional area and used electron microscopy imaging to explore intracellular muscle morphology. There were no differences in muscle fiber size and structure or mitochondrial size between genotypes (Fig. 1A and D). We also tested exercise capacity as well as muscle fatigability and capacity to generate force and found that these parameters were all comparable between genotypes (Supplementary Table 1 and Fig. 1C and D). Therefore, in contrast to the transgenic overexpression of SOCS3 (8,37), endogenous levels of skeletal muscle SOCS3 are not essential for regulating muscle development or performance.

SOCS3 MKO mice are not protected from diet-induced obesity. The overexpression of SOCS3 in skeletal muscle has been shown to lead to HFD-induced obesity (8) and suppress leptin activation of STAT3, AMPK, and...
fatty acid oxidation (15,17). We therefore hypothesized that SOCS3 MKO mice would not develop HFD-induced obesity. In chow-fed mice, SOCS3 expression in muscle was at the limits of detection of our assay in both WT and SOCS3 MKO mice (Fig. 2A). Obesity increases SOCS3 expression (6,7) and, as anticipated, SOCS3 expression was increased by approximately two-fold in muscle from WT but not SOCS3 MKO mice after 12 weeks of a HFD (Fig. 2A). SOCS1, which is homologous to SOCS3 and also regulates insulin sensitivity, was not altered by diet or genotype (Supplementary Table 2). There was no difference in body mass or adiposity when mice were fed a chow diet (Fig. 2B and C) and, as anticipated, the HFD induced substantial increases in these parameters, but there was no difference between genotypes (Fig. 2B and C). Female SOCS3 MKO mice also had similar body mass as WT littermates (Supplementary Fig. 2A).

**FIG. 1.** Muscle-specific deletion of SOCS3 does not alter muscle development or performance. A: Tibialis anterior muscle histology (top) and quantification of muscle fiber cross-sectional area (CSA) (n = 3). B: Electron microscopy image (top) and quantification of mitochondrial content (right) in subsarcolemmal (SS) and intramyofibrillar (IMF) regions of tibialis anterior muscle (n = 3, scale bar = 2 μm, total = SS+IMF). C: Treadmill running capacity. D: Peak absolute force of tibialis anterior muscles (n = 7–10). All data were obtained from 10-week-old chow-fed male WT and SOCS3 MKO mice. (A high-quality color representation of this figure is available in the online issue.)

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>SOCS3 MKO</th>
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<tbody>
<tr>
<td></td>
<td>Dark</td>
<td>Light</td>
</tr>
<tr>
<td>O2 (mL/kg/h)</td>
<td>2.993 ± 110</td>
<td>2.458 ± 103*</td>
</tr>
<tr>
<td>Activity (beam breaks/12 h)</td>
<td>37,742 ± 2,208</td>
<td>4,690 ± 794*</td>
</tr>
<tr>
<td>Food intake (g/12 h)</td>
<td>1.46 ± 0.14</td>
<td>0.81 ± 0.04*</td>
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Data are means ± SE over 12-h dark (1900–0700 h) and light (0700–1900 h) cycles. n = 8. V̇O₂, oxygen consumption. *P ≤ 0.05 vs. dark, same genotype.
glucose tolerance and insulin sensitivity (Fig. 2F and G). As expected, the HFD impaired whole-body glucose tolerance and insulin sensitivity in WT mice, but SOCS3 MKO mice had marked improvements in these parameters (Fig. 2F and G). Similar observations were made in female SOCS3 MKO mice (Supplementary Fig. 2B and C). Thus, deletion of skeletal muscle SOCS3 protects mice from obesity-induced glucose intolerance and insulin resistance.

To assess whether improvements in glucose homeostasis in HFD-fed SOCS3 MKO mice were related to alterations in whole-body energy expenditure, we measured oxygen consumption (VO2), substrate utilization, habitual physical activity, and the respiratory exchange ratio. There were no differences in oxygen utilization, activity levels, or food intake (Table 1). However, during the dark cycle, SOCS3 MKO mice had a higher respiratory exchange ratio, indicating greater carbohydrate oxidation during feeding (Fig. 2H), a finding that is indicative of enhanced metabolic flexibility and improved insulin sensitivity (38). SOCS3 MKO mice have normal muscle AMPK, mitochondrial content, and triglyceride. To assess potential mechanisms for the improvements in whole-body insulin sensitivity and glucose tolerance, we measured serum adipokines/cytokines, which are known to increase SOCS3 expression. Consistent with increased adiposity in HFD mice, we found that serum tumor necrosis factor-alpha and resistin were elevated by 35% and 220%, respectively, but there were no differences between genotypes (Table 2). Serum interleukin-6 and nonesterified fatty acids were not affected by either diet or genotype.

**TABLE 2**

Serum adipokines, nonesterified fatty acids, and hyperinsulinemic–euglycemic clamp parameters in WT and SOCS3 MKO mice fed a chow diet or an HFD

<table>
<thead>
<tr>
<th></th>
<th>Chow</th>
<th>SOCS3 MKO</th>
<th>HFD</th>
<th>SOCS3 MKO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td>8.55 ± 0.90</td>
<td>1.81 ± 0.41*</td>
<td>14.76 ± 1.45†</td>
<td>13.8 ± 0.85†</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>3.17 ± 0.20</td>
<td>2.98 ± 0.37</td>
<td>4.53 ± 0.82†</td>
<td>3.96 ± 0.56†</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>3.46 ± 0.86</td>
<td>3.39 ± 1.01</td>
<td>3.61 ± 0.75</td>
<td>5.08 ± 0.422</td>
</tr>
<tr>
<td>Resistin (ng/mL)</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.17</td>
<td>4.2 ± 0.59†</td>
<td>4.1 ± 0.39†</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>1.25 ± 0.18</td>
<td>1.26 ± 0.12</td>
<td>1.24 ± 0.08</td>
<td>1.28 ± 0.06</td>
</tr>
<tr>
<td>Preclamp glucose (mmol/L)</td>
<td>5.7 ± 0.3</td>
<td>5.5 ± 0.6</td>
<td>6.3 ± 0.50†</td>
<td>6.1 ± 0.50†</td>
</tr>
<tr>
<td>Clamp glucose (mmol/L)</td>
<td>5.9 ± 0.1</td>
<td>5.8 ± 0.1</td>
<td>6.0 ± 0.13</td>
<td>5.8 ± 0.12</td>
</tr>
<tr>
<td>Basal glucose disposal rate (mg/kg/min)</td>
<td>33.5 ± 4.5</td>
<td>40.5 ± 5.7</td>
<td>26.7 ± 2.5†</td>
<td>30.4 ± 1.4†</td>
</tr>
<tr>
<td>Suppression hepatic glucose output (%)</td>
<td>92.7 ± 1.3</td>
<td>91.2 ± 2.1</td>
<td>84.1 ± 4.9†</td>
<td>84.6 ± 3.04†</td>
</tr>
</tbody>
</table>

Data are means ± SE for n = 6–8. IL, interleukin; NEFA, nonesterified fatty acid; TNF, tumor necrosis factor. *P < 0.05 vs. WT from same dietary condition. †P ≤ 0.05 vs. chow, same genotype.
[37x49]Data are mean weeks (Gastrocnemius muscle mitochondrial protein expression (T172, and acetyl-CoA carboxylase (ACC2) S212 phosphorylation in male WT and SOCS3 MKO mice fed a chow diet or an HFD for 12 weeks.

3 ratio, indicating an increase in fatty acid oxidation to findings, leptin modestly reduced respiratory exchange ratio at the start of the light cycle (0700 h). In contrast to our in vitro metabolic cages and injected with saline or leptin at the start of the light cycle. These findings, we found that leptin increased fatty acid oxidation in chow-fed but not HFD-fed soleus muscle from WT mice (Fig. 3A). However, in contrast to WT mice leptin-stimulated fatty acid oxidation was maintained in muscles from HFD-fed SOCS MKO mice (Fig. 3A). To examine whether HFD-fed SOCS MKO mice also had enhanced sensitivity to leptin in vivo, mice were placed in metabolic cages and injected with saline or leptin at the start of the light cycle (0700 h). In contrast to our in vitro findings, leptin modestly reduced respiratory exchange ratio, indicating an increase in fatty acid oxidation to a similar degree in both WT and SOCS3 MKO mice (Fig. 3B). Food intake also was modestly reduced by ~10% in both genotypes (Fig. 3C). We subsequently examined phosphorylation of STAT3 Y705, AMPK T172, and its downstream substrate acetyl-CoA carboxylase S212 and found that they were not altered by either genotype or diet (Fig. 3D). These data suggest that leptin increases whole-body rates of fatty acid oxidation through mechanisms independent of skeletal muscle SOCS3, potentially involving increases in adipose tissue lipolysis (39). Because AMPK (40,41) and leptin (42) are important for regulating muscle mitochondrial content, we measured a number of mitochondrial markers and found that they also were not different between genotypes with the exception of complex IV activity, which was increased in chow-fed SOCS3 MKO mice (Fig. 3E and F). Consistent with normal levels of muscle AMPK and mitochondrial content, we found that muscle triglyceride while increased several fold with the HFD was not different between WT and SOCS3 MKO mice (Fig. 3G). Taken together, this suggests that improved insulin sensitivity in SOCS3 MKO mice is not attributable to alterations in skeletal muscle AMPK.

SOCS3 MKO mice have enhanced skeletal muscle insulin sensitivity. To determine the tissues that contribute to the improved whole-body insulin sensitivity of SOCS3 MKO mice, we performed hyperinsulinemic–euglycemic clamps. Serum glucose concentrations before and during the clamp were similar between WT and SOCS3 MKO mice (Table 2). Under chow-fed conditions, we detected no differences in the glucose infusion rate (Fig. 4A), glucose disposal rate (Fig. 4B), or hepatic glucose production (Fig. 4C) between WT and SOCS3 MKO mice. As anticipated, the HFD caused insulin resistance evinced by suppression in both the glucose infusion rate and the glucose disposal rate in WT mice, but this effect was blunted in the HFD-fed SOCS3 MKO mice (Fig. 4A and B). There were no changes in hepatic glucose production or insulin suppression of hepatic glucose production between genotypes (Fig. 4C and Table 2). These data indicate that deletion of SOCS3 from skeletal muscle improves whole-body insulin sensitivity because of enhanced glucose disposal.

To examine if an increase in muscle insulin-stimulated glucose uptake was responsible for the enhanced glucose disposal in HFD-fed SOCS MKO mice we conducted PET imaging in mice injected with insulin (0.5 units/kg), and FDG as. FDG uptake was comparable between WT and SOCS3 MKO epididymal white adipose, liver, heart (P = 0.12), kidney, and brain (Supplementary Table 3), but we found that the gastrocnemius muscle of SOCS3 MKO mice accumulated ~30% more FDG than WT littermates (Fig. 4D). To determine whether improved insulin sensitivity in obese SOCS3 MKO animals was attributable to intrinsic changes in the muscle and not attributable to a combination of insulin and potentially other circulating factors, we measured insulin-stimulated 2-deoxyglucose uptake in isolated muscles. Insulin

![Figure 3](image-url)
increased 2-deoxyglucose uptake by ~100% in isolated extensor digitorium longus muscle from chow-fed WT and SOCS3 MKO mice, and although the HFD suppressed this effect, muscles from SOCS3 MKO were more insulin-sensitive compared with WT littermates (Fig. 4E). This demonstrates that the deletion of skeletal muscle SOCS3 directly improves skeletal muscle insulin sensitivity in obesity.

**Skeletal muscle SOCS3 inhibits IRS1 tyrosine phosphorylation.** To elucidate potential mechanisms for the improved skeletal muscle insulin sensitivity in HFD-fed SOCS3 MKO mice, we performed a genome-wide expression analysis. In this analysis we found no differentially expressed genes between the WT and the SOCS3 MKO mice fed either a chow diet or a HFD (Fig. 5A). In contrast, 2,406 genes were differentially expressed as a consequence of diet (Fig. 5A). To further assess potential mechanisms, we measured total expression and phosphorylation of insulin signaling proteins. We found that IR, IRS1, and Akt expression were unaltered by SOCS3 deficiency (Fig. 5C). However, the IRS1-associated P85 subunit of PI3 kinase was increased in HFD-fed SOCS3 MKO mice after insulin treatment (Fig. 5D). Similarly, insulin-stimulated Akt T308 and S473 phosphorylation were increased in SOCS3 MKO mice (Fig. 5E). These data demonstrate that skeletal muscle SOCS3 inhibits activating phosphorylation of IRS1 without altering global gene expression or the total protein expression of insulin signaling proteins.

**DISCUSSION**

Insulin resistance associated with obesity is a well-established forerunner for the development of type 2 diabetes and has been linked to both ectopic lipid accumulation (43) and low-grade chronic inflammation (44). Studies in hepatocytes and adipocytes have shown that overexpression of SOCS3 antagonizes proximal insulin signaling (3–5,24,45,46). However, surprisingly, when SOCS3 is genetically deleted from the liver, it propagates the development of obesity and fatty liver disease (20,21). These studies highlighted the need to investigate the role of endogenous levels of SOCS3 under physiological conditions such as obesity. Given that SOCS3 is elevated in skeletal muscle with obesity, and that skeletal muscle plays a major role in controlling energy expenditure and glucose homeostasis, we generated mice with muscle-specific deletion of SOCS3. The SOCS3 MKO mice did not express any major phenotypic abnormalities as assessed by growth and survival, organ weights, food intake, energy expenditure, or habitual physical activity levels. We specifically addressed muscle function and found that skeletal muscles from SOCS3 MKO mice had normal morphology and function. SOCS3 whole-body heterozygous mice are protected against the development of obesity (12). The leptin receptor is expressed in skeletal muscle (47) and high levels of leptin increase skeletal muscle fatty acid oxidation in lean, but not in obese, rodents (14) and humans (15). Because the overexpression of SOCS3 inhibits leptin-induced activation of both STAT3 and AMPK in skeletal muscle myotubes (17),
and because muscle-specific SOCS3 transgenic mice are obese (8), we hypothesized that SOCS3 MKO mice might have increased muscle AMPK, elevated rates of fatty acid oxidation, and increased energy expenditure. However, we found that irrespective of diet, both genotypes increased body weight and adiposity over time to a similar degree. Consistent with this, we found that energy expenditure, activity levels, food intake, mitochondrial capacity, as well as skeletal muscle STAT3 and AMPK phosphorylation were comparable between genotypes. The SOCS3 MKO mice also had similar levels of muscle triglyceride compared with WT littermates. Thus, there is no absolute requirement for skeletal muscle SOCS3 in regulating whole-body energy expenditure in vivo.

The deletion of muscle SOCS3 improved glucose tolerance and insulin sensitivity in obese mice. The development of insulin resistance with obesity is a complex cascade of detrimental events involving several tissue types. We therefore assessed insulin-regulated glucose metabolism using the hyperinsulinemic–euglycemic clamp to determine the relative roles of peripheral tissues (skeletal muscle and adipose tissue) and the liver. Under control chow diet conditions, there were no differences in insulin sensitivity between genotypes. These data suggest that skeletal muscle SOCS3 does not play a major role in regulating insulin-stimulated negative feedback of the insulin signaling pathway (19), which is in contrast to findings in liver-specific SOCS3-null mice (20,21). SOCS3 MKO mice were partially protected against the development of HFD-induced insulin resistance, an effect that was attributable to improved peripheral glucose disposal as a result of enhanced glucose uptake into skeletal muscle, which was assessed both in vivo using PET and ex vivo in isolated skeletal muscle. Thus, deletion of skeletal muscle SOCS3 improves whole-body glucose tolerance and insulin sensitivity in obesity by restoring skeletal muscle insulin sensitivity.

To elucidate the mechanism responsible for muscle insulin sensitization in SOCS3 MKO mice, we performed a genome-wide expression analysis; however, no differences were observed, and thus we also assessed proximal components of the insulin signaling pathway. Previous studies have suggested that SOCS3 antagonizes insulin signaling by blocking phosphorylation of the IR, IRS1, P85-subunit of PI3 kinase, and Akt from HFD-fed male WT and SOCS3 MKO mice injected with saline. IR Y1150 phosphorylation (C), IRS1-associated P85-subunit of PI-3 kinase (D), and Akt T308 and S473 phosphorylation (E) in HFD-fed male WT and SOCS3 MKO mice injected with either saline or insulin. Data are mean ± SE, n = 6–8. *P ≤ 0.05 relative WT.
expression levels. Consistent with increased PI3 kinase activation, we found that Akt phosphorylation was enhanced in HFD-fed SOCS3 MKO mice. Therefore, skeletal muscle SOCS3 does not affect the expression of the IR or IRS1, but instead impairs insulin sensitivity by inhibiting the activating phosphorylation of IRS1.

In summary, deletion of SOCS3 in skeletal muscle does not alter muscle contractile performance or the development of obesity but does protect mice from obesity-related glucose intolerance and insulin resistance. Enhanced skeletal muscle insulin sensitivity was attributable to increases in IRS1 activation. Previous studies have shown that liver-specific deletion of SOCS3 promotes lipogenesis, hepatic insulin resistance, and obesity (20,21); however, in the current study we did not detect any detrimental metabolic effects in response to muscle-specific deletion of SOCS3. We conclude that in obesity, muscle SOCS3 is an important contributing factor to muscle insulin resistance and impairments in glucose homeostasis, suggesting that the inhibition of muscle SOCS3 could be a favorable strategy to restore insulin action in patients with type 2 diabetes.

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