Interleukin-6 acts as insulin sensitizer on glycogen synthesis in human skeletal muscle cells by phosphorylation of Ser\(^{473}\) of Akt

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Interleukin-6 acts as insulin sensitizer on glycogen synthesis in human skeletal muscle cells by phosphorylation of Ser\(^{473}\) of Akt. Am J Physiol Endocrinol Metab 289:E251–E257, 2005. First published March 8, 2005; doi:10.1152/ajpendo.00448.2004.—Previous studies showed an insulin–“desensitizing” action of IL-6 on glycogen synthesis in hepatocytes. We recently found no inhibition of the proximal steps of the insulin signal cascade in human skeletal muscle cells. Because these data indicate a possible tissue-specific effect of IL-6, we investigated the influence of IL-6 on insulin-stimulated glycogen synthesis in these cells. At first, we found that incubation of the cells with 20 ng/ml IL-6 alone induced phosphorylation of Ser\(^{473}\) of Akt, but not of Thr\(^{308}\) time dependently and we observed that IL-6 augments insulin-induced Ser\(^{473}\) and Thr\(^{308}\) phosphorylation in the low nanomolar range of insulin. Moreover, IL-6 increased insulin-stimulated phosphorylation of glycogen synthase kinase-3. Accordingly, IL-6 enhanced glycogen synthesis in the presence of 3 and 10 \(\mu\)M insulin, whereas IL-6 alone had only a marginal effect. IL-6 treatment of C57Bl/6 mice readily stimulated phosphorylation of Ser\(^{473}\) in skeletal muscle. Our result that IL-6 did not induce Ser\(^{473}\) phosphorylation in the liver of these mice suggests a tissue-specific effect. Together, our data demonstrate a novel insulin-sensitizing function of IL-6 on glycogen synthesis in skeletal muscle cells and indicate that IL-6 exerts cell/tissue-specific effects on insulin action.

insulin signaling; signaling; Akt; muscle; liver

There is increasing evidence that the cytokine interleukin-6 (IL-6), besides its role in the inflammatory network, may influence metabolism and insulin action. The observed correlation of IL-6 plasma concentrations with fat tissue mass and insulin resistance provides the first clue for IL-6 as a metabolic factor (1, 20). Ten to thirty-five percent of the body’s basal circulating IL-6 is derived from adipose tissue (7, 17, 21). In individuals with obesity and overt type 2 diabetes, IL-6 plasma levels are two- to three-fold higher than in control subjects and are associated with reduced insulin action (1, 8, 12, 15, 20). By studying the interaction of IL-6 with insulin-signaling pathways, it has been shown, both by IL-6 infusion in vivo in mice (14) and on the cellular level in hepatocytes (26, 27), that IL-6 induces hepatic insulin resistance. Chronic exposure to IL-6 causes impairment of early insulin receptor signaling in the liver of mice, resulting in reduced whole body insulin sensitivity (14). In both human hepatocarcinoma cell line Hep G2 and primary hepatocytes, IL-6 inhibits insulin receptor signal transduction and downstream insulin action, particularly glycogen synthesis (26). An IL-6-induced cellular insulin resistance was also observed in human subcutaneous adipose cells, e.g., reduction of insulin-stimulated tyrosine phosphorylation of insulin receptor substrate (IRS-1), a decrease in insulin-mediated glucose uptake, and long-term inhibitory action on the gene transcription of IRS-1, glucose transporter-4 and peroxisome proliferator-activated receptor-γ (24). Thus increases in plasma concentrations of IL-6 may accelerate the development of the metabolic syndrome and insulin resistance, which are characterized by hyperinsulinemia and hypertriglyceridemia.

Moreover, IL-6 has been shown to exert antiobesity effects and to increase blood glucose. In fact, IL-6-deficient mice develop mature-onset obesity and show decreased glucose tolerance, which could be partly reversed by IL-6 replacement (34). IL-6 infusion stimulated lipolysis and whole body fat oxidation in humans (33). In the liver, IL-6 elevated glucose output by inhibition of glycogen synthase and activation of glycogen phosphorylase (11, 32). Thus IL-6 counteracts the effects of insulin in adipose tissue and liver on lipolysis, glycogenolysis, and gluconeogenesis.

On the other hand, the markedly increased (\(\leq\)100-fold) expression of IL-6 in skeletal muscle during prolonged exercise suggests further biological functions of IL-6 (5, 6, 18, 19). It could be demonstrated that IL-6 contributes to the increase in whole body glucose disposal rate and in the metabolic clearance rate of glucose during exercise (4). Thus IL-6 may act on glucose uptake and glucose metabolism in skeletal muscle in an insulin-like manner. Accordingly, we (35) found no inhibitory action of IL-6 on proximal insulin signaling events in human myotubes. Therefore we hypothesize that the cross talk of IL-6 with insulin-signaling pathways in skeletal muscle cells differs from the observed IL-6-mediated cellular insulin resistance in hepatocytes and adipocytes.

Following this line, in the present study we investigated the effect of IL-6 on insulin action in human myotubes. We were able to demonstrate a novel insulin-sensitizing function of IL-6 on glycogen synthesis in skeletal muscle cells and we were able to show that IL-6 exerts tissue-specific effects on insulin-signalin molecules probably leading to the opposite results found in hepatocytes compared with the data of the present study.

RESEARCH DESIGN AND METHODS

Materials. Cell culture media and supplements were from Gibco (Eggenstein, Germany). Phosphatase inhibitors and human and mouse recombinant IL-6 were from Sigma (Munich, Germany); protease...
Inhibitor mixture was purchased from Roche (Mannheim, Germany), antibodies against phospho-STAT3 (signal transducer and activator of transcription-3) Tyr705, STAT3, phospho-Akt Ser473, phospho-Akt Thr308, and phospho-glycogen synthase kinase-3 (GSK-3) Ser921 were from Cell Signaling (Frankfurt, Germany); antibodies against Akt were from BD Biosciences (San Diego, CA); antibodies against GSK-3α/β were from Santa Cruz Biotechnology (Santa Cruz, CA); and α-[U-14C]glucose (250–360 mCi/mmol) was from PerkinElmer Life Sciences (Belgium).

In vivo IL-6 treatment of mice. Ten-week-old male C57Bl/6 mice were obtained from the Jackson Laboratory and studied after 2 wk of acclimatization. They were maintained on a normal light-dark cycle and kept on a regular diet. For in vivo stimulation, a bolus of 50 ng of mouse recombinant IL-6 was injected intraperitoneally. For short-term IL-6 or insulin effects, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body wt) and xylazine (10 mg/kg body wt), and a total of 50 ng of mouse recombinant IL-6 or 5 IE insulin were injected into the inferior vena cava. Controls received a comparable amount of diluent. Tissues (liver, muscle) were removed at the indicated time points and homogenized at 4°C by Potter homogenization in lysis buffer (50 mM Tris, pH 7.6, 150 mM NaCl, and 1% Triton X-100 containing phosphatase inhibitors and protease inhibitor mixture) (10). Homogenates were allowed to solubilize for 30 min on ice and clarified by centrifugation at 12,000 g for 20 min. The supernatant was then collected and protein content determined by the Bradford method. All procedures were approved by the local Animal Care and Use Committee.

Cell culture. Primary skeletal muscle cells were grown from satellite cells obtained from percutaneous needle biopsies performed on the lateral portion of quadriceps femoris (vastus lateralis) muscle, as recently described (16). The donors were normal-weight, healthy Caucasian subjects. The study was approved by the local ethics committee, and informed written consent had been obtained from all subjects before the biopsy. All experiments were performed on the first pass of subcultured cells that were plated at ~5 × 10^4 cells in 60-cm² dishes in a 1:1 mixture of α-MEM and Ham’s F-12 supplemented with 20% fetal calf serum (FCS), 1% chicken embryo extract, and 0.2% antibiotic antimycotic solution (growth medium), as described (16). When myoblasts reached 80–90% confluence, the cells were fused for 5 days in α-MEM containing 5.5 mM glucose with 2% FBS and 0.2% antibiotic antimycotic solution (fusion medium). Stimulation of the myotubes with IL-6 and insulin was performed in fusion medium. Hep G2 hepatoma cells were cultured in DMEM containing nonessential amino acids supplemented with 2 mM glutamine, 1 mM pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 10% FCS. Phorbol 12-myristate 13-acetate (50 ng/ml) was used as a differentiation factor. 3T3-L1 preadipocytes were differentiated into adipocytes by incubation in DMEM containing 10% FBS and 0.2% antibiotic antimycotic solution (growth medium), as described (30). When the adipocytes reached 80–90% confluence, the cells were fused for 5 days in DMEM containing 0.5% FCS and 0.2% antibiotic antimycotic solution (fusion medium). Stimulation of the adipocytes with IL-6 and insulin was performed in fusion medium. D12 rat myoblasts were cultured in MEM containing nonessential amino acids supplemented with 2 mM glutamine, 1 mM pyruvate, 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 10% FCS. A549 lung epithelial cells were cultured in DMEM containing 10% FCS and 0.2% antibiotic antimycotic solution (growth medium), as described (30).

Western blotting. Cells were lysed with 600 μl of lysis buffer/10-cm dish (50 mM Tris, pH 7.6, 150 mM NaCl, and 1% Triton X-100, containing protease and phosphatase inhibitors). Cytosolic extracts of myotubes were separated by sodium dodecyl sulfate-polyacrylamide (7.5%) gel electrophoresis. Proteins were transferred to nitrocellulose by semidry electroblotting, and immunodetection was performed as described (35).

Glycogen synthesis. Glycogen synthesis assay was as described (16), with modifications. Fused myotubes in six-well plates were pretreated for 90 min with IL-6 (20 ng/ml) in serum-free α-MEM containing 5.5 mM glucose. Cells were then incubated with 1–100 nM insulin for 30 min before addition of α-glucose/α-[U-14C]glucose (10 mM final concentration, 0.3 μCi/well). After 60 min at 37°C, the supernatants were aspirated, and the cells were washed three times with ice-cold PBS and lysed in 300 μl of 30% KOH. Aliquots (10 μl/well) were removed for determination of protein content. The extracts were heated for 30 min at 95°C, and glycogen (2 mg/ml final concentration) was added as carrier. Glycogen was precipitated with 550 μl of 95% ethanol and collected by centrifugation for 10 min at 10,000 g. The glycogen pellet was resuspended in 500 μl of water and counted by liquid scintillation counting. Glycogen synthesis was expressed as counts per minute per well per microgram of protein.

Statistical analysis. Results presented are derived from at least three independent experiments. Means ± SE were calculated, and groups of data were compared using Student’s t-test. Statistical significance was set at P < 0.05.

RESULTS

IL-6 induces Ser473 phosphorylation of Akt. The serine/threonine kinase Akt is a key signaling molecule, e.g., regulating insulin effects on glycogen synthesis and protein synthesis (25). Phosphorylation of Akt on Ser473 and Thr308 by insulin leads to phosphorylation and to inactivation of GSK-3 and subsequently to activation of glycogen synthase (25). The phosphorylation and activation of Akt by IL-6 have been described; however, opposite results were also reported (9). Thus we studied first the Ser473 phosphorylation of Akt in IL-6-stimulated myotubes. IL-6 treatment of myotubes induced phosphorylation of Ser473 of Akt in a time-dependent manner with a maximum increase after 20 min (Fig. 1A). The phosphorylation was still detectable after 180 min and was completely blocked in the presence of 100 nM wortmannin (Fig. 1A). The phosphorylation was clearly observed using 10 and 30 ng/ml IL-6 (Fig. 1B). For further studies we used 20 ng/ml IL-6. In contrast, no IL-6-mediated increase in basal Thr308 phosphorylation of Akt was detectable (Fig. 1C), whereas insulin stimulated the phosphorylation of Thr308 in human myotubes as expected dose dependently (Fig. 1D).

IL-6 enhances insulin-stimulated phosphorylation of Akt and GSK-3. Next, we investigated the effect of IL-6 on insulin-stimulated Akt phosphorylation. Preincubation of the myotubes with IL-6 increased insulin-induced phosphorylation of both Ser473 (Fig. 2A) and Thr308 (Fig. 2B) of Akt. This insulin-sensitizing action of IL-6 was clearly detectable using concentrations of insulin in the nanomolar range, whereas after stimulation with 100 nM insulin no further increase by IL-6 was detectable (data not shown). Accordingly, we found also that IL-6 pretreatment of myotubes augmented the phosphorylation of the Akt substrate GSK-3α on Ser21 (Fig. 2C). This effect is clearly detectable after stimulation with 3 and 10 nM insulin. We observed no phosphorylation of Ser9 of GSK-3β in the myotubes, although this isofrom is present in the myotubes (data not shown).

IL-6 increases insulin-stimulated glycogen synthesis in human myotubes. Next, we investigated the effect of IL-6 on glycogen synthesis. As shown in Fig. 3A, preincubation of myotubes with 20 ng/ml IL-6 for 90 min increased insulin-stimulated glycogen synthesis. This effect was significant using 3 and 10 nM of insulin, whereas the effect of 100 nM insulin on glycogen synthesis could not be further enhanced by IL-6. IL-6 alone had only a marginal effect. When we added IL-6 and insulin simultaneously, we found similar results (Fig. 3B).

IL-6 induces Ser473 phosphorylation of Akt in mouse skeletal muscle but not in liver and hepatoma cells. The insulin-sensitizing action of IL-6 on glycogen synthesis is in clear contrast to the results obtained in hepatocytes, hepatoma cells (Hep G2), and rat liver (14, 26, 27). According to those reports, IL-6 inhibits insulin-stimulated glycogen synthesis. Because our data suggest that Ser473 phosphorylation of Akt is the prerequisite for the IL-6-mediated augmentation of insulin
signaling, we studied whether this phosphorylation of Akt occurs also in Hep G2 cells and in the liver of IL-6-treated mice. Using identical conditions to those in the experiments with human myotubes, we detected no IL-6-inducible Ser473 phosphorylation of Akt in Hep G2 cells, whereas stimulation with insulin resulted in a strong, dose-dependent phosphorylation, as expected (Fig. 4A). To examine the IL-6 effect on Ser473 phosphorylation in intact animals, we treated C57Bl/6 mice with IL-6 intravenously for 5 min or intraperitoneally for 15–60 min. According to the results obtained in cell culture experiments, no increase in the basal phosphorylation of Ser473 was observed in the liver (Fig. 4B), whereas this was clearly visible after intravenous injection of insulin (Fig. 4C). In contrast, in skeletal muscle IL-6 induced the phosphorylation of Ser473 as well as after intravenous and intraperitoneal injection (Fig. 4D). Well in line with the results obtained in myotubes, no phosphorylation of Thr308 of Akt was detectable in muscle, whereas insulin treatment induced a strong phosphorylation of this site (Fig. 4E). We found in both tissues, liver and muscle, an IL-6-induced phosphorylation of STAT3, particularly in liver after intravenous injection of IL-6 (Fig. 4, B and D). Thus, although we could demonstrate in skeletal muscle and liver that IL-6 activated intracellular signaling pathways, it was only in skeletal muscle that we observed IL-6-dependent Ser473 phosphorylation of Akt.

**DISCUSSION**

In this study, we describe for the first time an insulin-sensitizing action of IL-6 in skeletal muscle cells. Using human myotubes as a well-established cell culture model, we found that IL-6 enhances insulin-stimulated glycogen synthesis and phosphorylation of both Akt and GSK-3. These insulin-sensitizing effects of IL-6 were found in the nanomolar range of insulin when the stimulatory effect of insulin on glycogen synthesis had not reached its maximum in the human myotubes (16).

The fact that the observed effects of IL-6 on insulin signaling and action (glycogen synthesis) were obtained with insulin levels in the nanomolar range may be explained by the low insulin responsiveness of human myotubes (16) compared with intact skeletal muscle, indicating that the cell culture data cannot be quantitatively compared with the in vivo situation. Considering the dose responses of IL-6, we observed maximal effects of IL-6 at 20 ng/ml, i.e., at much higher concentrations than serum IL-6 levels observed in human subjects. However, recent data clearly show that local concentrations of IL-6 are ~100-fold higher in tissues; in particular, the local concentrations of IL-6 in the interstitial muscles are increased ≥1 ng/ml after exercise (23). Furthermore, a recent report shows that the local IL-6 concentrations in the interstitial fat are 100 times higher than IL-6 plasma concentrations (29). Together with our
data obtained in intact animals, the present results indicate that the observed effects may be biologically relevant.

As a possible molecular mechanism for the insulin-sensitizing action of IL-6 in myotubes, our results implicate the IL-6-induced phosphorylation of Ser\(^{473}\) of Akt. IL-6 treatment of human myotubes induces the phosphorylation of Ser\(^{473}\) also in the absence of insulin. This phosphorylation was found as early as after 5 min of IL-6 treatment and increased within 20 min to its maximum, whereas Thr\(^{308}\) phosphorylation was unchanged. The time course of IL-6-induced Akt phosphorylation is well in line with our finding, that both preincubation with IL-6 for 90 min and simultaneous addition of IL-6 and insulin led to an essentially identical increase of insulin-stimulated glycogen synthesis.

IL-6 treatment alone had only marginal effects on glycogen synthesis. This could easily be explained by our observation that phosphorylation of Thr\(^{308}\) of Akt is not increased by IL-6. Thr\(^{308}\) of Akt is phosphorylated by phosphatidylinositol (PI) 3-kinase-mediated 3-phosphoinositide-dependent kinase-1 activation. Phosphorylation of Thr\(^{308}\) which is located within the activation T-loop of Akt, is important for enzymatic activity, whereas phosphorylation of Ser\(^{473}\) brings 3-phosphoinositide-dependent kinase-1 into proximity to Thr\(^{308}\). Thus, although phosphorylation of Thr\(^{308}\) strictly governs the activation of Akt, phosphorylation of Ser\(^{473}\) renders Thr\(^{308}\) more susceptible to phosphorylation (25). Therefore, IL-6-induced Ser\(^{473}\) phosphorylation alone is not sufficient to activate Akt, the prerequisite for the activation of glycogen synthase through inhibition of GSK-3. However, in the presence of nanomolar concentrations of insulin, this preactivated state of Akt led to an enhanced Thr\(^{308}\) phosphorylation and presumably to an increased activity of Akt and glycogen synthase compared with the stimulation with insulin alone.

![Image](53x110)
Our finding that IL-6-induced phosphorylation of Ser473 is wortmannin sensitive is well in line with previous reports demonstrating the activation of the PI 3-kinase cascade by IL-6 (3, 9). Of note, this activation is observed in a cell type-specific manner. In Hep G2 cells, no significant Akt activation was detected (9). These findings were accomplished by our data, demonstrating Ser473 phosphorylation of Akt in human myotubes, but not in Hep G2 cells. The presumed activation of PI 3-kinase by IL-6, however, did not result in Akt activation in the human myotubes, as phosphorylation of Thr308 is not enhanced. Thus other factors, which are activated for example through the insulin-signaling cascade, are necessary for the phosphorylation of Thr308 and subsequent Akt activation.

Because IL-6 has been shown to interfere with insulin-signaling pathways in the liver and adipocytes in an inhibitory manner and to reduce insulin-stimulated glycogen synthesis in hepatocytes, the cross talk of IL-6 with insulin-signaling pathways appears to be differently regulated depending on the cell type and the tissue. In fact, we observed the IL-6-mediated Akt phosphorylation only in myotubes and in skeletal muscle of IL-6 treated mice, but not in Hep G2 cells and liver. Thus Ser473 phosphorylation by IL-6 not only provides a possible mechanism for the insulin-sensitizing action of IL-6 but could also explain the different effects of IL-6 on insulin-stimulated glycogen synthesis in skeletal muscle and liver.

Although essentially all studies report that IL-6 impairs hepatic insulin action, some studies indicate that IL-6 may exert insulin-like actions. Stouthard et al. (31) found that acute IL-6 treatment increased both basal and insulin-stimulated glucose uptake in 3T3-L1 adipocytes and that the effects of IL-6 and insulin were additive. Febbraio et al. (4) suggest that during exercise IL-6 may play a role in enhancing whole body
glucose disposal. There were only a few reports on the effect of IL-6 and skeletal muscle insulin action. Kim et al. (13) demonstrated that IL-6 treatment reduced insulin-stimulated PI 3-kinase activation and glucose uptake in skeletal muscle whereas insulin-stimulated glycogen synthesis was not attenuated. Recently, Rieusset et al. (22) found a reduction of insulin-induced Ser^373 phosphorylation of Akt after IL-6 treatment in human skeletal myotubes. Data on phosphorylation of GSK-3 or glycogen synthesis were not reported in that study. However, studies of Klover et al. (14) showed that IL-6 infusion in mice failed to suppress skeletal muscle insulin receptor signal transduction. Their data demonstrate that IL-6 selectively impairs hepatic insulin signaling in vivo. Our data demonstrating a tissue-specific action of IL-6 on Ser^373 phosphorylation of Akt suggests a subtle modulation of insulin signaling by IL-6.

During exercise, when insulin plasma levels are usually low, skeletal muscle needs to increase the uptake of glucose and fatty acids for the generation of ATP and to quickly refill the glycogen pools (28). Thus one biological function of the contraction-induced production of IL-6 in skeletal muscle, leading to 100-fold increases in plasma IL-6 concentrations (5), may be to augment glycogen synthesis in skeletal muscle. Interestingly, IL-6 has been shown to induce glycogenolysis and increased glucose output in the liver (11, 32) and to enhance lipolysis in adipose tissue (33), but not tumor necrosis factor alpha, inhibit insulin-stimulated glycogen synthesis in rat hepatocytes. *Hepatology* 27: 1296–1303, 1998.

In conclusion, our finding is intriguing because previous results show an insulin—“desensitizing” effect of IL-6 in the tissues studied so far. The present results suggest that IL-6 may influence insulin action differently depending on the tissue: in energy-supplying tissues (e.g., liver, fat) the insulin signal is attenuated, whereas in energy-utilizing tissues (e.g., skeletal muscle) insulin action is improved.

**REFERENCES**


27. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, and Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a...


