Suppressor of Cytokine Signaling 3 Expression and Insulin Resistance in Skeletal Muscle of Obese and Type 2 Diabetic Patients

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Interleukin-6 (IL-6) could be a possible mediator of insulin resistance. We investigated whether IL-6 could inhibit insulin signaling in human skeletal myotubes and whether suppressor of cytokine signaling 3 (SOCS-3) could be related to insulin resistance in vivo in humans. IL-6 inhibited insulin signaling and induced SOCS-3 expression in differentiated myotubes. SOCS-3 mRNA levels were significantly increased in the skeletal muscle of type 2 diabetic patients compared with control subjects and correlated with reduced insulin-stimulated glucose uptake. In contrast, SOCS-3 mRNA levels were reduced in muscle of obese nondiabetic subjects compared with type 2 diabetic patients, despite similar circulating concentrations of IL-6. Increased SOCS-3 mRNA levels in diabetes were not attributable to hyperglycemia, as type 1 diabetic patients had normal SOCS-3 mRNA expression in muscle. However, the combination of high glucose and IL-6 levels in type 2 diabetic patients may induce SOCS-3 expression, as has been seen in human muscle cells. In subcutaneous adipose tissue, SOCS-3 mRNA levels were increased in obese individuals and strongly correlated with IL-6 expression, supporting a paracrine effect of IL-6 on SOCS-3 expression in fat. Taken together, our results showed that SOCS-3 expression in human skeletal muscle in vivo is not related to insulin resistance in the presence of elevated IL-6 concentrations and suggest that cytokine action could differ in type 2 diabetic patients and nondiabetic obese subjects. Diabetes 53:2232–2241, 2004

Obesity and type 2 diabetes are important metabolic disorders characterized by impaired insulin action (1,2). However, the relations among obesity, insulin resistance, and type 2 diabetes are not well understood. A large body of evidence demonstrates that obesity is associated with a chronic state of low-grade inflammation (3), which could be one of the important parameters in the development of insulin resistance (4,5). Indeed, elevated levels of proinflammatory cytokines can produce insulin resistance (6); furthermore, it has been clearly demonstrated that adipose tissue is able to synthesize and secrete several cytokines such as leptin, tumor necrosis factor-α (TNF-α), or interleukin-6 (IL-6) (7,8). These proinflammatory cytokines have also been shown to be elevated in type 2 diabetes (9,10), suggesting that adipocytokines might be a possible link among insulin resistance, diabetes, and obesity (5). Among these cytokines, IL-6 presents the strongest correlation with insulin resistance and type 2 diabetes in humans (9–13).

The mechanisms of cytokine-induced insulin resistance are not clearly defined. One possible mechanism is the serine phosphorylation of insulin receptor substrate 1 (IRS-1) by cytokine-activated kinases and the subsequent direct inhibitory effect on the insulin-signaling cascade (14–16). An alternative mechanism is that cytokines induce the expression of cellular proteins, such as members of the suppressor of cytokine signaling (SOCS) family, that inhibit insulin receptor signal transduction (17–19). The SOCS proteins are induced by various cytokines, and participate in a classic feedback loop to modulate cytokine action (20). It has been demonstrated that SOCS proteins can also play a role in the negative regulation of the signaling of insulin and IGF-I receptors (17,18,21). Thus, SOCS proteins might be good candidates for cytokine-mediated insulin resistance.

Few data exist regarding the possible implication of the SOCS family in cytokine action in human skeletal muscle, which is the major site of insulin-dependent glucose disposal. Therefore, this study was conducted to 1) characterize the effect of IL-6 on insulin signaling and the expression of SOCS members in vitro in human differentiated myotubes and 2) determine the relations among IL-6 plasma levels, SOCS expression, and insulin sensitivity in obese nondiabetic subjects and type 2 diabetic patients.
TABLE 1
Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Middle-aged control subjects</th>
<th>Obese nondiabetic subjects</th>
<th>Type 2 diabetic subjects</th>
<th>Young control subjects</th>
<th>Type 1 diabetic subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>16</td>
<td>12</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Men/women</td>
<td>8/8</td>
<td>9/7</td>
<td>6/6</td>
<td>8/7</td>
<td>6/4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.2 ± 5.4</td>
<td>45.7 ± 3.3</td>
<td>54.2 ± 2.7</td>
<td>26.5 ± 1.4</td>
<td>32.8 ± 2.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23 ± 0.43</td>
<td>32.9 ± 0.78*</td>
<td>31.9 ± 1.4†</td>
<td>23.2 ± 0.49</td>
<td>23.5 ± 0.86</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>0.88 ± 0.02</td>
<td>0.96 ± 0.03</td>
<td>2.07 ± 0.40†</td>
<td>0.9 ± 0.02</td>
<td>2.38 ± 0.17*</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>5.75 ± 0.39</td>
<td>12.5 ± 1.4*</td>
<td>12.83 ± 1.66*</td>
<td>7.03 ± 0.55</td>
<td>—</td>
</tr>
<tr>
<td>Nonesterified fatty acids (µmol/L)</td>
<td>464.7 ± 62</td>
<td>553.4 ± 39.7</td>
<td>701.7 ± 49.58</td>
<td>493.1 ± 65.6</td>
<td>570.6 ± 101.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>61.0 ± 0.07</td>
<td>1.31 ± 0.21†</td>
<td>2.25 ± 0.48‡</td>
<td>0.66 ± 0.07</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>Glucose disposal rate (mg · kg⁻¹ · min⁻¹)</td>
<td>2.16 ± 0.11</td>
<td>1.7 ± 0.07§</td>
<td>2.23 ± 0.15*</td>
<td>2.06 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td>Glucose oxidation rate (mg · kg⁻¹ · min⁻¹)</td>
<td>1.44 ± 0.17</td>
<td>1.15 ± 0.14</td>
<td>1.24 ± 0.15</td>
<td>1.39 ± 0.18</td>
<td>1.49 ± 0.15</td>
</tr>
<tr>
<td>Nonesterified fatty acid (µmol/L)</td>
<td>39.56 ± 7.46</td>
<td>3.7 ± 10.5§</td>
<td>143.5 ± 33.6†</td>
<td>48.2 ± 10.4</td>
<td>51.4 ± 5.6</td>
</tr>
</tbody>
</table>

Data are means ± SE; *P < 0.01, †P < 0.001, ‡P < 0.0001 vs. respective control subjects; §P < 0.05, ¶P < 0.01 for type 2 diabetic vs. obese nondiabetic subjects. ND, not determined.

RESEARCH DESIGN AND METHODS

The characteristics of the 69 subjects involved in the study are presented in Table 1 and have also been presented in a previous study (22). All participants gave their written consent after being informed of the nature, purpose, and possible risks of the study. The experimental protocol was approved by the ethical committees of the Hospices Civils de Lyon and performed according to French legislation (Huriet law).

The 31 healthy lean volunteers were divided into two groups based on their age. None of them had impaired glucose tolerance or a familial or personal history of diabetes, obesity, dyslipidemia, or hypertension. One group of 16 control subjects (middle-aged control subjects; 8 men and 8 women, age 45 ± 5 years, BMI 23.3 ± 0.4 kg/m²) was age matched with 12 type 2 diabetic patients (6 men and 6 women, age 54 ± 3 years, BMI 31.9 ± 1.4 kg/m², HbA1c 10.2 ± 0.4%) and 16 obese nondiabetic subjects (9 men and 7 women, age 45 ± 3 years, BMI 32.9 ± 0.8 kg/m², HbA1c 5.5 ± 0.3%). An unrelated group of 15 healthy lean subjects (young control subjects; 8 men and 7 women, age 27 ± 1 year, BMI 23.2 ± 0.5 kg/m²) served as control subjects for a group of 10 type 1 diabetic patients (6 men and 4 women, age 35 ± 2 years, BMI 25.9 ± 0.9 kg/m², HbA1c 9.2 ± 0.3%).

All studies were performed after an overnight fast. To investigate insulin action on glucose metabolism, the subjects were submitted to a 3-h euglycemic-hyperinsulinemic clamp with an insulin infusion rate of 450 pmol · m⁻² · min⁻¹, as previously described (22,23). The expression of target genes was determined in skeletal muscle 4 days after differentiation was initiated (26,27).

**Determination of insulin receptor and IRS-1 tyrosine phosphorylation.** After being incubated overnight without serum, myotubes were treated for 90 min with IL-6 and stimulated by insulin (100 nmol/l) for 10 min (IRS-1 phosphorylation). Cells were lysed at 4°C in 200 mmol/l NaF, 20 mmol/l Na2HPO4, 150 mmol/l NaCl, 50 mmol/l HEPES, 4 mmol/l NaVO4, 10 mmol/l EDTA, 1% Triton X, 10% glycerol, and 2 mmol/l phenylmethylsulfonyl fluoride. Insulin receptor or IRS-1 was immunoprecipitated with specific antibodies (Upstate Biotechnology, Lake Placid, NY). Immunoprecipitates were separated by SDS-PAGE, and immunoblotting was performed with a specific antiphosphotyrosine antibody (PY99; Santa Cruz Biotechnology, Santa Cruz, CA), as previously reported (27).

**Determination of IRS-1—associated phosphatidylinositol 3-kinase protein amount.** After being incubated overnight with serum, myotubes were treated for 90 min with IL-6 and stimulated by insulin (100 nmol/l) for 10 min. Lysates were centrifuged (12,000g for 15 min), and 100 µg of supernatant proteins were used for immunoprecipitation with a specific antibody against human IRS-1 (Upstate Biotechnology). After being washed, immunoprecipitates were separated by SDS-PAGE and immunoblotted using a specific antibody directed against human p85α phosphatidylinositol (PI) 3-kinase (Upstate Biotechnology).

**Determination of phosphorylated protein kinase B.** After being incubated overnight without serum, myotubes were treated for 90 min with IL-6 and stimulated by insulin (100 nmol/l) for 20 min. Cells were lysed, and proteins (50 µg) were separated by SDS-PAGE. Phosphorylated protein kinase B (PKB) was detected using an anti–phospho-Ser473 antibody (Upstate Biotechnology). To normalize for equal protein amount, the blots were stripped and reprobed again with anti-PKB antibody (Upstate Biotechnology).

**Quantification of messenger RNAs.** Total RNA from skeletal muscle biopsies was prepared according to a procedure based on the method of Chomczynsky and Sacchi (28). Total RNA from differentiated myotubes and adipose tissue samples was prepared using the RNeasy kit (Qiagen, Courtabœuf, France). The absolute concentration of cytokine-inducible SH2-containing protein (CIS); SOCS-1, -2, and -3; and IL-6 mRNAs were determined by RT-quantitative PCR (RT-qPCR) using a light cycler (Roche Diagnostics, Meylan, France), as previously described (29). The list of the primers and PCR assay conditions are available upon request (jennifer.rieusset@univ-lyon1.fr). The results were expressed as the percent of the cyclophilin mRNA concentra-
Serum IL-6 measurement. Fasted serum levels of IL-6 were determined by enzyme-linked immunosorbent assay (Quantikine IL-6; R&D Systems, Oxford, U.K.), as previously reported (30). The sensitivity of the assay was 0.70 pg/ml.

Statistical analysis. All data are presented as means ± SE. Statistical significance was determined using Kruskal-Wallis analysis followed by the nonparametric Mann-Whitney test when comparing groups of subjects. A nonparametric Wilcoxon's test for paired values was used when comparing mRNA levels before and after the clamp in the same group of subjects. Correlations were analyzed using Spearman's rank correlation test. The threshold for significance was set at *P < 0.05.

RESULTS

IL-6 inhibits insulin signaling and induces SOCS-3 mRNA levels in human myotubes. To assess the effect of IL-6 treatment on insulin signaling, muscle cells were preincubated with IL-6 (20 ng/ml) for 90 min and then

**FIG. 1.** Effect of IL-6 pretreatment on insulin signaling. Differentiated myotubes were serum starved overnight before being treated with IL-6 (20 ng/ml) for 90 min. Cells were then treated with (●) or without (□) insulin (100 nmol/l) for 3 (A), 10 (B and C) or 20 (D) min and lysed. The effect of IL-6 on insulin-stimulated tyrosine phosphorylation (pTyr) of the insulin receptor (A) and IRS-1 (B), on insulin-mediated association of the p85α subunit of PI 3-kinase with IRS-1 (C), and on insulin-dependent activation of PKB (D) was measured as described in RESEARCH DESIGN AND METHODS. Blots shown are representative of three (A, C, and D) or four (B) independent experiments. Results are expressed as fold increase over insulin-free basal conditions. Data represent means ± SE. Anti-IR, anti-insulin receptor; IB, immunoblotting; IP, immunoprecipitation. *P < 0.05.
stimulated by insulin (100 nmol/l). As shown in Fig. 1A, insulin increased insulin receptor autophosphorylation in untreated cells; this result was not affected by IL-6. Insulin also induced a 2.4-fold increase in the tyrosine phosphorylation of IRS-1 and a strong association of the p85 subunit of PI 3-kinase with IRS-1 in untreated cells. IL-6 completely prevented the effect of insulin on IRS-1 phosphorylation (Fig. 1B) and suppressed the insulin-stimulated association of the p85 subunit of PI 3-kinase with IRS-1 (Fig. 1C). The amounts of insulin receptor and IRS-1 proteins were not modified after a 90-min incubation in the presence of IL-6 (data not shown). In the absence of IL-6, insulin promoted a marked increase in the amount of phosphorylated PKB/Akt (Fig. 1D). This effect was strongly attenuated in myotubes preincubated with IL-6 (Fig. 1D).

In the absence of IL-6, the mRNA concentration of the different SOCS was very low in myotubes, with SOCS-3 mRNA being higher than the other SOCSs (0.93 ± 0.14, 0.27 ± 0.06, 0.73 ± 0.14, and 0.31 ± 0.14 amol/µg total RNA for SOCS-3, CIS, and SOCS-1 and -2, respectively; n = 3). Figure 2A shows that incubation of human myotubes for 90 min with IL-6 (20 ng/ml) specifically induced the mRNA levels of SOCS-3. CIS and SOCS-1 and -2 mRNAs were unaffected. The effect of IL-6 on SOCS-3 mRNA was barely detectable after 30 min, but reached values ~7 and 10 times above basal levels after 60 and 90 min of treatment. After that time point, SOCS-3 expression levels declined.

To verify whether IL-6–dependent inhibition of insulin signaling paralleled SOCS-3 induction, the time-course effect of IL-6 on insulin-stimulated phosphorylation of PKB/Akt was determined. As shown in Fig. 2B, the inhibitory effect of IL-6 was apparent after 1 h of treatment and was measurable after 90 min. The maximal effect was found after 2 h of treatment with IL-6 and persisted when cells were pretreated for 4 h.

**Plasma IL-6 concentrations and expression of SOCS-3 mRNA in the skeletal muscle of insulin-resistant patients.** We next investigated the associations of IL-6 plasma levels, SOCS-3 mRNA expression in skeletal muscle, and insulin sensitivity in healthy lean, obese nondiabetic, and type 2 diabetic subjects. The characteristics of the three groups of subjects are summarized in Table 1. Circulating IL-6 levels were significantly higher in the two groups of insulin-resistant subjects than in the group of healthy subjects, with a 2.9- and 2.6-fold increase in IL-6 concentrations in obese nondiabetic (P = 0.0019) and type 2 diabetic (P < 0.0018) subjects, respectively (Fig. 3A). Plasma concentrations of IL-6 significantly correlated with BMI and fasting triglyceride levels when all of the subjects were analyzed together (r = 0.51, P = 0.0012, and r = 0.59, P = 0.0002, respectively). In addition, Fig. 4A
shows that IL-6 concentrations correlated negatively with insulin-stimulated glucose disposal during the euglycemic-hyperinsulinemic clamp \( (r = -0.56, P = 0.002) \).

The SOCS-3 mRNA concentration was significantly increased in the skeletal muscle of type 2 diabetic subjects (SOCS-3/cyclophilin: 4.2 \pm 0.7 vs. 1.9 \pm 0.3; \( P = 0.0001\)) (Fig. 3B). In contrast, there was a marked reduction in SOCS-3 mRNA expression in the skeletal muscle of obese nondiabetic subjects (SOCS-3/cyclophilin: 0.8 \pm 0.1 vs. 1.9 \pm 0.3; \( P = 0.0029\)) (Fig. 3B). The mRNA levels of cyclophilin did not differ among control, obese, and type 2 diabetic subjects (13 \pm 2, 16 \pm 2, and 11 \pm 2 amol/\mu g total RNA, respectively; NS). We also measured the expression of another reference gene (hypoxanthine phosphoribosyltransferase-1 [HPRT-1]). As for cyclophilin, HPRT-1 mRNA levels were similar among control, obese, and type 2 diabetic subjects (1.7 \pm 0.4, 2.1 \pm 0.3, and 1.3 \pm 0.2 amol/\mu g total RNA, respectively; NS). The presentation of SOCS-3 mRNA data by reference to HPRT-1 (12 \pm 2, 4 \pm 0.5, and 23 \pm 3% of HPRT-1 mRNA) was similar in control, obese, and type 2 diabetic subjects, respectively; \( P = 0.001\) confirmed the results obtained with cyclophilin.

SOCS-3 mRNA levels in muscle were not correlated with insulin-stimulated glucose disposal (\( P = 0.37\)) (Fig. 4B) or IL-6 plasma concentrations (\( P = 0.48\)) when the three groups of subjects were analyzed together. However, strong positive correlations between SOCS-3 mRNA levels and IL-6 concentrations (\( r = 0.63, P = 0.002\)) and between SOCS-3 mRNA and glucose uptake rate (\( r = -0.69, P = 0.001\)) were observed when obese nondiabetic subjects were excluded from the analysis.

Regarding the other members of the SOCS family, levels of CIS and SOCS-1 mRNAs were barely detectable in skeletal muscle and were under the limit of the quantification by real-time PCR in a majority of the samples, independent of insulin resistance or obesity (data not shown). SOCS-2 mRNA was measurable in human muscle, but there was no significant difference among lean, nondiabetic obese, and type 2 diabetic subjects (SOCS-2/cyclophilin: 1.2 \pm 0.3, 1.0 \pm 0.3, and 1.1 \pm 0.4, respectively; \( P = 0.55\)).

To confirm the observed reduction in SOCS-3 mRNA concentrations in the muscle of obese nondiabetic subjects, we analyzed SOCS-3 expression in biopsies obtained from an unrelated group of 12 obese subjects (5 men and 7 women, age 35 \pm 3 years, BMI 32.6 \pm 1.0 kg/m\(^2\)), matched by age with the group of young control subjects presented in Table 1. We found similar expression of SOCS-3 between the young and middle-aged control subjects (SOCS-3/cyclophilin: 1.5 \pm 0.1 vs. 1.9 \pm 0.3; \( P = 0.55\)). The young obese subjects had normal glucose tolerance, but were insulin resistant during the hyperinsulinemic clamp (insulin-induced glucose disposal rate 5.5 \pm 0.8 mg \cdot kg\(^{-1}\) \cdot min\(^{-1}\)). The middle-aged obese subjects had a significant reduction in SOCS-3 mRNA levels in skeletal muscle (SOCS-3/cyclophilin: 0.8 \pm 0.16 vs. 1.5 \pm 0.1 in obese vs. control subjects; \( P = 0.01\)).

**Effect of hyperglycemia on the regulation of SOCS-3 expression.** To verify whether chronic hyperglycemia could be implicated in the differential expression of SOCS-3, mRNA levels were measured in the skeletal muscle of type 1 diabetic subjects. There was no difference in SOCS-3 mRNA levels between type 1 diabetic and age-matched control subjects (1.3 \pm 0.2 vs. 1.5 \pm 0.1; \( P = 0.58\)) (Fig. 3B), suggesting that SOCS-3 expression is not directly regulated by glucose levels in the diabetic state. It was notable that type 1 diabetic subjects had circulating IL-6 levels similar to those of age-matched control subjects (Fig. 3A) and had normal insulin-induced glucose disposal (Table 1).

The possibility that a combination of high glucose and elevated IL-6 concentrations, as seen in the type 2 diabetic subjects only, could have enhanced SOCS-3 mRNA levels in muscle was verified in cultured human myotubes. Muscle cells were incubated for 90 min with different concentrations of IL-6 in the presence of 5 or 15 mmol/l glucose. In the absence of IL-6, glucose alone had no effect on SOCS-3 expression (Fig. 5). However, increasing the glucose concentration from 5 to 15 mmol/l promoted a twofold increase in the effect of IL-6 at all tested concentrations (Fig. 5).

**Expression of SOCS-3 in adipose tissue.** As shown in Fig. 6A, IL-6 mRNA levels were not different in subcutaneous adipose tissue of type 2 diabetic and control subjects, but were significantly increased in obese subjects. IL-6 mRNA levels were also measured in fat biopsies from an unrelated group of massively obese subjects obtained during surgery for weight reduction (one man and five women, age 36 \pm 2 years, BMI 43.5 \pm 1.6 kg/m\(^2\)). IL-6
mRNA expression was further increased in the adipose tissue of the massively obese subjects (Fig. 6A). There was a positive correlation between IL-6 mRNA levels and the BMI of the subjects \((r = 0.34, P = 0.043)\). However, the correlation between IL-6 mRNA expression in adipose tissue and serum levels of the cytokine did not reach significance \((P = 0.07, r = 0.44)\).

SOCS-3 mRNA levels tended to be increased in adipose tissue of type 2 diabetic patients (Fig. 6B), but the difference did not reach significance. In contrast, SOCS-3 expression was significantly higher in nondiabetic obese subjects (Fig. 6B). There was a positive correlation between SOCS-3 mRNA levels and BMI \((r = 0.58, P = 0.0002)\) when all the subjects were analyzed; \(r = 0.77, P < 0.0001\) without the type 2 diabetic subjects. Finally, as is shown in Fig. 7, there was a positive correlation between IL-6 and SOCS-3 mRNA levels in human subcutaneous adipose tissue \((r = 0.53, P = 0.0017)\).

**DISCUSSION**

**Effect of IL-6 on insulin signal transduction and SOCS expression.** Proinflammatory cytokines are thought to contribute to insulin resistance in both humans and animals (31,32). We demonstrated here that IL-6 affects proximal events of insulin signal transduction in human muscle cells. Because the insulin-dependent tyrosine phosphorylation of the insulin receptor was not altered, whereas the tyrosine phosphorylation of IRS-1 was inhibited, it appears that IL-6 acts primarily at the level of the insulin receptor/IRS-1 interaction in human muscle cells. Similar results have been recently reported in mouse hepatocytes and HepG2 cells (33). Several mechanisms could be envisaged for this effect, such as the serine/threonine phosphorylation of IRS-1, leading to inhibition of tyrosine phosphorylation in response to insulin, as demonstrated with TNF-α (15), or the interaction of IL-6–dependent signaling proteins with insulin receptors and/or IRS-1, such as the proteins of the SOCS family (17–20).

In human muscle cells, we found that IL-6 exposure specifically induced SOCS-3 expression without affecting CIS or SOCS-1 or -2 mRNAs. The induction of SOCS-3 paralleled the inhibition by IL-6 of insulin-stimulated phosphorylation of PKB. This result suggests a possible role of SOCS-3 in the effect of IL-6 on insulin signaling. However, because of the poor specificity of available antibodies, we were not able to determine the expression of SOCS-3 at the protein level. It might be noted that the studies in which SOCS protein expression has been evaluated generally showed that the amounts of SOCS correlate well with the levels of the corresponding mRNA (34–36).

**SOCS-3 and insulin signal transduction.** It has been recently reported that ectopic expression of SOCS-3 in-
SOCS-3 EXPRESSION AND INSULIN RESISTANCE

FIG. 5. Effect of glucose on IL-6-induced SOCS-3 expression in human myotubes. Differentiated myotubes were serum starved overnight and then treated with IL-6 (20 ng/ml) in the presence of 5 or 15 mmol/l of glucose. Results are expressed by reference to the effect obtained with 200 ng/ml of IL-6 (10⁻³ mol/l) in the presence of 5 mmol/l glucose. Data represent means ± SE of three different experiments performed in triplicate. *P < 0.05.

... induces a reduction in insulin-dependent phosphorylation of IRS-1 on tyrosine and a decrease in the association of the p85 subunit of PI 3-kinase with IRS-1 (37,38). These effects of SOCS-3 were very similar to those of IL-6 observed in the present study in human myotubes or recently shown in HepG2 cells (33). Several mechanisms have been proposed to explain the effects of SOCS-3 on insulin signal transduction. SOCS-3 may inhibit signaling by targeting its binding partners to proteosomal degradation (39). In hepatocytes, for example, SOCS-3 was able to induce IRS-1 and -2 degradation by the ubiquitin-proteasome system (39). In the current study, there was no modification in the protein level of IRS-1 in the presence of IL-6, suggesting that another mechanism is involved. It has also been shown that SOCS-3 can interact directly with phosphotyrosine residues of insulin receptors and thereby inhibit the downstream signaling events by preventing IRS-1 tyrosine phosphorylation (17,18). In human myotubes, the observation that insulin-induced IRS-1 phosphorylation is inhibited by IL-6, whereas insulin receptor phosphorylation remains unaffected, supports such a mechanism.

**Implication of SOCS-3 in insulin resistance in vivo in humans.** As has been seen in previous studies (11,12), we found that circulating IL-6 levels were two- to threefold higher in insulin-resistant subjects. However, SOCS-3 expression in skeletal muscle biopsies was not related to insulin resistance. SOCS-3 mRNA levels were markedly increased in the muscle of type 2 diabetic patients, but significantly reduced in insulin-resistant, but nondiabetic, obese subjects. Because nondiabetic obese subjects were normoglycemic, whereas type 2 diabetic patients are characterized by chronic hyperglycemia, we tested the possible implication of hyperglycemia on the differential expression of SOCS-3. Normal levels of SOCS-3 mRNA were found in skeletal muscle of hyperglycemic type 1 diabetic patients. Nevertheless, incubation of human muscle cells with a high glucose concentration (15 mmol/l) promoted a marked increase in the potency of IL-6 to induce SOCS-3 expression, even at a low concentration of the cytokine. It was notable that in the presence of 5 mmol/l glucose, physiological concentrations of IL-6 had at most a marginal effect on SOCS-3 expression in cultured human muscle cells. Increasing the concentration of glucose in the medium was associated with about a twofold increase in SOCS-3 mRNA abundance at all concentrations of IL-6. We cannot, therefore, exclude the possibility that the combination of hyperglycemia and IL-6, in a synergistic way, participate in the increased SOCS-3 expression observed in the skeletal muscle of type 2 diabetic patients.

Because muscle can produce IL-6 (40), the difference in SOCS-3 mRNA levels in muscle might be also related to differences in the local production of IL-6 between diabetic and nondiabetic subjects. However, we were not able to detect a significant amount of IL-6 mRNA in the muscle samples of any groups (data not shown), indicating that IL-6 is not produced in the muscle at rest, an observation supported by previous reports (41,42). Increased SOCS-3 mRNA levels in muscle of diabetic patients could be the consequence of another hormone or cytokine than IL-6. For example, TNF-α is known to induce SOCS-3 expression (43). However, the plasma level of TNF-α is generally increased to a similar extent in obese nondiabetic and type 2 diabetic subjects (11). A number of other hormones, such as growth hormone, leptin, and the β-adrenergic agonist isoproterenol, have also been able to induce SOCS-3 expression in various cell systems (44–46). Their possible contribution to the differential expression of SOCS-3 in the muscle of type 2 diabetic and nondiabetic obese subjects remains to be defined.

**SOCS-3 and IL-6 expression in adipose tissue.** Previous reports have demonstrated that IL-6 is expressed in human adipose tissue (13). We confirmed these data in the present work and report a strong positive correlation between IL-6 mRNA levels in subcutaneous adipose tissue and BMI. Recently, increased expression of SOCS-3 has been reported in the adipose tissue of obese rodents (37). Moreover, it has been shown that IL-6 is able to upregulate the mRNA expression of SOCS-3 in 3T3-F442A adipose cell line (47). We demonstrated here that human obesity is also associated with exaggerated expression of SOCS-3 in subcutaneous adipose tissue. An important observation was that SOCS-3 mRNA levels were positively related with IL-6 mRNA, strongly suggesting that IL-6 may govern SOCS-3 expression through an autocrine/paracrine mechanism in human fat.

Although the correlation between IL-6 and SOCS-3 expression in adipose tissue was found in all subjects, it was notable that the type 2 diabetic subjects were characterized by lower levels of IL-6 and SOCS-3 mRNAs in fat than the obese nondiabetic subjects, despite the former having similar BMIs and circulating concentrations of IL-6. This suggests that the contribution of adipose tissue to plasma IL-6 concentrations might differ between diabetic and nondiabetic obese subjects. A previous study has indicated that visceral adipose tissue is a major contributor to IL-6...
production in humans (48). In the present study, only subcutaneous abdominal adipose tissue was investigated. The lower expression of IL-6, and consequently of SOCS-3, in subcutaneous adipose tissue of type 2 diabetic subjects could be related to different characteristics of the superficial depot, as type 2 diabetes is generally associated more with visceral fat expansion than with subcutaneous obesity (49).

In conclusion, the present work shows that the induction of SOCS-3 by IL-6 is related to a strong inhibition of insulin signaling in cultured human myotubes, suggesting that elevated expression of SOCS-3 in the skeletal muscle...
may contribute to insulin resistance. However, nondiabetic obese individuals, characterized by increased expression of IL-6 in subcutaneous adipose tissue and elevated circulating IL-6 concentrations, had a reduced expression of SOCS-3 in skeletal muscle, suggesting that SOCS-3 does not contribute to IL-6-associated insulin resistance in muscle of these subjects. In contrast, type 2 diabetic subjects, with levels of obesity and circulating IL-6 similar to those of the nondiabetic obese subjects, displayed significant increases in SOCS-3 mRNA expression in muscle, which was negatively associated with insulin-induced glucose disposal. These results suggest differences in the mechanisms by which cytokines could affect insulin sensitivity in type 2 diabetic subjects and nondiabetic obese subjects with normal glucose tolerance.

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